

The 21st International Conference of the
**WORLD ASSOCIATION FOR THE
ADVANCEMENT OF VETERINARY
PARASITOLOGY**

“From EPG to Genes”

August 19-23, 2007

GENT, BELGIUM

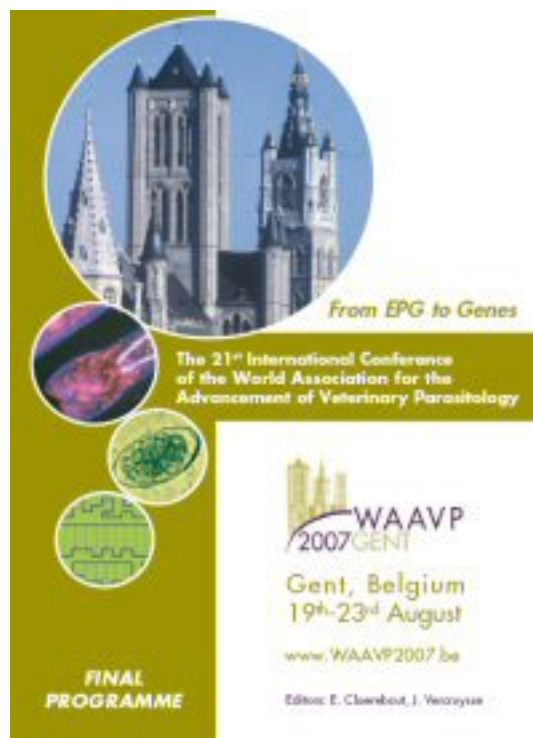


TABLE OF CONTENTS

Welcome Address	5
Conference information	6
Committees	7
Acknowledgments	9
Program WAAVP 2007	15

Abstracts: Oral Communications

Monday, 20 August 2007 130

Plenary Session I	130
EPVC Symposium: Helminth immunoregulation- An issue for veterinary clinicians?	131
Diagnosis of Parasitic Diseases	133
Biological Control	136
Mechanisms of Action of Parasiticides	139
Echinococcosis and Cysticercosis	142
Intervet Industry Forum	145
Symposium: PARASOL/DELIVER programmes on the sustainable control of nematodes and liver fluke in farm animals	146
Neospora	149
Babesia	152
Parasites of Companion Animals	155
Molecular Systematics & Phylogenetics	158
Non-human Primates	161
Meril Corporate Symposium	163

Tuesday, 21 August 2007 164

Symposium: Evidence based strategies for control of worms in dogs and cats	164
Anthelmintic Resistance	167
Epidemiology and Control of Cattle Parasites	170
Vector-Borne Protozoa	172
Haemonchus Antigens & Immunity	175
Plenary Session II	178
Janssen Corporate Symposium	
Symposium: Geospatial animal health: new ways to model the ecology of parasitism	179
Coccidia	182
Cysticercosis	185
Mechanisms of Action of Parasiticides	188
Ostertagia Antigens & Immunity	190
Symposium: Application of the three Rs in the regulation of parasiticides	188
Anthelmintic Resistance	196
Arthropods	198
Biological Control	201
Parasites of Horses	203
Bayer Corporate Symposium	206

Wednesday, 22 August 2007 207

Plenary Session III	207
Symposium: Distance learning in veterinary parasitology: a reality	208
Anthelmintic Resistance	211
Fasciola & Trematodes	214

Diseases caused by Arthropods	217
Coccidia	220
Novartis Corporate Symposium	223

Thursday, 23 August 2007

224

Symposium: Global incidence of anthelmintic resistance in sheep and cattle	224
Vector-Borne Protozoa	228
Diagnosis of Parasitic Diseases	230
Cryptosporidium & Giardia	233
Exotic Animals & Wild Life	236
Workshop: New developments in the field of Toxoplasmosis	239
Plenary Session IV	241
Fort Dodge Corporate Symposium	242
Symposium: Molecular approaches to the tick-host-pathogen interface	243
Workshop: Giardia in veterinary medicine: to consider or not to consider?	245
Epidemiology and Control of Helminth Infections in Small Ruminants	245
Fasciola & Trematodes	249
Exploiting Genomics	252
Pfizer Corporate Symposium	255

Abstracts: Poster Communications

Poster Session 1

258

Poster group 1: Helminth Infections of Small Ruminants	258
Poster group 2: Helminth Infections of Cattle	291
Poster group 3: Fasciolicides	299
Poster group 4: Miscellaneous	307
Poster group 5: Arthropods of Companion Animals	316
Poster group 6: Neospora	327
Poster group 7: Vector-Borne Protozoa	338
Poster group 8: Protozoa, miscellaneous	345
Poster group 9: Vector-Borne Protozoa	357
Poster group 10: Parasites of Poultry and Swine	365

Poster Session 2

377

Poster group 11: Helminth Infections of Horses	377
Poster group 12: Anthelmintic Resistance	388
Poster group 13: Helminths of Companion Animals	399
Poster group 14: Echinococcosis	416
Poster group 15: Fasciola and Trematodes	423
Poster group 16: Arthropods of Large Animals	442
Poster group 17: Arthropods, miscellaneous	459
Poster group 18: Coccidia	461
Poster group 19: Toxoplasmosis	471
Poster group 20: Cryptosporidium and Giardia	482

Authors' Index

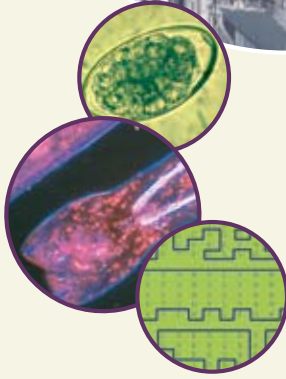
497





WELCOME ADDRESS

First, I would like to extend a warm welcome to all the participants of the 21st WAAVP conference. Many have travelled thousands of kilometres to participate but I am sure you will enjoy the meeting and appreciate the choice of excellent sessions, and that you will also have time to enjoy the beautiful city of Gent.



A WAAVP conference is not only the largest event in veterinary parasitology, but also provides all of you with the best of science, education and entertainment. It is difficult to select highlights within this programme, which has so much to offer. Abstract submissions ($n = 750$) exceeded expectation and have been accommodated by increasing the number of posters. Overall there are 8 invited lectures, 8 symposia, 32 sessions for oral presentations, 2 workshops, 6 corporate symposia and 1 industry forum, 2 poster sessions and continuous poster power point presentations.

Second, I would like to express my sincere appreciation to the authors of invited and contributed papers and to all conference participants for their active participation. I am very grateful to Edwin Claerebout, chairman of the scientific committee, the local organising and scientific committees and all the people who have worked hard in preparing the conference and the Proceedings and making the conference successful.

Finally, I would like to thank the VLIR-UOS and FWO for their financial contributions, which have allowed us to bring many young researchers from developing countries and the invited speakers to the meeting.

Quotes you may think about during the conference:

Everything you always wanted to know about why becoming a member of WAAVP

But were afraid to ask...

Find a member of the WAAVP Executive Committee, he/she will explain

Everything you always wanted to know about parasites

But were afraid to ask...

After the WAAVP conference you will confirm that the more you know, the less you understand

Everything you always wanted to know about methods for predicting the future

But were afraid to ask...

Those who have knowledge, don't predict. Those who predict, don't have knowledge. (Lao Tzu, 6th Century BC Chinese Poet)

Everything you always wanted to know about what made Albert Einstein a genius

But were afraid to ask.....

Any fool can make things bigger, more complex and more violent. It takes a touch of genius and a lot of courage to move in the opposite direction.

Everything you always wanted to know about happiness

But were afraid to ask.....

Happiness is good health and a bad memory (*Ingrid Bergman*)

Happiness doesn't come from doing what we like to do but from liking what we have to do (*Wilfred A. Peterson*)

Everything you always wanted to know about friendship among parasitologists

But were afraid to ask.....

Just find a soul mate, have a few beers on a terrace and be sure to attend all social events

Everything you always wanted to know about Gent,

But were afraid to ask...

Just take the map of Gent, sneak out of the conference building, and go for a walk

Everything you always wanted to know about Belgian beers

But were afraid to ask...

Read the leaflet in your conference bag, taste at least 10 Belgian beers each day and don't be afraid to ask for a (free) paracetamol the next morning at the registration desk

Everything you always wanted to know about the consequences of joining Jozef V one night in Gent

But were afraid to ask...

Ask everybody who tried before, don't do it!

IT IS AN HONOUR FOR ME TO WELCOME YOU IN GENT AND WE WILL TRY TO MAKE YOU NEVER FORGET YOUR STAY

JOZEF VERCRUYSE
President of WAAVP
Chairman of the 21st WAAVP conference



Committees

The Organizing and Scientific Committees of WAAVP 2007 comprise the following individuals:



Chairman Jozef Vercruysse

Local Organizing Committee

Edwin Claerebout

Pierre Dorny

Dirk de Graaf

Bertrand Losson

Administration Dirk Demeulenaere
Mieke Godefroid

Scientific Committee

Edwin Claerebout (Belgium) - **Chairman**

Dirk Berkvens (Belgium)

Fred Borgsteede (The Netherlands)

Dirk de Graaf (Belgium)

Pierre Dorny (Belgium)

Lou Gasbarre (USA)

Bruno Goddeeris (Belgium)

Dave Knox (United Kingdom)

Bertrand Losson (Belgium)

Theo Schetters (The Netherlands)

Jozef Vercruysse (Belgium)

Peter Willadsen (Australia)

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Acknowledgments

The Committees of WAAVP 2007 and the World Association for the Advancement of Veterinary Parasitology gratefully acknowledge the support of the following institutions and companies and wish to thank them for their contribution:

Institutions



VLIR-UOS Cooperation



Fund for Scientific Research (FWO) - Flanders



Louisiana State University (LSU) Faculty of Parasitology

WAAVP African Foundation

Major Partners



Bayer Healthcare Animal Health



Fort Dodge Animal Health



Janssen Animal Health



Merial



Novartis Animal Health



Pfizer Animal Health

Partner



Intervet International BV

Others



City of Ghent



Ghent University



Brussels airlines

GENERAL INFORMATION

Venue and Dates

The 21st Conference of the World Association for the Advancement of Veterinary Parasitology takes place at the ICC - International Convention Centre Gent, 19-23 August 2007

ICC- International Convention Centre
Citadelpark, 9000 Gent, Belgium

During the Conference:

Phone: +32 (0)9 233 86 60//+32 (0)9 242 89 45

Fax: +32 (0)9 242 89 01

Mobile Phone Policy

Delegates are advised that, in accordance with WAAVP policy, mobile phones always have to be switched off in the meeting rooms.

Language

The official language of the Conference is English.

Registration Desk, Information Desk and Conference Secretariat

OPENING HOURS:

Sunday, 19 August	14:00-19:00
Monday, 20 August	08:00-18:00
Tuesday, 21 August	08:00-18:00
Wednesday, 22 August	08:00-14:00
Thursday, 23 August	08:00-18:10

Information on Conference Social Events is available at desks.

Tickets for booked social events are included in your registration documentation. If you cannot attend an event, please, return your ticket to the Information Desk. Tickets for the Accompanying Persons Program can also be booked here.

Cloakroom

A cloakroom will be open throughout the Conference. It will be possible to store luggage in this cloakroom area.

Lost and Found

For lost and found personal belongings, please, contact the Information Desk in the registration area.

Name badge

Your personal badge is your entrance ticket to all sessions and the exhibition. Please, remember always to wear your badge at the ICC and the social events. If you lose your badge, a new one can be purchased against proof of your original registration, from the Conference secretariat at a cost of 4.00 Euro.

First Aid

In case of emergency, please, contact the Information Desk.

Coffee breaks and Meals

Coffee/tea and lunches are included in the registration fee and will be served daily in the Exhibition Area (Artevelde Forum).

Climate and Dress

The weather in Ghent at this time of the year is usually sunny with temperatures around 18-22°C. An umbrella might be useful as showers can occur. Dress will be informal throughout the Conference.

Time Zone

The time zone in Ghent is GMT + 1 hour.

Banks and Post Office

Most banks open at 09.00 and close around 16.00 Monday through Friday. They are generally closed for lunch between 12.30-14.00. Post Offices are generally open between 09.00 and 16.00.

Please, note that there will be no exchange facilities at the International Convention Centre.

Electricity

The voltage in Belgium is 220V, 50 Hz.

Exhibition

The Exhibition is held at the Artevelde Forum.

Opening Hours:

Sunday, 19 August	19:00-20:30
Monday, 20 August	08:30-18:00
Tuesday, 21 August	08:30-18:00
Wednesday, 22 August	08:30-14:40
Thursday, 23 August	08:30-18:00

SOCIAL PROGRAM

Welcome Reception

Sunday, 19 August (19:30-21:00)

(ICC, Citadelpark, 9000 Gent)

During this welcome reception, everybody will have the opportunity to meet old friends and make new ones.

Medieval Evening at the Castle of the Counts

Monday, 20 August (20:00-23:45)

(Gravensteen, Sint-Veerleplein-Gent)

WAAVP 2007 and Merial



invites you to take part in this event.

Evening at the Eskimo Site

Tuesday, 21 August (20:00-23:00)

(Wiedauwkaai 25, Gent)

WAAVP 2007 and Bayer



invites you to take part in this event.

Banquet at the St. Peter's Abbey

Thursday, 23 August

(20:00-01:00)

(St. Pietersabdij, St. Pietersplein, Gent)

Meeting Point: 19:00: Graslei- Gent

WAAVP FUN RUN GENT

Wednesday 22 August

(16:00-17:30)

(Blaarmeersen, Zuiderlaan 5-Gent)

Everyone completing the 5 km fun run course will receive an exclusive 'I survived the Gent Fun Run' T-shirt and special Belgian prizes will be awarded on the basis of individual and team performances. The teams will consist of 4 runners and can be composed per country, laboratory, department etc. The time of each participant over the 5 km will be registered and used to calculate the combined team time. The event is kindly sponsored by Bayer who will donate 2 Euros to the Peter Nansen Fund for every km completed by the participants



ACCOMPANYING PERSONS' PROGRAM

Brussels Sightseeing

Monday 20 August

Departure Point: ICC, Gent

Departure Time: 09:00

Arrival Back: 18:00, ICC

EURO: 77 per person (lunch included, beverage not included)

Visit to Antwerp:

Rubens and Diamonds

Tuesday, 21 August

Departure Point: ICC, Gent

Departure Time: 09:00

Arrival Back: 18:00, ICC

EURO: 78 per person (lunch included, beverage not included)

Guided Walk of Gent

Wednesday 22 August

Departure Time at Meeting Point: 09:20

Meeting Point for persons lodged in the Inner City:

Tourists Office – Belfry (Belfort)

Departure Point for persons accommodated outside the Inner City: ICC, Gent

Departure Time Coach: 09:00

Arrival Back: 13:00, ICC

Included in the registration fees for delegates and registered accompanying persons.

Bruges, the Venice of the North

Thursday 23 August

Departure Point: ICC, Gent

Departure Time: 09:00

Arrival Back: 17:00, ICC

EURO: 77 per person (lunch included, beverage not included)

SCIENTIFIC INFORMATION

Abstract Book

All accepted abstracts are published in the WAAVP Abstract Book, included in your Conference Bag.

Certificate of attendance

A certificate of attendance is included in your registration documentation.

Slide Preview Room

The slide preview room (Bauwens Room) is located at the second floor and will be open from one hour before the first session until half an hour after the last session of the day.

Please, hand in your presentation on CD-ROM (or USB-stick) at least two hours or even better the day before your presentation is scheduled.

Poster exhibition

All posters are displayed at the "Artevelde-Pedro de Gante Forum" for two consecutive days. Materials required to mount posters will be available. The maximum height of the poster boards is 2.4 m and the maximum width is 0.95 m.

Instructions for Poster Presenters

- Posters for Poster sessions I on Monday (20 August) and Tuesday (21 August) should be mounted at the latest on Monday (20 August) before 10:00 and left on display till Tuesday (21 August) 16:00.
- Posters for Poster sessions II on Wednesday (22 August) and Thursday (23 August) should be mounted on Wednesday (22 August) before 10:00 and left on display till Thursday (23 August) 16:00.

Poster Discussion Sessions

Presenting authors are asked to attend their poster during the Poster Sessions, as indicated here:

- For Poster Groups 1 through 4: on Monday 20 August, 12:30-13:30.
- For Poster Groups 5 through 10: on Tuesday 21 August, 13:00-14:00.

- For Poster Groups 11 through 15: on Wednesday 22 August, 13:10-14:10.
- For Poster Groups 16 through 20: on Thursday 23 August, 13:00-14:00.

Instructions for Oral Presenters

- Please, hand in your presentation on CD-ROM (or USB-stick) in the slide preview (Bauwens) room at least two hours or better the day before your presentation is scheduled.
- Speakers are requested to come to the meeting room at least 10 minutes prior to the start of the session and identify themselves to the chair.
- Speakers of regular oral communications have been allocated 15 minutes (including discussion).

Instructions for Session Chairs

- Before the session, check with the room technician to see if there are no-shows or last-minute changes to the program.
- You must be present in the room 10 minutes before the start of your session to confirm the names of the presenting authors.
- You should instruct each author as to the time allocated to his/her paper. Each regular presentation has been allocated 15 minutes (including discussion).
- Your task is to moderate the session to facilitate both the presentations and the ensuing discussion.
- You should introduce each speaker.
- If a presenter fails to show up, you may either continue discussion of the previous paper or call a break - **PLEASE, DO NOT call any paper before its scheduled time.**
- Please, keep absolutely to the schedule - this allows people to move between sessions with a minimum of confusion.

Outline of the Scientific Information

A lay-out of all sessions day by day introduces the complete Scientific Program.

CONFERENCE PROGRAM

MONDAY 20 AUGUST



ROOM	CONGRESS ROOM					
09h00	Opening Ceremony					
10h30	Coffee Break					
11h00	African trypanosomiasis: from immune escape and immuno- pathology to immune intervention Patrick De Baetselier	001				
11h30	The effect of nematode infections on vaccination to protozoal and bacterial infection Joe Urban	002				
12h00	Lunch Break& Poster Session I : PG 1-PG 4		VAN RYSELBERGHE	BAECKELANDT	JAN VAN EYCK	HUBERT VAN EYCK
13h30	EPVC Symposium: Helminth immunoregulation - An issue for veterinary clinicians?		Diagnosis of Parasitic Diseases	Biological Control	Mechanisms of Action of Parasiticides	Echinococcosis and Cysticercosis
13h30	Interactions between helminths and tuberculosis in man	003	Egg excretion by gastrointestinal strongyles in dairy goats: circadian rhythm and relation with worm burden	Tanniferous forage plants with anthelmintic properties: the example of sainfoin (<i>Onobrychis viciifolia</i>)	Chemical genetic approach to novel neurological receptor discovery in <i>Caenorhabditis elegans</i>	Canine echinococcosis in Kyrgyzstan
13h45	Helminths and malaria - interactions in man	004	Sequential patterns of serum and milk antibodies following experimental infection with <i>Dictyocaulus viviparus</i> in lactating dairy cows	Effects of sainfoin (<i>Onobrychis viciifolia</i>) extracts on the establishment of abdominal nematode larvae in fundic explants	Modulation of nematode and arthropod reproductive systems by anthelmintics, with special focus on ivermectin	Cross-sectional survey on the prevalence of <i>Echinococcus multilocularis</i> and <i>E. granulosus</i> infections in domestic dogs in Germany
14h00	The immunoepidemiology of nematode infections in humans	005	A survey to determine the range of bulk tank milk antibody titres to <i>Ostertagia ostertagi</i> in West European countries	Influence of inulin on <i>Trichuris suis</i> infection in pigs: elimination by metabolic products	The nicotinic acetylcholine receptors of <i>Ascaris suum</i>	Prevalence of <i>Echinococcus multilocularis</i> in wild and domestic hosts in Lithuania
14h15	Immunomodulation in <i>Fasciola hepatica</i> infection; implications for animal health	006	An allergen protein for <i>Fasciola hepatica</i> diagnosis	The effect of dietary fructans on <i>Oesophagostomum dentatum</i> and <i>Ascaris suum</i> infections in pigs	Temporal synergism renews efficacy of ivermectin in resistant roundworms	Endogenous development in the countermeasure of multilocular echinococcosis in red foxes: a successful and sustainable approach
14h30	<i>Dirofilaria immitis</i> - clinically relevant immunoregulation in dogs?	007	Towards a quantitative interpretation of the existing coprological and serological techniques for the diagnosis of fasciolosis in cattle	An increasing level of maize supplementation provides better control of gastrointestinal nematodes in browsing Criollo goats	Modulation of P-glycoprotein enhances ivermectin and moxidectin systemic availabilities and their efficacy against resistant nematodes in cattle	Evaluation of a serological method for the detection of <i>Taenia saginata</i> cysticercosis using serum and meat-juice samples
14h45	Discussion	008	Estimating the prevalence of <i>Fasciola hepatica</i> in cattle: which method is the best diagnostic test?	Effect of <i>Senecio jacobaea</i> hay feeding on growth rate of goats infected with gastrointestinal nematodes	Discussion	Risk factors for bovine cysticercosis in Switzerland
15h00	Coffee Break	009	Coffee Break	Coffee Break	Coffee Break	Coffee Break
						Intervet Industry Forum
						031 032

	CONGRESS ROOM	VAN RYSSELBERGHE	BAECKELANDT	JAN VAN EYCK	HUBERT VAN EYCK	JAN VAN DER GOES
15h30	Symposium: PARASOL/DELIVER programmes on the sustainable control of nematodes and liver fluke in farm animals	15h30	Babesia	Parasites of Companion Animals	Molecular Systematics & Phylogenetics	Non-human Primates
15h30	Targeted selective treatments an innovative strategy for the control of nematode infections in sheep and goats	15h30	A descriptive epidemiological study on canine babesiosis in the Lake Geneva region	Molecular-based prediction of the geographical spread of <i>Angiostrongylus vasorum</i> in dog and fox populations	Genetic and phenotypic divergence in Australian <i>Haemonchus contortus</i>	Zoonotic nematode parasites in wild caught African green monkeys (<i>Chlorocebus sabaeus</i>) on St Kitts, West Indies
15h40	Assessment of the repeatability and reproducibility of a serum- pepsinogen assay and an indirect milk ELISA to monitor gastrointestinal-nematode infections in cattle	033	039	051	057	063
15h50	The development and standardisation of methods for detecting anthelmintic resistance in gastrointestinal nematodes of ruminants	034	040	052	058	064
16h00	The epidemiology of fasciolosis in Europe	035	041	053	059	065
16h10	Fasciolicide resistance in liver fluke	036	042	054	060	066
16h20	Development of vaccines against <i>Fasciola hepatica</i>	037	043	055	061	067
16h30	Discussion	038	044	056	062	
17h00	Transition time	Transition time	Transition time	Transition time	Transition time	Transition time
17h10	Merial Corporate Symposium	068-069				
18h10	End					
20h00- 24h00	Medieval Evening Castle of the Counts					



CONFERENCE PROGRAM

MONDAY 20 AUGUST



Tuesday 21 August

	VAN RYSSELBERGHE	BAECKELANDT	JAN VAN EYCK	HUBERT VAN EYCK	JAN VAN DER GOES
	Symposium: Evidence based strategies for control of worms in dogs and cats	Anthelmintic Resistance	Epidemiology and Control of Cattle Parasites	Vector-Borne Protozoa	Haemonchus Antigens & Immunity
08h30	Toxocara canis in Swiss dogs: temporal aspects of different anthelmintic treatment procedures 070	P-glycoproteins in <i>Haemonchus contortus</i> : ivermectin and moxidectin select for constitutive overexpression and induce further overexpression of P-glycoprotein 075	Horn fly (<i>Haematobia irritans</i>) olfactometric responses to cattle host odour in a Y-tube 081	Characterization of South African <i>Theileria equi</i> and <i>Babesia caballi</i> isolates based on 18S rRNA gene sequences 086	Immune cell kinetics in sheep abomasal tissues following infection with <i>Haemonchus contortus</i> L3 larvae 092
08h45	Induction of patent <i>Toxocara canis</i> infections in previously exposed and in helminth free, regularly treated or untreated young dogs after low dose inoculations with embryonated eggs 071	Investigations into the effects of P-glycoprotein and/or cytochrome P450 inhibitors on the phenotypic responses of various developmental stages of ovine nematodes 076	Effect of cypermethrin selection pressure and substitution with amitraz on phenotypic and genotypic measures of cypermethrin resistance in field populations of <i>Beophilus microphilus</i> in the Mexican Tropics 082	Genetic diversity of South African <i>Theileria parva</i> isolates 087	Immune responses to <i>Haemonchus contortus</i> primary infection in susceptible (IMRA 401) and resistant (Barbados Black belly) breeds of lambs 093
09h00	Geographical information systems in health application: experience on filariasis and other vector-borne diseases 072	Pyrosequencing of two loci in the beta-tubulin gene, codons 167 and 200, in cyathostomin populations of known benzimidazole resistance status 077	Effectiveness of a community-based strategic anthelmintic treatment programme in the control of gastrointestinal nematodes and <i>Fasciola gigantica</i> in cattle in Kilolo district, Tanzania 083	The epidemiology of theileriosis in Rwanda and the significance of the Poly- morphic Immunodominant Molecule (PIM) of <i>Theileria parva</i> in the vaccine development approach 088	Vaccination against <i>Haemonchus contortus</i> : performance of native parasite gut membrane glycoproteins in Merino lambs grazing contaminated pasture 094
09h15	Infection biology of <i>Angiostrongylus vasorum</i> in foxes (<i>Vulpes vulpes</i>) with emphasis on the impact of host age, infection dose and exposure 073	Flow cytometry in anthelmintic resistance research 078	A case-control study on outbreaks of lungworm disease in dairy herds in The Netherlands 084	Impact of tsetse-transmitted trypanosomiasis: variations on a theme 089	Effect of vaccination of goats with H-9ah-GP and H11 antigens from intestinal membrane cells of <i>Haemonchus contortus</i> 095
09h30	The ESCCAP Guideline for worm control in dogs and cats 074	Single or multiple appearance of benzimidazole resistance: a microsatellite investigation of goat nematodes in two French regions 079	Original systematic combination of moxidectin pour-on and long action intestinal strongidosis. Control of the efficacy of the protocol by dosing blood pepsinogen 085	Epimastigote morphogenesis and the cell cycle of <i>Trypanosoma brucei</i> in the tsetse vector revisited 090	Characterization of the intestinal confortin structure in <i>Haemonchus contortus</i> 096
09h45	Discussion 074	Macrocyclic lactones selection on beta-tubulin in <i>Haemonchus contortus</i> : implications for cross-resistance selection to benzimidazole and macrocyclic lactone anthelmintics 080	Discussion 085	Tsetse habitat fragmentation dynamics and bovine trypanosomiasis epidemiology in the Oti river basin of Northern Togo 091	Speciation of ovine gastric nematodes using two molecular based techniques, SSCP and Pyrosequencing 097
10h30	CONGRESS ROOM				
	Coffee Break				
10h30	The biological significance and practical implications of antigenic variability in parasite populations 098				
11h00	From parasite genome to parasite control 099				
11h30	Janssen Corporate Symposium				
12h30	Lunch Break & Poster Session I: PG 5-PG 10				
	JANSSEN ANIMAL HEALTH				

Tuesday 21 August

	VAN RYSSELBERGHE	BAECKELANDT	JAN VAN EYCK	HUBERT VAN EYCK	JAN VAN DER GOES
14h00	Symposium: Geospatial animal health: new ways to model the ecology of parasitism	Coccidia	Cysticercosis	Mechanisms of Action of Parasitocides	Ostertagia Antigens & Immunity
14h00	New insights into the application of geographical information systems and remote sensing in veterinary parasitology ¹⁰⁰	Treatment of <i>Neospora caninum</i> and other apicomplexan parasites with nitazoxanide and other thiazolides interferes in protein disulfide isomerase enzyme activity ¹⁰⁵	<i>Taenia solium</i> cysticercosis in western Kenya ¹¹¹	Interaction of macrocyclic lactones (MLs) with multidrug resistance transporters ¹¹⁷	Local cellular response of sheep to infection with <i>Teladorsagia circumcincta</i> ¹²²
14h15	Climate, satellites and the ecology of vector-borne parasites ¹⁰¹	Inoculation of balb/c mice with live attenuated tachyzoites protects against a lethal challenge with <i>Neospora caninum</i> ¹⁰⁶	Prevalence of porcine cysticercosis and human taeniasis/cysticercosis in Nepal ¹¹²	Latrophilin-like proteins in parasitic nematodes ¹¹⁸	Proteomic characterisation of mucus derived from gastric mucosa during the immune exclusion of <i>Teladorsagia circumcincta</i> ¹²³
14h30	Spatial modeling tools in parasitology: ready for consumption? ¹⁰²	Eimeriosis in camelids of Miandoab region, Iran ¹⁰⁷	Taeniasis/cysticercosis in Vietnam: more questions than answers ¹¹³	In vitro and in vivo activity of the anti-cancer agent 2-methoxyestradiol (2ME), either alone or in combination with albendazole, against <i>Echinococcus multilocularis</i> metacystodes ¹¹⁹	Local cellular changes in serial abomasal biopsy samples and their association with the cost of immunity in <i>Teladorsagia circumcincta</i> infected sheep ¹²⁴
14h45	Predictive mapping of the habitat suitability for ticks ¹⁰³	Innate immune reactions against <i>Eimeria bovis</i> ¹⁰⁸	Porcine antibody isotype responses in oxfendazole treated and non treated pigs naturally infected with <i>Taenia solium</i> cysticercosis ¹¹⁴	In vitro analysis of glutathione transferases from the mite <i>Sarcoptes scabiei</i> ¹²⁰	Vaccination of calves against <i>Ostertagia ostertagi</i> with subfractions of the protective ES-thiol fraction ¹²⁵
15h00	PARAGIS - Assessing eco-climatic factors affecting the spatial distribution of helminth parasite burdens in dairy herds ¹⁰⁴	Cellular immune response to <i>Isospora suis</i> infections in piglets ¹⁰⁹	Nanobodies® as a tool for specific diagnosis of porcine cysticercosis (<i>Taenia solium</i>) ¹¹⁵	Relative activity of triclabendazole metabolites against the liver fluke, <i>Fasciola hepatica</i> ¹²¹	A comparative analysis of immunogenic excretory/secretory proteins from <i>Teladorsagia circumcincta</i> harvested at 1, 3 and 5 days post infection ¹²⁶
15h15	Discussion	Metaphylactic efficacy of diclazuril against naturally acquired <i>Eimeria alabamensis</i> infection in calves ¹¹⁰	Optimisation of the cysticercosis diagnosis in pigs through Bayesian modelling ¹¹⁶	Discussion	A calcium-dependent, structural homologue of a novel salivary apyrase from blood-feeding arthropods has been identified in <i>Ostertagia</i> third stage larvae ¹²⁷
15h30	Coffee Break	Coffee Break	Coffee Break	Coffee Break	Coffee Break

Tuesday 21 August

	VAN RYSSSELBERGHE	BAECKELANDT	JAN VAN EYCK	HUBERT VAN EYCK	JAN VAN DER GOES
16h00	Symposium: Application of the three Rs in the regulation of parasiticides: current status and prospects for innovation	Anthelmintic Resistance	Arthropods	Biological Control	Parasites of Horses
16h00	The three Rs: setting the context	Resistance of <i>Parascaris equorum</i> against ivermectin due to frequent anthelmintic treatment of foals in The Netherlands	Gene silencing of the tick protective antigens, Bm86, Bm91 and subolesin, in the one-host tick <i>Boophilus microplus</i> by RNA interference	Candidate endoparasitic fungi for use in the biological control of ovine parasites	Epidemiology and Control of Gastrointestinal Nematodes of Horses in Ukraine
16h15	Statistical support for the three Rs	Experimentally induced ivermectin resistance in <i>Ostertagia ostertagi</i> in cattle	Immunization studies in Rabbits using Gut Membrane-Bound Proteins derived from <i>Rhipicephalus appendiculatus</i> , <i>R. evertsii</i> and <i>Amblyomma variegatum</i>	Control of the sheep scab mite <i>Psoroptes ovis</i> in vivo and in vitro using fungal pathogens	Evaluation of the association between <i>Anoplocephala perfoliata</i> infection and colic in horses in Ontario, Canada
16h30	Regulatory process	The identification of cattle nematode parasites resistant to multiple classes of anthelmintics in a commercial cattle population in the US	Identification of novel antigens from the sheep scab mite, <i>Psoroptes ovis</i>	The acaricidal effect of the essential oil of <i>Artemisia siberi</i> and <i>Zataria multivlora</i> extract against adult stage of <i>Rhipicephalus (Boophilus) annulatus</i> in Iran	Age clustered pooled faecal samples as monitoring tool in cyathostomiasis in horses
16h45	The perspective from industry	Statistical approach to measure efficacy of anthelmintic treatment on horse farms	Epidemiology of <i>Dermatophytosis</i> and acaricidal efficacy of phoxim 50% in alternative housing systems during the laying period of hens	Genetic resistance to <i>Heterakis gallinarum</i> in two chicken layer lines following a single dose infection	Isolation and characterization of stage-specific antigens of <i>Gasterophilus intestinalis</i>
17h00	The CR0 perspective	Evidence of macrocyclic lactone-resistant <i>Parascaris equorum</i> on a Turkish horse farm	Genetic differences in <i>Dermatophytosis</i> <i>gallinae</i> from wild birds and layers	Length of <i>Ascaris suum</i> larvae is affected by parental genotype and location in the intestine in pigs	Larval viability and serological response in horses with long-term <i>Trichinella spiralis</i> infection
17h15	CONGRESS ROOM	Transition Time	Transition Time	Transition Time	Transition Time
17h25	Bayer Corporate Symposium 153-155				
18h25	End				
20h00-24h00	Cultural Evening				



CONGRESS ROOM	
08h30	Genetic diversity and infection levels of anisakid nematode parasites of aquatic organisms of Boreal and Austral regions Simonetta Mattiucci ¹⁵⁶
09h00	Veterinary epidemiology and Bayes: Commensalism, not parasitism Emmanuel Lesaffre ¹⁵⁷
09h30	WAAVP Awards Peter Nansen Young Scientist Award
10h00	Coffee Break

WEDNESDAY 22 AUGUST

WAAVP
2007 GENT

VAN RYSELBERGHE		BAECKELANDT	JAN VAN EYCK	HUBERT VAN EYCK	JAN VAN DER GOES
10h30	Symposium: Distance learning in veterinary parasitology: a reality!	Anthelmintic Resistance	Fasciola & Trematodes	Diseases caused by Arthropods	Coccidia
10h30	A web-based MSc course in veterinary tropical diseases: an impression of the first two years ¹⁵⁸	Why is anthelmintic resistance more common in <i>Teladorsagia circumcincta</i> than <i>Trichostrongylus colubriformis</i> ? ¹⁶³	Discovery of new molecular targets for drug & vaccine design against liver fluke ¹⁶⁹	Epidemiology of blowfly myiasis in domestic rabbits in the UK – a nationwide study ¹⁷⁵	Is toltrazuril-based prevention of piglet coccidiosis always economically justifiable? ¹⁸¹
10h45	The present and coming mobile learning ¹⁵⁹	Development of genetic crossing methods to identify genes associated with macrocyclic lactone resistance in <i>Haemonchus contortus</i> ¹⁶⁴	The alternative way of vaccine targeting, phosphoglycerate kinase (PhPGK) from the adult <i>Fasciola hepatica</i> - evaluation of the cDNA and recombinant protein vaccine protectivity ¹⁷⁰	Prevalence of <i>Anaplasma phagocytophilum</i> infection in dogs in Germany ¹⁷⁶	Revitalisation of anticoccidial sensitivity in poultry flocks by the use of a live attenuated vaccine ¹⁸²
11h00	Radically new approach required for countering progressively failing information transfer in the field of parasite management ¹⁶⁰	In vitro detection of anthelmintic resistance in <i>Haemonchus contortus</i> by larval development test ¹⁶⁵	Molecular discrimination of <i>Fascioloides magna</i> and <i>Fasciola hepatica</i> based on the species-specific ITS regions ¹⁷¹	Immunological and Serological study on the infectivity of host animals and ticks (Ixodidae, Argasidae) to CCHF virus in west Azerbaijan, Iran ¹⁷⁷	Immunostimulating complexes incorporated coccidia antigens and low toxicity plant saponins as a novel delivery system for coccidia vaccine immunization ¹⁸³
11h15	Canine vector born diseases: from the dog to the parasite. A new teaching approach to clinical parasitology ¹⁶¹	The development of diagnostic assays to evaluate triclabendazole resistance in the field ¹⁶⁶	SEM observations on the impact of a methimazole on triclabendazole (TCBZ) action against TCBZ-resistant and -susceptible isolates of <i>Fasciola hepatica</i> ¹⁷²	Seasonal and geographic variation in the epidemiology of <i>Anaplasma phagocytophilum</i> and <i>Rickettsia</i> spp. in the hard tick <i>Ixodes ricinus</i> in Bavaria, Germany ¹⁷⁸	Molecular-based identification of sarcocysts in bovine meat ¹⁸⁴
11h30	The tick game: using an interactive board game for teaching tick biology and chemical resistance counter-strategies ¹⁶²	Anthelmintic resistance survey in French dairy goat nematodes in four main breeding areas ¹⁶⁷	Uptake of triclabendazole by the liver fluke: trans-tegumental or oral? ¹⁷³	Distribution and phylogeny of intramitochondrial bacteria in Ixodid ticks ¹⁷⁹	The development of conventional and real-time PCR for the detection of <i>Besnoitia besnoiti</i> ¹⁸⁵
11h45	Discussion ¹⁶⁸	The search for mutations associated with ivermectin resistance in the ligand-gated chloride channel genes of <i>Teladorsagia circumcincta</i> ¹⁶⁸	Molecular identification and genotypic characterization of <i>Fasciola</i> spp first isolated in goats in Vietnam ¹⁷⁴	Detection of <i>Borrelia</i> spp. in natural vector <i>Ixodes ricinus</i> in the Hannover region with genus-specific and species specific PCR – a representative sampling approach of public greens ¹⁸⁰	Re-emerging cattle besnoitiosis (<i>Besnoitia besnoiti</i>) in France: update on clinical and epidemiological aspects ¹⁸⁶
CONGRESS ROOM		Transition Time	Transition Time	Transition Time	Transition Time

12h10 Novartis Corporate Symposium ¹⁸⁷⁻¹⁸⁸13h10 Lunch & Poster Session II:
PG 11-PG 15

16h00 WAAVP Fun Run



	CONGRESS ROOM	VAN RYSSSELBERGHE	BAECKELANDT	JAN VAN EYCK	HUBERT VAN EYCK	JAN VAN DER GOES
08h30	Symposium: Global incidence of anthelmintic resistance in sheep and cattle and best practices to counter resistance development	Vector-Borne Protozoa	Diagnosis of Parasitic Diseases	Cryptosporidium & Giardia	Exotic Animals & Wild Life	Workshop: New developments in the field of Toxoplasmosis
08h30	Anthelmintic resistance in small ruminants and its diagnosis in the field	First report on population structure for the <i>Leishmania major</i> vector, <i>Phlebotomus papatasi</i> sandfly using microsatellite loci	Phylogenetic analysis of filarial worm <i>Setaria digitata</i> of Sri Lanka based on CO1 and 12s rDNA genes	Molecular characterization, longitudinal prevalence and impact on performance of <i>Cryptosporidium andersoni</i> and <i>Giardia</i> infections in feedlot cattle of Western Australia and Alberta, Canada	Biodiversity and systematics of haemosporidia infecting south African tortoises	New developments in the field of Toxoplasmosis
08h45	Anthelmintic resistance in cattle - a developing problem?	189	195	201	203	213
09h00	Alternatives to anthelmintics in parasite control in sheep	190	196	202	208	214
09h15	Targeted anthelmintic treatment of sheep for resistance management: early experience in Australia and future directions	191	197	203	209	215
09h30	Parasite control in the face of existing resistance in New Zealand	192	198	204	210	216
09h45	Dissemination of best practice advice to producers and advisors	193	199	205	211	217
10h00		194	200	206	212	218
10h30	Ectoparasites: Future challenges in a changing world	221				
11h00	Structural biology in search of new therapeutics for protozoal diseases	222				
11h30	Fort Dodge Corporate Symposium	223-225				
12h30	Lunch Break & Poster Session II: PG 16-PG 20					

THURSDAY 23 AUGUST

	VAN RYSELBERGHE	BAECKELANDT	JAN VAN EYCK	HUBERT VAN EYCK	JAN VAN DER GOES
14h00	Symposium: Molecular approaches to the tick - host- pathogen interface	Workshop: Giardia in veterinary medicine: to consider or not to consider?	Epidemiology and Control of Helminth Infections in Small Ruminants	Fasciola & Trematodes	Exploiting Genomics
14h00	Identification and molecular characterization of a Vitellogenin receptor from the hard tick <i>Haemaphysalis longicornis</i> 226	Discussion	Control of parasitic gastroenteritis in sheep: modelling worm control strategies 231	<i>Fasciola hepatica</i> : a study of the vectorial capacity of four different species of lymnaeids snails commonly found in Belgium 237	The <i>Haemonchus contortus</i> genome project: an update and the issue of genome polymorphism 243
14h15	Tick-vertebrate host interface in the tick-borne flavivirus transmission 227		The past and future of <i>Nematodirus battus</i> 232	The population dynamics of <i>Lymnaea truncatula</i> in the west of Ireland and the prevalence of <i>Fasciola hepatica</i> within the intermediate host 238	Stage-specific gene expression in <i>Teladorsagia circumcincta</i> 244
14h30	A tick cysteine protease, longipain, from the babesial parasite vector <i>Haemaphysalis longicornis</i> , with possible multifunctional roles during parasite transmission 228		Metazoan-protozoan parasite co-infections and host fitness in St. Kilda Soay sheep 233	The dramatic spread of the giant liver fluke in Croatia 239	Functional genomic analyses of excretory/secretory proteins from the parasitic stages of <i>Haemonchus contortus</i> 245
14h45	Approaches to improved understanding of vector-parasite interaction within the genus <i>Theileria</i> and their Ixodid tick vectors, using genomics 229		The integration of anthelmintic treatment and grazing management for the provision of clean lambing paddocks in a summer rainfall region of Australia 234	Mechanism of <i>Centrocestus armatus</i> (digenea: heterophyidae) cercariae attachment and penetration to gills of second intermediate fish hosts, <i>Zacco temminckii</i> 240	Differentially expressed genes in infective larvae of <i>Ascaris suum</i> identified by microarray and sequencing analyses of an expressed sequence tag library 246
15h00	Discussion		Animal production performance and treatment frequency using the selective/famacha and suppressive strategy of parasite control in ewes: report of a five-year period 235	Fishborne zoonotic trematodes in domestic animals in Nghe An province, Vietnam 241	Analysis of adult <i>Fasciola gigantica</i> expressed sequence tags 247
15h15		229	Prevalence and seasonal incidence of helminth parasite infections of sheep and goats in eastern Ethiopia: Nematodes and trematodes 236	The effect of triclabendazole sulphoxide on egg production and hatch rate in the liver fluke, <i>Fasciola hepatica</i> 242	An analysis of differential transcription between adult female and male <i>Haemonchus contortus</i> using an oligonucleotide microarray platform 248
15h30	Coffee Break				
16h00	CONGRESS ROOM Pfizer Corporate Symposium 249-250				
17h00	WAAVP 2009				
17h10	WAAVP General Assembly				
18h10	End				
20h00-	Banquet St. Peter's Abbey	 Bayer HealthCare Animal Health  FORT DODGE  JANSSEN ANIMAL HEALTH   			

THURSDAY 23 AUGUST

SUNDAY 19 AUGUST

14:00-20:00 Registration
19:30-21:00 Welcome Reception (Artevelde Forum)

MONDAY 20 AUGUST

Main Congress Room

09:00-10:30 Opening Ceremony

Welcome Address	J. Vercruysse
Presidential Opening	J. Vercruysse
Honorary Member Award	R.C. Krecek
Memoriam Des Hennessy	N. Sangster, R. Pritchard
Conference Announcements	E. Claerebout

Main Congress Room

11:00-12:00 Plenary Session I: Invited lectures
Chair: Vercruysse J.

- 11:00 001 **African trypanosomiasis: from immune escape and immunopathology to immune intervention**
De Baetselier P., *Free University of Brussels, Belgium*
- 11:30 002 **The effect of nematode infections on vaccination to protozoal and bacterial infection**
Urban J., *US Department of Agriculture, USA*

Main Congress Room

13:30-15:00 EPVC Symposium:
Helminth immunoregulation- An issue for veterinary clinicians?
Chair: Mulcahy G.

The propensity of helminths to modulate immune responsiveness of their hosts is well-known. Helminth-mediated immunoregulation can lead to chronicity of helminth infections, and has also been implicated in balancing unwanted or pathological immune responses - the “Hygiene Hypothesis”. In man, bystander immunoregulation induced by helminths is beginning to be recognized as an important factor in the epidemiology of infectious diseases such as HIV-Aids, malaria and tuberculosis, and hence of direct concern to clinicians. The potential “every-day” impact of helminth-mediated immunoregulation on infectious diseases important in veterinary medicine is to a large extent unexplored, and is the topic of this symposium.

- 13:30 003 **Interactions between helminths and tuberculosis in man**
Elias D.
ACE BioScience, Odense, Denmark
- 13:45 004 **Helminths and malaria - interactions in man**
Booth M.
University of Cambridge Dept of Pathology, Tennis Court Rd, Cambridge, U.K.
- 14:00 005 **The immunoepidemiology of nematode infections in humans**
Bradley J.E.
School of Biology, University of Nottingham, University Park, Nottingham, UK
- 14:15 006 **Immunomodulation in *Fasciola hepatica* infection; implications for animal health**
Flynn R.J., and Mulcahy G.
School of Agriculture Food Science and Veterinary Medicine, University College Dublin, Delfield, Dublin 4 Ireland
- 14:30 007 ***Dirofilairia immitis* - clinically relevant immunoregulation in dogs?**
Kramer L.
Faculty of Veterinary Medicine, University of Parma, Italy
- 14:45 **Discussion**

Van Rysselberghe Room

13:30-15:00 Diagnosis of Parasitic Diseases
Chairs: Schneider T.
Bauer C.

- 13:30 008 **Egg excretion by gastrointestinal strongyles in dairy goats: circadian rhythm and relation with worm burden**
Veneziano V. 1, Rinaldi L. 1, Santaniello M. 1, Pennacchio S. 1,
Morgoglione M.E. 1, Rubino R. 2, and Cringoli G. 1
1 Department of Pathology and Animal Health, University of Naples "Federico II", CREMOPAR Regione Campania, Naples, Italy; 2 CRA-Istituto Sperimentale per la Zootecnia, Muro Lucano (PZ), Italy
- 13:45 009 **Sequential patterns of serum and milk antibodies following experimental infection with *Dictyocaulus viviparus* in lactating dairy cows**
Strube C. 1, Fiedor C. 1, Forbes A.B. 2, von Samson-Himmelstjerna G. 1, and Schnieder T. 1
1 Institute for Parasitology, University of Veterinary Medicine Hannover, Hannover, Germany; 2 Merial, Lyon, France
- 14:00 010 **A survey to determine the range of bulk tank milk antibody titres to *Ostertagia ostertagi* in West European countries**
Forbes A.B. 1, Charlier J. 2, and Vercruysse J. 2
1 Merial, Lyon, France; 2 Ghent University, Department of Virology, Parasitology and Immunology, Ghent, Belgium

- 14:15 011 **An allergen protein for *Fasciola hepatica* diagnosis**
Conceição M.A.P. 1, Castro A. 2, Silva E. 2, Correia da Costa 2, Costa I. 1, Viana A.B. 1, Senos J.C. 3, and Jorge R. 3
1 Escola Superior Agrária de Coimbra, Instituto Politécnico de Coimbra, Bencanta, Coimbra, Portugal; 2 Instituto Nacional de Saúde Dr. Ricardo Jorge.CIBP- Laboratório de Parasitologia, Porto, Portugal; 3 Organização de Produtores Pecuários de Aveiro Ílhavo e Vagos, Aveiro
- 14:30 012 **Towards a quantitative interpretation of the existing coprological and serological techniques for the diagnosis of fasciolosis in cattle**
Charlier J. 1, De Meulemeester L. 2, Claerebout E. 1, Williams D.J.L. 3, and Vercruysse J. 1
1 Ghent University, Department of Virology, Parasitology and Immunology, Merelbeke, Belgium; 2 Flanders Animal Health Center, Torhout, Belgium; 3 Veterinary Parasitology, Liverpool School of Tropical Medicine/Faculty of Veterinary Science, University of Liverpool, Liverpool, UK
- 14:45 013 **Estimating the prevalence of *Fasciola hepatica* in cattle: which method is the best diagnostic test?**
 Salem A. 1, Jacquet P. 1, Chauvin A. 2, and Dorchies P. 1
1 UMR INRA/DGER 1225, Ecole Nationale Vétérinaire de Toulouse, France; 2 UMR INRA/DGER 1034, Ecole Nationale Vétérinaire de Nantes, France

Baeckelandt Room

13:30-15:00

Biological Control

Chairs: Hoste H.

Torres-Acosta F.

- 13:30 014 **Tanniferous forage plants with anthelmintic properties: the example of sainfoin (*Onobrychis viciifolia*)**
Heckendorn F. 1, Maurer V. 1, Haering D. 2, Langhans W. 3, and Hertzberg H. 4
1 Research Institute of Organic Agriculture (FiBL), Department Veterinary Parasitology, Frick, Switzerland; 2 Research Station ART, Zurich, Switzerland; 3 ETH Zurich, Department Physiology and Animal Husbandry, Schwerzenbach, Switzerland; 4 University Zurich, Institute of Parasitology, Zurich, Switzerland
- 13:45 015 **Effects of sainfoin (*Onobrychis viciifoliae*) extracts on the establishment of abomasal nematode larvae in fundic explants**
Brunet S. 1, Jackson F. 2, and Hoste H. 1
1 Unité Mixte de Recherches 1225 INRA/DGER, Interactions Hôte Agents Pathogènes. École Nationale Vétérinaire de Toulouse, Toulouse, France; 2 Moredun Research Institute, Penicuik, Scotland, UK
- 14:00 016 **Influence of inulin on *Trichuris suis* infection in pigs: elimination by metabolic products**
Petkevicius S. 1, Thomsen L.E. 2, Bach Knudsen K.E. 3, Murrell K.D. 2, Roepstorff A. 2, and Boes J. 4
1 Lithuanian Veterinary Academy, Department of Infectious Diseases, Kaunas, Lithuania; 2 Danish Centre for Experimental Parasitology, University of Copenhagen, Faculty of Life Sciences, Copenhagen, Denmark; 3 Aarhus University, Faculty of Agricultural Sciences, Department of Animal Health, Welfare and Nutrition, Tjele, Denmark; 4 Danish Meat Association, Danish Pig Production, Kjellerup, Denmark

- 14:15 017 **The effect of dietary fructans on *Oesophagostomum dentatum* and *Ascaris suum* infections in pigs**
Mejer H. 1, Thamsborg S.M. 1, Bach Knudsen K.E. 2, and Roepstorff A. 1
 1 University of Copenhagen, Faculty of Life Sciences, Department of Veterinary Pathobiology, Frederiksberg, Denmark; 2 University of Aarhus, Faculty of Agricultural Sciences, Department of Health, Welfare and Nutrition, Tjele, Denmark
- 14:30 018 **An increasing level of maize supplementation provides better control of gastro-intestinal nematodes in browsing Criollo goats**
 Garate-Gallardo L., Torres-Acosta J.F.J., Aguilar-Caballero A.J., Sandoval-Castro C.A., Cámara-Sarmiento R., Canul-Ku H.L., Cob-Galera L., and May-Martínez M.
 Universidad Autónoma de México, Facultad de Medicina Veterinaria y Zootecnia, Mérida, México
- 14:45 019 **Effect of *Sericea lespedeza* hay feeding on growth rate of goats infected with gastrointestinal nematodes**
Terrill T.H. 1, Moore D.A. 1, Kouakou B. 1, Shaik S.A. 1, Vanguru M. 1, Mosjidis J.A. 2, Miller J.E. 3, and Burke J.M. 4
 1 Fort Valley State University, Agricultural Research Station, Fort Valley, Georgia, United States; 2 Auburn University, Department of Agronomy and Soils, Auburn, Alabama, United States; 3 Louisiana State University, Department of Pathobiological Sciences, School of Veterinary Medicine, Baton Rouge, Louisiana, United States; 4 United States Department of Agriculture, Agricultural Research Service, Dale Bumpers Small Farms Research Center, Booneville, Arkansas, United States

Jan van Eyck Room

13:30-15:00 Mechanisms of Action of Parasiticides
Chairs: Wolstenholme A.
Alvinerie M.

- 13:30 020 **Chemical genetic approach to novel neurological receptor discovery in *Caenorhabditis elegans***
Ball G. 1, Woods D. 2, Greenwood K. 2, and Gilleard J. 1
 1 University of Glasgow, Faculty of Veterinary Medicine, Bearsden Road, Glasgow; 2 Pfizer Animal Health, Sandwich, Kent
- 13:45 021 **Modulation of nematode and arthropod reproductive systems by anthelmintics, with special focus on ivermectin**
Kidd E., Mousley A., Atkinson L., Marks N.J., and Maule A.G.
 Parasitology, School of Biological Sciences, Queen's University Belfast, Belfast, UK
- 14:00 022 **The nicotinic acetylcholine receptors of *Ascaris suum***
Williamson S. 1, Walsh T. 1, Martin R. 2, Williams T. 3, and Wolstenholme A. 1
 1 The University of Bath, Department of Biology and Biochemistry, Bath, UK; 2 Iowa State University, Department of Biomedical Sciences, College of Veterinary Medicine, Ames, USA; 3 Pfizer Animal Health
- 14:15 023 **Temporal synergism renews efficacy of ivermectin in resistant roundworms**
Bingham G. 1, Jackson F. 2, Bartley D. 2, Gordon Y. 2, and Moores G. 1
 1 Rothamsted Research, Biological Chemistry, Harpenden, Herts. UK; 2 Moredun Research, Institute, Pentlands Science Park, Edinburgh. UK

- 14:30 024 **Modulation of P-glycoprotein enhances ivermectin and moxidectin systemic availabilities and their efficacy against resistant nematodes in cattle**
 Lifschitz A. 1,2, Sallovitz J. 1, Imperiale F. 1,2, Suarez V. 3, Cristel S. 3, Ahoussou S. 3, and Lanusse C. 1,2
1 Lab. Farmacología, FCV, UNCPBA, Tandil, Argentina; 2 CONICET, Argentina; 3 EEA INTA Anguil, La Pampa, Argentina

14:45 **Discussion**

Hubert van Eyck Room

13:30-15:00 Echinococcosis and Cysticercosis
Chairs: Dorny P.
Lightowlers M.

- 13:30 025 **Canine echinococcosis in Kyrgystan**
 Ziadinov I. 1,2, Mathis A. 1, Trachsel D. 1, Rysmuhambetova A. 3, Abdykerimov K. 2, Abdyjaparov T. 4, Deplazes P. 1, and Torgerson P.R. 1
1 Institute for Parasitology, University of Zürich, Zürich, Switzerland; 2 Kyrgyz research Institute of Livestock, Veterinary and Pastures, Bishkek, Kyrgyzstan; 3 Institute of Zoology, Kazakh Academy of Sciences, Akademgorodok, Almaty, Kazakhstan; 4 Bishkek Medical Academy, Bishkek, Kyrgyzstan
- 13:45 026 **Cross-sectional survey on the prevalence of *Echinococcus multilocularis* and *E. granulosus* infections in domestic dogs in Germany**
 Dyachenko V. 1, Pantchev N. 2, Gawlowska S. 1, Globokar M. 2, and Bauer C. 3
1 University of Leipzig, Institute of Parasitology, Leipzig, Germany; 2 Vet Med Labor, Division of IDEXX, Ludwigsburg, Germany; 3 Justus Liebig University Giessen, Institute of Parasitology, Giessen, Germany
- 14:00 027 **Prevalence of *Echinococcus multilocularis* in wild and domestic hosts in Lithuania**
 Sarkunas M. 1, Bruzinskaite R. 1, Marcinkute A. 2, Mathis A. 3, and Deplazes P. 3
1 Lithuanian Veterinary Academy, Department of Infectious Diseases, Kaunas, Lithuania.; 2 Hospital of Tuberculosis and Infectious Diseases, Vilnius, Lithuania; 3 University of Zurich, Institute of Parasitology, Zürich, Switzerland
- 14:15 028 **Endogenous development in the countermeasure of multilocular echinococcosis in red foxes: a successful and sustainable approach**
 Kamiya M. 1, and Lagapa J.T. 1,2
1 Rakuno Gakuen University, OIE Reference Laboratory for Echinococcosis and Laboratory of Environmental Zoology, Ebetsu, Japan; 2 Central Mindanao University, College of Veterinary Medicine, Musuan, Philippines
- 14:30 029 **Evaluation of a serological method for the detection of *Taenia saginata* cysticercosis using serum and meat-juice samples**
 Epe C. 3, Abuseir S. 1, Kühne M. 2, Schnieder T. 1, and Klein G. 3
1 Department for Infectious Diseases, Institute for Parasitology. University of Veterinary Medicine Hannover, Hannover, Germany; 2 Lower Saxony Federal State Office for Consumer Protection and Food Safety, Oldenburg, Germany; 3 Centre for Food Science. Institute for Food Quality and Safety. University of Veterinary Medicine, Hannover, Hannover, Germany

- 14:45 030 **Risk factors for bovine cysticercosis in Switzerland**
 Flüttsch F. 1, Heinzmann D. 1, Mathis A. 1, Hertzberg H. 1, Stephan R. 2, and Deplazes P. 1
1 Institute of Parasitology, University of Zürich, Switzerland; 2 Institute for Food Safety and Hygiene, University of Zürich, Switzerland

Jan van der Goes Room

14:30-15:00 Intervet Industry Forum

- 14:30 031 ***Cryptosporidium parvum*: major aetiological agent of neonatal diarrhoea in dairy calves**
 Martins S. 1, Madeira de Carvalho L.M., 2, and Cannas da Silva J. 1,2
1 Laboratório de Medicina Veterinária, Ltd., Santarém, Portugal; 2 Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade Técnica de Lisboa, Av. Universidade Técnica, Pólo Universitário do Alto da Ajuda, Lisbon, Portugal; 3 Escola Universitária Vasco da Gama, Coimbra, Portugal
- 14:45 032 **Efficacy of halofuginone lactate for the treatment and prevention of cryptosporidiosis in lambs and kids: An extensive field trial**
 Giadinis N.D. 1, Papadopoulos E. 2, Lafi S.Q. 3, Panousis N.K. 1, Papazahariadou M. 2, and Karatzias H. 1
1 Clinic of Farm Animals; 2 Laboratory of Parasitology and Parasitic Diseases, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece; 3Department of Pathology and Animal Health, Faculty of Veterinary Medicine, Jordan University of Science and Technology, Irbid, Jordan

Main Congress Room

15:30-17:00 Symposium: **PARASOL/DELIVER programmes on the sustainable control of nematodes and liver fluke in farm animals** **Chairs: Mulcahy G.** **Coles G.**

The European Union has funded two international projects designed to improve the control of parasitic helminths of sheep, goats and cattle, PARASOL (Parasite Solutions – Novel solutions for the sustainable control of nematodes in ruminants) and DELIVER (Design of Effective and Sustainable Control Strategies for Liver Fluke in Europe).

To conserve the efficacy of anthelmintics by leaving populations of nematodes in refugia whilst at the same time reducing the incidence of helminthoses, Targeted Selective Treatment (TST) is being evaluated within PARASOL focussing on those animals or herds that require treatment. From present evidence with sheep TST is likely to be based on weight gain using electronic weighing of tagged sheep. In cattle it will be based on blood pepsinogen at housing as an indicator of exposure of first season grazing calves to nematodes (*Ostertagia*) and on milk ELISAs for *Ostertagia* antigens which will indicate the likely improvement in milk yield from treatment during lactation. From this information recommendations on changing worm control management will be suggested and evaluated. In addition methods for detection of anthelmintic resistance are being developed and

ring tested. In DELIVER methods of sustainable control are being developed for *Fasciola* taking into account its increasing prevalence and the development of drug resistance. Trials with potential candidate vaccines are being undertaken and drug resistance is being studied. The symposium will report on recent developments in the two programmes.

- 15:30 033 **Targeted selective treatments an innovative strategy for the control of nematode infections in sheep and goats**
Jackson F. 1, Coles G. 2, Cabaret J. 3, Varady M. 4, Van Wyk J. 5, Berrag B. 6, Papadopoulos E. 7, and Cringoli G. 8
1 Moredun Research Institute, UK; 2 University of Bristol, U.K.; 3 INRA, Nouzilly, France; 4 Slovak Academy of Sciences, Kosice, Slovak Republic; 5 University of Pretoria, South Africa; 6 University of Rabat, Morocco; 7 Aristotle University, Thessaloniki, Greece; 8 University of Naples, Italy
- 15:40 034 **Assessment of the repeatability and reproducibility of a serum-pepsinogen assay and an indirect milk ELISA to monitor gastrointestinal-nematode infections in cattle**
 Vercruysse J. 1, Höglund J. 2, von-Samson Himmelstjerna G. 3, Coles G. 4, Merza M. 5, Demeler J. 3, Dorny P. 1, and Charlier J. 1
1 Ghent University, Department of Virology, Parasitology and Immunology, Merelbeke, Belgium; 2 SWEPAR, Swedish University of Agricultural Sciences, Uppsala, Sweden; 3 Hannover University of Veterinary Medicine, Hannover, Germany; 4 University of Bristol, Bristol, UK; 5 Svanova Biotech, Uppsala, Sweden
- 15:50 035 **The development and standardisation of methods for detecting anthelmintic resistance in gastrointestinal nematodes of ruminants**
Wolstenholme A. 1, Coles G. 2, von Samson-Himmelsjerna G. 3, Vercruysse J. 4, Jackson F. 5, Kerboeuf D. 6, Höglund J. 7, and Várady M. 8
1 University of Bath, U.K.; 2 University of Bristol, U.K.; 3 University of Veterinary Medicine, Hannover, Germany; 4 University of Gent, Belgium; 5 Moredun Research Institute, U.K.; 6 INRA, IASP-213, Nouzilly, France; 7 SWEPAR, Uppsala, Sweden; 8 Slovak Academy of Sciences, Kosice, Slovak Republic
- 16:10 036 **The epidemiology fasciolosis in Europe**
 Williams D.J.L.
Liverpool School of Tropical Medicine/Faculty of Veterinary Science, University of Liverpool, UK
- 16:20 037 **Fasciolicide resistance in liver fluke**
 Fairweather I.
School of Biological Sciences, Queens University, Belfast, UK
- 16:30 038 **Development of vaccines against *Fasciola hepatica***
 Mulcahy G.
School of Agriculture, Food and Veterinary Medicine, University College Dublin, Ireland

Van Rysselberghe Room**15:30-17:00****Neospora****Chairs: Trees S.
Losson B.**

- 15:30 039 ***Neospora caninum* – lack of evidence of human exposure in an English population**
McCann C.M. 1, Vyse A.J. 2, Williams D.J.L. 1, McGarry J.W. 1, Pebody R. 2, and Trees A.J. 1
1 Veterinary Parasitology, Liverpool School of Tropical Medicine and Faculty of Veterinary Science, University of Liverpool, Pembroke Place, Liverpool, UK; 2 Health Protection Agency Sero-Epidemiology Unit, Communicable Disease Surveillance Centre, London, UK
- 15:45 040 **Investigations into the involvement of *Neospora caninum* in unexplained sheep abortions**
Howe L.J., Pomroy W.E., West D.M., Collett M.G., Kenyon P.R., Morris S.T., and Pattison R.S.
Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North
- 16:00 041 **No evidence of transplacental transmission in pregnant heifers inoculated with *Neospora caninum* tachyzoites by conjunctival route**
Moore D.P. 1, Chiapparrone M.L. 2, Lischinky T. 2, Posso M.A. 2, and de Yaniz M.G. 1, Odeón A.C. 2, Leunda M.R. 2, and Campero C.M. 2
1 National Research Council, Argentina; 2 National Institute of Agrarian Technology, Balcarce, Argentina
- 16:15 042 **Usefulness of the recombinant proteins rNcSAG4 and rNcGRA7-based ELISA tests to distinguish primo-infection, recrudescence and chronic bovine neosporosis**
Aguado-Martínez A. 1, Álvarez-García G. 1, Fernández-García A. 1, Risco-Castillo V. 1, Arnaiz-Seco I. 2, Rebordosa-Trigueros X. 3, Navarro-Lozano V. 1, and Ortega-Mora L.M. 1
1 SALUVET, Animal Health Department, Faculty of Veterinary Sciences, Complutense University of Madrid, Ciudad Universitaria s/n, Madrid, Spain; 2 Laboratorio Regional de Sanidad y Producción Animal, Lugo, Spain; Hipra, Amer (Girona) Spain
- 16:30 043 **The magnitude of cytokine expression in the placenta of cattle in naturally and experimentally infected cattle and its association with foetal death**
Rosbottom A. 1, Gibney E.H. 1, Smith R.F. 2, Kipar A. 3, Guy C.S. 1, Trees A.J. 1, and Williams D.J.L. 1
1 Liverpool School of Tropical Medicine / Faculty of Veterinary Science, University of Liverpool, UK; 2 Department of Clinical Science, Faculty of Veterinary Science, Liverpool, UK, 3 Department of Pathology, Faculty of Veterinary Science, Liverpool, UK
- 16:45 044 **A molecular in vitro assay to assess the parasitocidal activity of toltrazuril against *Neospora caninum***
Strohbusch M. 1, Mueller N. 1, Hemphill A. 1, Greif G. 2, and Gottstein B. 1
1 Institute of Parasitology, University of Berne, Berne, Switzerland; 2 Bayer HealthCare AG, Leverkusen.

Baeckelandt Room**15:30-17:00****Babesia****Chairs: Schetters T.
Chauvin A.**

- 15:30 045 **A descriptive epidemiological study on canine babesiosis in the Lake Geneva region**
 Porchet M.-J., Sager H., Muggli L., Oppliger A., Mueller N., Frey C., and Gottstein B.
Institute of Parasitology, University of Berne, Berne, Switzerland
- 15:45 046 **Molecular characterization of a *Babesia* sp. from sable antelope**
 Oosthuizen M.C. 1, Collins N.E. 1, Zwegarth E. 2, and Penzhorn B.L. 1
1 Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; 2 Onderstepoort Veterinary Institute, Onderstepoort, South Africa
- 16:00 047 **Molecular diagnostic method for *Babesiosis*; Direct detection of *Babesia microti* DNA from heat-treated blood by loop-mediated isothermal amplification**
Iseki H. 1, Alhassan A. 1, Kim C. 1, Inokuma H. 2, Kawazu S. 1, Masuzawa T. 3, Yokoyama N. 1, and Igarashi I. 1
1 National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan; 2 Department of Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan; 3 Faculty of Pharmaceutical Sciences, Chiba Institute of Science, Choushi, Japan
- 16:15 048 **Epidemiological and molecular genetic investigations of *Babesia divergens*/*B. capreoli*-like parasite findings in free-ranging wild ruminants in eastern Switzerland**
 Schmid N. 1, Deplazes P. 1, Hoby S. 2, Ryser M-P. 2, Edelhofer R. 3, and Mathis A. 1
1 Institute of Parasitology, University of Zurich, Switzerland; 2 Centre for Fish and Wildlife Health, Institute of Animal Pathology, University of Berne, Switzerland; 3 Departement of Parasitology and Zoology, Veterinary Medical University, Vienna, Austria
- 16:30 049 **Correlation between *Babesia rossi* genotypes and clinical canine babesiosis**
Matijila P.T. 1, Penzhorn B.L. 1, Leisewitz A.L. 1, Schetters T. 2, Carcy B. 3, and Jongejan F. 1,4
1 Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa ; 2 Parasitology R&D Department, Intervet International B.V., Boxmeer, The Netherlands; 3 Laboratoire de Biologie Cellulaire et Moléculaire, Vaccination antiparasitaire, UFR des Sciences Pharmaceutiques et Biologiques, Montpellier France; 4 Utrecht University, Department of Infectious Diseases and Immunology, Utrecht Centre for Tick-borne Diseases (UCTD), Faculty of Veterinary Medicine, Utrecht, the Netherlands
- 16:45 050 **Effects of protein kinase inhibitors on the in vitro growth of *Babesia bovis***
Bork-Mimm S. 1,2, Das S. 1,3, Okubo K. 1, Yokoyama N. 1, Igarashi I. 1, and Pfister K. 2.
1 National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido, Japan; 2 Institute for Comparative Tropical Medicine and Parasitology, Veterinary Faculty, Ludwig-Maximilians-University, Munich, Germany; 3 Institute of Animal Health and Veterinary Biologicals, Animal Research Development Department, Kolkata, Government of West Bengal, India

Jan van Eyck Room

15:30-17:00

Parasites of Companion Animals

**Chairs: Genchi C.
Jacobs D.**

- 15:30 051 **Molecular-based prediction of the geographical spread of *Angiostrongylus vasorum* in dog and fox populations**
Jefferies R., Shaw S., Viney M., and Morgan E.
University of Bristol, School of Biological Sciences, Bristol, UK
- 15:45 052 **Predicting the spread of the canid heartworm *Angiostrongylus vasorum***
Morgan E.R. 1,2, Ward P. 1, Krajewski M. 1, and Shaw S.E. 2
1 School of Biological Sciences, University of Bristol, UK; 2 School of Clinical Veterinary Science, University of Bristol, UK
- 16:00 053 **Prevalence of haemotrophic *Mycoplasma* and *Bartonella* spp. in fleas of companion animals from Germany and France**
Just F.T. 1, Gilles J. 1, Pradel I. 1, Pfalzer S. 1, Lengauer H. 2, Hellmann K. 2, and Pfister K. 1
1 Institute for Comparative Tropical Medicine and Parasitology, Veterinary Faculty, Ludwig-Maximilians-University Munich, Munich, Germany; 2 KlifovetAG, Munich, Germany
- 16:15 054 **Development of a challenge model and efficacy of 5% ponazuril (toltrazuril sulfone) oral suspension against experimental *Isospora canis* infection in puppies**
Charles S.D. 1, Lindsay D.S. 2, Mitchell S.M. 2, Reinemeyer C.R. 3, Mundt H.C. 4, and Settje T.L. 1
1 Bayer HealthCare LLC, Animal Health Division, Kansas, USA; 2 Virginia Tech, Department of Biomedical Sciences and Pathobiology, Blacksburg, Virginia, USA; 3 East Tennessee Clinical Research, Rockwood, Tennessee, USA; 4 Bayer HealthCare AG, Animal Health Division, Leverkusen, Germany
- 16:30 055 **Autochthonous transmission of *Thelazia callipaeda* in southern Switzerland**
Schnyder M. 1, Malacrida F. 1, Bacciarini L. 2, Otranto D. 3, and Deplazes P. 1
1 Institute of Parasitology, Vetsuisse Faculty, University of Zurich, Switzerland; 2 University of Zurich, Institute of Parasitology, Zürich, Switzerland
- 16:45 056 ***Dirofilaria immitis* and *Wolbachia*: Effects of treatment of dogs with ivermectin, doxycycline and/or melarsomine**
McCall J.W. 1, Genchi C. 2, Kramer L. 3, Dzimiński M.T. 1, Guerrero J. 4, and McCall S.C. 5
1 Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, Athens, USA; 2 Dipartimento di Patologia Animale, Igiene e Sanità Pubblica Veterinaria, Università degli Studi di Milano, Milano, Italy; 3 Dipartimento di Produzioni Animale, Università di Parma, Parma, Italy; 4 Department of Pathobiology, University of Pennsylvania, Philadelphia, Pennsylvania, USA; 5 TRS Labs, Inc., Athens, Georgia, USA

Hubert van Eyck Room

15:30-17:00 Molecular Systematics & Phylogenetics
Chair: Zarlenga D.

- 15:30 057 **Genetic and phenotypic divergence in australian *Haemonchus contortus***
Hunt P.W., Le Jambre L.F., McNally J., Anderson L., and Knox M.R.
Moredun Research Institute, Penicuik, UK
- 15:45 058 **Genetic characterisation of *Haemonchus contortus* laboratory isolates and a rapid method of genetic fingerprinting using microsatellites**
Redman L. 1, Packard E. 1, Grillo T. 1, Smith J. 1, Jackson F. 2, and Gilleard J. 1
1 Division of Infection and Immunity, Inst. Comparative Medicine, Faculty of Veterinary medicine, University of Glasgow, UK; 2 Moredun Research Institute, Edinburgh, UK
- 16:00 059 **A practical and cost effective mutation scanning approach for investigating the population genetics and epidemiology of *Cryptosporidium* from humans**
Jex A.R. 1, Whipp M. 2, Campbell B.E. 1, Cacciò S.M. 3, Stevens M. 4, Hogg G. 2, and Gasser R.B. 1
1 The University of Melbourne, Department of Veterinary Science, Werribee, Australia; 2 Melbourne Diagnostic Unit, Royal Parade, Melbourne, Australia; 3 Istituto Superiore di Sanità, Rome, Italy; 4 Melbourne Water Corporation, East Melbourne, Australia
- 16:15 060 **Diversity in mitochondrial lineages of liver fluke (*Fasciola hepatica*) populations**
Walker S.M., Prodöhl P.A., Fletcher H.L., Fairweather I., Brennan G., Hoey E.M. and Trudgett A.
The Queen's University of Belfast, School of Biological Sciences
- 16:30 061 **Molecular characterization and phylogenetic studies of *Steganinae* (Diptera, Drosophilidae) inferred by the mitochondrial cytochrome C oxidase subunit I**
Cantacessi C. 1, Stevens J.R. 2, Testini G. 1, Máca J. 3, and Otranto D. 1
1 Department of Animal Health and Welfare, Faculty of Veterinary Medicine, Bari, Italy; 2 School of Biosciences, Exeter, England; 3 Czech Agency of Nature Conservation (AOPK), České Budějovice, Czech Republic
- 16:45 062 **Molecular identification and geographic distribution of food and water-borne parasitic zoonoses in Vietnam focusing on (hybrid) *Fasciola gigantica*; *Opisthorchis viverrini*; *Clonorchis sinensis*; *Fasciolopsis buski*; *Haplorchis pumilio*; *H. taichui* and *Paragonimus heterotremus***
Le T.H. 1, Nguyen D.V. 2, Ngo T.H. 3, Van V.K. 4, Nguyen T.G.T. 5, Agatsuma T. 6, Blair D. 7, and McManus D.P. 8
1 Institute of Biotechnology, Vietnam; 2 Hanoi Medical University, Vietnam; 3 Institute of Malariaology, Parasitology and Entomology Qui Nhon, Vietnam; 4 Hanoi Agricultural University, Vietnam; 5 Institute of Veterinary Research, Vietnam; 6 Kochi Medical School, Japan; 7 James Cook University, Australia; 8 Queensland Institute of Medical Research, Australia

Jan van der Goes Room**15:30-17:00 Non-human Primates****Chair: Claerebout E.**

- 15:30 063 **Zoonotic nematode parasites in wild caught African green monkeys (*Chlorocebus sabaeus*) on St Kitts, West Indies**
Hunt P.W., Le Jambre L.F., McNally J., Mitchell B. 1, 2, McNeal-Hutchinson P. 1, 2, and Krecek R.C. 1, 3
1 Ross University School of Veterinary Medicine (RUSVM), St Kitts, West Indies; 2 St Kitts Biomedical Research Foundation, St Kitts, West Indies; 3 University of Johannesburg, South Africa
- 15:45 064 **Ocular oxyspirosis of lower primates in zoos: intermediate host, worm morphology, and probable origin of the infection in Moscow zoo**
 Ivanova E. 1, Spiridonov S. 1, and Bain O. 2
1 Institut of Parasitology, Russian Academy of Sciences, Moscow, Russia; 2 Museum National d'Histoire Naturelle, Parasitologie comparée et Modèles expérimentaux, Paris, France
- 16:00 065 **Mixed *Giardia* infections in captive non-human primates in Belgium and the Netherlands**
Levecke B. 1, Geurden T. 1, Geldhof P. 1, Vercammen F. 2, Dorny P. 1,3, Claerebout E. 1, and Vercruysse J. 1
1 Department of Virology, Parasitology & Immunology, Faculty of Veterinary Medicine, Ghent University, Belgium; 2 Veterinary Department, Royal Zoological Society of Antwerp, Belgium; 3 Department of Animal Health, Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium
- 16:15 066 **Molecular characterisation of *Blastocystis* isolates found in animals and zoo-keepers at the Perth Zoo**
Parkar U. 1, Traub R.J. 2, Vitali S. 3, and Thompson R.C.A. 1
1 Murdoch University, School of Veterinary and Biomedical Sciences, Perth, Australia; 2 University of Queensland, School of Veterinary Science, St Lucia, Australia; 3 Perth Zoo, Perth, Australia
- 16:30 067 **Molecular evidence of two species of *Oesophagostomum* in fecal samples from wild chimpanzees in Uganda**
 Vermeulen B. 1, 2, Krief S. 3, Kasenene J. 4, Nieguitsila A. 1, Berthelemy M. 1, L'Hostis M. 2, Bain O. 5, and Guillot J. 1
1 UMR INRA, AFSSA, ENVA, UPVM 956 BIPAR, Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort, France; 2 Service de Parasitologie, Ecole Nationale Vétérinaire de Nantes, France; 3 Eco-anthropologie et ethnobiologie, Muséum National d'Histoire Naturelle de Paris, France; 4 Makerere University Biological Field Station, Uganda; 5 Laboratoire de Parasitologie comparée et modèles expérimentaux, Muséum National d'Histoire Naturelle de Paris, France
- 16:45 **Discussion**

Main Congress Room**17:10-18:10****Merial Corporate Symposium:
Change and spatial shifts in Parasitic Diseases:
Fact or Fiction**17:10 **Introduction**17:20 068 **Emergence and vector borne diseases in Livestock - a bright future for veterinary entomologists**

Hendrickx G.

*Avia-GIS, Zoersel, Belgium*17:45 069 **Emergence of vector borne zoonosis, focus on granulocytic anaplasmosis (*A. phagocytophilum*), tick borne encephalitis and flea transmitted rickettsiosis (*R. Felis*)**

Davoust B., and Marié J.L.

General French Army

ICC - International Convention Centre Gent

TUESDAY 21 AUGUST

Van Rysselberghe Room

08:30-10:00 **Symposium:**
Evidence based strategies for control of worms in dogs and cats
Chairs: Thamsborg S.
Deplazes P.

Recent epidemiological investigations in different areas in Europe have revealed high nematode prevalences in the normal domestic dog and cat population. At least for some of the endemic areas this result is surprising. Based on highly efficient chemotherapeutic agents and detailed biological and epidemiological knowledge of the key nematodes, optimal control strategies can theoretically be designed. However, even in the parasitological community the aim of such strategies is discussed controversially. For example agreement on “zero-tolerance” for the excretion of zoonotic helminths in dogs and cats is of veterinary public health importance, but not significant for veterinarians arguing with the low pathogenicity of these parasites in adult dogs. The symposium aims to present some recent epidemiological and experimental data focussed on intestinal nematodes (especially *Toxocara*) and heartworms (*Dirofilaria* and *Angiostrongylus*) as a basis for the discussion of control strategies as proposed by the last presentation of the ESCCAP group in a first guideline focussing on worms in dogs and cats.

- 08:30 070 ***Toxocara canis* in Swiss dogs: temporal aspects of different anthelmintic treatment procedures**
Gottstein B. 1, Sager H. 1, and Deplazes P. 2
1 Institute of Parasitology, Vetsuisse Faculty, University of Bern, Switzerland; 2 Institute of Parasitology, Vetsuisse Faculty, University of Zurich, Switzerland
- 08:45 071 **Induction of patent *Toxocara canis* infections in previously exposed and in helminth free, regularly treated or untreated young dogs after low dose inoculations with embryonated eggs**
Fahrion A. , Stäbler S., and Deplazes P.
Institute of Parasitology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland
- 09:00 072 **Geographical information systems in health application: experience on filariosis and other vector-borne diseases**
Rinaldi L. 1, Musella V. 1, Marzatico G. 1, Genchi C. 2, and Cringoli G. 1
1 Dipartimento di Patologia e Sanità Animale, Settore di Parassitologia Veterinaria, Università degli Studi di Napoli “Federico II”, Napoli, Italy; 2 Dipartimento di Patologia Animale, Igiene e Sanità Pubblica Veterinaria, Sezione di Patologia Generale e Parasitologia, Università degli Studi di Milano, Milano, Italy
- 09:15 073 **Infection biology of *Angiostrongylus vasorum* in foxes (*Vulpes vulpes*) with emphasis on the impact of host age, infection dose and exposure**
Webster P. 1, Monrad J. 1, Kapel C.M.O. 2, and Thamsborg S.M. 1
1 Danish Centre for Experimental Parasitology, Dep. of Veterinary Pathobiology, University of Copenhagen, Denmark; 2 Section for Zoology, Dep. of Ecology, University of Copenhagen, Denmark

- 09:30 074 **The ESCCAP guideline for worm control in dogs and cats**
Epe C. 1,9, Deplazes P. 2,9, Guillot J. 3,9, Fisher M. 4,9, Genchi C. 5,9, Miro G. 6,9, van Knapen F. 7,9, and Thamsborg S.M. 8,9
1 Institute for Parasitology, Department of Infectious Diseases, University of Veterinary Medicine Hannover, Hannover, Germany; 2 Institute for Parasitology, University of Zurich, Zurich, Switzerland; 3 Parasitologie-Mycologie, Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort, France; 4 Shernacre Cottage, Lower Howsell Road, Malvern Worcs, UK; 5 Department of Animal Pathology, Università degli Studi di Milano, Milano, Italy; 6 Universidad Complutense de Madrid Facultad de Veterinaria Madrid, Spain; 7 Faculty of Life Sciences, Copenhagen University, Institute for Risk Assessment Sciences, Division of Public Health and Food Safety, Utrecht, The Netherlands; 8 Danish Centre for Experimental Parasitology, Department of Veterinary Pathobiology, Faculty of Life Sciences, University of Copenhagen; 9 ESCCAP (Reg. No. 5821601), ESCCAP Secretariat: Shernacre Cottage, Lower Howsell Road, Malvern, Worcestershire, UK

09:45 **Discussion**

Baeckelandt Room

08:30-10:00 Anthelmintic Resistance
Chairs: Jackson F.
Besier B.

- 08:30 075 **P-glycoproteins in *Haemonchus contortus*: Ivermectin and moxidectin select for constitutive overexpression and induce further overexpression of P-glycoproteins**
Roulet A., and Prichard R.K.
Institute of Parasitology and Centre for Host-Parasite Interactions, McGill University, Montreal, Canada
- 08:45 076 **Investigations into the effects of p-glycoprotein and/or cytochrome P450 inhibitors on the phenotypic responses of various developmental stages of ovine nematodes**
Bartley D.J. 1, Lespine A. 2, Dupey J. 2, Alvenerie M. 2, McAllister H. 1, Gordon Y. 1, and Jackson F. 1
1 Moredun Research Institute, Pentland Science Park, PENICUIK, Scotland; 2 INRA-UR66 Pharmacologie-Toxicologie, Toulouse, France
- 09:00 077 **Pyrosequencing of two loci in the beta-tubulin gene, codons 167 and 200, in *Cyathostomin* populations of known benzimidazole resistance status**
Lake S. 1, Clark H. 1, Dowdall S. 1, Matthews J.B. 2, Kaplan R.M. 3, and Hodgkinson J. 1
1 Veterinary Parasitology, Liverpool School of Tropical Medicine, Liverpool, UK; 2 Moredun Research Institute, Penicuik, Midlothian Scotland; 3 Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, Athens GA, USA
- 09:15 078 **Flow cytometry in anthelmintic resistance research**
McBean D., Jackson F., and Jackson E.
Moredun Research Institute, Pentlands Science Park, Scotland
- 09:30 079 **Single or multiple appearance of benzimidazole resistance: a microsatellite investigation of goat nematode in two French regions**
Silvestre A., and Cabaret J.
UR1282 Infectious animal disease and public health, F-37380 Nouzilly, France

- 09:45 080 **Macrocyclic lactones selection on beta-tubulin in *Haemonchus contortus*: Implications for cross-resistance selection to benzimidazole and macrocyclic lactone anthelmintics**
Mottier M.L., and Prichard R.K.
Institute of Parasitology and Centre for Host-Parasite Interactions, McGill University, Montreal, Canada

Jan van Eyck Room

08:30-10:00 **Epidemiology and Control of Cattle Parasites** **Chairs: Höglund J.** **Pomroy B.**

- 08:30 081 **Horn fly (*Haematobia irritans*) olfactometric responses to cattle host odour in a Y-tube**
Oyarzún M.P. 1, Alberti E. 2, Palma R. 1, Birkett M. 3, and Quiroz A. 2
1 Doctorado en Ciencias de Recursos Naturales, Universidad de La Frontera, Temuco, Chile; 2 Laboratorio de Química Ecológica, Universidad de La Frontera, Temuco, Chile; 3 Biological Chemistry Division, Rothamsted Research, Harpenden, U.K.
- 08:45 082 **Effect of cypermethrin selection pressure and substitution with amitraz on phenotypic and genotypic measures of cypermethrin resistance in field populations of *Boophilus microplus* (Acari: Ixodidae) in the Mexican Tropics**
Rodriguez-Vivas R.I. 1, Hodgkinson J.E. 2, Rosado-Aguilar J.A. 1, Villegas P.S.L. 1, and Trees A.J. 2
1 Facultad de Medicina Veterinaria y Zootecnia, Universidad Autonoma de Yucatan, Merida, Yucatan, Mexico; 2 Department of Veterinary Parasitology, Liverpool School of Tropical Medicine and Faculty of Veterinary Science. University of Liverpool, Liverpool, UK
- 09:00 083 **Effectiveness of a community-based strategic anthelmintic treatment programme in the control of gastrointestinal nematodes and *Fasciola gigantica* in cattle in Kilolo district, Tanzania**
Keyyu J.D. 1,2, Kassuku A.A. 1, Kyvsgaard N.C. 3, and Monrad J.M. 4
1 Sokoine University of Agriculture, Department of Veterinary Microbiology and Parasitology, Tanzania; 2 Tanzania Wildlife Research Institute (TAWIRI), Arusha, Tanzania; 3 Department of Veterinary Pathobiology, Royal Veterinary and Agricultural University, Frederiksberg C, Denmark; 4 Danish Centre for Experimental Parasitology, The Royal Veterinary and Agricultural University, Frederiksberg C, Denmark
- 09:15 084 **A case-control study on outbreaks of lungworm disease in dairy herds in The Netherlands**
Feenstra P.B. 1, Holzhauer M. 2, and Ploeger H.W. 1
Utrecht University, Infectious Diseases and Immunology, The Netherlands
- 09:30 085 **Original systematic combination of moxidectin pour-on and long action in beef cattle for control of gastro intestinal strongylosis. Control of the efficacy of the protocol by dosing blood pepsinogen**
Camuset Ph.
Commission Parasitologie SNGTV, France
- 09:45 **Discussion**

Hubert van Eyck Room

08:30-10:00 Vector-Borne Protozoa

**Chairs: Geerts S.
Goddeeris B.**

- 08:30 086 **Characterization of South African *Theileria equi* and *Babesia caballi* isolates based on 18S rRNA gene sequences**
Bhoora R. 1, Zweggarth E. 2, Guthrie A.J. 3, Franssen L. 1,4, Jongejan F. 1,4, Oosthuizen M.C. 1, Penzhorn B.L. 1, and Collins N.E. 1
 1 University of Pretoria, Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, Onderstepoort, South Africa; 2 Onderstepoort Veterinary Institute, Onderstepoort, South Africa; ; 3 Equine Research Centre, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; ; 4 Utrecht University, Department of Infectious Diseases and Immunology, Utrecht Centre for Tick-borne Diseases (UCTD), Faculty of Veterinary Medicine, Utrecht, the Netherlands
- 08:45 087 **Genetic diversity of South African *Theileria parva* isolates**
Sibeko K.P. 1, Collins N.E. 1, Oosthuizen M.C. 1, and Geysen D. 2
 1 University of Pretoria, Veterinary Tropical Diseases, Pretoria, South Africa; 2 Institute for Tropical Medicine, Department of animal health, Antwerp, Belgium
- 09:00 088 **The epidemiology of theileriosis in Rwanda and the significance of the polymorphic immunodominant molecule (PIM) of *Theileria parva* in the vaccine development approach**
Bazarusanga T. 1, Vercruysse J. 2, and Geysen D. 3
 1 National Veterinary Laboratory of Rwanda, Kigali- Rwanda; 2 University of Ghent, Faculty of veterinary medicine, Merelbeke, Belgium; 3 Institute for Tropical Medicine, Department of animal health, Antwerp, Belgium
- 09:15 089 **Impact of tsetse-transmitted trypanosomiasis: variations on a theme**
Van den Bossche P. 1,2, Masumu J. 3, Simukoko H. 4, De Deken R. 1, and Geerts S. 1
 1 Institute of Tropical Medicine, Animal Health Department, Antwerp, Belgium; 2 Department of Veterinary Tropical Diseases, University of Pretoria, South Africa; 3 Laboratoires Vétérinaires de Kinshasa, Kinshasa, Democratic Republic of Congo; 4 University of Zambia, School of Veterinary Medicine, Lusaka, Zambia
- 09:30 090 **Epimastigote morphogenesis and the cell cycle of *Trypanosoma brucei* in the tsetse vector revisited**
Sharma R. 1,2, Peacock L. 3, Gluenz E. 4, Gull K. 4, Gibson W. 3, and Carrington M. 2
 1 Faculty of Veterinary Medicine, Universiti Putra Malaysia ,Selangor, Malaysia; 2 Department of Biochemistry, University of Cambridge, Cambridge, UK; 3 School of Biological Sciences, University of Bristol, Bristol, UK; 4 Sir William Dunn School of Pathology, University of Oxford, Oxford, UK
- 09:45 091 **Tsetse habitat fragmentation dynamics and bovine trypanosomosis epidemiology in the Oti river basin of Northern Togo**
Dao B. 1, Hendrickx G. 2, Sidibé I. 1, Bélém A. 3, and De la Rocque S. 4
 1 Institut Togolais de Recherche Agronomique, Lomé, Togo; 2 Avia-GIS, Zoersel, Belgium

Jan van der Goes Room

08:30-10:00 **Haemonchus Antigens & Immunity**

**Chairs: Skuce P.
Stear M.**

- 08:30 092 **Immune cell kinetics in sheep abomasal tissues following infection with *Haemonchus contortus* L3 larvae**
Robinson N. 1, Piedrafita D. 1, Snibson K. 2, and Meeusen E. 1
 1 Department of Physiology, Monash University, Melbourne, Australia; 2 Faculty of Veterinary Science, University of Melbourne, Australia
- 08:45 093 **Immune responses to *Haemonchus contortus* primary infection in susceptible (INRA 401) and resistant (Barbados Black belly) breeds of lambs**
 Terefe G. 1, Lacroux C. 2, Grisez C. 2, Prevot F. 2, Bergeaud J.P. 2, Gruner L. 3, Bouix J. 4, and Jacquiet P. 2
 1 Faculty of Veterinary Medicine, Debre Zeit, Ethiopia; 2 UMR INRA/DGER 1225, Ecole Nationale Vétérinaire de Toulouse, France; 3 INRA, Tours Nouzilly, France; 4 INRA, UR 631, Station d'Amélioration Génétique des Animaux, Toulouse, France
- 09:00 094 **Vaccination against *Haemonchus contortus*: performance of native parasite gut membrane glycoproteins in Merino lambs grazing contaminated pasture**
 LeJambre L.F. 1, Windon R.G. 1, and Smith W.D. 2
 1 CSIRO Livestock Industries, F.D. McMaster Laboratory, Armidale, NSW 2350, Australia; 2 Moredun Research Institute, Midlothian, Scotland, UK
- 09:15 095 **Effect of vaccination of goats with H-gal-GP and H11 antigens from intestinal membrane cells of *Haemonchus contortus***
Dupree-Olcott D. 1, Weeks B. 2, Shakya K. 2, Smith D. 3, and Miller J. 2
 1 Cypress Animal Hospital, Orange, Texas, USA; 2 Louisiana State University, Department Pathobiological Sciences, Baton Rouge, Louisiana, USA; 3 Moredun Research Institute, Midlothian, Scotland, UK
- 09:30 096 **Characterization of the intestinal contortin structure in *Haemonchus contortus***
Geldhof P. 1,2, Munn E., and Knox D. 2
 1 Laboratory of Parasitology, Faculty of Veterinary Medicine, Ghent University, Gent, Belgium; 2 Moredun Research Institute, Penicuik, UK
- 09:45 097 **Speciation of ovine gastric nematodes using two molecular based techniques, SSCP and Pyrosequencing**
Donnan A.A. 1, Walsh T. 2, Wolstenholme A. 2, Jackson E. 1, McBean D. 1, Jackson F. 1, and Skuce P.J. 1
 1 Moredun Research Institute, Scotland, UK; 2 School of Biology and Biochemistry, University of Bath, Bath, UK

Main Congress Room**10:30-11:30 Plenary Session II: Invited lectures**
Chair: Goddeeris B.

- 10:30 098 **The biological significance and practical implication of antigenic variability in parasite populations**
Morrison I., *University of Edinburgh, United Kingdom*
- 11:00 099 **From parasite genome to parasite control**
Mitrevva M., *Washington University, USA*

Main Congress Room**11:30-12:30 Janssen Corporate Symposium:**
Change and spatial shifts in Parasitic Diseases:
Fact or Fiction

- 11:30 **Welcome**
- 11:35 **Applying genomics for drug discovery in parasitology**
Winkler H.
Johnson & Johnson – Biotechnology, Immunology, Oncology Beerse, Belgium
- 12:00 **Can proteomics support parasite drug discovery?**
Brophy P.
Institute of Rural Studies – Institute of Biological Sciences University of Wales, Aberystwyth, UK

Van Rysselberghe Room**14:00-15:30 Symposium: Geospatial animal health: new ways to model the ecology of parasitism**
Chairs: Malone J.
Rinaldi L.

- 14:00 100 **New insights into the application of geographical information systems and remote sensing in veterinary parasitology**
Rinaldi L., Musella V., and Cringoli G.
Dipartimento di Patologia e Sanità Animale, Settore di Parassitologia Veterinaria, Università degli Studi di Napoli "Federico II", Napoli, Italy
- 14:15 101 **Climate, satellites and the ecology of vector-borne parasites**
Malone J. 1, Nieto P. 1, and Bavia M. 2
1 Louisiana State University, Pathobiological Sciences, Baton Rouge, LA USA; 2 Federal University of Bahia, Preventive Medicine, Salvador, Bahia, Brasil

- 14:30 102 **Spatial modeling tools in parasitology: ready for consumption?**
Hendrickx G., and Ducheyne E.
Avia-GIS, Zoersel, Belgium
- 14:45 103 **Predictive mapping of the habitat suitability for ticks**
 Estrada-Peña A.
University of Zaragoza, Spain
- 15:00 104 **PARAGIS - Assessing eco-climatic factors affecting the spatial distribution of helminth parasite burdens in dairy herds**
Charlier J. 1, Ducheyne E. 2, Claerebout E. 1, Williams D.J.L. 3, Hendrickx G. 2, and Vercruysse J. 1
1 Ghent University, Department of Virology, Parasitology and Immunology, Merelbeke, Belgium; 2 Avia-GIS, Zoersel, Belgium; 3 Veterinary Parasitology, Liverpool School of Tropical Medicine/Faculty of Veterinary Science, University of Liverpool, Liverpool, UK
- 15:15 **Discussion**

Baeckelandt Room

14:00-15:30 **Coccidia**
Chairs: Penzhorn B.
Williams D.

- 14:00 105 **Treatment of *Neospora caninum* and other apicomplexan parasites with nita-zoxanide and other thiazolides interferes in protein disulfide isomerase enzyme activity**
Hemphill A. 1, Esposito M. 1, Mathis A. 2, Cortes H. 3, Müller N. 1, and Müller J. 1
1 Institute of Parasitology, Vetsuisse Faculty, University of Berne, Switzerland; 2 Institute of Parasitology, Vetsuisse Faculty, University of Zürich, Switzerland; 3 Laboratório de Parasitologia Victor Caeiro, Universidade de Évora, Évora, Portugal
- 14:15 106 **Inoculation of balb/c mice with live attenuated tachyzoites protects against a lethal challenge with *Neospora caninum***
Bartley P.M., Wright S.E., Chianini F., Buxton D., and Innes E.A.
Moredun Research Institute, Edinburgh, UK
- 14:30 107 **Eimerioidosis in camelids of Miandoab region, Iran**
Yakhchali M., and Cheraghi E.
Faculty of Veterinary medicine, Nazlu campus, Urmia University, Urmia city, West Azerbaijan province, Iran
- 14:45 108 **Innate immune reactions against *Eimeria bovis***
Hermosilla C., Behrendt J.H., Zahner H., and Taubert A.
Justus Liebig University Giessen, Institute of Parasitology, Giessen, Germany
- 15:00 109 **Cellular immune response to *Isospora suis* infections in piglets**
Worliczek H.L. 1,2, Buggelsheim M. 1, Gerner W. 2, Schmidt P.1, Saalmüller A. 2, and Joachim A. 1
1 Department of Pathobiology, University of Veterinary Medicine, Vienna, Austria; 2 Clinical Department for Diagnostic Imaging, Infectious Diseases and Clinical Pathology, University of Veterinary Medicine Vienna, Vienna, Austria

- 15:15 110 **Metaphylactic efficacy of diclazuril (vecoxan) against naturally acquired *Eimeria alabamensis* infection in calves**
Veys P., and Goossens L.
Janssen Animal Health, Beerse, Belgium

Jan van Eyck Room

14:00-15:30 Cysticercosis
Chairs: Willingham L.
Krecek R.C.

- 14:00 111 ***Taenia solium* cysticercosis in western Kenya**
Willingham A.L. 1, Mutua F. 2, Massawe S. 3, and Downie-Ngini K. 4
1WHO/FAO Collaborating Center for Parasitic Zoonoses, Department of Veterinary Pathobiology, Faculty of Life Sciences, University of Copenhagen, Frederiksberg, Denmark
2University of Nairobi, Nairobi, Kenya; 3International Livestock Research Institute, Nairobi, Kenya; 4 University of Edinburgh, Edinburgh, Scotland
- 14:15 112 **Prevalence of porcine cysticercosis and human taeniasis/ cysticercosis in Nepal**
Joshi D.D. 1, Oommen A. 2, Dorny P. 3, Bista P.R. 1, Rajshekhar V. 2, and Vercruysse J. 3
1 NZFHRC, Kathmandu, Nepal; 2 Christine Medical College, Vellore, India; 3 Ghent University Ghent, Belgium
- 14:30 113 **Taeniasis/cysticercosis in Vietnam: more questions than answers**
Somers R. 1, Dorny P. 1,2, Lan Anh N.T. 3, Linh B.K. 3, and Vercruysse J. 1
1 Laboratory of Parasitology, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium;
2 Institute of Tropical Medicine Antwerp, Belgium; 3 National Institute of Veterinary Research, Hanoi, Vietnam
- 14:45 114 **Porcine antibody isotype responses in oxfendazole treated and non treated pigs naturally infected with *Taenia solium* cysticercosis**
Sikasunge C.S. 1, Johansen M.V. 2, Phiri I.K. 1, Willingham III A.L. 3, and Ørnbjerg N. 2
1 School of Veterinary Medicine, University of Zambia, Lusaka, Zambia; 2 DBL – Centre for Health Research and Development, Charlottenlund, Denmark; 3 WHO/FAO Collaborating Centre for Parasitic Zoonoses, Faculty of Life Sciences, University of Copenhagen, Frederiksberg C, Denmark
- 15:00 115 **Nanobodies® as a tool for specific diagnosis of porcine cysticercosis (*Taenia solium*)**
Deckers N. 1,2, Conrath K. 3, Kanobana K. 1, Victor B. 1, Muyltermans S. 3, Vercruysse J. 2, and Dorny P. 1,2
1 Institute of Tropical Medicine, Department of Animal Health, Antwerp, Belgium; 2 Ghent University, Faculty of Veterinary Medicine, Laboratory of Parasitology, Merelbeke, Belgium; 3 Free University of Brussels, Department of Cellular and Molecular Immunology, Etterbeek, Belgium
- 15:15 116 **Optimisation of the cysticercosis diagnosis in pigs through Bayesian modelling**
Praet N. 1, Saegerman C. 3, Berkvens D. 1, Lesaffre E. , Speybroeck N. 1, and Dorny P. 1
1 Institute of Tropical Medicine, Animal Health Department, Antwerp, Belgium; 2 Biostatistical Centre, Catholic University of Leuven, Leuven, Belgium; 3 Department of Infectious and Parasitic Diseases, Epidemiology and Risk Analysis Applied to Veterinary Sciences, Faculty of Veterinary Medicine, University of Liege, Liege, Belgium

Hubert van Eyck Room**14:00-15:30 Mechanisms of Action of Parasiticides****Chairs: Fairweather I.
Sangster N.**

- 14:00 117 **Interaction of macrocyclic lactones (MLs) with multidrug resistance transporters**
Lespine A., and Alvinerie M.
INRA-UR66 Pharmacologie-Toxicologie, Toulouse, France
- 14:15 118 **Latrophilin-like proteins in parasitic nematodes**
Welz C. 1, Fischer N. 1, Harder A. 2, Schnieder T. 1, and von Samson-Himmelstjerna G. 1
1 University of Veterinary Medicine, Hannover, Germany; 2 Bayer Health Care AG, Animal Health, R&D Parasiticides, Monheim, Germany
- 14:30 119 **In vitro and in vivo activity of the anti-cancer agent 2-methoxyestradiol (2ME), either alone or in combination with albendazole, against *Echinococcus multilocularis* metacestodes**
Spicher M., Naguleswaran A., and Hemphill A.
University of Berne, Vetsuisse Faculty, Institute of Parasitology, Berne, Switzerland
- 14:45 120 **In vitro analysis of glutathione transferases from the mite *Sarcoptes scabiei***
Molin E.U., and Mattsson J.G.
Department of Parasitology (SWEPAR), National Veterinary Institute and Swedish University of Agricultural Sciences, Sweden
- 15:00 121 **Relative activity of triclabendazole metabolites against the liver fluke, *Fasciola hepatica***
Fairweather I., Brennan G.P., Shaw E., Shaw L., Halferty L., and Toner E.M.
School of Biological Sciences, Queens University Belfast, Ireland
- 15:15 **Discussion**

Jan van der Goes Room**14:00-15:30 Ostertagia Antigens & Immunity****Chairs: Claerebout E.
Smith D.**

- 14:00 122 **Local cellular response of sheep to infection with *Teladorsagia circumcincta***
Halliday A.M. 1, Morrison W.I. 2, Miller H.R.P. 2, and Smith W.D. 1
1 Moredun Research Institute, UK; 2 RDVS, University of Edinburgh, Easter Bush, Roslin
- 14:15 123 **Proteomic characterisation of mucus derived from gastric mucosa during the immune exclusion of *Teladorsagia circumcincta***
Athanasiadou S. 1,2, Jackson F. 2, Pemberton A.M. 3, Miller H.R.P. 3, and Huntley J. 2
1 Scottish Agricultural College, Midlothian, UK; 2 Moredun Research Institute, Midlothian, UK; 3 University of Edinburgh, Midlothian, UK.

- 14:30 124 **Local cellular changes in serial abomasal biopsy samples and their association with the cost of immunity in *Teladorsagia circumcincta* infected sheep**
Greer A.W. 1,2, Huntley J.F. 2, Mackellar A. 2, McAnulty R.W. 1, Stankiewicz M. 1, and Sykes A.R. 1
1 Lincoln University, Canterbury, New Zealand; 2 Moredun Research Institute, Edinburgh, Scotland
- 14:45 125 **Vaccination of calves against *Ostertagia ostertagi* with subfractions of the protective ES-thiol**
Meyvis Y., Geldhof P., Vercruysse J., and Claerebout E.
Laboratory for Parasitology, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium
- 15:00 126 **A comparative analysis of immunogenic excretory/secretory proteins from *Teladorsagia circumcincta* harvested at 1, 3 and 5 days post infection**
Smith S., Nisbet A., and Matthews J.
Division of Parasitology, Moredun Research Institute, Midlothian, Scotland
- 15:15 127 **A calcium-dependent, structural homologue of a novel salivary apyrase from blood-feeding arthropods has been identified in *Ostertagia* third stage larvae**
Zarlenga D.S., and Gasbarre L.C.
USDA, ARS, ANRI, Bovine Functional Genomics Laboratory, Beltsville, USA

Van Rysselberghe Room

16:00-17:15 Symposium:
Application of the three Rs in the regulation of parasiticides: current status and prospects for innovation
Chair: Fisher M.

The process for the regulation of parasiticides, in fulfilling its mandate to protect the well-being of humans, animals and the environment, can appear to provide little lee-way for innovation. Against this, many national and international laws controlling the use of animals in research express objectives of replacing the use of animals in experiments and science as a whole. Steps have already been made in the regulation of parasiticides to reduce duplication of studies by creating international harmonisation and guidelines to ensure that, having followed sound and well-established practices, useful and acceptable data is produced. But that is not the end of the process, rather a staging point along the way, and this symposium is aimed at identifying the current drivers and what could be the next stages of the process.

The symposium will begin by setting the context for the three Rs and will then examine the status quo from the perspective of the different parties involved in the regulatory process: the statistician, the regulator, the pharmaceutical industry, the contract research organisation (CRO) and the parasitologist. The participants will then explore the possibilities for innovation in the short and longer term for replacement, reduction and refinement in this area.

- 16:00 128 **The three Rs: setting the context**
 Morton D.B.
School of Biosciences, University of Birmingham, Edgbaston, Birmingham, UK
- 16:15 129 **Statistical support for the three Rs**
 Fisher M.A.
Shernacre Enterprise,, Shernacre Cottage,, Lower Howsell Road, Malvern, Worcestershire, UK

- 16:30 130 **Regulatory process**
Dean S.P.
Veterinary Medicines Directorate, Woodham Lane, New Haw , Addlestone Surrey, UK
- 16:45 131 **The perspective from industry**
Altreuther G. 1, and Kennedy T. 2
1 Bayer HealthCare AG, Animal Health Division, R&D Parasitocides, Leverkusen, Germany; 2 R&D Central Life Sciences , Phoenix, USA
- 17:00 132 **The CRO perspective**
Ketzis J.K.
Charles River Laboratories Biolabs Europe Ltd., Carrentrila, Ballina, Co. Mayo, Ireland

Baeckelandt Room

16:00-17:15 Anthelmintic Resistance Chairs: Geary T. Rojo-Vazquez F.

- 16:00 133 **Resistance of *Parascaris equorum* against ivermectin due to frequent anthelmintic treatment of foals in The Netherlands**
van Doorn D.C.K., Lems S., Weteling A., Ploeger H.W., and Eysker M.
Utrecht University, Infectious Diseases and Immunology, The Netherlands
- 16:15 134 **Experimentally induced ivermectin resistance in *Ostertagia ostertagi* in cattle**
Van Zeveren A.M. 1, Geldhof P. 1, Alvinerie M. 2, Prichard R. 3, Claerebout E. 1, and Vercruysse J. 1
1 Laboratory for Parasitology, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium; 2 Laboratoire de Pharmacologie-Toxicologie, INRA, Toulouse, France; 3 Institute of Parasitology, McGill University, Sainte-Anne-de-Bellevue, Quebec, Canada
- 16:30 135 **The identification of cattle nematode parasites resistant to multiple classes of anthelmintics in a commercial cattle population in the US**
Gasbarre L.C. 1, and Smith L.L. 2
1 USDA, ARS, ANRI, Bovine Functional Genomics Laboratory, Beltsville MD, USA; 2 Larry L. Smith Research and Development Inc, Lodi WI, USA
- 16:45 136 **Statistical approach to measure efficacy of anthelmintic treatment on horse farms**
Kaplan R.M. 1, Vidyashankar A.N. 2, and Chan S. 2
1 Dept of Infectious Diseases, College of Veterinary Medicine, University of Georgia, Athens, Georgia, USA; 2 Dept of Statistical Science, Cornell University, Ithaca, New York, USA
- 17:00 137 **Evidence of macrocyclic lactone-resistant *Parascaris equorum* on a Turkish horse farm**
Cirak V.Y. 1, Girisgin O. 1, Karabacak A. 2, Sonmez F. 1, and Balkaya I. 3
1 University of Uludag, Faculty of Veterinary Medicine, Dep. Parasitology, Bursa, Turkey; 2 Military Veterinary School and Training Centre, Gemlik-Bursa, Turkey; 3 Central Veterinary Control and Research Institute, Etlik-Ankara, Turkey

Jan van Eyck Room

16:00-17:15

Arthropods

**Chairs: Wall R.
Huntley J.**

- 16:00 138 **Gene silencing of the tick protective antigens, Bm86, Bm91 and subolesin, in the one-host tick *Boophilus microplus* by RNA interference**
Nijhof A.M. 1, Taoufik A. 1, de la Fuente J. 2,3, Kocan K.M. 2, de Vries E. 1, and Jongejan F. 1,4
 1 Utrecht Centre for Tick-borne Diseases (UCTD), Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands; 2 Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, USA; 3 Instituto de Investigación en Recursos Cinegéticos IREC (CSIC-UCLM-JCCM), Ciudad Real, Spain; 4 Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, South Africa
- 16:15 139 **Immunization studies in rabbits using gut membrane-bound proteins derived from *Rhipicephalus appendiculatus*, *R. evertsi evertsi* and *Amblyomma variegatum* female ticks**
Kutima H.L. 1, Amoo O. 2, Imbuga M. 3, and Kutima P.M. 4
 1 Jomo Kenyatta University of Agriculture and Technology (JKUAT), Department of Zoology, NAIROBI, Kenya; 2 University of Ibadan, Department of Veterinary Parasitology, Nigeria; 3 JKUAT, Department of Biochemistry; 4 JKUAT, Department of Food Science and Post Harvest Technology
- 16:30 140 **Identification of novel antigens from the sheep scab mite, *Psoroptes ovis***
McNair C.M. 1, Nisbet A.J. 1, Billingsley P.F. 2, and Knox D.P. 1
 1 Moredun Research Institute, Edinburgh, UK; 2 Sanaria Inc, Rockville, Maryland, USA
- 16:45 141 **Epidemiology of *Dermanyssus gallinae* and acaricidal efficacy of phoxim 50% (ByeMite®) in alternative housing systems during the laying period of hens**
Meyer-Kühling B. 1, Heine J. 2, Müller-Lindloff J. 3, and Pfister K. 4
 1 BWE Brüterei Weser-Ems, PHW Zentrallabor, Visbek, Germany; 2 Bayer HealthCare AG, Monheim, Germany; 3 Veterinary Practice for Poultry and Pigs "Am Bergweg", Lohne, Germany; 4 Institute for Comparative Tropical Medicine and Parasitology, Faculty of Veterinary Medicine, Ludwig-Maximilians-University, Munich, Germany
- 17:00 142 **Genetic differences in *Dermanyssus gallinae* from wild birds and layers**
Arvidsson S., Chirico J., Morrison D., and Mattsson J.G.
 National Veterinary Institute and Swedish University of Agricultural Sciences, Department of Parasitology (SWEPAR), Uppsala, Sweden

Hubert van Eyck Room

16:00-17:15

Biological Control

**Chairs: Panchadcharam C.
Thamsborg S.**

- 16:00 143 **Candidate endoparasitic fungi for use in the biological control of ovine parasites**
Kelly P. 1, 2, Good B. 1, Hanrahan J.P. 1, Mulcahy G. 2., and De Waal D.T. 2
 1 Teagasc, Animal Production Research Centre, Athenry, Co. Galway, Ireland; 2 University College Dublin, School of Agriculture Food Science and Veterinary Medicine, Belfield, Dublin 4, Ireland

- 16:15 144 **Control of the sheep scab mite *Psoroptes ovis* in vivo and in vitro using fungal pathogens**
Abolins S. 1, Thind B. 2, Luke B. 3, Moore D. 3, Jackson V. 2, Wall R. 1, and Taylor M.A. 2
 1 School of Biological Sciences, University of Bristol, Bristol, UK; 2 Veterinary Surveillance Team, Central Science Laboratory, York, UK; 3 CABI Europe-UK, Ascot, UK
- 16:30 145 **The acaricidal effect of the essential oil of *Artemisia siberi* and *Zataria multiflora* extract against adult stage of *Rhipicephalus (Boophilus) annulatus* in Iran**
 Pirali-Kheirabadi Kh.
 University of Shahrekord, Faculty of Veterinary Medicine, Department of Pathobiology, Shahrekord, Iran
- 16:45 146 **Genetic resistance to *Heterakis gallinarum* in two chicken layer lines following a single dose infection**
 Kanan A. 1, Brandt H. 2, Weigend S. 3, Moors E. 1, Erhardt G. 2, and Gauly M. 1
 1 Institute of Animal Breeding and Genetics, Albrecht Thaer Weg 3, University of Goettingen, Germany; 2 Department of Animal Breeding and Genetics, Justus Liebig University Giessen, Germany; 3 Institute for Animal Breeding Mariensee, Federal Agricultural Research Center, Germany
- 17:00 147 **Length of *Ascaris suum* larvae is affected by parental genotype and location in the intestine in pigs**
Nejsum P. 1,2, Thamsborg S.M. 1, Jørgensen C. 2, Fredholm M. 2, and Roepstorff A. 1
 1 Danish Centre for Experimental Parasitology, Department of Veterinary Pathobiology, University of Copenhagen, Denmark; 2 Genetics and Bioinformatics, Department of Animal and Veterinary Basic Sciences, Faculty of Life Sciences, University of Copenhagen, Denmark

Jan van der Goes Room

16:00-17:15 Parasites of Horses

**Chairs: Duncan J.
Eysker M.**

- 16:00 148 **Epidemiology and control of gastrointestinal nematodes of horses in Ukraine**
Starovir O., Syvak V., and Verzhikhovskiy O.
 Department of Veterinary Parasitology and Tropic Veterinary on National Agricultural University, Kyiv, Ukraine
- 16:15 149 **Evaluation of the association between *Anoplocephala perfoliata* infection and colic in horses in Ontario, Canada**
 Trotz-Williams L. 1, Physick-Sheard P. 2, McFarlane H. 1, Pearl D.L. 2, Martin S.W. 2, and Peregrine A.S. 1
 1 Department of Pathobiology and 2 Department of Population Medicine, Ontario Veterinary College, University of Guelph, Ontario, Canada
- 16:30 150 **Age clustered pooled faecal samples as monitoring tool in cyathostomiasis in horses**
Eysker M., Bakker J., van den Berg M., Doorn D.C.K., and van Ploeger H.W.
 Utrecht University, Department of Infectious Diseases and Immunology, Division of Clinical Infectiology

- 16:45 151 **Isolation and characterization of stage-specific antigens of *Gasterophilus intestinalis***
Roelfstra L. 1, Deeg C. 2, Betschart B. 1, Kopacek P. 4, and Pfister K. 3,1
 1 Institute of Zoology, University of Neuchâtel, Switzerland; 2 Institute of Physiology, Ludwig-Maximilians University, Munich, Germany; 3 Institute of Comparative Tropical Medicine and Parasitology, Ludwig-Maximilians University, Munich, Germany; 4 Academy of Science of the Czech Republic, Ceske Budejovice, Czech Republic
- 17:00 152 **Larval viability and serological response in horses with long-term *Trichinella spiralis* infection**
Hill D.E. , Forbes L., Kramer M., Gajadhar A., and Gamble H.R.
 USDA, Agricultural Research Service, Animal Parasitic Diseases Laboratory, Beltsville, USA

Main Congress Room

17:10-18:10

**Bayer Corporate Symposium
 Bayer Parasite Solutions Symposium**



- 17:10 153 ***Bartonella* spp. – new pathogens, old diseases**
 Kempf V.A.J.
 University Hospital of Tuebingen, Institute for Medical Microbiology and Hygiene, German Consiliary Laboratory for Bartonella-infections, Tuebingen, Germany
- 17:30 154 **Efficacy of a combination of 10% imidacloprid / 50% permethrin for the prevention of Leishmaniosis in kennelled dogs in endemic area**
Otranto D. 1, Paradies P. 1, Lia R.P. 1, Cantacessi C. 1, Mencia N. 2, Capelli G. 3, and Stanneck D. 2
 1 Department of Animal Health and Welfare, Faculty of Veterinary Medicine, Valenzano, Bari, Italy; 2 Bayer Health Care AG, Animal Health Division Leverkusen, Germany; 3 Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy
- 17:50 155 **Spread of companion animal vector-borne parasitic disease in US and lessons to be learned**
 Bowman D.D.
 Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, USA

WEDNESDAY 22 AUGUST

Main Congress Room

08:30-09:30 **Plenary Session III: Invited lectures**
Chair: Claerebout E.

- 08:30 156 **Genetic diversity and infection levels of anisakid nematode parasites of aquatic organisms of Boreal and Austral regions**
 Mattiucci S., *University of Rome “La Sapienza”, Italy*
- 09:00 157 **Veterinary epidemiology and Bayes: Commensalism, not parasitism**
 Lesaffre E., *Catholic University of Leuven, Belgium*

Main Congress Room

09:30-10:00 **WAAVP Awards**
Peters Nansen Young Scientist Award

Van Rysselberghe Room

10:30-12:00 **Symposium:**
Distance learning in veterinary parasitology: a reality
Chair: Madder M.

Distance learning in all its facets has become part of our life-long learning and especially in the domain of veterinary parasitology it successfully made its introduction in the last years. This symposium aims at portraying all existing and new initiatives in this domain, from complete web-based MSc courses up to smaller initiatives such as short courses or interactive course material and furthermore wants to show the development and use of new technologies and communication tools that facilitate distance learning. The last decade a variety of mobile devices have been developed from ordinary cell-phones up to powerful pocket PC's and smart phones. Several initiatives and projects that focus on the use of these mobile devices in resource poor settings where internet access is not always functional or even present will be presented as well.

- 10:30 158 **A web-based MSc course in veterinary tropical diseases: an impression of the first two years**
Madder M. 1, Venter L. 2, and Coetzer J.A.W. 2
1 ITMA, Belgium; 2 University of Pretoria, Department of Veterinary Tropical Diseases, Pretoria, South Africa
- 10:45 159 **The present and coming mobile learning**
 de Waard I. 1, Correwyn S. 1, Iluyemi A. 2, Kiyan C. 1, and Zolfo M. 1
1 Institute of Tropical Medicine, Antwerp Belgium; 2 Centre for Healthcare Modelling & Informatics, UK

- 11:00 160 **Radically new approach required for countering progressively failing information transfer in the field of parasite management**
van Wyk J.A.
Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, 0110 Onderstepoort, South Africa
- 11:15 161 **Canine Vector-Borne Diseases: from the dog to the parasite. A new teaching approach to clinical parasitology**
Paradies P., Arndt N., Sasanelli M., de Caprariis D., Lia R.P., Mencke N., and Otranto D.
Faculty of Veterinary Medicine, Department of Animal Health and Welfare, Bari, Italy
- 11:30 162 **The tick game: using an interactive board game for teaching tick biology and chemical resistance counter-strategies**
Playford M., and Lean I.J.
Strategic Bovine Services
- 11:45 **Discussion**

Baeckelandt Room

10:30-12:00

Anthelmintic Resistance

**Chair: von Samson-Himmelstjerna G.
Cabaret J.**

- 10:30 163 **Why is anthelmintic resistance more common in *Teladorsagia (Ostertagia) circumcincta* than *Trichostrongylus colubriformis*?**
Dobson R.J. 1, and Besier R.B. 2
1 School of Veterinary & Biomedical Sciences, Murdoch University, South Street Murdoch, Western Australia; 2 Department of Agriculture and Food Western Australia, Albany, Western Australia
- 10:45 164 **Development of genetic crossing methods to identify genes associated with macrocyclic lactone resistance in *Haemonchus contortus***
Sargison N.D. 1, Jackson F. 2, and Gilleard J.S. 3
1 University of Edinburgh, Royal (Dick) School of Veterinary Studies, Large Animal Practice, Easter Bush Veterinary Centre, Roslin, Midlothian, Scotland; 2 Moredun Research Institute, Penicuik, Midlothian, Scotland; 3 University of Glasgow, Faculty of Veterinary Medicine, Bearsden, Glasgow, Scotland
- 11:00 165 **In vitro detection of anthelmintic resistance in *Haemonchus contortus* by larval development test**
Várady M., and Corba J.
Parasitological Institute, Slovak Academy of Sciences, Hlinkova Košice, Slovakia
- 11:15 166 **The development of diagnostic assays to evaluate triclabendazole resistance in the field**
Flanagan A. 1, Fairweather I. 1, Brennan G. 1, Edgar H.W.J. 2, and Hanna R.W. 2
1 Parasite Proteomics and Therapeutics Research Group, Queen's University Belfast, Ireland; 2 Veterinary Sciences Division, AFBINI, Belfast, Ireland

- 11:30 167 **Anthelmintic resistance survey in French dairy goat nematodes in four main breeding areas**
Chartier C. 1, Pors I. 1, Rehby L. 2, and Kerboeuf D. 3
 1 French Food Safety Agency (AFSSA), Laboratoire d'Etudes et de Recherches Caprines, Niort Cedex, France; 2 Veterinary Technical Group of Burgundy, Gueugnon, France; 3 INRA, IASP-213, Nouzilly, France
- 11:45 168 **The search for mutations associated with ivermectin resistance in the ligand-gated chloride channel genes of *Teladorsagia circumcincta***
Skuce P.J., Kenyon F., Donnan A.A., and Jackson F.
 Moredun Research Institute, Edinburgh, UK

Jan van Eyck Room

10:30-12:00 Fasciola & Trematodes

**Chairs: Dorchies P.
Pfister K.**

- 10:30 169 **Discovery of new molecular targets for drug & vaccine design against liver fluke**
Piedrafita D. 1, Estuningsih E. 2, Pleasance J. 1, Raadsma H.W. 3, Meeusen E. 1, and Spithill T. 4
 1 Animal Biotechnology Research Laboratories (ABRL), Department of Physiology, Monash University, Clayton, Australia; 2 Research Institute for Veterinary Science, Bogor, West Java, Indonesia; 3 Faculty of Veterinary Science, University of Sydney, Australia; 4 Institute of Parasitology and Centre for Host-Parasite Interactions, McGill University, Montreal, Canada
- 10:45 170 **The alternative way of vaccine targeting, phosphoglycerate kinase (FhPGK) from the adult *Fasciola hepatica* – evaluation of the cDNA and recombinant protein vaccine protectivity**
Jaros S. 1, Januszkiewicz K. 1, Smaga-Kozłowska K. 1, Wesolowska A. 1, Zygnier W. 2, and Wedrychowicz H. 1, 2
 1 Department of Molecular Biology, Laboratory of Molecular Parasitology, W. Stefanski Institute of Parasitology, Polish Academy of Sciences, Warsaw, Poland; 2 Division of Parasitology, Department of Preclinical Sciences, Warsaw Agricultural University, Warsaw, Poland
- 11:00 171 **Molecular discrimination of *Fascioloides magna* and *Fasciola hepatica* based on the species-specific ITS regions**
Králová-Hromadová I., Spakulová M., and Turceková L.
 Parasitological Institute, Slovak Academy of Sciences, Kosice, Slovakia
- 11:15 172 **SEM observations on the impact of a methimazole on triclabendazole (TCBZ) action against TCBZ-resistant and -susceptible isolates of *Fasciola hepatica***
Devine C., Brennan G., Hoey E., Trudgett A., and Fairweather I.
 Parasite Proteomics and Therapeutics Research Group, Queen's University Belfast, Ireland
- 11:30 173 **Uptake of triclabendazole by the liver fluke: trans-tegumental or oral?**
Toner E.M., McConvery F.P., Brennan G.P., Meaney M.M., and Fairweather I.
 Queen's University of Belfast, School of Biological Sciences, Belfast, Northern Ireland

- 11:45 174 **Molecular identification and genotypic characterization of *Fasciola spp* first isolated in goats in Vietnam**
Nguyen T.G.T. 1,3, Nguyen V.D. 2, Dorny P. 3, Vercruysse J. 3, Blair D. 4, and Hoa Le T. 5
 1 Institute of Veterinary Research, Vietnam; 2 Hanoi Medical University, Vietnam; 3 Faculty of Veterinary Medicine, Ghent University, Belgium; 4 James Cook University, Australia; 5 Institute of Biotechnology, Vietnam

Hubert van Eyck Room

10:30-12:00 Diseases caused by Arthropods

**Chairs: Bates P.
Bourdeau P.**

- 10:30 175 **Epidemiology of blowfly myiasis in domestic rabbits in the UK – a nationwide study**
Fox M.T. 1, Kluiters G. 1, Cousquer G. 2, Hall M.J.R. 3, MacLean C. 4, and Wall R. 5
 1 Department of Pathology and Infectious Diseases, Royal Veterinary College, London, UK; 2 Ar Lan A Mor, Allée de la Salorge, Domaine de Rostu, Mesquer, France; 3 Department of Entomology, The Natural History Museum, London, UK; 4 Department of Medical Genetics, University of Cambridge, Cambridge, UK; 5 School of Biological Sciences, University of Bristol, Bristol, UK
- 10:45 176 **Prevalence of *Anaplasma (A.) phagocytophilum* infection in dogs in Germany**
Pfister K. 1, Galke D. 2, Fumi C. 2, Beelitz P. 1, and Kohn B. 2
 1 Institute for Comparative Tropical Medicine and Parasitology, LMU Munich; 2 Clinic for Small Animals, Free University of Berlin, Germany
- 11:00 177 **Immunological and serological study on the infectivity of host animals and ticks (*Ixodidae*, *Argasidae*) to CCHF virus in west Azerbaijan, Iran**
Zelmadarraiy Z. 1, Tighi S., Chinikar S. 2, Vatandoost H. 1, Oshaghi M.A. 1, and Rassi Y. 1
 1 School of Public Health & Institute of Health Research, Tehran University of Medical Sciences, Tehran, Iran; 2 Pasteur Institute of Iran
- 11:15 178 **Seasonal and geographic variation in the epidemiology of *Anaplasma Phagocytophilum* and *Rickettsia spp.* in the hard tick *Ixodes ricinus* in Bavaria, Germany**
Silaghi C., Gilles J., and Pfister K.
 Institute for Comparative Tropical Medicine and Parasitology, Veterinary Faculty, Ludwig – Maximilians – University, Munich, Germany
- 11:30 179 **Distribution and phylogeny of intramitochondrial bacteria in Ixodid ticks**
Epis S. 1, Sassera D. 1, Beninati T. 2, Lo N. 2, Sacchi L. 3, Bandi C. 1, and Genchi C. 1
 1 University of Milano, Dipartimento di Patologia Animale, Igiene e Sanità, Milano, Italy
- 11:45 180 **Detection of *Borrelia spp.* in natural vector *Ixodes ricinus* in the Hannover region with genus specific and species specific PCR – a representative sampling approach of public greens**
Epe C. 1, Montenegro V.M. 1, Schnieder T. 1, and Kreienbrock L. 2
 1 Department for Infectious Diseases, Institute for Parasitology. University of Veterinary Medicine Hannover, Hannover, Germany; 2 Institute of Biometry & Epidemiology. University of Veterinary Medicine Hannover, Germany

Jan van der Goes Room**10:30-12:00****Coccidia****Chairs: Daugschies A.
Joachim A.**

- 10:30 181 **Is toltrazuril-based prevention of piglet coccidiosis always economically justifiable?**
Skampardonis V. 1,2, Sotiraki S. 2, Kostoulas P. 1, Alexopoulos C. 3, and Leontides L. 1
1 University of Thessaly, Faculty of Veterinary Medicine Laboratory of Biostatistics, Epidemiology and Economics of Animal Production, Karditsa, Greece; 2 NAGREF, Veterinary Research Institute, Thessaloniki, Greece; 3 Aristotle University, Faculty of Veterinary Medicine, Clinic of Large Animals, Thessaloniki, Greece
- 10:45 182 **Revitalisation of anticoccidial sensitivity in poultry flocks by the use of a live attenuated vaccine**
Marshall R.N. 1, Rennie S. 2, and Marshall J.A. 1
1 Veterinary Laboratories Agency (Weybridge), Woodham Lane, New Haw, Addlestone, Surrey, UK; 2 Schering-Plough Animal Health, Middlesex, UK
- 11:00 183 **Immunostimulating complexes incorporated coccidia antigens and low toxicity plant saponins as a novel delivery system for *Coccidia* vaccine immunization**
Berezin V. 1, Bogoyavlenskiy A. 1, Tolmacheva V. 1, Makhmudova N. 1, Fetterer R. 2, Barfield R. 2, Miska K. 2, and Jenkins M. 2
1 Institute of Microbiology and Virology, Almaty, Kazakhstan; 2 Animal Parasitic Diseases Laboratory, USDA-ARS, Beltsville, MD, USA
- 11:15 184 **Molecular-based identification of sarcocysts in bovine meat**
Vangeel L. 1, Houf K. 1, Chiers K. 1, Vercruysse J. 1, D'Herde K. 2, and Ducatelle R. 1
1 Research Group Veterinary Public Health and Zoonosis, Faculty of Veterinary Medicine, Ghent University, Belgium; 2 Department of Human Anatomy, Embryology, Histology and Medical Physics, Faculty of Medicine and Health Sciences, Ghent University, Belgium
- 11:30 185 **The development of conventional and real-time PCR for the detection of *Besnoitia besnoiti***
Cortes H.C.E. 1, Reis Y. 2, Gottstein B. 3, Hemphill A. 3, Leitao A. 2, and Müller N. 3
1 Laboratório de Parasitologia Victor Caeiro, Núcleo da Mitra, ICAM, Universidade de Évora, Évora, Portugal; 2 Instituto de Investigação Científica Tropical, CIISA, Faculdade de Medicina Veterinária, Lisboa, Portugal; 3 Institute of Parasitology, Berne, Switzerland
- 11:45 186 **Re-emerging cattle besnoitiosis (*Besnoitia besnoiti*) in France: update on clinical and epidemiological aspects**
Alzieu J.P.
Clinique Vétérinaire Les Althéas, Pamier, France

Main Congress Room

12:10-13:10 **Novartis Corporate Symposium:**  **Delivering Innovation**
Chair: Geary T.

12:10 **Welcome**

12:15 187 **Ticks of dogs and cats and their importance as disease vectors in Europe**
Epe C.
*Department for Infectious Diseases, Institute for Parasitology. University of Veterinary Medicine
Hannover, Germany*

12:40 188 **Delivering a new class of anthelmintics**
Kaminsky R.
Novartis, Centre de Recherche Santé Animale SA, St-Aubin (FR), Switzerland

THURSDAY 23 AUGUST

Main Congress Room

08:30-10:00

Symposium:

Global incidence of anthelmintic resistance in sheep and cattle and best practices to counter resistance development

**Chairs: von Samson-Himmelstjerna G.
Prichard R.**

The widespread and frequent use of mainly benzimidazole and macrocyclic lactone anthelmintics in ruminants has led to the selection of resistant helminth populations in many countries. However, to date the prevalence and intensity of anthelmintic resistance varies considerably between different geographical regions. Most severe situations have developed in the southern hemisphere, e.g. in Australia, New Zealand, Argentina or South Africa. In contrast, for Europe and North America it seems that the problem is not as much advanced. However, there clearly is a considerable lack of scientific information on the actual scale of AR in the field for most of the countries and on best practices to handle the problem. Several issues contribute to this, like insufficient diagnostic tools, lack of research funding but also only limited exchange of information between researchers and end users. This symposium will therefore bring together experts from different countries to present and discuss the current status of AR in ruminants and the views on how to avoid or postpone resistance development. Specifically the following topics will be addressed: Diagnosis of AR in the field, AR in cattle: a developing problem world wide, alternatives to anthelmintics in parasite control, identification of animals that need or need not treatment, parasite control in the face of existing resistance, dissemination of best practice to producers and advisors.

- 08:30 189 **Anthelmintic resistance in small ruminants and its diagnosis in the field**
Kaplan R.M.
Dept. of Infectious Diseases, College of Veterinary Medicine, University of Georgia, Athens, Georgia, USA
- 08:45 190 **Anthelmintic resistance in cattle - a developing problem?**
Demeler J. 1, Van Zeveren A.M. 2, Coles G. 3, Höglund J. 4, Kleinschmidt N. 5, Koopmann R. 5, von Samson-Himmelstjerna G. 1, and Vercruysse J. 2
1 Hannover School of Veterinary Medicine, Institute for Parasitology, Germany; 2 Ghent University, Faculty of Veterinary Medicine, Laboratory for Parasitology, Mellebeke, Belgium; 3 SWEPPAR, Swedish University of Agricultural Sciences, Uppsala, Sweden; 4 University of Bristol, England; 5 Institute of Organic Farming of the Federal Agricultural Research Centre Trenthorst, Germany
- 09:00 191 **Alternatives to anthelmintics in parasite control in sheep**
Sayers G., and Sweeney T.
School of Agriculture, Food Science and Veterinary Medicine, University College Dublin, Ireland
- 09:15 192 **Targeted anthelmintic treatment of sheep for resistance management: early experience in Australia and future directions**
Besier R.B.
Animal Health Laboratories, Department of Agriculture and Food Western Australia, Albany, Australia

- 09:30 193 **Parasite control in the face of existing resistance in New Zealand**
 Pomroy W.E.
Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand
- 09:45 194 **Dissemination of best practice advice to producers and advisors**
Jackson F., Bartley D., Kenyon F., and Sargison N.
Moredun Research Institute, Edinburgh, Scotland

Van Rysselberghe Room

08:30-10:00 **Vector-Borne Protozoa**
Chairs: Van den Bossche P.
Bourdeau P.

- 08:30 195 **First report on population structure for the *Leishmania* major vector, *Phlebotomus papatasi* sandfly using microsatellite loci**
Hamarsheh O. 1,2, Presber W. 1, Abdeen Z. 2, and Schönián G.
1 Institut für Mikrobiologie und Hygiene, Humboldt Universität, Charité Universitätsmedizin Berlin, Germany; 2 Laboratory of Leishmaniasis, Faculty of Medicine, Al-Quds University, Jerusalem, Palestine
- 08:45 196 **Canine leishmaniasis candidate vaccines containing ribosomal and histone antigens could not protect dogs from experimental infection with *Leishmania infantum***
Poot J., Janssen L., Schaap T., Vermeij P., Sondermeijer P., and Vermeulen A.
Intervet International B.V., Boxmeer, the Netherlands
- 09:00 197 **Detection of natural *Trypanosoma vivax* infections in pigs with microhaematocrit centrifugation and amplification of ITS1 rDNA**
Biryomumaisho S. 1, Melville S.E. 2, Katunguka-Rwakishaya E. 1, and Lubega G. 3
1 Makerere University, Department of Veterinary Medicine, Kampala, Uganda; 2 University of Cambridge, Department of Pathology, Cambridge, United Kingdom; 3 Makerere University, Department of Veterinary Parasitology and Microbiology, Kampala, Uganda
- 09:15 198 **Estimating the impact of *Trypanosoma evansi* (surra) on buffalo populations in the Philippines using data from cross-sectional surveys**
Dobson R.J. 1, Dargantes A.P. 1-2, Reid S.A. 1, Hood G.M. 3, and Mercado P. 2
1 School of Veterinary & Biomedical Sciences, Murdoch University, Western Australia; 2 College of Veterinary Medicine, Central Mindanao University, Musuan, Bukidnon, Philippines; 3 Department of Agriculture and Food Western Australia, Albany, Western Australia
- 09:30 199 **Trypanocidal drug resistance in trypanosomes of livestock in sub-Saharan Africa: an update**
Geerts S., Delespaux V., and Van den Bossche P.
Institute of Tropical Medicine, Antwerp, Belgium
- 09:45 200 **Immunization of mice with formalin inactivated *Trypanosoma evansi***
Tewari A.K., Rao J.R., and Mishra A.K.
Division of Parasitology, Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India

Baeckelandt Room**08:30-10:00 Diagnosis of Parasitic Diseases****Chairs: Zarlenga D.
Geldhof P.**

- 08:30 201 **Phylogenetic analysis of filarial worm *Setaria digitata* of Sri Lanka based on CO1 and 12s rDNA genes**
Yatawara L. 1, Wickramasinghe S. 1, Nagataki M. 1, Rajapakse R.P.V.J. 2, and Agatsuma T. 1
 1 Department of Environmental Health Sciences, Kochi Medical School, Kochi, Japan; 2 Department of Veterinary Pathobiology, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Peradeniya, Sri Lanka
- 08:45 202 **Development of real-time polymerase chain reaction assays (PCR) for detection and quantification of pathogen DNA within vector tissues**
Robinson M. 1, Morgan E. 2, Williams T. 3, and Shaw S. 1
 1 School of Clinical Veterinary Sciences, University of Bristol, Langford, UK; 2 School of Biological Sciences, University of Bristol, Bristol, UK; 3 Pfizer, Sandwich, Kent, UK
- 09:00 203 **Detection and quantification of *Strongylus vulgaris* DNA in equine faeces by real-time PCR**
Nielsen M.K. 1, Kaplan R.M. 2, and Peterson D.S. 2
 1 Department of Large Animal Sciences, Faculty of Life Sciences, University of Copenhagen, Denmark; 2 Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, Athens, Georgia, USA
- 09:15 204 **Specific detection of *Histomonas meleagridis* in turkeys by a PCR assay with an internal amplification control**
Bleyen N. 1, De Gussem K. 2, De Gussem J. 3, and Goddeeris B.M. 1
 1 K.U.Leuven, Faculty of Bioscience Engineering, Department of Biosystems, Division of Gene Technology, Heverlee, Belgium; 2 Alpharma Animal Health Inc., Antwerpen, Belgium; 3 Degulab, Izegem, Belgium
- 09:30 205 **First evaluation of a Reverse Line Blot hybridization assay for the speciation of drug resistant equine cyathostomes (Nematoda, Cyathostominae)**
Traversa D. 1, Klei T.R. 2, Iorio R. 1, Mauro A. 1, Di Lucente A. 1, and Sparagano O.A.E. 3
 1 Department of Comparative Biomedical Sciences, Faculty of Veterinary Medicine, Teramo, Italy; 2 Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, USA
 3 School of Agriculture, Food and Rural Development, Newcastle University, Newcastle upon Tyne, UK
- 09:45 206 **A rapid and simple field test to detect *Haemonchus contortus* infections in sheep**
Besier R.B. 1, Le Jambre L.F. 2, and Colditz I.G. 2
 1 Animal Health Laboratories, Department of Agriculture and Food Western Australia, Albany Western Australia, Australia; 2 FD McMaster Laboratory, CSIRO, Armidale New South Wales, Australia

Jan van Eyck Room

08:30-10:00 *Cryptosporidium* & *Giardia*

**Chair: Thompson A.
De Graaf D.**

- 08:30 207 **Molecular characterization, longitudinal prevalence and impact on performance of *Cryptosporidium andersoni* and *Giardia* infections in feedlot cattle of Western Australia and Alberta, Canada**
Ralston B.J. 1, Thompson R.C.A. 2, Pethick D. 2, McAllister T.A. 3, and Olson M.E. 4
 1 Alberta Agriculture and Food, Airdrie, Alberta Canada; 2 Division of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, Australia;
 3 Agriculture and Agri-Food Canada Research Centre, Lethbridge, Alberta, Canada; 4 Bow Valley Research, Calgary, Alberta, Canada
- 08:45 208 **Molecular epidemiology of *Cryptosporidium* in the Netherlands**
Wielinga P. 1, de Vries A. 1, Mank T. 2, Mars J. 3, Kortbeek L.M. 4, and van der Giessen J.W.B. 1
 1 RIVM, Laboratory for zoonoses and environmental microbiology (LZO), Bilthoven, the Netherlands;
 2 Animal Health Service (GD), Deventer, the Netherlands; 3 Laboratory of Public Health, Department of Parasitology, Haarlem, The Netherlands; 4 RIVM, Laboratory for Infectious Diseases Surveillance and Screening, Bilthoven the Netherlands
- 09:00 209 **New genotypes of *Cryptosporidium* species and their impact to the drinking water supplies**
 Karanis, P.
 National Research Center for Protozoan Diseases, Unit for Diseases Control and Genetics, Obihiro, Japan
- 09:15 210 **Occurrence, viability and genotyping of *Cryptosporidium* and *Giardia* in influent water and harvested shellfish in the Varano Lagoon (Italy)**
Giangaspero A. 1, Lonigro A. 2, Lacasella V. 3, Cavallo P. 3, Marangi M. 1, Cirillo R. 1, Berrilli F. 4, and Brandonisio O. 3
 1 Dipartimento di Scienze delle Produzioni, dell'Ingegneria e della Meccanica e dell'Economia Applicate ai Sistemi Agro-Zootecnici, University of Foggia, Italy;
 2 Dipartimento di Scienze delle Produzioni Vegetali, University of Bari, Italy; 3 Dipartimento di Clinica Medica, Immunologia e Malattie Infettive, University of Bari, Italy; 4 Dipartimento di Sanità Pubblica e Biologia Cellulare, University of Tor Vergata, Rome, Italy
- 09:30 211 **Giardiasis in cattle - a zoonanthroponosis not a zoonosis**
O'Handley R.M. 1, Uehlinger F.D. 2, Coklin T. 3, Greenwood S.J. 2, Dixon B.R. 3, and Barkema H.W. 4
 1 Murdoch University, Veterinary and Biomedical Sciences Perth, Australia; 2 University of Prince Edward Island, Atlantic Veterinary College, Charlottetown, Canada; 3 Health Canada, Microbiology Research Division, Ottawa, Canada; 4 University of Calgary, Faculty of Veterinary Medicine, Calgary, Canada
- 09:45 212 **Resazurin as a viability indicator for *Giardia duodenalis* trophozoites**
Bénére E. 1, Inocêncio da Luz R. 1, Vermeersch M. 1, Matheeußen A. 1, Cos P. 1 and, Maes L. 1,2
 1 University Antwerp, Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, Antwerp, Belgium; 2 Institute for Tropical Medicine, Antwerp, Belgium

Hubert van Eyck Room

08:30-10:00 Exotic Animals & Wild Life

**Chairs: Borgsteede F.
Taira N.**

- 08:30 213 **Biodiversity and systematics of *Haemosporidia* infecting South African tortoises**
Cook C. 1, Smit N.J. 1, and Davies A.J. 2
 1 Department of Zoology, University of Johannesburg, South Africa; 2 School of Life Sciences, Kingston University, Surrey, UK
- 08:45 214 **Prevalence of liver flukes (*Fasciola jacksoni*) in free ranging elephants in Sri Lanka**
Perera B.V.P. 1, and Rajapakse R.P.V.J. 2
 1 Department of Wildlife Conservation, Sri Lanka; 2 University of Peradeniya, Sri Lanka
- 09:00 215 **Intestinal nematode distributions in wild and captive populations of giant Galápagos tortoises (*Geochelone nigra*)**
Fournié G. 1, Goodman S. 1,2, Cedeño V. 1,3, Cunningham A. 1,4, Cruz M. 1, Millins C. 1, Haddad N. 5, and Guillot J. 5
 1 Galapagos Genetics Epidemiology and Pathology Laboratory, University of Guayaquil, Galápagos Islands, Ecuador; 2 Department of Biology, University of Leeds, UK; 3 Program of Biotechnology, University of Guayaquil, Ecuador; 4 Institute of Zoology, London, UK; 5 UMR INRA, AFSSA, ENVA, UPVM 956 BIPAR, Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort, France
- 09:15 216 **Lungworms in porpoises and seals do have vertebrate intermediate hosts: molecular tools determine lifecycle**
Lehnert K. 1, von Samson-Himmelstjerna G. 2, Fonfara S. 3, Wohlsein P. 4, and Siebert U. 1
 1 Research and Technology Center Westcoast, Christian-Albrechts-Universität zu Kiel, Buesum, Germany; 2 Institute for Parasitology, Stiftung Tierärztliche Hochschule Hannover, Hannover, Germany; 3 GKSS Forschungszentrum, Geesthacht, Germany; 4 Institute for Pathology, Stiftung Tierärztliche Hochschule Hannover, Hannover, Germany
- 09:30 217 **Chorioptes ear mites in moose (*Alces alces*): pathological and taxonomic investigations**
Zahler-Rinder M. 1, Bornstein S. 2, Hestvik G. 3, Mattsson R. 4, Lindberg R. 3, Gavier-Widén D. 5, and Morrison D. 2
 1 Bavarian Health and Food Safety Authority, Oberschleissheim, Germany, and Institute for Comparative Tropical Medicine and Parasitology, Ludwig Maximilian University, Munich, Germany; 2 Department of Parasitology, National Veterinary Institute, Uppsala, Sweden; 3 Department of Biomedical Sciences and Veterinary Public Health, Division of Pathology, Pharmacology and Toxicology, Swedish University of Agricultural Sciences, Uppsala, Sweden; 4 Department of Wildlife, Fish and Environment, National Veterinary Institute, Uppsala, Sweden
- 09:45 218 ***Toxoplasma* in Australian Marsupials: analysis of pouch young of naturally infected wild kangaroos for evidence of vertical transmission**
O'Handley R. 1, Fenwick S. 1, and Thompson R.C.A. 1
 1 Murdoch University, Department of Veterinary Biology & Biomedical Science, Perth, Western Australia

Jan van der Goes Room

08:30-10:00 Workshop:
New developments in the field of Toxoplasmosis
Chairs: Kijlstra A.
Jongert E.

08:30 **Introduction**

08:30 219 **New developments in the field of toxoplasmosis**
 Jongert E.
Pasteur Institute of Belgium

08:45 220 **Discussion**

Main Congress Room

10:30-11:30 Plenary Session IV: Invited lectures
Chair: Willadsen P.

10:30 221 **Ectoparasites: Future challenges in a changing world**
 Wall R., *University of Bristol, United Kingdom*

11:00 222 **Structural biology in search of new therapeutics for protozoal diseases**
 Hol W., *University of Washington, USA*

Main Congress Room

11:30-12:30 Fort Dodge Corporate Symposium:
Novel Options in Ectoparasite Control



11:30 223 **The mechanism of action of metaflumizone and other veterinary ectoparasiticides**
 Salgado V.L.
BASF Corporation, North Carolina, USA

11:50 224 **Safety and efficacy of ProMeris® for cats and ProMeris /ProMeris Duo® for dogs: the development of new products for the control of fleas in cats and fleas and ticks in dogs**
Rugg D., Holzmer S., Heaney K., DeLay R., and Rock D.
Fort Dodge Animal Health, New Product Development, Princeton, NJ, USA

12:10 225 **Efficacy of ProMeris® for cats and ProMeris Duo® for dogs in Europe**
Hellmann K. 1, Blond F. 2, Parker L. 3, DeLay R. 4, and Rugg D. 4
1 Klifovet AG, Munich, Germany; 2 Fort Dodge Animal Health, Betton, France; 3 Fort Dodge Animal Health, Southampton, UK; 4 Fort Dodge Animal Health, Princeton, USA

Van Rysselberghe Room**14:00-15:30****Symposium:****Molecular approaches to the tick-host-pathogen interface****Chair: Willadsen P.**

Ticks are major vectors of diseases of livestock, wildlife and humans, transmitting pathogenic viruses, bacteria and protozoa. They establish and manipulate a feeding site on mammalian hosts, creating an environment that is exploited by many tick-borne diseases. Ticks themselves are also intimately involved in the developmental processes of many pathogens.

The tick-host-pathogen interface is therefore both exceedingly complex and critically important to animal and human health. Molecular tools and genomic data are beginning to revolutionise our ability to dissect and understand that interface, though their full utilisation lies in the future. Examples of the way molecular approaches are increasing our knowledge will be given in this symposium. The aim of the symposium discussion will be to identify current opportunities and constraints to progress.

- 14:00 226 **Identification and molecular characterization of a vitellogenin receptor from the hard tick *Haemaphysalis longicornis***
 Boldbaatar D. 1, and Fujisaki K. 1,2
1 Kagoshima University, Department of Frontier Veterinary Science, Kagoshima, Japan; 2 Obihiro University of Agriculture and Veterinary Medicine, National Research Center for Protozoan Diseases, Obihiro, Japan- Laboratory of Emerging Infectious Diseases, Department of Frontier Veterinary Science, Faculty of Agriculture, Kagoshima University, Kagoshima, Japan
- 14:15 227 **Tick-vertebrate host interface in the tick-borne flavivirus transmission**
 Labuda M.
Institute of Zoology, Slovak Academy of Sciences, Bratislava, Slovakia
- 14:30 228 **A tick cysteine protease, longipian, from the babesial parasite vector *Haemaphysalis longicornis*, with possible multifunctional roles during parasite transmission**
 Tsuji N. 1, and Fujisaki K. 2,3
1 Laboratory of Parasitic Diseases, National Institute of Animal Health, National Agricultural and Food Research Organization, Tsukuba, Japan; 2 National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan; 3 Department of Emerging Infectious Diseases, School of Veterinary Medicine, Kagoshima University, Kagoshima, Japan
- 14:45 229 **Approaches to improved understanding of vector-parasite interaction within the genus *Theileria* and their Ixodid tick vectors, using genomics**
 Bishop R. 1, Musoke A. 1, Skilton R. 1, Odongo D. 1, Scoles D., Knowles D., and Nene V. 2
1 The International Livestock Research Institute (ILRI), Nairobi Kenya; 2 The Institute for Genomic Research, Rockville Maryland, USA; 3 USDA-ARS, Pullman, Washington State, USA
- 15:00 **Discussion**

Baeckelandt Room

14:00-15:30 Workshop:
Giardia in veterinary medicine:
to consider or not to consider?
Chairs: O'Handley R.
Geurden T.

14:00 230 **Discussion**

Jan van Eyck Room

14:00-15:30 Epidemiology and Control of Helminth Infections
in Small Ruminants
Chairs: Miller J.
Eysker M.

- 14:00 231 **Control of parasitic gastroenteritis in sheep: modelling worm control strategies**
Taylor M.A. 1, Learmount J. 1, Morgan C. 1, Craig B. 1, and Stubbings L. 2
1 Central Science Laboratory, Sand Hutton, York, UK; 2 LSCC Ltd Aldwincle, Kettering, UK
- 14:15 232 **The past and future of *Nematodirus battus***
van Dijk J., and Morgan E.R.
University of Bristol, School of Biological Sciences, Bristol, UK
- 14:30 233 **Metazoan-protozoan parasite co-infections and host fitness in St. Kilda Soay sheep**
Craig B.H., Tempest L.J., Pilkington J.G., and Pemberton J.M.
Central Science Laboratory, York, UK
- 14:45 234 **The integration of anthelmintic treatment and grazing management for the provision of clean lambing paddocks in a summer rainfall region of Australia**
Bailey J. 1, Walkden-Brown S. 1, Kahn L. 1, and Besier B. 2
1 University of New England, Centre for Animal Health and Welfare, Armidale, Australia; 2 Department of Agriculture and Food, Western Australia
- 15:00 235 **Animal production performance and treatment frequency using the selective/famacha and suppressive strategy of parasite control in ewes: report of a five-year period**
Molento M. 1, Gavião A. 2, Depner R. 2, and Pires C. 2
1 Universidade Federal do Paraná, Laboratório de Doenças Parasitárias, Curitiba, Brazil; 2 Universidade Federal de Santa Maria, Centro de Ciências Rurais, Santa Maria, Brazil
- 15:15 236 **Prevalence and seasonal incidence of helminth parasite infections of sheep and goats in eastern Ethiopia: Nematodes and trematodes**
Sissay M.M. 1, Ugglä A. 1, and Waller P.J. 1
1 Department of Parasitology (SWEPAR), National Veterinary Institute and Swedish University of Agricultural Sciences, Uppsala, Sweden

Hubert van Eyck Room**14:00-15:30 Fasciola & Trematodes****Chairs: Losson B.
Rojo-Vazquez F.**

- 14:00 237 ***Fasciola hepatica*: a study of the vectorial capacity of four different species of lymnaeids snails commonly found in Belgium**
Caron Y., Lasri S., and Losson B.
University of Liège, Faculty of Veterinary Medicine, Department of Infectious and Parasitic Diseases, Liège, Belgium
- 14:15 238 **The population dynamics of *Lymnaea truncatula* in the west of Ireland and the prevalence of *Fasciola hepatica* within the intermediate host**
Relf V. 1, 2, Good B. 2, Gray J. 3, Mulcahy G. 1, Forbes A. 4, Murphy T. 5, and de Waal T. 1
1 School of Agriculture, Food Science and Veterinary Medicine and; 3 School of Biology and Environmental Science, University College Dublin, Ireland; 2 Teagasc, Animal Production Research Centre, Athenry, Co Galway, Ireland; 4 Merial, Lyon, France; 5 Central Veterinary Laboratory, Department of Agriculture and Food, Co. Kildare, Ireland
- 14:30 239 **The dramatic spread of the giant liver fluke in Croatia**
Marinculic A. 1, Beck R. 1, Florijancic T. 2, Kuticic V. 1, and Lucinger S. 1
1 Faculty of Veterinary Medicine University of Zagreb, Zagreb, Croatia; 2 Faculty of Agronomy University of Osijek, Osijek, Croatia
- 14:45 240 **Mechanism of *Centrocestus armatus* (Digenea: Heterophyidae) cercariae attachment and penetration to gills of second intermediate fish hosts, *Zacco temminckii***
Paller V.G.V., and Uga S.
1 Kobe University School of Medicine, Department of Parasitology, Faculty of Health Sciences, Kobe, Japan
- 15:00 241 **Fishborne zoonotic trematodes in domestic animals in Nghe An province, Vietnam**
Lan Anh T.N. 1,2, Phuong T.N. 1, Johansen M.V. 3, Murrell D.K. 2, Thu T.T. 1, and Thamsborg S.M. 2
1 Parasitology Department, National Institute of Veterinary Research, Hanoi, Vietnam; 2 Danish Centre for Experimental Parasitology, Department of Veterinary Pathobiology, University of Copenhagen, Denmark; 3 DBL-Centre for Health Research and Development, Department of Veterinary Pathobiology, University of Copenhagen, Denmark
- 15:15 242 **The effect of triclabendazole sulphoxide on egg production and hatch rate in the liver fluke, *Fasciola hepatica***
Shaw L., Kirkwood D., Fairweather I., Trudgett A., Hoey L., and Brennan. G
Queen's University Belfast, School of Biological Sciences, Belfast, Northern Ireland

Jan van der Goes Room

14:00-15:30 Exploiting Genomics

**Chairs: Gilleard J.
Gasbarre L.**

- 14:00 243 **The *Haemonchus contortus* genome project; an update and the issue of genome polymorphism**
Gilleard J., Quail M., Rajandream M.-A., Mungall K., Barrell B., Walker D., White B., Coughlan A., Redman L., Pachabet J., Dear P., and Berriman M.
Univeristy of Glasgow, Veterinary Infection and Immunity, Glasgow, UK
- 14:15 244 **Stage-specific gene expression in *Teladorsagia circumcincta***
Nisbet A.J. 1, Redmond D.L. 1, Matthews J.B. 1, Watkins C. 1, Jones J.T. 2, and Knox D.P. 1
Moredun Research Institute, Parasitology, Edinburgh, UK
- 14:30 245 **Functional genomic analyses of excretory/secretory proteins from the parasitic stages of *Haemonchus contortus***
Zawadzki J.L., Binos S., Walker C.L., and Presidente P.J.A.
Department of Primary Industries Victoria, Animal Genetics and Genomics Platform, Attwood, Australia
- 14:45 246 **Differentially expressed genes in infective larvae of *Ascaris suum* identified by microarray and sequencing analyses of an expressed sequence tag library**
Huang C.Q. 1, Lin R.Q. 1, Chen N. 1, Zou F.C. 2, Song H.Q. 1, and Zhu X.Q. 1
1 College of Veterinary Medicine, South China Agricultural University, Guangzhou, Guangdong Province, PR China; 2 College of Animal Science and Technology, Yunnan Agricultural University, Kunming, Yunnan Province, PR China
- 15:00 247 **Analysis of adult *Fasciola gigantica* expressed sequence tags**
Huang W.Y., Zhang W.Y., and Wang D.Y.
Guangxi University, College of Animal Science and Technology, Nanning, PRChina
- 15:15 248 **An analysis of differential transcription between adult female and male *Haemonchus contortus* using an oligonucleotide microarray platform**
Campbell B.E. 1, Nagaraj S. 2, Hu M. 1, Ong B. 3, Loukas A. 4, Beveridge I. 1, Ranganathan S. 2, and Gasser R.B. 1
1 Department of Veterinary Science, The University of Melbourne, Werribee, Victoria, Australia; 2 Department of Chemistry and Biomolecular Sciences & Biotechnology Research Institute, Macquarie University, Sydney, New South Wales, Australia; 3 Museum Victoria, Melbourne, Victoria, Australia; 4 Helminth Biology Laboratory, Division of Infectious Diseases and Immunology, Queensland Institute of Medical Research, Queensland, Australia

Main Congress Room**16:00-17:00****Pfizer Corporate Symposium:****Pill or Poke-which anti-parasitic will reign supreme?**

16:00 249 **Better living through chemistry: new drugs must be found**

Geary T.

McGill University, Institute of Parasitology, Canada

16:30 250 **Evolution backs the vaccinologist: Why vaccines will win the battle of the anti-parasitics**

Lightowlers M.

The University of Melbourne, Veterinary Clinical centre, Victoria, Australia

Main Congress Room**17:00-17:10****WAAVP 2009****17:10-18:10****WAAVP General Assembly**

POSTER SESSION 1

Poster group 1: Helminth Infections of Small Ruminants

- 251 **Effect of the consumption of a tropical tannin-rich plant (*Tzalam, Lysiloma latisiliquum*) on the larval establishment of parasitic nematodes in goats**
 Brunet S. 1, Torres-Acosta J.F.J. 2, Sandoval-Castro C.A. 2, Aguilar-Caballero A.J. 2, Capetillo-Leal C. 2, Hoste H. 1, and Martinez-Ortiz de Montellano C. 2
1 Unité Mixte de Recherches 1225 INRA/DGER, Interactions Hôte Agents Pathogènes, École Nationale Vétérinaire de Toulouse, Toulouse, France; *2* Area de Pequeños Rumiantes, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Yucatán, Mérida, Yucatán, Mexico
- 252 **Use of condensed tannins against gastro-intestinal parasites in naturally infected grazing lambs**
 Fouquet A. 1, Leonard V. 2, Delmotte Ch. 3, and Kirschvink N. 1
1 Animal Physiology, Department of Veterinary Medicine, University of Namur, Namur, Belgium; *2* Centre d'essai biologiques, Gembloux, Belgium; *3* Région wallonne DGA33 Ciney, Belgium
- 253 **Effects of *Azadirachta indica* on sheep infected naturally with helminthes**
 Macedo F.R. 1, Louvandini H. 1, Paludo G.R. 1, McManus C.M. 1, Dell'Porto A. 1, Martins R.F.S. 1, Viana P.G. 1, and Coelho M.M.S. 1
 Universidade de Brasilia, Faculdade de Agronomia e Medicina Veterinária, Brasilia, Brazil
- 254 ***Drimys brasiliensis* against nematode parasites of sheep**
 Molento M. 1, Lemos R. 1, Runcos L. 1, Corrêa D. 1, Pinheiro D. 1, Bannach E. 1, and Almeida F. 2
1 Universidade Federal do Paraná, Laboratório de Doenças Parasitárias, Curitiba, Brazil; *2* Universidade Federal do Paraná, Departamento de Zootecnia, Curitiba, Brazil
- 255 **African plant-based treatment for gastrointestinal nematodes in sheep**
 Grade J.T. 1,2, and Arble B.L. 3
1 University Gent, Department Ethnobotany Gent Belgium; *2* Christian Veterinary Mission Seattle USA; *3* University of Wisconsin-Madison, School of Medicine and Public Health
- 256 **In vitro and in vivo anthelmintic activity of *Caesalpinia crista* (L.) seed kernels against gastrointestinal nematodes of sheep**
 Zaman M. 1, Iqbal Z. 1, Jabbar A. 1, Arshad M. 2, and Abbas R. 1
1 Department of Veterinary Parasitology, University of Agriculture, Pakistan; *2* Department of Veterinary Microbiology, University of Agriculture Faisalabad, Pakistan
- 257 **Anthelmintic effects of *Newbouldia laevis* on parasitic nematodes of the gastrointestinal tract of sheep**
 Olounlade P. 1, Attakpa E. 2, Azando E. 1, Hoste H. 3, and Hounzangbe-Adote M.S. 1
1 Faculté des Sciences Agronomiques, Université d'Abomey-Calavi, Bénin; *2* Faculté d'Agronomie / Université de Parakou, Bénin; *3* UMR 1225 INRA / DGER Ecole Nationale Vétérinaire de Toulouse, France
- 258 **Evaluation of anthelmintic activity of *Lippia sidoides* essential oil on sheep gastrointestinal nematodes**
 Camurça-Vasconcelos A.L.F. 1, Bevilaqua C.M.L. 1, Morais S.M. 1, Maciel M.V. 1, Costa C.T.C. 1, Macedo I.T.F. 2, Oliveira L.M.B. 2, Braga R.C. 2, Silva R.A. 2, Navarro A.M.C. 3, Aragão W.R. 3, and Vieira L.S. 3
1 Programa de Pós-Graduação em Ciências Veterinárias/Universidade Estadual do Ceará, Brazil; *2* Faculdade de Medicina Veterinária/Universidade Estadual do Ceará, Brazil; *3* EMBRAPA/CNPC, Brazil

- 259 **The effect of sericea lespedeza, fed as pellets, on established infection of *Haemonchus contortus* in sheep**
 Miller J.E. 1, Mosjidis J.A. 2, Terrill T.H. 3, and Burke J.M. 4
1 Louisiana State University, Department Pathobiological Sciences, Baton Rouge, LA USA; 2 Auburn University, Department Agronomy and Soils, Auburn, AL USA; 3 Fort valley State University, Department Animal Science, Fort Valley, GA USA; 4 USDA ARS, Booneville, AR USA
- 260 **Larvicidal activity of butanol extract from fiber water of green Coconut (*Cocos nucifera* Linn.) on *Haemonchus contortus***
 Costa C.T.C., Bevilaqua C.M.L., Maciel M.V., Camurça-Vasconcelos A.L.F., Braga R.R., Silva R.A., and Morais S.M.
Universidade Estadual do Ceará, Faculdade de Veterinaria, Fortaleza, Brazil
- 261 **Relationship between the origin of sainfoin samples, the tannin content and the anthelmintic activity against *Haemonchus contortus***
 Manolaraki F. 1, Brunet S. 3, Luminet S. 2, Sotiraki S. 1, and Hoste H. 3
1 NAGREF-VRI NAGREF Campus, Thermi 57001 PO Box 60272 Thessaloniki, Greece; 2 Société Caussade Semences, ZI de Meaux, France; 3 UMR 1225 INRA / DGER Ecole Nationale Vétérinaire de Toulouse, France
- 262 **In vitro anthelmintic activity of plant extracts from tropical tanniniferous trees against *Haemonchus contortus***
 Alonso-Díaz M.A. 1,2, Torres-Acosta F.J. 1, Sandoval-Castro C.A. 1, Aguilar-Caballero A.J. 1, and Hoste H. 3
1 Universidad Autónoma de Yucatán, FMVZ, Mérida, México; 2 Universidad Nacional Autónoma de México, CEIEGT-FMVZ, Veracruz, México; 3 UMR 1225 INRA/DGER. Ecole Nationale Vétérinaire Toulouse, Toulouse, France
- 263 **In vitro studies using bioassays to examine the effect of aqueous plant extracts on *Teladorsagia circumcincta***
 Gordon Y. 1, Jackson E. 1, Bartley D. 1, and Jackson F. 1
1 Moredun Research Institute, Bush Loan, Penicuik, Edinburgh, Scotland
- 264 **Potential use of Cassava (*Manihot esculenta*) leaves for worm control for small ruminants in the tropics**
 Nurulaini R. 1, Khadijah S. 1, 2, Adnan M. 1, Zaini C.M. 1, Jamnah O. 1, Waller P. J. 3, and Chandrawathani P. 1
1 Department of Parasitology and Haematology, Veterinary Research Institute, Ipoh, Perak, Malaysia; 2 School of Biological Sciences, University Science of Malaysia, Minden, Penang, Malaysia; 3 National Veterinary Institute, Uppsala, Sweden
- 265 **Aprisco-seridó project: a new perspective for goat and sheep farmers on a Brazilian dry area**
 Molento M. 1, Cavalcanti W. 2, Pontes J. 2, Cardoso M. 2, Silva G. 2, Wanderley E. 2, Leal R. 2, Pessoa-Filho P. 2, and Brum E. 1
1 Universidade Federal do Paraná, Laboratório de Doenças Parasitárias, Curitiba, Brazil; 2 Governo do Estado do Rio Grande do Norte, Secretaria de Estado da Agricultura, Pecuária e da Pesca, Natal, Brazil
- 266 **Development and evaluation of a decision support model for the identification of animals that would benefit from anthelmintic treatment**
 Greer A.W., Kenyon F., Bartley D.B., Jackson E.B., Gordon Y., Donnan A.A., McBean D., and Jackson F.
Moredun Research Institute, Edinburgh, EH26 0PZ, Scotland

- 267 **Colour of mucous membrane as an indicator of endoparasite infections in sheep**
Moors E., Fasshauer C., and Gauly M.
Institute of Animal Breeding and Genetics, Goettingen, Germany
- 268 **Nematode targeted selective treatments in goats under temperate climate using body condition scoring and milk yield records**
Paraud C. 1, Pors I. 1, and Chartier C. 1
1 French Food Safety Agency (AFSSA), Laboratoire d'Etudes et de Recherches Caprines, Niort Cedex, France
- 269 **The basis for anthelmintic-targeted selective treatment in high value Sardi sheep in Morocco: Questionnaire and actual infection**
Berrag B. 1, Allaoui R. 1, and Cabaret J. 2
1 Institut Agronomique et Vétérinaire Hassan II, Rabat, Morocco; 2 INRA, Bio-Agresseurs, Santé Environnement, Nouzilly, France
- 270 **How can targeted selective treatment be applied in commercial sheep flocks in south-west England?**
Stafford K.A., Morgan E.R., and Coles G.C.
School of Clinical Veterinary Science, University of Bristol, Langford House, Bristol, UK
- 271 **Parasite control practices on lowland sheep farms in Ireland**
Patten T. 1,2, Good B. 1, Hanrahan J.P. 1, Mulcahy G. 2, and De Waal D.T. 2
1 Teagasc, Animal Production Research Centre, Athenry, Co. Galway, Ireland; 2 School of Agriculture, Food Science and Veterinary Medicine, UCD, Belfield, Ireland
- 272 **Alternatives to anthelmintics in parasite control in sheep**
Sayers G., and Sweeney T.
School of Agriculture, Food Science and Veterinary Medicine, University College Dublin, Ireland
- 273 **Survey of nematophagous fungi on sheep farms in Ireland**
Kelly P. 1,2, Good B. 1, Fitzpatrick R. 1, Hanrahan J.P. 1, and De Waal D.T. 2
1 Teagasc, Animal Production Research Centre, Athenry, Co. Galway, Ireland; 2 University College Dublin, School of Agriculture Food Science and Veterinary Medicine, Belfield, Dublin 4, Ireland
- 274 **Biological control of gastrointestinal nematodes of goat with a nematophagous fungus-*Dactylaria* sp.**
Adhikari B.B. 1, and Divina B.P. 2
1 Institute of Agriculture & Animal Science, Rampur campus, Chitwan, Nepal; 2 College of Veterinary medicine, University of the Philippines, The Philippines
- 275 **Efficacy of the nematode-trapping fungi *Duddingtonia flagrans* and *Arthrobotrys robusta* against infections by gastrointestinal nematodes in sheep at pasture**
Carrijo Mauad J.R. 1, Silva B.F. 1, Araújo J.V. 2, Braga F.R. 2, and Amarante A.F.T. 1
1 Universidade Estadual Paulista, Departamento de Parasitologia, Botucatu, Brazil; 2 Universidade Federal de Viçosa, Departamento de Veterinária, Viçosa, Brazil
- 276 **Development of a PCR diagnostic test for detection of *Duddingtonia flagrans***
Kelly P. 1,2, Good B. 1, Fitzpatrick R. 1, Hanrahan J.P. 1, and De Waal D.T. 2
1 Teagasc, Animal Production Research Centre, Athenry, Co. Galway, Ireland; 2 University College Dublin, School of Agriculture Food Science and Veterinary Medicine, Belfield, Dublin 4, Ireland

- 277 **Using a conventional nematode egg counting technique for the estimation of *Duddingtonia flagrans* chlamydospores in sheep faeces**
 Ojeda-Robertos N.F. 1, Torres-Acosta J.F.J. 1, Aguilar-Caballero A.J. 1, Mendoza-de-Gives P. 2, Cob-Galera L.A. 1, and Ayala-Burgos A. 1
1 Universidad Autónoma de Yucatán, Facultad de Medicina Veterinaria y Zootecnia, Mérida, México; 2 INIFAP, Centro Nacional de Investigaciones Disciplinarias en Parasitología Veterinaria, Jiutepec, México
- 278 **FLOTAC, a novel apparatus for a multivalent faecal egg count technique**
 Cringoli G.
Department of Pathology and Animal Health, University of Naples “Federico II”, CREMOPAR Regione Campania, Naples, Italy
- 279 **Phenotyping selection of resistance Pelibuey lambs to the main tropical nematode *Haemonchus contortus*: Preliminary study**
 Gonzalez R.J.L. 1, Lopez A.M.E. 2, Olazaran J.S. 1, Liebano H.E. 2, Mendoza de G.P. 2, Vega M.V. 1, Vazquez P.V.M. 2, and Calderon R.R. 1
2 Inst. Nal. Invest. Forestales Agrícolas y Pecuarias, Helminthology, Jiutepec, Mexico
- 280 **Copper oxide wire particles as an adjunct in the control of *Haemonchus contortus* in sheep and goats of pastoralist and smallholder farmers in Kenya**
 Githiori J.B. 1, Vatta A.F. 2, Waller P.J. 3, and Medley G.F. 4
1 International Livestock Research Institute (ILRI), Nairobi, Kenya; 2 Onderstepoort Veterinary Institute (OVI), Onderstepoort, South Africa; 3 National Veterinary Institute (SVA), Department of Parasitology (SWEPAR), Uppsala, Sweden; 4 University of Warwick, Department of Biological Sciences, Coventry, UK
- 281 **Copper oxide wire particles as an adjunct in the control of *Haemonchus contortus* in goats of resource-poor farmers in South Africa**
 Vatta A.F. 1, Waller P.J. 2, Githiori J.B. 3, and Medley G.F. 4
1 Onderstepoort Veterinary Institute, Onderstepoort, South Africa; 2 National Veterinary Institute, Department of Parasitology (SWEPAR), Uppsala, Sweden; 3 International Livestock Research Institute, Nairobi, Kenya; 4 University of Warwick, Department of Biological Sciences, Coventry, UK
- 282 **In vitro predatory activity of “cannibal” nematodes against *Haemonchus contortus* and *Rhabditis* sp.**
 Rodríguez-Esparza J.R. 1,2, Mendoza-de Gives P. 1, Liébano-Hernández E. 1, López-Arellano Ma.E. 1, Cid-Del Prado I. 3, Franco-Navarro F. 3, and López-Martínez V. 2
1 CENID-Parasitología Veterinaria, INIFAP, Depto. de Helminthología, Morelos, México; 2 Facultad de Ciencias Agropecuarias de la Universidad Autónoma del Estado de Morelos, México; 3 Colegio de Posgraduados, Centro de Fitopatología, Depto. de Nematología, Edo. de México, México
- 283 **Move-than-treat: a new pasture rotation management strategy as an aid to prevent parasite resistance in calves**
 Molento M. 1, Heck I. 2, Leandro A. 2, Leite C. 2, Gindri J. 2, Souza M. 2, and Depner R. 2
1 Universidade Federal do Paraná, Laboratório de Doenças Parasitárias, Curitiba, Brazil; 2 Universidade Federal de Santa Maria, Laboratório de Doenças Parasitárias, Santa Maria, Brazil
- 284 **Analysis of systemic and mucosal responses to *Teladorsagia circumcincta* in immune and naïve sheep**
 Meikle L.I., Nisbet A.J., Smith S.K., and Matthews J.B.
Moredun Research Institute, Edinburgh, UK
- 285 **Identification of antigens from *Teladorsagia circumcincta***
 Murphy L., Eckersall P.D., and Stear M.J.
Division of Animal Production and Public Health, Glasgow University Veterinary School, Glasgow, Scotland

- 286 **The acute phase protein response to *Teladorsagia circumcincta***
 Johnston G.M. 1,3, Eckersall P.D. 2, Pemberton A.D. 3, and Smith W.D. 1
1 Moredun Research Institute, Pentlands Science Park, Edinburgh, UK; 2 Division of Animal Production and Public Health, Institute of Comparative Medicine, Faculty of Veterinary Medicine, University of Glasgow, Glasgow, UK; 3 Division of Veterinary Clinical Studies, University of Edinburgh, Easter Bush Veterinary Centre, Roslin, Midlothian, UK
- 287 **The pathogenicity of nematodiasis in sheep and goats in Pakistan: 1, Parasitological, haematological and clinical findings**
 Ahmed I. 1, Miller J.E. 2, Chaudahry F.R. 1, Munir W. 1, Mehmood S. 1, and Qayyum M. 1
1 Department of Zoology, University of Arid Agriculture, Rawalpindi, Pakistan; 2 Department of Pathobiological Sciences, School of Veterinary Sciences, Louisiana State University, Baton Rouge, USA
- 288 **Analysis of mhc class II genes in Scottish blackface sheep**
 Rennie K., Fitton L., Murphy L., and Stear M.
Division of Animal Production and Public Health, Glasgow University Veterinary School, Glasgow, Scotland
- 289 **Eosinophil - *Haemonchus contortus* infective larvae interaction: demonstration of larval killing after intra-abomasal infection**
 Terefe G. 1, Grisez C. 2, Prevot F. 2, Bergeaud J.P. 2, François D. 3, Dorchie P. 2, Fourqueaux I. 4, and Jacquet P. 2
1 Faculty of Veterinary Medicine, Debre Zeit, Ethiopia; 2 UMR INRA/DGER 1225, Ecole Nationale Vétérinaire de Toulouse, France; 3 INRA, Station d'Amélioration Génétique des Animaux, Toulouse, France; 4 Département de microscopie électronique, Faculté de Médecine de Rangueil, Toulouse, France
- 290 **Relationship between abomasal histology and parasitological parameters following *Haemonchus contortus* infection in two sheep breeds native to the Canary Islands**
 González J.F. 1, Hernández A. 1,2, Rodríguez F. 2, Molina J.M. 1, Andradá M. 2, Raadsma H. 3, Meeusen E.N.T. 4, and Piedrafita D. 4
1 Veterinary Faculty/University of Las Palmas de Gran Canaria, Animal Pathology, Arucas, Spain; 2 Faculty of Veterinary Science, University of Sydney, Australia; 3 Department of Physiology, Monash University, Melbourne, Australia
- 291 **Comparison of different antigens for the diagnosis of *Haemonchus contortus* in experimentally infected sheep by western blotting**
 Meshgi B., and Hossieni S.H.
Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
- 292 **Quantification of *Bos indicus* cytokines mRNA levels and its relationship with the cellular and humoral responses in infections by *Haemonchus placei***
 Zaros L.G. 1, Bricarello P.A. 2, Amarante A.F.T. 2, Rocha R.A. 2, Kooyman F.N.J. 3, De Vries E. 3, and Coutinho L.L. 1
1 Escola Superior de Agricultura Luiz de Queiroz - ESALQ/USP, Departamento de Zootecnia, Laboratório de Biotecnologia Animal, Piracicaba, SP, Brazil; 2 Universidade Estadual Paulista, UNESP, Instituto de Biociências, Departamento de Parasitologia, Botucatu, SP, Brazil; 3 Department of Infectious Diseases and Immunology, Utrecht University, Utrecht, The Netherlands
- 293 **Detection of circulating and purified *Cysticercus tenuicollis* antigens in experimental and natural infections of sheep with cysticercosis**
 Kandil O.
Department of Parasitology and Animal Diseases, National Research Center, Dokki, Giza, Egypt

- 294 **Validation of an indirect ELISA for the diagnosis of the infection by *Teladorsagia circumcincta* in sheep**
Martínez-Valladares M., Vara-Del Río M.P., and Rojo-Vázquez F.A.
Dept Sanidad Animal, Facultad de Veterinaria, University of León, Spain
- 295 **Species-specific PCR for differential detection of the gastro-intestinal nematode *Ashworthius sidemi***
Ljunggren E.L., and Pastusiak K.
W. Stefanski Institute of Parasitology, PAS, Warsaw, Poland
- 296 **Use of Bayesian MCMC to improve analysis of faecal egg count data**
Denwood M.J., Stear M.J., Love S., and Innocent G.T.
Division of Animal Production and Public Health, University of Glasgow, Scotland
- 297 **Comparison of plasma pharmacokinetic profile of ivermectin following administration of subcutaneous injection (baymec®) and oral tablet (efektin®) in goats**
Gokbulut C. 1, Karademir U. 1, and Boyacioglu M. 1
1 Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Adnan Menderes, Aydin, Turkey
- 298 **Effect of pregnancy on the pharmacokinetic of ivermectin in sheep**
Pérez R., Palma C., Cox J., and Núñez M.J.
Laboratorio de Farmacología, Facultad de Medicina Veterinaria, Universidad de Concepción, Chile
- 299 **Efficacy and persistent prophylactic efficacy of long-acting moxidectin (inj. sol. 2%) for control of Trichostrongylid infections of sheep**
Papadopoulos E. 1, Fragkou I.A. 2, Mavrogianni V.S. 2, Gougoulis D.A. 2, Orfanou D.C. 2, Gallidis E. 1, Parker L. 3, and Fthenakis G.C. 2
1 School of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece; 2 Veterinary Faculty, University of Thessaly, Karditsa, Greece; 3 Fort-Dodge Animal Health, Southampton, UK
- 300 **Reproductive benefits in ewe-lambs after administration of long-acting moxidectin (inj. sol. 2%) before mating**
Mavrogianni V.S. 1, Papadopoulos E. 2, Fragkou I.A. 1, Gougoulis D.A. 1, Orfanou D.C. 1, Ptochos S. 2, Parker L. 3, and Fthenakis G.C. 1
1 Veterinary Faculty, University of Thessaly, Karditsa, Greece; 2 School of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece; 3 Fort-Dodge Animal Health, Southampton, United Kingdom
- 301 **Effect of gastrointestinal parasitism on the patterns of moxidectin tissue residue depletion, in sheep**
Pérez R., Palma C., Araneda M., Cabezas I., Rubilar L., and Núñez J.
Lab. Farmacología, Facultad Medicina Veterinaria, Universidad de Concepción, Chillán, Chile
- 302 **Effect of multiple anthelmintic drugs on live weight gain in Awassi sheep in subtropical region of Pakistan**
Munir W. 1, Miller J.E. 2, Riaz F.Ch. 1, Ahmad I. 1, Mehmood S. 1, and Qayyum M. 1
University of Arid Agriculture Rawalpindi, Pakistan
- 303 **Efficacy of albendazole against *Coenurus cerebralis* in goats**
Afonso S.M.S. 1,2, Neves L. 1, Domingos A. 3, Sousa P.D. 1, Capece B.P.S. 3, and Cristófol C. 2
1 Secção de Parasitologia, Departamento de Paraclinicas, Faculdade de Veterinária, Universidade Eduardo Mondlane, Mozambique; 2 Servei d'Anàlisi de Fàrmacs, Departament de Farmacologia, Terapeutica i

Toxicología, Universitat Autònoma de Barcelona, Barcelona, Spain; 3 Secção de Farmacologia e Toxicologia, Departamento de Paraclínicas, Faculdade de Veterinária, Universidade Eduardo Mondlane, Mozambique

- 304 **Influence of pasture type and sheep breed on gastrointestinal parasitism in the Brazilian Savannah**
Santos V.R.V. 1, Louvandini H. 1, McManus C.M. 1, Torres S.E.F.A. 1, and Sereno F. 1
Universidade de Brasília, Faculdade de Agronomia e Medicina Veterinária, Campus Universitário Darcy Ribeiro, Brazil
- 305 **Population dynamics of gastrointestinal nematodes in organic sheep farms in Canada – first year**
Fernández S. 1,2, Mederos A. 1,3, Sudeman R. 2, Bond E. 2, Peregrine A. 2, Menzies P. 3, VanLeeuwen J. 4, and Leboeuf A. 5
1 Organic Agriculture Centre of Canada, Nova Scotia Agricultural College, Truro, Canada; 2 Dept. of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Canada; 3 Dept. of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, Canada; 4 Dept. of Health Management, Atlantic Veterinary College, Charlottetown, Canada; 5 Centre d'expertise en production ovine du Québec, La Pocatière, Canada.
- 306 **Cross-sectional surveys on helminth infections of goats in Bali, Indonesia**
Damriyasa I.M. 1, and Bauer C. 1,2
1 Udayana University, Centre for Studies on Animal Diseases, Bali, Indonesia; 2 Justus Liebig University Giessen, Institute of Parasitology, Giessen, Germany
- 307 **The first record of the nematode *Ashworthius sidemi* in Sweden**
Höglund J. 1, Christensson D. 1, Holmdahl J. 3, Mörner T. 2, Osterman E. 1, and Uhlhorn H. 2
1 Swedish University of Agricultural Sciences and National Veterinary Institute, Department of Parasitology (SWEPAR), Uppsala, Sweden; 2 National Veterinary Institute, Department of Wildlife, Uppsala, Sweden; 3 The District Veterinarians, Swedish Board of Agriculture, Sweden
- 308 **Gastro-intestinal nematodes of small ruminants in Shahrekord district of Chaharmahal and Bakhtiari province of Iran**
Manouchehri Naeini K. 1, Gandji A. 1, and Zebardast N. 2
1 Azad University, Department of Veterinary Parasitology, Shahrekord, Iran; 2 Faculty of Medicine, Department of Parasitology and Mycology, Shahrekord, Iran
- 309 **Study of genetic variability of *Haemonchus contortus* in alpine Italian ruminant populations**
Cerutti M.C. 1, Citterio C.V. 3, Bazzocchi C. 1, D'Amelio S. 2, and Lanfranchi P. 1
1 Dipartimento di Patologia animale, Igiene e Sanità pubblica veterinaria - Università degli Studi di Milano, Italy; 2 Dipartimento di Scienze di Sanità pubblica - Università di Roma "La Sapienza", Italy; 3 Istituto Zooprofilattico Sperimentale delle Venezie - Università degli Studi di Milano, Italy
- 310 **Seasonal trend of *Haemonchus contortus* in small ruminants breed grazed under semi-extensive farming system, Pakistan**
Qayyum M., Chaudary F.R. 1, and Miller J.E. 2
*1 Department of Zoology, University of Arid Agriculture, Rawalpindi, Pakistan
2 Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, USA*
- 311 **Development of free-living stages of *Teladorsagia* (= *Ostertagia*) *circumcincta* at varying temperatures**
Abdalla M.E. 1, and Pomroy W.E. 1
1 Massey University, Institute of Veterinary Animal and Biomedical Sciences, Palmerston North, New Zealand

- 312 **A survey of seasonal infestation with *Przhevalskiana* larvae in slaughtered goats and sheep in South-western Iran**
 Darabi S. 1, Azizi H.R. 2, Asadian F. 1, Pourjafar M. 3, and Baradaran H.R. 1
 1 Graduated from veterinary medicine, University of Shahrekord, Shahrekord, Iran; 2 Dept. of Pathobiology, Faculty of Veterinary Medicine, University of Shahrekord, Shahrekord, Iran; 3 Dept. of clinical science, Faculty of Veterinary Medicine, University of Shahrekord, Shahrekord, Iran
- 313 **Use of *Caenorhabditis elegans* to study the metabolism of ivermectin by nematodes**
 Laing S. 1, Laing R. 1, Redman L. 1, Ball G. 1, Woods D. 2, Ivens A. 3, and Gilleard J. 1
 1 University of Glasgow Veterinary School Department of Infection and Immunity, Glasgow, Scotland; 2 Pfizer Animal Health, Sandwich, Kent, England; 3 Sanger Genome Institute, Cambridge, England
- 314 **Comparative genomics of nematodes: *Caenorhabditis elegans* as a tool to study the *Haemonchus contortus* genome**
 Saunders G. 1, Berriman M. 2, Britton C. 1, and Gilleard J. 1
 1 University of Glasgow, Department Veterinary Parasitology, Glasgow, UK; 2 Pathogen Sequencing Unit, The Wellcome Trust Sanger Institute, London, UK
- 315 **Investigation of the cytochrome P450 family in the parasitic nematode *Haemonchus contortus***
 Laing R. 1, Laing S. 1, Redman L. 1, Ball G. 1, Woods D. 2, Berriman M. 3, and Gilleard J. 1
 1 Division of Infection and Immunity, Faculty of Veterinary Medicine, University of Glasgow; 2 Veterinary Medicine Drug Discovery, Pfizer Animal Health, Sandwich, Kent; 3 Pathogen Sequencing Unit, Sanger Genome Institute, Hinxton, Cambridge
- 316 **In vitro development of the fourth stage of *Haemonchus contortus* as a biological study model**
 Ramirez V.G. 1, Lopez A.M.E. 1, Liebano H.E. 1, Mendoza de G.P. 1, and Arteaga N.G. 1
 Inst. Nal. Invest. Forestales Agrícolas y Pecuarias, Helminthology, Jiutepec, Morelos, Mexico
- 317 **Transcription studies on desiphsilins in different stages of *Ancylostoma caninum*, *Haemonchus contortus* and *Ostertagia ostertagi* using quantitative real-time PCR**
 Fischer N. 1, Welz C. 1, Harder A. 2, and von Samson- Himmelstjerna G.
 1 Institute for Parasitology, University of Veterinary Medicine Hannover, Hannover, Germany; 2 Bayer HealthCare AG, Animal Health Division, Leverkusen, Germany

Poster group 2: Helminth Infections of Cattle

- 318 **Decision trees for worm control in cattle, sheep and horses as teaching tools**
 Eysker M., Doorn D.C.K., and van Ploeger H.W.
 Utrecht University, Department of Infectious Diseases and Immunology, Division of Clinical Infectiology, The Netherlands
- 319 **Delivery of anthelmintic drug combinations – A rumen delivery technology to meet current needs**
 Wunderlich M., Rennie G., Troncoso C., Weller K.M., and Rathbone M.J.
 InterAg, Hamilton, New Zealand

- 320 **Pharmacokinetics of moxidectin long-acting formulation in cattle: disposition in plasma and hair**
Dupuy J., Sutra J.F., and Alvinerie M.
INRA – UR66, Laboratoire de Pharmacologie-Toxicologie, Toulouse Cedex 9, France
- 321 **Toxicity evaluation of a 2.25% ivermectin + 1,25% abamectin solution in calves younger than four months of age**
Rodrigues D.C. 1, Buzzulini C. 2, Santana A.E. 3, Santos T.R. 3, Bergamin L.K.S. 3, Arantes T.P. 1, Santos E. 1, and Costa A.J. 3
1 Intervi do Brasil Veterinária Ltda; 2 CPPAR/FCAV/UNESP, Center for Research in Animal Health, Jaboticabal, São Paulo, Brazil ; 3 São Paulo State University, Jaboticabal, São Paulo, Brazil
- 322 **Preliminary evaluation of the efficacy of albendazole and ivermectin against gastrointestinal nematodes of cattle and goats in south of Mozambique**
Afonso S.M.S. 1,3, Capece B.P.S. 2, José L. 1, Castells G. 2., Arboix M. 2, Cristófol C. 2, and Neves L. 1
1 Secção de Parasitologia, Departamento de Paraclinicas, Faculdade de Veterinária, Universidade Eduardo Mondlane, Mozambique; 2 Secção de Farmacologia e Toxicologia, Departamento de Paraclinicas, Faculdade de Veterinária, Universidade Eduardo Mondlane, Mozambique; 3 Servei d'Anàlisi de Fàrmacs, Departament de Farmacologia, Terapeutica i Toxicologia, Universitat Autònoma de Barcelona, Spain
- 323 **Evaluation of cymiazole+abamectin pour-on formulation against bovine gastrointestinal nematodes and *Rhipicephalus (Boophilus) microplus* ticks under experimental and field conditions in Brazil**
Costa A.J. 1, Oliveira G.P. 1, Santos T.R. 1, Lima R.C.A. 1, Camargo G. 2, Bhushan C. 2, Mendonça, R.P. 1, and Borges F.A. 1
1 CPPAR/FCAV/UNESP, Center for Research in Animal Health, Jaboticabal, São Paulo, Brazil; 2 Bayer S.A., Bayer AG
- 324 **Evaluation of the 0 + 8 doramectin pour-on programme against lungworm in cattle**
Höglund. J. 1, Rydzik A. 1, and Ljungström B. 2
1 Swedish University of Agricultural Sciences and National Veterinary Institute, Department of Parasitology (SWEPAR), Uppsala, Sweden; 2 Vidilab AB, Enköping, Sweden
- 325 **Parasitic infections in dairy cattle around Hanoi, Vietnam**
Vien L.V. 1, Somers R. 1, Thanh N.T.G. 3, Dorny P. 1,2, Giao H.K. 1, Vercruysse J. 1, and Geurden T. 1
1 Department of Virology, Parasitology & Immunology, Faculty of Veterinary Medicine, Ghent University, Belgium; 2 Institute of Tropical Medicine Antwerp, Belgium; 3 Institute of Veterinary Research, Vietnam
- 327 **Morphological differentiation among three *Thelazia* species (Nematoda: Thelaziidae) by scanning electron microscopy**
Naem S.
Department of Pathobiology, College of Veterinary Medicine, Urmia University, Urmia, Iran
- 328 **Occurrence and pathology of gongylonema infection in cattle in north of Iran, Mazandaran Province**
Halajian A. 1, Eslami A. 2, and Tavakkol S. 3
1 Islamic Azad university, Science and research branch, Veterinary faculty, Department of parasitology, Tehran; 2 Tehran university, Veterinary faculty, Department of parasitology, Tehran; 3 Tonekabon Azad University, Science faculty, Iran

- 329 **Immunological responses and cytokines gene expression analysis to *Cooperia punctata* infections in resistant and susceptible Nelore cattle**
Bricarello P.A. 1, Zaros L.G. 2, Coutinho L.L. 2, Rocha R.A. 1, Kooyman F.N.J. 3, De Vries E. 3, Yatsuda A.P. 4, and Amarante A.F.T. 1
1 Universidade Estadual Paulista, IB, Departamento de Parasitologia, Botucatu, SP, Brazil; 2 Universidade de São Paulo, ESALQ, Departamento de Zootecnia, Piracicaba, SP, Brazil; 3 Department of Infectious Diseases and Immunology, Utrecht University, Utrecht, The Netherlands; 4 Faculdade de Ciências Farmacêuticas, USP, Ribeirão Preto, SP, Brazil.
- 330 **Gender specific transcription of asp antigens in *Ostertagia ostertagi***
Visser A. 1, Van Zeveren A.M. 1, Meyvis Y. 1, Peelaers I. 1, Van den Broeck W. 2, Vercruysse J. 1, Claerebout E. 1, and Geldhof P. 1
1 University Ghent, Department Veterinary Parasitology, Gent, Belgium; 2 University Ghent, Department Veterinary Morphology, Gent, Belgium
- 331 **Identification and characterisation of a novel ASP-like protein (OoAL1) from *Ostertagia ostertagi***
Saverwyns H., Peelaers I., Van Zeveren A.M., Vercruysse J., Claerebout E., and Geldhof P.
University Ghent, Department Veterinary Parasitology, Gent, Belgium
- 332 **Identification of galectins involved in the infection process of *Ostertagia ostertagi* in cattle**
Hoorens P.R., Geldhof P., Vercruysse J., and Claerebout E.
Laboratory for Parasitology, Faculty of Veterinary Medicine, Ghent University, Belgium
- 333 **No antigenic variation in the major sperm protein (MSP) of the nematode *Dictyocaulus viviparus***
Engström A., Mattsson J.G., and Höglund J.
SWEPAR, Uppsala, Sweden
- 334 **In vivo transfer of young adult *Dictyocaulus viviparus***
Ploeger H.W., Kooyman F.N.J., Koning F., Den Toom M., Dankers I., Van der Kuur A., and Eysker M.
Utrecht University, Department of Infectious Diseases and Immunology, Division of Clinical Infectiology, The Netherlands

Poster group 3: Fasciolicides

- 335 **A recombinant-based ELISA for the evaluation of two fasciolicides in autochthonous *Rubia gallega* bovine**
Arias M. 1, Sánchez-Andrade R. 1, Suárez J.L. 1, Francisco I. 1, Morrondo P. 1, Calvo E. 2, Díez-Baños P. 1, and Paz-Silva A. 1
1 Animal Pathology Department, Epidemiology, Zoonoses and Parasitic diseases, Veterinary Faculty, Santiago de Compostela University, Lugo, Spain; 2 Schering-Plough S.A., Madrid, Spain
- 336 **Metabolic interference enhances the plasma profiles of triclabendazole metabolites in sheep**
Virkel G. 1,2, Lifschitz A. 1,2, Ballent M. 1,2, Sallovitz J. 1,3, Scarcella S. 1, and Lanusse C. 1,2
1 Laboratorio Farmacología, FCV-UNCPBA, Argentina; 2 CONICET, Argentina; 3 CICPBA, Buenos Aires, Argentina

- 337 **A comparison of the efficacy of doramectin, closantel and levamisole in the treatment of the “oriental eye fluke”, *Philopthalmus gralli* in commercially reared ostriches (*Struthio camelus*)**
Mukaratirwa S. 1, Chimbwanda T.M. 1, and Matekwe N. 1
1 Faculty of Veterinary Science, University of Zimbabwe, Harare, Zimbabwe
- 338 **In vitro activity of some plant extracts agasint newly excysted *Fasciola hepatica* metacercariae**
Vera Montenegro Y. 1, Ibarra V.F. 1, Ramírez A.G. 2, and Munguía X.J. 3
1 Universidad Nacional Autónoma de México, Facultad de Medicina Veterinaria y Zootecnia, Departamento de Parasitología, México; 2 CIBIS, Biología Celular, Xochitepec, Mor. México; 3 Instituto Tecnológico de Sonora, México
- 339 **Fasciocidal activity of artemisinin derivatives**
Keiser J. 1, Rinaldi L. 2, Cringoli G. 2, Veneziano V. 2, Vennerstrom J. 3, and Utzinger J. 1
1 Swiss Tropical Institute, Basel, Switzerland; 2 Dipartimento di Patologia e Sanità Animale, Facoltà di Medicina Veterinaria, Napoli, Italy; 3 University of Nebraska, Nebraska, USA
- 340 **Pharmacokinetic evaluation of the ivermectin-triclabendazole combination in sheep**
Lifschitz A. 1,2, Virkel G. 1,2, Ballent M. 1,2, Sallovitz J. 1, and Lanusse C. 1,2
1 Lab. Farmacología, FCV, UNCPBA, Tandil, Argentina; 2 CONICET, Argentina.
- 341 **The relative potential of verapamil and ivermectin in combination with triclabendazole, to overcome triclabendazole resistance**
Meaney M., Brennan G.P., Trudgett A., Hoey E.M., Savage J., and Fairweather I.
School of Biological Sciences, Queens University Belfast
- 342 **Susceptibility of a newly isolated strain of *Fasciola hepatica* to closantel in sheep**
Jacobs J. 1, Burden J. 2., Bendall H. 2, Goossens L. 1, and Veys P. 1
1 Janssen Animal Health, Beerse, Belgium; 2 Ridgeway Research Ltd, Gloucestershire, UK
- 343 **A field efficacy study to determine the efficacy of ivermectin/closantel injection (Norbrook Laboratories Limited, Produce code P-CIV-170) against naturally acquired infections of *Fasciola hepatica* and gastrointestinal nematodes in cattle**
Cromie L. 1, Couper A. 1, Borgsteede F. 2, and Taylor S. 3
1 Norbrook Laboratories Limited, Newry, Co. Down, BT35 6PU, Northern Ireland; 2 Animal Sciences Group, NL-8200 AB, Lelystad, The Netherlands; 3 Vetpar Services, Bangor, Co. Down, Northern Ireland
- 344 **The effect of a parenteral ivermectin/closantel injection on the growth and reproductive development of early immature *Fasciola hepatica* in cattle**
Hanna B. 1, Cromie L. 2, Taylor S. 3, and Couper A. 2
1 VSD, AFBINI, Stormont, Belfast, Northern Ireland; 2 Norbrook Laboratories Limited, Newry, Co. Down, Northern Ireland; 3 Vetpar Services, Bangor, Co. Down, Northern Ireland
- 345 **Evaluation of efficacy of moxidectin/triclabendazole oral drench against mixed infections of gastro-intestinal nematodes and liver fluke in sheep under field conditions in Spain**
Cordero-Pérez C. 1, Álvarez-Sánchez M.A. 1, Fernández-Pato N. 1, Meana A. 2, and Rojo-Vázquez F.A. 1
1 Dept Sanidad Animal, Facultad de Veterinaria, University of León, Spain; 2 Dept Sanidad Animal, Facultad de Veterinaria, Complutense University, Madrid, Spain

- 346 **The role of beta-tubulins in inferring resistance to triclabendazole in *Fasciola hepatica***
Fuchs M.-A. 1, Ryan L. 1, Trudgett A. 1, Fairweather I. 1, and Hoey E. 1
1 School of Biological Sciences, Queen's University Belfast
- 347 **An electron microscopical study to assess the effects of the 1,2,4- trioxolane, OZ78, at a surface and ultrastructural level on the parasitic trematode *Fasciola hepatica***
Halferty L. 1, O'Neill J. 1, Brennan G. 1, Vennerstrom J. 3, Keiser J. 2, and Fairweather I. 1
1 School of Biological Sciences, Queen's University Belfast; 2 Swiss Tropical Institute, Basel, Switzerland; 3 College of Pharmacy, University of Nebraska Medical Center, Nebraska, USA
- 348 **The evaluation of the activity of compound alpha against two isolates of *F. hepatica* in sheep**
McConville M. 1, Fairweather I. 1, Brennan G.P. 1, McCoy M.A. 2, Edgar H.W.J. 2, Ellison S. 2, Flanagan A. 2, Hernández-Campos A. 3, and Castillo R. 3
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- 349 **Fasciolicide activity in sheep of three experimental compounds selected by computer aided design**
Ibarra V.F. 1, Vera M.Y. 1, Cantó A.J.G. 2, Huesca G.A. 1, Alcala C.Y. 1, and Marrero P.Y. 3
1 Universidad Nacional Autónoma de México, Facultad de Medicina Veterinaria y Zootecnia. Departamento de Parasitología. México; 2 CENID-Fisiología-INIFAP, Ajuchitlán Oro; 3 Universidad de la Villas de Santa Clara, Cuba
- 350 **Comparative ovicidal effect of albendazole and triclabendazole on *Fasciola hepatica* eggs**
Moreno G. 1, Alvarez L. 1, Moreno L. 1, Ceballos L. 1, Shaw L. 2, Fairweather I. 2, and Lanusse C. 1
Queen's University Belfast, School of Biological Sciences, Belfast, Northern Ireland

Poster group 4: Miscellaneous

- 351 **International collaboration in support of standardization, validation, and harmonization of quality assured diagnostic parasitology testing for food safety and trade**
Scandrett B., Forbes L., and Gajadhar A.
Canadian Food Inspection Agency, Centre for Food-borne and Animal Parasitology, Saskatoon, Canada
- 352 **Veterinary parasite collections at Harvard University's Museum of Comparative Zoology**
Conn D.B. 1,2
1 Harvard University, Museum of Comparative Zoology, Cambridge, Massachusetts, USA; 2 Berry College, School of Mathematical and Natural Sciences, Mount Berry, Georgia, USA
- 353 **DNA barcoding for filarial nematodes: a web-based tool for the molecular identification of parasitic infections**
Ferri E. 1, Barbuto M. 2, Bain O. 3, Martin C. 3, Uni S. 4, Bandi C. 1, Genchi C. 1, and Casiraghi M. 2
1 University of Milano, Department of Veterinary Pathology, Milan, Italy; 2 University of Milano Bicocca, Department of Biotechnology and Bioscience, Milan, Italy; 3 Muséum National d'Histoire Naturelle. Paris, France; 4 Osaka City University Medical School, Department of Medical Zoology, Osaka, Japan

- 354 **Parasite contamination of soil samples taken from the household areas of a Mexican rural community endemic for neurocysticercosis**
Huerta M. 1, Avila R. 2, Jiménez H.H. 2, Díaz R. 1, Díaz J. 7, Díaz Huerta M.D. 1, Hernández M. 3, Martínez J.J. 4, Garate T. 5, Gómez E. 7, Abad T. 1, Fragoso 3, Fleury A. 6, and Sciutto E. 3
1 Facultad de Medicina, 2 Escuela de Medicina Veterinaria y Zootecnia, Benemérita Universidad Autónoma de Puebla, Puebla, Pue, México; 3 Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, México; 4 Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, México; 5 Ministerio de Sanidad y Consumo. Instituto de Salud Carlos III, Madrid, Spain; 6 Instituto Nacional de Neurología y Neurocirugía, Delegación Tlalpan, México; 7 Unidad Medico Familiar 57 IMSS- Puebla, México
- 355 **Prevalence of soil-transmitted helminths in women in developing country**
Joshi S.D. 1, and Panday K. 2
1 Nepal Medical college teaching hospital; 2 Community health and environmental society Nepal
- 356 **The effect of *Nigella sativa* oil against *Aspicularis tetraptera* and *Hymenolepis nana* in naturally infected mice**
Ayaz E. 1, Yilmaz H. 2, Ozbek H. 3, Tas Z. 2, and Orunc O. 2
1 Yenisehirbrahim Orhan Vocational School, Uluda University, Bursa, Turkey; 2 Department of Parasitology, Medical School of Yuzuncu Yl University, Van, Turkey; 3 Department of Pharmacology, Medical School of Yüzüncü Yl University, Van, Turkey
- 357 ***Nippostrongylus brasiliensis* infection in the lactating rat: A model to assess nutritional sensitivity of periparturient breakdown of immunity**
Jones L.A. 1, Houdijk J.G.M. 1, Normanton H. 1, Knox D.P. 2, and Kyriazakis I. 1,3
1 Animal Nutrition and Health Department, Scottish Agricultural College, Kings Buildings, Edinburgh, UK; 2 Parasitology Division, Moredun Research Institute, Bush Loan, Penicuik UK; 3 Veterinary Faculty, University of Thessaly, Karditsa, Greece
- 358 **Morphological description and molecular analysis of *Filaria martis* Gmelin 1790 (Spirurida, Filariidae) affecting beech marten (*Martes foina*)**
Lia R.P. 1, Cantacessi C. 1, Brianti E. 2, Giannetto S. 2, and Otranto D. 1
1 Department of Animal Health and Welfare, Faculty of Veterinary Medicine, Bari, Italy; 2 Department of Public Animal Health of the Faculty of Veterinary Medicine, Messina, Italy
- 359 **Residual determination of 5-chloro-2-methyltio-6-(1-naphtyloxy)-1h-benzimidazole in bovine tissue by HPLC**
Vera-Montenegro Y. 1, Ibarra V.F. 1, Bautista O.J. 2, Cantó A.G.J. 3, Rosiles M.R. 2, Castillo B.R. 4, Hernández C.A. 4
1 Universidad Nacional Autónoma de México, Facultad de Medicina Veterinaria y Zootecnia, Departamento de Parasitología; 2 CENID-Fisiología-INIFAP, Ajuchitlán Qro; 3 Universidad Nacional Autónoma de México, Facultad de Medicina Veterinaria y Zootecnia, Departamento de Toxicología; 4 Universidad Nacional Autónoma de México, Facultad de Química, Departamento de Biofarmacia, Mexico
- 360 **In vitro activity of the cyclooctadepsipeptides emodepside and PF1022A in comparison to ivermectin and levamisole in gastrointestinal nematodes**
Schürmann S. 1, Harder A. 2, Schnieder T. 1, and von Samson-Himmelstjerna G. 1
1 Institute for Parasitology, University of Veterinary Medicine Germany; 2 Bayer Health Care AG, Animal Health, R&D Parasitocides, Monheim, Germany
- 361 **Interactive effects between growth potential and protein nutrition on resilience and resistance to parasites in mice**
Houdijk J.G.M. 1, and Bünger L. 2
1 Animal Health and Nutrition Team, SAC, Edinburgh, UK; 2 Animal Breeding and Development Team, SAC, Edinburgh, UK

- 362 **Disseminated cestodiasis of mammals involving anomalous epithelial development in metacestodes**
 Conn D.B. 1, and Conn D.A. 2
1 Berry College, School of Mathematical and Natural Sciences, Mount Berry, Georgia, USA; 2 North American Scientific Enterprises (NASCENT), Monteagle, Tennessee, USA
- 363 **Trichinellosis in Switzerland**
 Frey C. 1, Zeeh F. 2, Müller N. 1, Zimmermann W. 2, Schuppers M.E. 3, and Gottstein B. 1
1 Institute for Parasitology, University of Bern; 2 Swine Clinic, University of Bern; 3 SAFOSO, Bern, Switzerland
- 364 **Heterogeneity of human trichinellosis and animal *Trichinella* infection in Romania: a geographical paradox due to ancestral food habits and political changes**
 Blaga R. 1,2, Durand B. 3, Stoichici A. 4, Antoniu S. 5, Gherman C. 2, Stefan N. 4, Cozma V. 2, and Boireau P. 1
1 JRU BIPAR INRA AFSSA ENVA UPVM, 23, Maisons-Alfort, France; 2 University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Parasitology Unit, Cluj-Napoca, Romania; 3 Epidemiology Unit, AFSSA, Maisons-Alfort, France; 4 Institute for Diagnosis et Animal Health, Bucharest, Romania; 5 University of Agronomical Sciences et Veterinary Medicine, Faculty of Veterinary Medicine, Parasitology Unit, Bucharest, Romania
- 365 **Molecular identification of *Trichinella* species circulating among wild and domestic animals in Romania**
 Blaga R. 1,2, Gherman C. 2, Cozma V. 2, Glemarec A. 1, Pozio E. 3, and Boireau P. 1
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- 366 **Isolation, biochemical analysis and localization of a 24 kDa Glutathione S-Transferase from *Trichinella***
 Bahuon C. 1, Blaga R. 1,3, Fu B. 4, Chiumiento L. 5, Liu M.Y. 2, Le Rhun D. 1, Glemarec A. 1, and Boireau P. 1
1 JRU BIPAR INRA AFSSA ENVA UPVM, 23, Maisons-Alfort, France; 2 Jilin University of Agriculture and Animal Sciences, Changchun, P.R. China; 3 University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania; 4 Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, P.R. China; 5 Department of Experimental Pathology, M.B.I.E, University of Pisa, Pisa, Italy
- 367 **Species identification of *Trichinella* isolates from China**
 Fu B. 1, Li W. 1, Li H. 1, Li Y. 1, Gai W. 1, Gou H. 1, Wang Y. 1, Zhang D. 1, Dou L., Liu M.Y. 2, Blaga R. 3, and Boireau P. 4
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- 368 **Sylvatic trichinella infection in Finland - red fox (*Vulpes vulpes*) and raccoon dog (*Nyctereutes procyonoides*) as indicator species**
 Saari S. 1, Airas N. 1, Virtala A.-M. 1, Mikkonen T. 1, Pellikka J. 2, Oksanen A. 3, Isomursu M. 3, and Sukura A. 1
1 Department of Basic Veterinary Sciences (FINPAR), Faculty of Veterinary Medicine, University of Helsinki, Helsinki, Finland; 2 Department of Forest Ecology, Faculty of Agriculture and Forestry, University of Helsinki, Helsinki, Finland; 3 Oulu Research Unit, Finnish Food Safety Authority Evira (FINPAR), Oulu, Finland

Poster group 5: Arthropods of Companion Animals

- 369 **Longitudinal systematic survey of fleas on dogs**
Rinaldi L., Veneziano V., Carbone S., Schioppi M., Maurelli M.P., Spera G., and Cringoli G.
Department of Pathology and Animal Health, University of Naples "Federico II", CREMOPAR Regione Campania, Naples, Italy
- 370 **Evolution of flea infestation in dogs on an eleven years period in west of France**
Bourdeau P., Bruet V., and Travers F.
Dermatology-Parasitology-Mycology, Veterinary School of Nantes, France
- 371 **Are corticosteroids involved in generalized demodicosis in dogs?: Informations from 55 cases**
Bruet V., Travers F., and Bourdeau P.
Dermatology- Parasitology-Mycology, Veterinary School Nantes, France
- 372 **Distribution of cat flea eggs and larvae in the carpeted environment**
Beck W. 1,2
1 Pfizer Animal Health, Karlsruhe, Germany; 2 Institute for Comparative Tropical Medicine and Parasitology, LMU Munich, Munich, Germany
- 373 **Flea infestation in cats: evolution on an eleven years period through prospective/retrospective evaluation**
Bourdeau P., Bruet V., and Travers F.
Dermatology - Parasitology – Mycology, Veterinary School of Nantes, France
- 374 **Investigation of the microscopic anatomy of *Ixodes ricinus* and *Ctenocephalides felis* with view to identification of sites for microbial pathogen localisation**
Robinson M.T. 1, Morgan E. 2, Williams T. 3, and Shaw S.E. 1
1 School of Clinical Veterinary Sciences, University of Bristol, Langford, UK; 2 School of Biological Sciences, University of Bristol, Bristol, UK; 3 Pfizer, Sandwich, Kent, UK.
- 375 **Clinical and immunity aspects in experimental infection and reinfection with *Microsporum canis* in cats**
Mircean V. 1, Czirjak G.A. 1, Mircean M. 1, and Cozma V. 1
1 University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania
- 376 ***Demodex gatoi* associated contagious pruritic dermatitis in cats – A report from six households in Finland**
Saari S. 1, and Saijonmaa-Koulumies L. 2
1 Department of Basic Veterinary Sciences (FINPAR), Faculty of Veterinary Medicine, University of Helsinki, Finland; 2 Department of Equine and Small Animal Medicine, Faculty of Veterinary Medicine, University of Helsinki, Finland
- 377 **Assessment of the ear mite, *Otodectes cynotis* infestation and the efficacy of Advocate® in the treatment of otoacariosis in a Hungarian cat shelter**
Farkas R. 1, Germann T., and Szeidemann Zs. 2
1 Department of Parasitology and Zoology, Faculty of Veterinary Science, Szent István University, Budapest, Hungary; 2 Bayer Hungaria Ltd., Budapest, Hungary
- 378 **Clinical evaluation of the efficacy and safety of topically applied advantage Multi™ (imidacloprid/moxidectin) against ear mites (*Otodectes cynotis*) in client owned cats**
Davis W., Arther R., and Settje T.
Bayer HealthCare, Animal Health Division, Department of Research and Development, Shawnee, Kansas, USA

- 379 **Efficacy of selected spot-on insecticides in the treatment of cats from animal shelters infested with ticks (Acarina) and fleas (Siphonaptera)**
Kocisova A. 1, Lukesova D. 2, and Letkova V. 1
1 University of Veterinary Medicine, Kofice, The Slovak Republic; 2 Czech University of Life Sciences Prague, Institute of Tropics and Subtropics, The Czech Republic
- 380 **0.4% dimeticone spray, a novel control material to kill fleas by physical means**
Jones I., Kidman E.R., and Burgess I.
Medical Entomology Centre, Insect Research & Development Limited, Royston, UK
- 381 **Pharmacokinetics of metaflumizone in the plasma and hair of cats following application in a topical spot-on formulation**
DeLay R. 1, Blond-Riou F. 2, Lacoste E. 3, Delprat S. 3, and Rugg D. 1
1 Fort Dodge Animal Health, USA; 2 Fort Dodge Sante Animale, Tours, France; 3 Avogrado, France
- 382 **Pharmacokinetics of metaflumizone and amitraz in the plasma and hair of dogs following application in a topical spot-on formulation**
DeLay R. 1, Blond-Riou F. 2, Lacoste E. 3, Messasalma T. 3, and Rugg D. 1
1 Fort Dodge Animal Health, USA; 2 Fort Dodge Sante Animale, France; 3 Avogrado, France
- 383 **The efficacy of pyriprole topical solution against the cat flea, *Ctenocephalides felis*, on dogs**
Barnett S. 1, Luempert L. 1, Quezada A. 2, Schuele G. 3, Strehlau G. 4, and Doherty P. 5
1 Novartis Animal Health US Inc., Greensboro, USA; 2 Stillmeadow Inc., Sugar Land, USA; 3 Novartis Animal Health, Basel, Switzerland; 4 Novartis Centre de Recherche Santé Animale SA, St. Aubin, Switzerland; 5 Charles River Laboratories BioLabs Europe, Ballina, Co. Mayo, Ireland
- 384 **The efficacy of pyriprole topical solution against tick (*Rhipicephalus sanguineus*, *Ixodes ricinus*, *Dermacentor reticulatus*) infestations on dogs**
Schuele G. 1, Barnett S. 2, Bapst B. 3, Luempert L. 2, Strehlau G. 3, Young D.R. 4, and Moran C. 5
1 Novartis Animal Health, Basel, Switzerland; 2 Novartis Animal Health US Inc., Greensboro, USA; 3 Novartis Centre de Recherche Santé Animale SA, St. Aubin, Switzerland; 4 Young Veterinary Research Services, Turlock, Canada; 5 Charles River Laboratories BioLabs Europe, Ballina, Co. Mayo, Ireland
- 385 **The safety profile of pyriprole and prac-tic®**
Helbig R.
Novartis Animal Health, Basel, Switzerland
- 386 **The effect of water and shampooing on the efficacy of pyriprole topical solution**
Schuele G. 1, Barnett S. 2, Luempert L. 2, Cavaliero T. 3, Bapst B. 3, Strehlau G. 3, Quezada A. 4, Doherty P. 5, and Young D.R. 6
1 Novartis Animal Health, Basel, Switzerland; 2 Novartis Animal Health US Inc., Greensboro, USA; 3 Novartis Centre de Recherche Santé Animale SA, St. Aubin, Switzerland; 4 Stillmeadow Inc., Sugar Land, TX; 5 Charles River Laboratories BioLabs Europe, Ballina, Co. Mayo, Ireland; 6 Young Veterinary Research Services, Turlock, Canada
- 387 **Efficacy of imidacloprid 10% and imidacloprid 10% plus moxidectin 2.5% against natural lice (*Trichodectes canis*) infestations in dogs**
Stanneck D. 1, Doyle J. 2, and Heine J. 1
1 Bayer HealthCare AG, Animal Health Clinical Development Parasitocides, Leverkusen, Germany; 2 Charles River Laboratories BioLabs Europe, Glenamoy, Ireland

- 388 **Efficacy of a novel formulation of metaflumizone plus amitraz (ProMeris® / ProMeris Duo®) for the treatment of demodectic and sarcoptic mange in dogs**
 Fourie L. 1, Kok D. 1, Rugg D. 2, and du Plessis A. 3
1 ClinVet International, Universitas, South Africa; 2 Fort Dodge Animal Health, Princeton, USA; 3 Fort Dodge Animal Health, Midrand, South Africa
- 389 **In vitro and in vivo bioassay systems of insecticides applied to dogs against the European house dust mite *Dermatophagoides pteronyssinus***
 Shin S.S., Jeong I.S., Lim S., Song O.H., Han H.J., and Cho K.O.
College of Veterinary Medicine, Chonnam National University, Gwangju, South Korea

Poster group 6: Neospora

- 390 **Pathological and immunological findings on placenta after infection with *Neospora caninum* in a pregnant mouse model**
 López-Pérez I.C. 1, Collantes-Fernández E. 1, Risco-Castillo V. 1, Pérez-Pérez V. 2, Pereira-Bueno J. 3, and Ortega-Mora L.M. 1
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- 391 ***Neospora caninum* infection in birds: experimental infections in chicken and embryonated eggs**
 Furuta P.I. 1, Mineo T.W.P. 1,2, Carrasco A.O.T. 1, Godoy G.S. 1, Pinto A.A. 1, and Machado R.Z. 1
1 Department of Veterinary Pathology, FCAV/UNESP, Jaboticabal, São Paulo, Brazil; 2 Department of Biochemistry and Immunology, FMRP/USP, Ribeirão Preto, São Paulo, Brazil
- 392 **Seroprevalence of *Neospora caninum* in cattle from western Australia**
 O'Handley R.M. 1, de Boer M.W. 2, Palmer D.G. 3, and Ryan U.M. 1
1 Murdoch University, Veterinary and Biomedical Sciences, Perth, Australia; 2 The Tern Veterinary Group, Market Drayton, United Kingdom; 3 Department of Agriculture and Food Western Australia, Perth, Australia
- 393 **Cross-sectional survey on the seroprevalence of *Neospora caninum* infection in Bali cattle (*Bos javanicus*) in Bali, Indonesia**
 Damriyasa I.M. 1, Schares G. 2, and Bauer C. 1,3
1 Udayana University, Centre for Studies on Animal Diseases, Bali, Indonesia; 2 Federal Research Institute for Animal Health, Institute of Epidemiology, Wusterhausen, Germany; 3 Justus Liebig University Giessen, Institute of Parasitology, Giessen, Germany
- 394 **Occurrence of *Neospora caninum* infection and other intestinal parasites in at-risk dog populations in Spain**
 Collantes-Fernández E. 1, Regidor-Cerrillo J. 1, Pedraza-Díaz S. 1, Vazquez-Moreno E. 1, Árnaiz I. 2, Aduriz G. 3, Gómez-Bautista M. 1, and Ortega-Mora L.M. 1
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- 395 **Antibodies to *Neospora caninum* and *Toxoplasma gondii* in domestic cats from Brazil**
 Bresciani K.D.S. 1, Gennari S.M. 2, Serrano A.C.M. 1, Rodrigues A.A.R. 2, Ueno T. 2, Franco L.G. 1, Táparo C.V. 1, and Amarante A.F.T. 3
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Veterinária Preventiva e Saúde Animal, São Paulo, SP, Brazil, 3 UNESP, Instituto de Biociências, Departamento de Parasitologia, Botucatu, SP, Brazil

- 396 **Detection of antibodies to *Neospora caninum* in the blood of bison (*Bison bonasus bonasus* L.) eliminated from the breeding program in 2004-2007 in Poland**
Gozdzik K., Bieñ J., Moskwa B., and Cabaj W.
Witold Stefanski Institute of Parasitology of the Polish Academy of Sciences, Warsaw, Poland
- 397 **Acute protozoal myopathy in a dog due to *Neospora caninum***
Grandi G. 1, Venzi C. 2, Zavattiero S. 2, Cantoni A.M. 2, Di Lecce R. 2, and Del Bue M. 2
1 Department of Animal Production - University of Parma, Italy; 2 Animal Health Department, University of Parma, Italy
- 398 **Effect of *Neospora caninum* infection on *Bos taurus* and *Bos indicus* retropharyngeal lymph node mRNA expression**
Viero L.M. 1, Gennari S.M. 2, Soares R.M. 2, Maiorka P. 3, and Nishi S.M. 2
1 Department of Parasitology, Institute of Biomedical Sciences; 2 Department of Preventive Veterinary Medicine and Animal Health; 3 Department of Pathology, Faculty of Veterinary Medicine, University of São Paulo, Brazil
- 399 ***Neospora caninum* infects the canine macrophage cell line dh8**
Grandi G. 1, Kramer L. 1, DeAngelis E. 2, Borghetti P. 2, Mortarino M. 3, Ferri E. 3, and Genchi C. 3
1 University of Parma, Department of Animal Production, Parma, Italy; 2 University of Parma, Department of Animal Health, Parma Italy; 3 University of Milan, Department of Veterinary Pathology, Milan, Italy
- 400 ***Neospora caninum* infection of bovine pbmc-derived macrophages**
Grandi G. 1, Kramer L. 1, De Angelis E. 2, Borghetti P. 2, Bazzocchi C. 3, Ferri E. 3, and Genchi C. 3
1 University of Parma, Department of Animal Production, Parma, Italy; 2 University of Parma, Department of Animal Health, Parma Italy; 3 University of Milan, Department of Veterinary Pathology, Milan, Italy
- 401 **Persistence of colostral antibodies to *Neospora caninum* in infection free calves**
Cardoso J.M.S., Funada M.R., Soares R.M., and Gennari S.M.
University of São Paulo, Department of Veterinary Preventive Medicine and Animal Health, São Paulo, Brazil
- 402 **Detection of antibodies to *Neospora caninum* in cattle by enzyme-linked immunosorbent assay with truncated NcSRS2 expressed in *Pichia pastoris***
Généreux C. 1, Alvarez-Garcia G. 2, Ortega-Mora L.M. 2, and Losson B. 1
1 University of Liège, Faculty of Veterinary Medicine, Department of Infectious and Parasitic Diseases, Belgium; 2 SALUVET, Animal Health Department, Faculty of Veterinary Sciences, Complutense University of Madrid, Ciudad Universitaria s/n, Madrid, Spain
- 403 **Phenotypic characterization of spleen dendritic cells in BALB/c mice infected with *Neospora caninum* tachyzoites**
Teixeira L. 1,2, Correia A. 1, Cerca F. 1,2, Botelho A. 1,2, and Vilanova M.
1 ICBAS, Instituto de Ciências Biomédicas de Abel Salazar, Porto, Portugal; 2 IBMC, Instituto de Biologia Molecular e Celular, Porto, Portugal
- 404 **Efficacy of the vaccination with killed whole *Neospora caninum* tachyzoite or tachyzoite-bradyzoite antigens in a pregnant BALB/c mouse model**
Rojo-Montejo S. 1, Collantes-Fernández E. 1, Rodríguez-Marcos S. 1, Pérez-Zaballos F. 1, Prenafeta A. 2, and Ortega-Mora L.M. 1
1 SALUVET, Animal Health Department, Faculty of Veterinary Sciences, Complutense University of Madrid, Madrid, Spain; 2 Hipra Laboratories, Girona, Spain

- 405 **Reduced parasitaemia after infection with *Neospora caninum* in mice immunised with NcSRS iscoms**
Pinitkiatisakul S., Mattsson J.G., and Lundén A.
Department of Parasitology (SWEPAR), Swedish University of Agricultural Sciences and National Veterinary Institute, Uppsala, Sweden
- 406 **Protective immunity induced by recombinant antigens rNcSAG4 and rNcGRA7 against chronic and congenital neosporosis in an experimental murine model**
Aguado-Martínez A. 1, Álvarez-García G. 1, Fernández-García A. 1, Risco-Castillo V. 1, Marugán-Hernández V. 1, and Ortega-Mora L.M. 1
1 SALUVET, Animal Health Department, Faculty of Veterinary Sciences, Complutense University of Madrid, Madrid, Spain
- 407 **Characterisation of the effects of pentamidine-derivatives in *Neospora caninum*-infected cell cultures**
Hemphill A. 1, Leepin A. 1, Stüdl A. 2, Brun R. 2, and Boykin D. 3
1 Institute of Parasitology, Vetsuisse Faculty, University of Bern, Switzerland; 2 Department of Medical Parasitology and Infection Biology, Swiss Tropical Institute, Basel, Switzerland; 3 Department of Chemistry and Center for Biotechnology and Drug Design, Georgia State University, USA
- 408 **Identification of polymorphic regions in several strain of *Neospora caninum***
Bazzocchi C. 1, Sassera D. 1, Ferri E. 1, Mortarino M. 1, Grandi G. 2, and Genchi C. 1
1 University of Milano, Department of Veterinary Pathology, Milan, Italy; 2 University of Parma, Department of Animal Production, Parma, Italy
- 409 **A BALB/c nu/nu mouse model to evaluate the viability of *Neospora caninum* tachyzoites in bovine extended semen**
Ferre I. 1, Mateos-Sanz A. 1, Aduriz G. 2, Ugarte-Garagalza C. 3, Mota R.A. 1, and Ortega-Mora L.M. 1
1 SALUVET, Animal Health Department, Faculty of Veterinary Sciences, Complutense University of Madrid, Madrid, Spain; 2 Instituto Vasco de Investigación y Desarrollo Agrario (NEIKER), Derio, Spain; 3 Aberekin, Centro de Inseminación, Derio, Spain
- 410 **Isolation and genetic characterization of *Neospora caninum* from asymptomatic calves in Spain**
Regidor-Cerrillo J. 1, Gómez-Bautista M. 1, Pedraza-Díaz S. 1, and Ortega-Mora L.M. 1
1 SALUVET, Animal Health Department, Faculty of Veterinary Sciences, Complutense University of Madrid, Madrid, Spain
- 411 **Anti-neosporal effect of *S. flavescens* and *T. japonica* extracts against *N. caninum* on mice, goats and dogs**
Seo H.S., Cho M.H., Kim J.H., and Youn H.J.
Seoul National University, Department of Parasitology, College of Veterinary Medicine, Seoul, South Korea
- 412 **Comparative *Bos taurus* and *Bos indicus* tissue mRNA response against *Neospora caninum* infection**
Nishi S.M. 1, Viero L.M. 2, Soares R.M. 1, Maiorka P. 3, and Gennari S.M. 1
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Poster group 7: Vector-Borne Protozoa

- 413 **Occurrence of *Borrelia* and *Babesia* spp. in hard ticks collected from dogs in Hungary**
Földvári G. 1, Márialigeti M. 1, Solymosi N. 2, Lukács Z. 3, Majoros G. 1, and Farkas R. 1
1 Department of Parasitology and Zoology, Faculty of Veterinary Science, Szent István University, Budapest, Hungary; 2 Department of Biomathematics, Faculty of Veterinary Science, Szent István University, Budapest, Hungary; 3 Bayer Hungary Ltd., Budapest, Hungary
- 414 **Isolation and in vitro culture of zoonotic *Babesia* sp. EU1 from roe deer (*Capreolus capreolus*) and evidence of parasite transmission in *Ixodes ricinus***
Bonnet S., Jouglin M., L'Hostis M., and Chauvin A.
UMR ENVN, INRA 1034, Ecole Nationale Vétérinaire de Nantes, Nantes, France
- 415 **Chemotherapeutic evaluation of babesicides products (IC 040, Berenil and Vivater LA) in calves experimentally infected with *Babesia bigemina* and *Babesia bovis* in Brazil**
Massard C.L. 1, Franque M.P. 1, Santos H.A. 1, Silva G.V.O. 1, Fernandes K.R. 1, Golinsky A.A. 1, Santos E. 2, and Vashist U. 1
1 Universidade Federal Rural do Rio de Janeiro, Department of Animal Parasitology, Rio de Janeiro Brazil; 2 Asko Nobel Ltda. Intervet- Division, Brazil
- 416 **Piroplasmosis as a possible cause of mortality in giraffes**
Oosthuizen M.C., Collins N.E., and Penzhorn B.L.
Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa
- 417 ***Trypanosoma evansi* in cattle farms in Malaysia**
Sani R.A. 1, Cheah T.S. 2, Sharifah-Salmah S.H. 1, and Chin P.L. 1
1 Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia; 2 Perak Royal Medical College, Ipoh, Malaysia
- 418 **Haematologic response of donkeys to *Trypanosoma evansi* infection**
Ismail A.A. 1, and Abdalla I.M. 2
1 Sudan University of Science and Technology, Department of Parasitology, Sudan; 2 University of Bahr el Ghazal, Department of Parasitology, Sudan
- 419 **Trypanosomiasis among free ranging Indian tigers: a global challenge for veterinarians**
Singh D.P.
Govt. Vety. Hospital, Department of Animal Husbandry, Sawai Madhopur, India
- 420 **A survey on the cardiac arrhythmias in the cows with theileriosis**
Hasanpoor A. 1, Refa M. 1, and Amoughli Tabrizi B. 1
1 Islamic Azad University of Tabriz department of clinical science of the veterinary medicine faculty, Tabriz, Iran
- 421 **The potential of East Coast Fever (ECF) vaccine on improved and indigenous cattle in Tanzania**
Magwisha H.B. 1, Ruheta M.R. 2, Mbwambo H.A. 1, Madege M. 3, Ranga E.E. 3, and Makungu L.S. 3
1 Central Veterinary Laboratory, Department of Parasitology Dar es Salaam, Tanzania; 2 Ministry of Livestock Development Dar es Salaam, Tanzania; 3 Veterinary Investigation Centre Mwanza, Tanzania

- 422 **Infectivity, immune response and possible protective capacity of *Leishmania infantum* strains isolated from HIV-*Leishmania* co infected patients in the canine model**
Miret J. 1, Nieto J. 1, Chicharro C. 1, Cañavate C. 1, Carrillo E. 1, González F. 2, and Moreno J. 3
1 WHO Collaborative Centre for Leishmaniasis. National Centre of Microbiology, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain; 2 University Complutense of Madrid, Department of Toxicology and Pharmacology, Veterinary Faculty, Madrid, Spain; 3 Centro de Investigaciones Biológicas, CSIC, Madrid, Spain
- 423 **Evaluation of enzyme-linked immunosorbent and flow cytometry assays for the serological diagnosis of visceral leishmaniasis using crude and recombinant *Leishmania* proteins**
Santarém N. 1, Silvestre R. 1, Cardoso L. 2, and Cordeiro-da-Silva A. 1
1 Serviço de Bioquímica, Faculdade de Farmácia e IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto, Portugal; 2 Department of Veterinary Sciences, CECAV, University of Trás-os-Montes e Alto Douro, Vila Real, Portugal
- 424 **Serological survey of *Leishmania* infection in dogs from 3 municipalities of central Portugal**
Rosa F. 1, Ribeiro S. 1, Maia F. 2, Varejão E. 3, Schallig H.D.F.H. 4, and Cardoso L. 5
1 Veterinary Medical Clinic, Alcanena, Portugal; 2 Municipal Council, Entroncamento, Portugal; 3 Department of Forestry, CEFE, University of Trás-os-Montes e Alto Douro (UTAD), Vila Real, Portugal, 4 KIT (Koninklijk Instituut voor de Tropen/Royal Tropical Institute), KIT Biomedical Research, Amsterdam, The Netherlands; 5 Department of Veterinary Sciences, CECAV, UTAD, Vila Real, Portugal
- 425 **Evaluation of a DOT-immunoblot assay for detecting Leishmanial antigen in *Phlebotomus papatasi* in Iran**
Nekouie H., Khabiri A., and Razavi M.R.
Pasteur Institute, Department Parasitology, Tehran, Iran

Poster group 8: Protozoa, miscellaneous

- 426 **Mapping of quantitative trait loci affecting resistance/susceptibility to *Sarcocystis miescheriana* in swine**
Reiner G. 1, Hepp S. 1, Berge T. 1, Willems H. 1, Kliemt D. 2, Dauschies A. 3, Mackenstedt U. 2, and Zahner H. 4
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- 427 **Molecular studies on *Sarcocystis* species in reindeer from Norway and Iceland**
Dahlgren S.S., and Gjerde B.
Norwegian School of Veterinary Science, Department of Food Safety and Infection Biology, Oslo, Norway
- 428 **Serodiagnosis of sarcocystosis in water buffalo by elisa test and its comparison with digestion method**
Hamidinejat H. 1, Nabavi L. 1, Khadje G.H. 2, Ghorbanpour M. 3, and Jalali M. 2
1 Shaid Chamran University, Department Veterinary Parasitology, Ahvaz, Iran; 2 Shaid Chamran University, Department Clinical Pathology, Ahvaz, Iran; 3 Shaid Chamran University, Department Veterinary Microbiology, Iran

- 429 **Tissue cyst forming protozoa of camels in Egypt**
Ashmawy K.I. 1, and El-Tayar A.A. 2
1 Alexandria university, Egypt, Department of parasitology, Faculty of Veterinary Medicine, Egypt; 2 Animal Health Research Institute, Damanhour branch, Egypt
- 430 ***Bartonella* and Bartonellosis - Forgotten and Coming Back**
Larionov S.V. 1, Gasanova T.A. 1, 2, and Kudinov A.B. 1
1 Vavilov Saratov State Agricultural University, Parasitology, Epizootiology and Veterinary and Sanitary Examination Department, Russia; 2 Saratov City Clinical Hospital, Russia
- 431 **Development of immunofluorescence and in situ hybridisation techniques for the detection of *Bartonella* and *Anaplasma* organisms within vector tissues**
Robinson M.T. 1, Morgan E. 2, Williams T. 3, and Shaw S.E. 1
1 School of Clinical Veterinary Sciences, University of Bristol, Langford, UK; 2 School of Biological Sciences, University of Bristol, Bristol, UK; 3 Pfizer, Sandwich, Kent, UK
- 432 **Molecular detection of *Ehrlichia canis* and *Anaplasma platys* in dogs living in rural areas of the State of Minas Gerais, Brazil**
Costa-Junior L.M. 1,2, Ribeiro M.F.B. 2, Rabelo E.M.L. 2, Rembeck K. 3, Hirzmann J. 3, Zahler-Rinder M. 3, Pfister K. 3, and Passos L.M.F. 3
1 Universidade Federal do Maranhão, Centro de Ciências Agrárias e Ambientais, Brazil; 2 Universidade Federal de Minas Gerais, Departamento de Parasitologia, ICB, Brazil; 3 Institute for Comparative Tropical Medicine and Parasitology, Veterinary Faculty, Ludwig-Maximilians-University, Munich, Germany
- 433 **Presence of erlichiosis and anaplasmosis in dogs in Sinaloa, Mexico**
Gaxiola S. 1, Sosa C. 1, Cota S. 1, Castro N. 1, Quintero T. 2, Càrcamo N. 1, Borbolla J. 1, and Barraza C. 1
1 FMVZ-University of Sinaloa; 2 FMVZ University National of Mexico
- 434 **Effect of a monthly imidacloprid-permethrin treatment against *Ixodes ricinus* on the seroprevalence of *Anaplasma phagocytophilum* in dogs**
Pfister K. 1, Galke D. 2, Beelitz P. 1, and Kohn B. 2
1 Institute for Comparative Tropical Medicine and Parasitology, LMU Munich, 2 Clinics for Small Animals, Free University Berlin, Germany
- 435 **Seroprevalence of *Encephalitozoon cuniculi* infection in pet rabbits in France**
Beaurin D. 1, 2, Delbac F. 2, Quinton J.F. 3, Wawrzyniak I. 2, Boussarie D. 4, Haddad N. 1, Vivarès C.P. 2, and Guillot J. 1.
1 UMR INRA, AFSSA, ENVA, UPVM 956 BIPAR, Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort, France; 2 Laboratoire Biologie des Protistes, UMR CNRS, Université Blaise Pascal, Aubière, France; 3 Clinique vétérinaire République, Paris, France; 4 Clinique vétérinaire Frégis, Arcueil, France
- 436 **Nf-cHsp70 is essential for proliferation and cytotoxicity of pathogenic *Naegleria fowleri***
Song K.J. 1, Yang A.H. 1, Hong J.K. 1, Choi K.J. 1, Lee Y.L. 1, Kim J.H. 1, Kwan D. 1, Im K.I. 2, and Shin H.J. 1
1 Department of Microbiology, and Molecular Science & Biotechnology, Ajou University School of Medicine, Suwon, Korea; 2 Department of parasitology, Yonsei University School of Medicine, Seoul, Korea
- 437 **Immune responses and mortality of mice immunized with the rNfa1 protein post-infection of pathogenic *Naegleria fowleri***
Lee Y.J. 1, Kim H.J. 1, Choi K.J. 1, Song A.R. 1, Sohn H.J. 1, Song K.J. 1, Im K.I. 2, and Shin H.J. 1
1 Department of Microbiology, and Department of Molecular Science & Technology, Ajou University School of Medicine, South Korea; 2 Department of Parasitology, Yonsei University College of Medicine, Seoul, South Korea

- 438 **Regurgitated pellets of *Merops apiaster* as reservoir of infective *Nosema ceranae* spores**
 Martín-Hernández R. 1, Garrido-Bailón E. 1, García-Palencia P. 2, Meana A. 2, and Higes M. 1
1 Regional Apicultural Center, Dirección General de la Producción Agropecuaria, Consejería de Agricultura, Junta de Comunidades de Castilla-La Mancha, Marchamalo, Guadalajara. Spain; 2 Faculty of Veterinary Medicine, Universidad Complutense de Madrid, Spain
- 439 **Detection of infective *Nosema ceranae* (Microsporidia) spores in corbicular pollen of forager honeybees**
 Higes M. 1, Martín-Hernández R. 1, Garrido-Bailón E. 1, García-Palencia P. 2, and Meana A. 2
1 Centro Apícola Regional, Dirección General de la Producción Agropecuaria, Consejería de Agricultura, Junta de Comunidades de Castilla-La Mancha, Marchamalo, Guadalajara. Spain; 2 Facultad de Veterinaria, Universidad Complutense de Madrid, Spain
- 440 **Life cycle of *Nosema ceranae* in *Apis mellifera***
 Meana A. 1, García-Palencia P. 1, Martín-Hernández R. 2, Garrido-Bailón E. 2, Fenoy S. 3, Del Aguila C. 3, and Higes M. 2
1 Facultad de Veterinaria, Universidad Complutense de Madrid, Spain; 2 Centro Apícola Regional, Dirección General de la Producción Agropecuaria, Consejería de Agricultura, Junta de Comunidades de Castilla-La Mancha, Marchamalo, Guadalajara. Spain; 3 Facultad de Farmacia, Universidad San Pablo CEU de Madrid, Spain
- 441 **Prevalence of *Tritrichomonas foetus* infection in cats in France**
 Brigui N. 1, Hénaff M. 2, and Polack B. 1,3
*1 Unité de Parasitologie-Mycologie; 2 Unité de Médecine de l'Élevage et du Sport
 3 INRA, AFSSA, ENVA, UPVM, UMR 956 BIPAR; École Nationale Vétérinaire d'Alfort, Maisons-Alfort cedex, France*
- 442 **The use of PCR to confirm non-*T. foetus* trichomonads in cultured preputial samples of a bull, and its modification for routine diagnostic use**
 Gajadhar A., Lalonde L., Huby F., and Scandrett B.
Centre for Foodborne and Animal Parasitology, Canadian Food Inspection Agency, Saskatoon, Canada
- 443 ***Trichomonas spp.* as a possible cause of a new recognized Canary Infraorbital Synusitis syndrome in Italy**
 Catania S. 1,2, Muliari R. 2, Pozzato N. 2, Vicari D. 1, Ferrantelli V. 1, and Capelli G. 1
1 Istituto Zooprofilattico Sperimentale della Sicilia, Palermo, Italy; 2 Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy
- 444 ***Balantidium coli* in dairy cattle: Clinical signs and successful treatment**
 Kojouri G.A. 1
1 Department of Clinical Sciences, School of Veterinary Medicine, Shahrekord University, Shahrekord, Iran
- 445 **Development and application of nested PCR assay for detection of dairy cattle-derived *Cyclospora sp.***
 Li G., Xiao S., Li W., and Zhou R.
College of Veterinary Medicine, South China Agricultural University, Guangzhou, China
- 446 **Standardization of a real time PCR for differential diagnosis of *Hepatozoon canis* and *H. americanum* in dogs**
 Costa-Junior L.M. 1,2, Ribeiro M.F.B. 2, Hirzmann J. 3, Zahler-Rinder M. 3, Passos L.M.F. 3, and Pfister K. 3
1 Universidade Federal do Maranhão, Centro de Ciências Agrárias e Ambientais, Brazil; 2 Universidade Federal de Minas Gerais, Departamento de Parasitologia, ICB, Brazil; 3 Institute for Comparative Tropical Medicine and Parasitology, Veterinary Faculty, Ludwig-Maximilians-University, Munich, Germany

- 447 **Prevalence of bovine theileriosis and babesiosis in irrigated area of Morocco**
 Sahibi H. 1, Rhalem A. 1, Ait Hamou S. 1, Rahali T. 1, Van Impe G. 2, El Jirari L. 1, Goff W. 3, and Losson B.
1 Département de Parasitologie et Maladies Parasitaires, Institut Agronomique et Vétérinaire Hassan II, BP. 6202, Rabat, Morocco; 2 Laboratoire de Biogéographie et d'Ecologie, Faculté des Sciences, Université catholique de Louvain, Belgium; 3 ARS Pullman, Washington State University USA; 4 Service de Parasitologie Faculté de Médecine Vétérinaire, Université de Liège, Belgium
- 448 **Canine Babesiosis (*Babesia canis*) in France : A national survey. Clinical presentation of the disease**
 Bourdeau P., Lasbleiz M., and Marchand A. (in mem.)
Dermatology - Parasitology - Mycology, Veterinary School, Nantes, France
- 449 **Canine Leishmaniosis (*Leishmania infantum*) in France : A national survey. Informations obtained on treatments used by veterinarians and criterias of efficacy**
 Bourdeau P., Mallet M., and Marchand A. (in mem.)
Dermatology - Parasitology - Mycology, Veterinary School, Nantes, France
- 450 **Bovine Theileriosis: Evaluation of 372 cases during spring and summer in south of Iran**
 Sakha M. 1, Radfar M.H. 2, and Janbazi M. 2
1 Faculty of specialised Veterinary Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran; 2 Faculty of Veterinary Medicine, Shahid Beheshti University of Kerman, Kerman, Iran

Poster group 9: Vector-Borne Protozoa

- 451 **Infection of the fishes with *Ligula intestinalis* and *Bothriocephalus acheilognathi* in Sattarkhan Dam, northwestern Iran**
 Mortazavi Tabrizi J. 1, Pazooki J. 2, and Javanmard A. 3
1 Islamic Azad university -Tabriz Branch, Tabriz, Iran; 2 Shahid Beheshti University, Tehran, Iran; 3 Agricultural Research and Natural Source Center, East-Azarbaijan, Tabriz, Iran
- 452 **On cestode and nematode parasites of cyprinoid fish from Zayandehrood dam, centre of Iran**
 Nematollahi A. 1, Azizi H. 2, and Sadeghi Z. 3
1 Shahrekord University, Aquatic Animal Health Division, Department of Food Hygiene and Quality Control, Shahrekord, Iran; 2 Shahrekord University, Department of Pathobiology, Shahrekord, Iran; 3 Shahrekord University, Shahrekord, Iran
- 453 **Parasites of wild and cultured fish from Sepidrood River, north of Iran**
 Nematollahi A. 1, Pirali K.H. 2, Nikpour S. 3, and Majidian F. 3
1 Shahrekord University, Department of Food Hygiene and Quality Control, Shahrekord, Iran; 2 Shahrekord University, Department of Pathobiology, Shahrekord, Iran; 3 Shahrekord University, Shahrekord, Iran
- 454 **Identification of anisakid nematodes with zoonotic potential by isotopic and non-isotopic SSCP of ITS-2 rDNA**
 Zhu X.Q.
1 College of Veterinary Medicine, South China Agricultural University, Guangzhou, Guangdong Province, PR China; 2 Huizhou Harbour Entry-exit Inspection and Quarantine, Huizhou, Guangdong Province, PR China; 3 Sea Fisheries Institute in Gdynia, Gdynia, Poland; 4 Laboratory of Animal Quarantine, Guangzhou Entry-exit Inspection and Quarantine, Guangzhou, Guangdong Province, PR China

- 455 **Survey on epicomensal parasites of *Penaeus indicus* in Bushehr province**
Mirbakhsh M. 1, Afshar nasab M. 2, and Ghaednia B. 1
1 Iran Shrimp Research Center; 2 Iran fishery Research Organization, Iran
- 456 **External fouling disease in shrimp culture *Fenneropenaeus indicus* of I.R.IRAN, Bushehr Province**
Dashtian Nasab A., Afsharnasab M., and Mirbakhsh M.
Iranian Shrimp Research Center, Aquatics Animal health & Disease, Bushehr, Iran
- 457 **Parasitic fauna of *Liza spp* (mugilidae) in Southern Caspian sea and freshwaters of Iran**
Tavakkol S. 1, Jalali B. 2, and Halajian A. 3
1 Islamic Azad University, Tonekabon branch, Aquaculture department, Tonekabon, Iran; 2 Islamic Azad University, Tehran science and research branch, Veterinary department, Tehran, Iran; 3 Islamic Azad University, Tehran science and research branch, Veterinary faculty, Parasitology department, Tehran, Iran
- 458 **Parasites of cultured trouts in Iran**
Tavakkol S. 1, and Jalali B. 2
1 Islamic Azad University, Tonekabon branch, Aquaculture department, Tonekabon, Iran; 2 Islamic Azad University, Science and research branch, Veterinary faculty, Tehran, Iran
- 459 ***Giardia duodenalis* in clams (*Ruditapes philippinarum*) and mussels (*Mytilus galloprovincialis*) from Northern Adriatic Sea**
Granato A. 1, Salvador A. 1, Caldon M. 1, Corrain C. 1, Paoletti B. 2, Danesi P. 1, Arcangeli G. 1, and Capelli G. 1
1 Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy; 2 Faculty of Veterinary Medicine, University of Teramo, Italy
- 460 **Gastrointestinal parasites in phocid and otarid populations from South Shetland Islands and Antarctic Peninsula**
Pedraza-Díaz S. 1, García-Moreno F.T. 2, Ferre I. 1, Gómez-Bautista M. 1, and Ortega-Mora L.M. 1
1 ISALUVET, Animal Health Department, Faculty of Veterinary Sciences, Complutense University of Madrid, Madrid, Spain; 2 Jefatura de Apoyo Veterinario. Inspección General de Sanidad de la Defensa, Clínicas Especiales, Madrid, Spain
- 461 **Host specificity and host range in *Dactylogyridae* (Monogenea) parasites of freshwater fishes of Iran**
Jalali B., and Barzegar M.
Islamic Azad University-Research and Sciences branch, Veterinary Dep., Tehran, Iran
- 462 **Parasites of fishes in Choghakhor Lake, Iran**
Raissy M. 1, Barzegar M. 2, Rahimi E. 1, and Jalali B. 3
1 Veterinary Faculty, Azad University of Shahrekord, Iran; 2 Asarab Consulting Engineering Co, Iran; 3 Veterinary Faculty, Azad university (Science and Research Branch), Iran
- 463 **Parasitic fauna of *Capoeta damascina* (valenciennes, 1842) in Beheshtabad river, Iran**
Hosseini Fard M. 1, and Raissy M. 2
1 Veterinary Faculty, Azad University, Babol Branch, Iran; 2 Veterinary Faculty, Azad university, Shahrekord Branch, Iran
- 464 **Characterization and Sequencing of ITS-1 gene of *Tylodelphys clavata* (von Nordman 1832)**
Raissy M. 1, and Doosti A. 2
1 Veterinary Faculty, Azad University, Shahrekord Branch, Iran; 2 Agriculture Faculty, Azad university, Shahrekord Branch, Iran

- 465 ***Cryptosporidium* in oysters sold in markets and commercial stores in Mexico-city**
Vázquez T.O. 1,2, Campos R.T. 2, Rondán Z.A. 1, Ponce M.M. 2, Martínez G.M.N. 2, Zaragoza A.D. 2, and Gutiérrez Q.M. 3
1 La Salle University; 2 National Institute-of-Pediatrics; 3 UNAM Mexico
- 466 **Helminths and copepods parasites of skip jack tuna *Katsuwonus pelamis* (Pisces, Scombridae) disembarked at the harbour of Abidjan (atlantic coast of Ivory Coast)**
Cissé M., Bédé O., Gnayoro M., Gourène G., Ouattara A., Ouattara M., and Manizan P.
Université d'Abobo-Adjamé, UFR Sciences et Gestion de l'Environnement, Abidjan, Ivory Coast

Poster group 10: Parasites of Poultry and Swine

- 467 **Prevalence of ecto and endoparasites in pregnant and weaning sows under four management systems**
Baranenko J. 1, Quijada J. 2, González C. 1, Araque H. 1, Vivas I. 3, Pérez A. 2, Bethencourt A. 2, and Pérez H. 2
1 Universidad Central de Venezuela (UCV), Facultad de Agronomía, Maracay, Venezuela; 2 UCV, Facultad de Ciencias Veterinarias (FCV), Departamento de Patología Veterinaria, Maracay, Venezuela; 3 UCV, FCV, Departamento de Producción Animal. Maracay, Venezuela
- 468 **Distribution of *Ascaris suum* genotypes within the pig host is affected by pig and location in the small intestine**
Nejsum P. 1,2, Thamsborg S.M. 1, Jørgensen C. 2, Fredholm M. 2, and Roepstorff A. 1
1 Danish Centre for Experimental Parasitology, Department of Veterinary Pathobiology, 2 Genetics and Bioinformatics, Department of Animal and Veterinary Basic Sciences, Faculty of Life Sciences, University of Copenhagen, Denmark
- 469 **Effect of *Ascaris suum* infection on performance of fattening pigs**
Boes J. 1, Jacobs J. 2, Havn K.T. 3, Christiansen S. 4, Vestergaard-Nielsen K. 5, and Alban L. 1
1 Danish Pig Production, Danish Meat Association, Kjellerup, Denmark; 2 Janssen Animal Health, Beerse, Belgium; 3 Swinevet, Haderslev, Denmark; 4 Roslev Animal Clinic, Roslev, Denmark; 5 Boehringer-Ingelheim Denmark A/S, Copenhagen, Denmark
- 470 **Effect of Solubenol® on *Ascaris suum* infections in fattening pigs**
Boes J. 1, Christiansen S. 3, Vestergaard-Nielsen K. 4, and Jacobs J. 2
1 Danish Pig Production, Danish Meat Association, Kjellerup, Denmark; 2 Janssen Animal Health, Beerse, Belgium; 3 Roslev Animal Clinic, Roslev, Denmark; 4 Boehringer-Ingelheim Denmark A/S, Copenhagen, Denmark
- 471 **The effect of *Ascaris suum* infection on the protective response to vaccination against *Mycoplasma hyopneumoniae* in pigs**
Steenhard N.R. 1, Jungersen G. 2, Kokotovic B. 2, Urban J. 3, Roepstorff A. 1, and Thamsborg S.M. 1
1 Copenhagen University, Danish Centre for Experimental Parasitology, Department of Veterinary Pathobiology, Copenhagen, Denmark; 2 Technical University of Denmark, National Veterinary Institute, Copenhagen, Denmark; 3 ARS/USDA, Nutrient Requirements and Functions Laboratory, Beltsville Human Nutrition Research Center, Beltsville, Maryland, USA

- 472 **Parasite specific IL-4 responses in *Ascaris suum* and *Trichuris suis* infected pigs evaluated by ELISPOT**
 Steenhard N.R. 1, Kringel H. 1, Roepstorff A. 1, Thamsborg S.M. 1, and Jungersen G. 2
 1 Copenhagen University, Danish Centre for Experimental Parasitology, Department of Veterinary Pathobiology, Copenhagen, Denmark; 2 Technical University of Denmark, National Veterinary Institute, Copenhagen, Denmark
- 473 **Prevalence of taeniasis swine in suburban localities of Ludhiana, India and its association with risk factors**
 Sharma R. 1, Sharma D.K. 2, Juyal P.D. 3, Gill J.P.S. 1, and Sharma J.K. 1
 1 Department of Veterinary Public Health, College of Veterinary Sciences, Guru Angad Dev Veterinary & Animal Sciences University, Ludhiana, Punjab, India; 2 Department of Livestock Products Technology, College of Veterinary Sciences, Guru Angad Dev Veterinary & Animal Sciences University, Ludhiana, Punjab, India; 3 Department of Veterinary Parasitology, College of Veterinary Sciences, Guru Angad Dev Veterinary & Animal Sciences University, Ludhiana, Punjab, India
- 474 **The Glutathione-S-transferase of *Oesophagostomum dentatum*: isolation, characterisation and possible biological function**
 Joachim A., Ruttkowski B., and Peschke R.
 Department of Pathobiology, University of Veterinary Medicine, Vienna, Austria
- 475 **Effects of parasitism due to *Metastrongylus* sp. in wild boar (*Sus scrofa*)**
 Vedrine B. 1, Bourdeau P. 1, Costiou P. 2, and Marchand A. (in mem)
 1 Dermatology - Parasitology – Mycology, Veterinary School Nantes, France; 2 Anatomy, Veterinary School Nantes, France
- 476 **Epidemiological study of *Dermanyssus gallinae* infestation in egg laying flocks of Mashhad area, Iran**
 Razmi G.R. 1, Moaveni M. 2, and Kalidari G.A. 3
 1 Graduated of School of Veterinary Medicine, Ferdowsi University of Mashhad; 2 Dept of Vet Pathobiology, School of Veterinary Medicine, Ferdowsi University of Mashhad; 3 Dept of Vet Clinical Science, School of Veterinary Medicine, Ferdowsi University of Mashhad, Iran
- 477 **Identification and localisation of tropomyosin and paramyosin in *Dermanyssus gallinae* (De Geer)**
 Wright H.W., Nisbet A.J., MacKellar A., and Huntley J.F.
 Moredun Research Institute, Pentland Science Park, Bush Loan, Penicuik, Scotland, UK
- 478 **In vitro effect of some synthetic acaricides and plant extracts against *Dermanyssus gallinae***
 Magdas C. 1, Cozma V. 1, and Briciu R. 1
 1 University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Department of Parasitology, Cluj-Napoca, Romania
- 479 **Persistence and efficacy of traps impregnated with phoxime for the control of *Dermanyssus gallinae***
 Chirico J.
 National Veterinary Institute, Department of Parasitology (SWEPAR), Uppsala, Sweden
- 480 **Evaluation of methods of control of poultry red mite infestations in two caged layer houses in the United Kingdom**
 Rankin M., Davies R.H., Carrique-Mas J., and Bates P.G.
 Veterinary Laboratories Agency (Weybridge), Addlestone, UK

- 481 **Investigating the residual toxicity of lavender essential oils as acaricides against the poultry red mite (*Dermanyssus gallinae*)**
 Callaghan K. 1,2, George D.R. 1, Guy J.H. 1, and Sparagano O.A.E. 1
1 School of Agriculture, Food and Rural Development; 2 School of Medical Sciences, Newcastle University, Newcastle upon Tyne, UK
- 482 **Temporal synergism controls highly resistant poultry red mite**
 Bingham G. 1, Bates P. 2, and Moores G. 1
1 Rothamsted Research, Harpenden, UK; 2 Veterinary Laboratory Agency, New Haw, Surrey, UK
- 483 **Leucocytozoon infection in poultry in Vietnam**
 Lam T.T.H.
University of Agriculture and Forestry, Veterinary Pathology and Parasitology, Ho Chi Min, Vietnam
- 484 **Prevalence and pathology of parasitic gastrointestinal infections of poultry in Punjab**
 Sandhu B.S. 1, Brar R.S. 1, Brar A.P.S. 1, Sood N.K. 1, and Singla L.D. 2
1 Department of Veterinary Pathology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India; 2 Department of Veterinary Parasitology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India
- 485 **Nematodes natural infection in Neotropical cormorant, *Phalacrocorax brasilianus* Gmelin, 1789 (Aves; Phalacrocoracidae) from Rio de Janeiro, Brazil**
 Rodrigues M.L.A. 1, Souza P.C.A. 2, Rodrigues R.L. 3, Noronha M.L.M. 3, Monteiro C.M. 4, Santos M.D. 4, and Brasil-Sato M.C. 5
1 Fellowship CNPq, CPGCV, DPA, UFRRJ, Unigranrio, RJ Brazil; 2 DESP, IV, UFRRJ, Brazil; 3 Inst. Munic. Med Vet Jorge Vaitsman, Unigranrio, Brazil; 4 CPGCV - UFRRJ, Brazil; 5 DBA, IB, CPGCV, UFRRJ, RJ, Brazil
- 486 **The role of preparation technique, culture media and incubation time for embryonation of *Heterakis gallinarum* eggs**
 Püllen U. 1, Cheat Sophal 2, Moors E. 1, and Gaulty M. 1
1 Institute of Animal Breeding and Genetics, Albrecht Thaer Weg 3, University of Goettingen, Goettingen, Germany; 2 Faculty of Animal Science and Veterinary Medicine, Royal University of Agriculture, Phnom Penh, Cambodia
- 487 **Bird caecal worm, *Heterakis gallinarum* (Ascarida: Heterakidae): ultrastructural observations**
 Naem S.
Department of Pathobiology, College of Veterinary Medicine, Urmia University, Urmia, Iran
- 488 **Studies on endoparasites of some migratory birds**
 El-Seify M.A., Desouky A.Y., El-Herawy A.Z., and Sattor N.A.
Department of Parasitology, Faculty of Veterinary Medicine, Kafr El-Sheikh University, Egypt

POSTER SESSION II

Poster group 11: Helminth Infections of Horses

- 489 **The prevalence of intestinal parasites of horses in NSW, Australia**
Sangster N.C. 1, and Al Anazi A. 2
1 Veterinary Pathobiology, School of Agricultural & Veterinary Sciences, Charles Sturt University, Australia; 2 Faculty of Veterinary Science, Sydney University, Australia
- 490 **Is *Strongylus vulgaris* still a problem in Sardinian horses?**
Pilo C. 1, Scala A. 1, Altea A. 1, Mula P. 1, Garippa G. 1, Genchi M. 2, and Genchi C. 2
1 Dip Biol Anim, Section of Parasitology and Parasitic Diseases, Università degli Studi di Sassari, Italy; 2 DIPAV, Section of General Pathology and Parasitology, Università degli Studi di Milano, Italy
- 491 **Development and migration of *Cyathostominae* infective larvae in Coast-cross grass (*Cynodon dactylon*) in a subtropical region of Rio de Janeiro, Brazil**
Couto M.C.M. 1, Bezerra S.Q. 1, Souza T.M. 2, Anjos D.H. 3, Sampaio I.M. 4, and Rodrigues M.L.A. 5
1 CPGCV, IV, UFRRJ, RJ; 2 Zootecnista; 3 Instituto de Biofísica, UFRJ, RJ; 4 IV, UFMG, MG, Brazil; 5 Fellowship CNPq, CPGCV, DPA, UFRRJ, RJ, Brazil
- 492 **Equine parascariasis under the tropical weather conditions of Ethiopia: a cross-sectional coprological and post-mortem study**
Getachew M. 1,3, Innocent G.T. 1, Trawford A.F. 2, Feseha G. 3, Reid S.J.W. 1, and Love S. 1
1 University of Glasgow, Faculty of Veterinary Medicine, Glasgow, UK; 2 Donkey Sanctuary, Devon, Sidmouth, EX10 0NU, England, UK; 3 Addis Ababa University, Faculty of Veterinary Medicine, Donkey Health and Welfare Project
- 493 **Acquired cervical scoliosis in 6 horses associated with dorsal grey column chronic myelitis caused by aberrant *Parelaphostrongylus tenuis* migration**
Van Biervliet J. 1, de Lahunta A. 2, Ennulat D. 3, Oglesbee M. 4, and Summers B. 2
1 Laboratory for neuronal cell biology and gene transfer, VIB11, KU Leuven, Belgium; 2 College of Veterinary Medicine, Cornell University, Ithaca, NY; 3 College of Veterinary Medicine, Ohio State University, Columbus, Ohio; 2 College of Veterinary Medicine, University of Pennsylvania, Pennsylvania, USA
- 494 **Epidemiology and control of strongyle infection in a native donkey breed in Portugal**
Gomes T. 1, Lucena G. 1, Gomes L. 1, Santos C. 1, Monteiro A. 2, and Madeira de Carvalho L.M. 1
1 CIISA/FMV/UTL, Pólo Universitário do Alto da Ajuda, Avenida da Universidade Técnica, 1300-477 Lisbon, Portugal; 2 Miranda do Douro donkey breed producer
- 495 **Identification of high egg shedding donkeys and associated risk factors**
Burden F. 1, and Trawford A. 1
1 The Donkey Sanctuary, Sidmouth, Devon, UK
- 496 **The strongylid nematodes of working donkeys from Ethiopia**
Kuzmina T.A. 1, Kharchenko V.A. 1, Trawford A. 2, and Getachew M. 3.
1 Institute of Zoology NAS of Ukraine, Department of Parasitology, Kyiv, Ukraine; 2 The Donkey Sanctuary, Sidmouth, Devon, UK; 3 Donkey Health and Welfare Project-DS, Faculty of Veterinary Medicine, Addis Ababa University, Ethiopia

- 497 **Ocular infection of donkeys with *Setaria equina***
Desouky A.Y. 1, and Marzok M.A. 2
1 Department of Parasitology, Faculty of Veterinary Medicine, Kafr El-Sheikh University ; 2 Department of Surgery, Faculty of Veterinary Medicine, Kafr El-Sheikh University, Egypt
- 498 **Cyathostomin infection of horses and donkeys in Portugal based on the study of L3 subpopulations of *Cyathostomum sensu latum***
Madeira de Carvalho L.M. 1, Martins S. 2, Sousa S. 2, Gersão S. 2, Gomes T. 1, Lucena G. 1, Fazendeiro M.I. 1, and Afonso-Roque M.M. 1
1 Centro Interdisciplinar de Investigação em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade Técnica de Lisboa (CIISA/FMV/UTL), Pólo Universitário do Alto da Ajuda, Av. Universidade Técnica, Lisbon, Portugal; 2 Escola Universitária Vasco da Gama, Estrada da Conraria, Castelo Viegas, Coimbra, Portugal
- 499 **Cyathostomin infection in a horse production system in Portugal using dry and irrigated pastures**
Madeira de Carvalho L.M. 1, Afonso-Roque M.M. 2, Gomes L. 1, and Fazendeiro M.I. 1
1 Centro Interdisciplinar de Investigação em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade Técnica de Lisboa (CIISA/FMV/UTL), Pólo Universitário do Alto da Ajuda, Av. Universidade Técnica, Lisbon, Portugal; 2 Centro de Veterinária e Zootecnia Tropical/Instituto de Investigação Científica Tropical, FMV, Pólo Univ. Ajuda, Avenida da Universidade Técnica, Lisboa, Portugal
- 500 **Preliminary detection of infection by gastrointestinal nematodes in horses from NW Spain**
Francisco I., Arias M., Sánchez-Andrade R., Paineira A., Díaz P., Morrondo P., Díez-Baños P., and Paz-Silva A.
Animal Pathology Department, Epidemiology, Zoonoses and Parasitic diseases, Veterinary Faculty, Santiago de Compostela University, Lugo, Spain
- 501 **Annual survey on oribatid mites soil density in Spain and possible link with the risk of tapeworm infection in horses**
Estrada-Pena A. 1, Bousquet E. 2, Sanquer A. 2, and Sanchez-Sanz N. 1
1 Department of Parasitology, Veterinary Faculty, Zaragoza, Spain; 2 Virbac, Carros, France
- 502 **Coprological and serological diagnosis of *Anoplocephala perfoliata* in horses from Southern Alberta, Canada**
Skotarek S.L. 1, Colwell D.D. 2, and Goater C.P. 1
1 Department of Biological Sciences, University of Lethbridge, Lethbridge, Alberta, Canada; 2 Lethbridge Research Centre, Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada
- 503 **The prevalence of *Anoplocephala perfoliata* in abattoir and stabled horses**
Davies P. 1, Wells L. 1, Cringoli G. 2, and Coles G.C. 3
1 Dept. Anatomy, University of Bristol; 2 Faculty of Veterinary Medicine, University of Naples; 3 School of Clinical Veterinary Science, Langford House, Bristol, UK
- 504 **Some factors related to the infection by *Anoplocephala magna***
Meana A. 1, Luzón M. 1, Bohórquez A. 1, Fernández-Pato N. 2, Sanquer A. 3, and Bousquet E. 3
1 Facultad de Veterinaria, Universidad Complutense de Madrid, Spain; 2 Facultad de Veterinaria, Universidad de León, Spain; 3 Virbac, Carros, France
- 505 ***Anoplocephala perfoliata* infections in horses: parasitic burdens and pathology**
Meana A. 1, Luzón M. 1, Marin P. 1, Bohórquez A. 1, Fernández-Pato N. 2, Sanquer A. 3, and Bousquet E. 3
1 Facultad de Veterinaria, Universidad Complutense de Madrid, Spain; 2 Facultad de Veterinaria, Universidad de León, Spain; 3 Virbac, Carros, France

- 506 ***Trichinella* sp. infection in horses of Romania: serological survey and parasitological examination**
 Blaga R. 1,2, Cretu C.M. 3, Gherman C. 2, Pozio E. 4, Noeckler K. 5, Kape C.M.O. 6, Cozma V. 2, and Boireau P. 1
1 JRU BIPAR INRA, AFSSA, ENVA, UPVM, Maisons-Alfort, France; 2 University of Agricultural Sciences et Veterinary Medicine, Faculty of Veterinary Medicine, Parasitology Unit, Cluj-Napoca, Romania; 3 Department of Infectious, Parasitic and Immunomediated Diseases, Istituto Superiore di Sanità, Rome, Italy
- 507 **Easiness of administration and underdosing risk of endectocide oral pastes in horses**
 Bousquet E. 1, Ribot X. 2, Besson B. 3, Sanquer A. 1, and Demonceau T. 1
1 Virbac, Carros, France; 2 Service de Santé des Armées, Paris, France; 3 Vignoles, France
- 508 **A survey of parasite control methods used by horse owners in Portugal**
 Martins S. 1, Sousa S. 1, and Madeira de Carvalho L.M. 2
1. Escola Universitária Vasco da Gama, Estrada da Conraria, Castelo Viegas, Coimbra, Portugal; 2. Centro Interdisciplinar de Investigação em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade Técnica de Lisboa (CIISA/FMV/UTL), Pólo Universitário do Alto da Ajuda, Lisbon, Portugal
- 509 **Time of re-appearance of faecal tapeworm eggs output in horses treated with an ivermectin-praziquantel combination and left in their contaminated environment**
 Mercier P. 1, Bousquet E. 1, Sanquer A. 1, and Ludwig R. 2
1 Virbac, Carros, France; 2 Berlin, Germany
- 510 **The activity of GenesisTM Equine in naturally infected ponies**
 Coles G.C. 1, Taylor F.G.R. 1, Hillyer M.H. 1, and Shubber A. 2
1 School of Clinical Veterinary Science, Langford House, Bristol, UK; 2 Ancare New Zealand Ltd., Takapuna, Auckland, New Zealand.
- 511 **Plasma disposition and faecal excretion of netobimin following oral administration in horses**
 Gokbulut C. 1, Cirak V.Y. 2, Senlik B. 2, Yildirim F. 3, and McKellar Q.A. 4
1 Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Adnan Menderes, Aydin, Turkey; 2 Department of Parasitology, Faculty of Veterinary Medicine, University of Uludag, Bursa, Turkey; 3 General Directory of Agricultural Farms, Karacabey Agricultural Farm, Turkey; 4 The Royal Veterinary College, Hawkshead Lane, Hatfield, Hertfordshire, UK

Poster group 12: Anthelmintic Resistance

- 512 **Analysis of the L256F mutation in a new ivermectin resistant *Cooperia oncophora* isolate**
 El-Abdellati A. 1, Vercruysse J. 1, Wolstenholme A.J. 2, Skuce P. 3, Donnan A. 3, Van Zeveren A.M. 1, Claerebout E. 1, and Geldhof P. 1
1 Laboratory for Parasitology, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium; 2 Department of Biology and Biochemistry, University of Bath, Bath, UK; 3 Moredun Research Institute, Penicuik, UK
- 513 **Ivermectin resistance in cyathostomins in UK horses**
 Campbell C. 1, Cringoli G. 2, and Coles G.C. 3
1 Dept. Anatomy, University of Bristol; 2 Faculty of Veterinary Medicine, University of Naples; 3 School of Clinical Veterinary Science, Langford House, Bristol BS40 5DU, UK
- 514 **Ivermectin resistant *Parascaris equorum* in Sweden**
 Osterman Lind E., and Christensson D.
National Veterinary Institute, Department of Parasitology (SWEPAR), Uppsala, Sweden

- 515 **Selection of a benzimidazole-treated *Ostertagia ostertagi* strain**
 Pachnicke S. 1, Blackhall W. 1, von Samson-Himmelstjerna G. 1, van Zeveren A. 2, and Vercruysse J. 2
1 Institute for Parasitology, School of Veterinary Medicine Hannover; 2 Laboratory of Parasitology, Faculty of Veterinary Medicine, Gent University
- 516 **Genotyping benzimidazole-resistant *Cylicocyclus nassatus* from an isolated herd of horses from the Ukraine**
 Blackhall W. 1, Kuzmina T. 2, and von Samson-Himmelstjerna G. 1
1 University of Veterinary Medicine Hannover, Institute for Parasitology, Hannover, Germany; 2 Department of Parasitology, Schmalhausen Institute of Zoology, National Academy of Sciences, Kiev, Ukraine
- 517 **The cyathostominae species resistant to benzimidazole anthelmintics in Ukraine**
 Kuzmina T.A., Slivinska K.A., and Mamona O.A.
Institute of Zoology NAS of Ukraine, Department of Parasitology, Kyiv, Ukraine
- 518 **Multiple anthelmintic resistance against mixed gastrointestinal nematodes in dairy goats**
 Jabbar A., Iqbal Z., Saddiqi H.A., Babar W., Masood S., and Saeed M.
Department of veterinary Parasitology, University of Agriculture, Faisalabad, Pakistan
- 519 **Anthelmintic resistance in Ireland: current status**
 Good B. 1, Patten T. 1,2, Hanrahan J.P. 1, Mulcahy G. 2, and De Waal D.T. 2
1 Teagasc, Animal Production Research Centre, Athenry Co, Galway, Ireland; 2 School of Agriculture Food Science and Veterinary Medicine, UCD, Belfield, Dublin, Ireland
- 520 **Preliminary observations on drug resistance in sheep gastrointestinal *Trichostrongyles* in central Italy**
 Paoletti B. 1, Traversa D. 1, and Miller J. 2
1 Department of Comparative Biomedical Sciences, Faculty of Veterinary Medicine, Teramo, Italy; 2 Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, USA
- 521 **The efficacies of three macrocyclic lactones against nematodes in sheep with confirmed macrocyclic resistance**
 McAnulty R.W. 1, and Poff G. 2
1 Lincoln University, Animal & Life sciences Division, Canterbury, New Zealand; 2 North Canterbury Veterinary Clinic, Waikari, New Zealand
- 522 **A field study on the effect of targeted selective treatment (TST) on lamb performance and the phenotypic expression of anthelmintic resistance in nematodes exposed to ivermectin treatment**
 Kenyon F., Bartley D., Donnan A.A., Greer A., Gordon Y., Jackson E., McBean D., and Jackson F.
Moredun Research Institute, Pentlands Science Park, Bush Loan, Midlothian, UK
- 523 **Drug combination against multiple resistant nematodes: albendazole-ivermectin kinetic interaction and clinical efficacy in lambs**
 Entrocasso C. 1, Alvarez L. 2, Manazza J. 1, Lifschitz A. 2, Borda B. 1, Virkel G. 2, Mottier L. 2, and Lanusse C. 2
1 Laboratorio de Parasitología, INTA, Balcarce, Argentina; 2 Laboratorio de Farmacología, Facultad de Ciencias Veterinarias, Universidad Nacional del Centro, Tandil, Argentina and CONICET, Argentina

- 524 **Faecal egg count reduction test on a new commercial goat farm in Malaysia**
Khadijah S. 1,2, Chandrawathani P. 2, Nurulaini R. 2, Jamnah O. 2, Adnan M. 2, Zaini C.M. 2, and Waller P.J 3
1 University Science Malaysia, School of Biological Sciences, Penang, Malaysia; 2 Veterinary Research Institute, Department of Parasitology and Haematology, Ipoh, Malaysia; 3 National Veterinary Institute, Uppsala, Sweden
- 525 **Effect of lipids on the active transport of xenobiotics in nematodes**
Garnier M. 1, Riou M. 1,2, Koch C. 1, Guegnard F. 1, Le Vern Y. 1, and Kerboeuf D. 1
1 INRA, UR-1282, Animal Infectiology and Public Health (IASP), Nouzilly, France; 2 Institut for Research of Biology of Insects (IRBI), UMR CNRS 6035, University Tours, Parc de Grandmont, France
- 526 **Effect of the metabolic inhibitor methimazole on the drug susceptibility of a triclabendazole-resistant isolate of *Fasciola hepatica***
Brennan G. 1, Devine C. 1, Hoey E. 1, Trudgett A. 1, and Fairweather I. 1
1 Queen's University Belfast
- 527 **The efficacy and persistent activity of moxidectin, abamectin and ivermectin against susceptible ML resistant and F1 cross *Ostertagia (Teladorsagia) circumcincta* in sheep**
Vlassoff A., and McMurtry L.
Auckland, New Zealand
- 528 **P-gp interfering agents potentiate ivermectin susceptibility in nematode ivermectin-resistant strains**
Lespine A. 1, Bartley D. 2, Dupuy J. 1, Alvinerie M. 1, and Jackson F. 2
1 INRA-UR66 Pharmacologie-Toxicologie, Toulouse, France; 2 Moredun Institute, Edinburgh, UK
- 529 **Identification of putative QTL for nematode parasite resistance in sheep**
Hadfield T. 1, Miller J. 2, Wu C. 1, Bishop S. 3, Davies G. 3, and Cockett N. 1
1 Utah State University, Logan, UT, USA; 2 Louisiana State University, Baton Rouge, LA, USA; 3 Roslin Institute, Midlothian, UK
- 530 **Reversion of ivermectin resistance in *Haemonchus contortus* by verapamil: in vitro and in vivo studies**
Borges F.A. 1, Velludo P. 1, Rossini J. 1, Buzzulini C. 1, Molento M. 2, Silva H.C. 1, Soares V.E. 1, and Costa A.J. 1
1 CPPAR/FCAV/UNESP, Center for Research in Animal Health, Jaboticabal, São Paulo, Brazil; 2 Laboratório de Doenças Parasitárias, Universidade Federal do Paraná, Curitiba, Brazil
- 531 **Induction of resistance in *Haemonchus contortus* to thiabendazole and ivermectin by in vitro exposure**
Rufener L. 1, and Kaminsky R. 1
1 Novartis, Centre de Recherche Santé Animale SA, St-Aubin (FR), Switzerland
- 532 **A novel approach for combining the use of in vitro and in vivo data to measure and detect emerging moxidectin resistance in gastrointestinal nematodes of goats**
Kaplan R.M. 1, Vidyashankar A.N. 2, Howell S.B. 1, Neiss J.M. 1, Williamson L.H. 3, and Terrill T.H. 4
1 Dept of Infectious Diseases, College of Veterinary Medicine, University of Georgia, Athens, Georgia, USA; 2 Dept of Statistical Science, Cornell University, Ithaca, New York, USA; 3 Dept of Large Animal Medicine, College of Veterinary Medicine, University of Georgia, Athens, Georgia, USA; 4 College of Agriculture, Home Economics and Allied Programs, Agricultural Research Station, Fort Valley State University, Fort Valley, Georgia USA

Poster group 13: Helminths of Companion Animals

- 533 **Intestinal parasites in shelter dog populations from Northeastern Spain**
Ortuño A. 1, and Castellà J. 1
1 Universitat Autònoma de Barcelona, Facultat de Veterinària, Dept. Sanitat i Anatomia Animals, Barcelona, Spain
- 534 **Examinations in endoparasite prevalences in dogs and cats in animal shelters in Lower-Saxony and investigations of anthelmintic resistance**
Epe C. 1, Rohen M. 1, and Kreienbrock L. 2
1 Department for Infectious Diseases, Institute for Parasitology, University of Veterinary Medicine Hannover, Hannover, Germany; 2 Institute of Biometry & Epidemiology, University of Veterinary Medicine Hannover, Hannover, Germany
- 535 **Coprolological survey of intestinal parasites in dog breeding kennels in Flanders, Belgium**
Claerebout E. 1, Casaert S. 1, Geurden T. 1, De Wilde N. 1, De Croock F. 1, Carels B. 1, Vercruysse J. 1, and Dalemans A.-C. 2
1 Department of Virology, Parasitology & Immunology, Faculty of Veterinary Medicine, Ghent University, Belgium
- 537 **Zoonotic potential of the helminths infection of stray dogs from Havana City**
Hernández R. 1, Nuñez F. 1, Pelayo L. 1, Hernández R. 2, and Ramos F. 2
1 "Pedro Kourí" Tropical Medicine Institute; 2 Canine Observation and Control Centre, MINSAP, Cuba
- 538 **Zoonosis in companion animal in Sinaloa, Mexico**
Cota-Guajardo S. 1, Sosa G. 1, Gaxiola C.S. 1, Castro C.N. 1, and Barraza T.C. 1
1 Universidad Autónoma de Sinaloa. Facultad de Medicina Veterinaria y Zootecnia, Sinaloa, México
- 539 **A comparative study into the prevalence of intestinal parasites in owned dogs in Grenada, West Indies**
Erskine A. 1, Jacobs D. 1, Pinckney R. 2, Mofya S. 3, McKibben J. 3, and Field C. 4
1 The Royal Veterinary College, University of London, Herts, UK; 2 St. George's University, Department of Paraclinical Studies, Grenada, West Indies; 3 St. George's University, Small Animal Clinic, Grenada, West Indies; 4 Bayer Animal Health, Newbury, UK
- 540 **Toxocariasis in dogs, its zoonotic importance and chemotherapy**
Azhar M., Shakeela A., Sabiqaa M., and Awais A.
University of Veterinary and Animal Sciences, Department of Parasitology, Lahore Pakistan
- 541 **Patterns of parasitic infections in faecal samples from stray cat populations in Qatar**
Abu-Madi M.A. 1, and Lewis J.W. 2
1 Department of Health Sciences, College of Arts & Sciences, Qatar University, Doha-Qatar; School of Biological Sciences, Royal Holloway, University of London, Egham, Surrey, UK
- 542 **Study of intestinal helminthes of stray cats in Sari, north of Iran**
Ziapour S.-P. 1,2, Alipour-espehkolaei M. 3, Sharif M. 2, Gholami Sh. 2, Ziaei H. 2, Daryani A. 2, Esfandiari B. 1, and Mobedi I. 4
1 Pasteur Institute of Iran-Amol Research Center; Dep. of Parasitology & Mycology, Amol, Iran; 2 Mazandaran University of Medical Sciences, Dep. of Parasitology & Mycology, Sari, Iran; 3 Babol University of Medical Sciences, Shahid Yahyanejad hospital, Clinical Laboratory, Babol, Iran; 4 Tehran University of Medical Sciences, Dep. of Parasitology & Mycology, Tehran, Iran

- 543 **Prevalence of gastrointestinal nematodes in stray cats from Shiraz, Iran**
Zibaei M. 1, Sadjjadi S.M. 1, Sarkari B. 2, and Jahadi Hosseini S.H. 3
1 Shiraz University of Medical Sciences, Department of Medical Parasitology and Mycology, Shiraz, Iran; 2 Yasuj University of Medical Sciences, Department of Microbiology and Immunology, Yasuj, Iran; 3 Shiraz University of Medical Sciences, Department of Ophthalmology, Shiraz, Iran
- 544 **Epidemiological studies on the gastrointestinal helminth parasites of dogs in Ibadan, Nigeria**
Sowemimo O.A., and Asaolu S.O.
Department of Zoology, Obafemi Awolowo University, Ile-Ife, Nigeria
- 545 **Prevalence and risk factors for *Toxocara canis* infection in dogs in Ile-Ife and Ibadan, southwest Nigeria**
Sowemimo O.A., and Asaolu S.O.
Department of Zoology, Obafemi Awolowo University, Ile-Ife, Nigeria
- 546 **Nation wide survey of intestinal parasites in dogs and cats in Australia**
Palmer C.S. 1, Robertson I.D. 1, Traub R.J. 2, Rees R. 3, Mencke N. 4, and Thompson R.C.A. 1
1 School of Veterinary and Biomedical Sciences, Murdoch University, Australia; 2 School of Veterinary Science, University of Queensland, St Lucia, Queensland; 3 Bayer HealthCare, Animal Health Division, Tingalpa, Queensland; 4 Bayer AG, BHC- Business Group Animal Health, Germany
- 547 **Design and testing of a natural *Dirofilaria immitis* infection model to evaluate the S-safety of Advocate®/Advantage Multi™ for cats**
Arther R. 1, Atkins C. 2, Ciszewski D. 1, Davis W. 1, and Settje T. 1
1 Bayer HealthCare LLC, Animal Health Division, Shawnee Mission, KS, USA; 2 College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA
- 548 **Imidacloprid/Moxidectin (Advocate®) to prevent heartworm infection (*Dirofilaria immitis*) in ferrets**
Schaper R. 1, Heine J. 1, Arther R. 2, Charles S. 2, and McCall J. 3
1 Bayer Healthcare Animal Health, Leverkusen, Germany; 2 Bayer Healthcare LLC, Shawnee Mission, Shawnee, Kansas, USA; 3 TRS Labs, Inc., Athens, USA
- 549 **Inhibitory efficacy on mosquito-feeding and experimental infection of *Dirofilaria immitis* by administration of topical imidacloprid-permethrin compound to dogs**
Hayasaki M. 1, and Saeki H. 2
1 School of Veterinary Medicine, Yamaguchi University, Yamaguchi, Japan; 2 College of Environmental Health, Azabu University, Sagami-hara, Japan
- 550 **Combined ivermectin and doxycycline treatment has microfilaricidal and adulticide activity against *Dirofilaria immitis* in experimentally infected dogs**
Kramer L. 1, Grandi G. 1, Bazzocchi C. 2, Mortarino M. 2, Genchi M. 3, and McCall J.W. 4
1 University of Parma, Department of Animal Production, Parma, Italy; 2 University of Milan, Department of Veterinary Pathology, Milan, Italy; 3 University of Pavia, Department of Animal Biology, Pavia Italy; 4 TRS Laboratories, Athens, Georgia USA
- 551 **The use of imidacloprid / ixidectin spot-on in the treatment of canine angiostrongylosis**
Willesen J.L. 1, Kristensen A.T. 1, Jensen A.L. 1, Heine J. 2, and Koch J. 1
1 Department of Small Animal Clinical Sciences, Faculty of Life Sciences, University of Copenhagen, Denmark; 2 Bayer Healthcare, Animal Health, Research & Development Parasitocides, Monheim, Germany

- 552 **Efficacy of an imidacloprid/moxidectin spot-on solution against fleas (*Ctenocephalides felis*) and hookworms (*Ancylostoma spp*) in Brazilian cats and dogs**
Silva H.C. 1, Bresciani K.D.S. 2, Mendonça R.P. 1, Mencke N. 3, Duran M. 4, Camargo G. 4, Oliveira G.P. 1, and Costa A.J. 1
1 CPPAR/FCAV/UNESP, Center for Research in Animal Health, São Paulo, Brazil; 2 FOA – UNESP, Araçatuba, São Paulo, Brazil; 3 Bayer HealthCare AG, Germany; 4 Bayer S.A., Brazil
- 553 **Anthelmintic efficacy of the emodepside + praziquantel spot-on association against *Ancylostoma braziliense* in experimentally infected cats**
Bergamin L.K.S. 1, Silva H.C. 1, Nascimento A.A. 1, Cunha T.R. 1, Sakamoto C.A.M. 1, Mencke N. 2, Duran M. 3, and Costa A.J. 1
1 CPPAR/FCAV/UNESP, Center for Research in Animal Health, São Paulo, Brazil; 2 Bayer HealthCare AG, Germany, 3 Bayer S.A.
- 554 **Tools for exploring pyrantel resistance in *Ancylostoma caninum***
Kopp S.R. 1, Coleman G.T. 1, McCarthy J.S. 2, Traub R.J. 1, and Kotze A.C. 3
1 School of Veterinary Science, University of Queensland, Brisbane, Australia; 2 Queensland Institute of Medical Research, Brisbane, Australia; 3 CSIRO Livestock Industries, Brisbane, Australia
- 555 **Efficacy of emodepside + praziquantel topical solution (Profender® Spot on) against immature stages of nematodes (*Ancylostoma sp.* and *Toxocara sp.*) in cats**
Schaper R. 1, Altreuther G. 1, and Hopkins T. 2
1 Bayer Healthcare Animal Health, Leverkusen, Germany; 2 Bayer Australia Ltd Animal Health, Pymble, Australia
- 556 **Comparative plasma disposition of fenbendazole, oxfendazole and albendazole in dogs**
Gokbulut C. 1, Bilgili A. 2, Hanedan B. 3, and McKellar Q.A. 4
1 Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine and Research and Development Laboratory, University of Adnan Menderes, Aydin, Turkey; 2 Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Ankara, Turkey; 3 Department of Internal Diseases, Faculty of Veterinary Medicine, University of Ankara, Turkey; 4 The Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield, Hertfordshire, UK
- 557 **The dog as an erratic intermediate host for *Taenia crassiceps***
Nijse E.R. 1, Cremers H.J.W.M. 3, Rofina J.E. 4, Vos J.H. 5, Wielinga P. 2, Smits R.A.J. 6, and van der Giessen J.W.B. 2
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- 558 **Comparison between four coproparasitological techniques for the efficiency in the diagnosis of eggs or cysts of enteroparasites in dogs**
Táparo C.V. 1, Amarante A.F.T. 2, Perri S.H.V. 2, Serrano A.C.M. 1, Ishizaki M.N. 1, Costa T.P. 1, and Bresciani K.D.S. 1
1 UNESP - Universidade Estadual Paulista, Departamento de Apoio, Produção e Saúde Animal, Faculdade de Odontologia, Araçatuba, SP, Brazil; 2 UNESP, Instituto de Biociências, Departamento de Parasitologia, Botucatu, SP, Brazil
- 559 **Active *Strongyloides stercoralis* infection in dogs from Brittany, France**
Polack B. 1,2, Guillot J. 1,2, Bickel T. 1, Fontbonne A. 3, and Chermette R. 1,2
1 Parasitology-Mycology Unit; 2 INRA, AFSSA, ENVA, UPVM, UMR 956 BIPAR; 3 Reproduction; Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort cedex, France

- 560 ***Ancylostoma braziliense* experimental infection in domestic cats: Clinical and biological aspects**
Sakamoto C.A.M. 1, Silva H.C. 1, Bergamin L.K.S. 1, Prette N. 1, Cunha T.R. 1, Nascimento A.A. 1, Borges F.A. 1, and Costa A.J. 1
1 CPPAR/FCAV/UNESP, Center for Research in Animal Health, São Paulo, Brazil
- 561 ***Toxocara cati* infections in a mice model: migratory pattern and pathological responses**
Cardillo N. 1, López C. 2, Ribicich M. 1, and Sommerfelt I. 2
1 University of Buenos Aires, Faculty of Veterinary Medicine, Cathedra of Parasitology, Buenos Aires, Argentina; 2 University of Buenos Aires, Faculty of Veterinary Medicine, Cathedra of Veterinary Public Health, Buenos Aires, Argentina
- 562 **Preliminary result of the natural infection with *Toxocara cati* in cats**
Cardillo N. 1, Rosa A. 1, and Sommerfelt I. 2
1 University of Buenos Aires, Faculty of Veterinary Medicine, Cathedra of Parasitology, Buenos Aires, Argentina; 2 University of Buenos Aires, Faculty of Veterinary Medicine, Cathedra of Veterinary Public Health, Buenos Aires, Argentina
- 563 **Nested PCR assay for the specific detection of *Aelurostrongylus abstrusus* (Nematoda, Strongylida) DNA in faeces and pharyngeal swabs from naturally infected cats**
Traversa D. 1, Iorio R. 1, Pampurini F. 2, Paoletti B. 1, Gatti A. 1, and Heine J. 3
1 Department of Comparative Biomedical Sciences, Faculty of Veterinary Medicine, Teramo, Italy; 2 Bayer Animal Health, Milano, Italy; 3 Bayer HealthCare AG, Leverkusen, Germany
- 564 **A study on intestinal helminthes of jackals in the western provinces of Iran**
Sattari A. 1, Dalimi A. 2, and Moshiri F. 1
1 Department of Laboratory Sciences, Islamic Azad University, Gorgan branch, Gorgan, I.R.Iran; 2 Department of Parasitology, Medical Sciences Faculty, Tarbiat Modarres University, Tehran, I.R.Iran
- 565 **A new technique for worm burden assessment of *Angiostrongylus vasorum* in experimentally infected foxes (*Vulpes vulpes*)**
Monrad J. 1, Webster P. 1, Johansen M.V. 2, and Thamsborg S.M. 1
1 Danish Centre for Experimental Parasitology, Department of Veterinary Pathobiology, University of Copenhagen, Denmark; 2 DBL Centre for Health Research and Development, University of Copenhagen, Denmark
- 566 **Competence of wolf (*Canis lupus*) as host of *Thelazia callipaeda* (Spirurida, Thelaziidae) eyeworms**
Otranto D. 1,2
1 Department of Animal Health and Welfare, Faculty of Veterinary Medicine, Bari, Italy; 2 National Park of Gallipoli Cognato, Basilicata, Italy
- 567 **Important helminthoosonoses circulating in red fox (*Vulpes vulpes*) as wildlife reservoir**
Miterpáková M., Hurníková Z., Antolová D., and Hromadová I.
Parasitological Institute, Slovak Academy of Sciences, Košice, Slovakia

Poster group 14: Echinococcosis

- 568 **Echinococcosis/hydatidosis prevalence in animal and human in Nepal**
Joshi D.D. 1, Khatri P.S. 1, Bista P.R. 1, and Dorny P. 2
1 NZFHRC, Kathmandu, Nepal; 2 Ghent University Ghent, Belgium

- 569 **Hydatids in the garden: encroachment of *Echinococcus granulosus* into urban areas in Australia**
 Jenkins D. 1, Allen L. 2, and Goullet M. 3
1 Australian Hydatid Control & Epidemiology Program, Canberra, Australia; 2 Biosecurity Queensland, Department of Primary Industry, Toowoomba, Australia; 3 Ferals Out, Burpengary, Australia
- 570 **Canine alveolar echinococcosis: report on 20 cases in Germany diagnosed between 1995 and 2007**
 Bauer C. 1, Dyachenko V. 1,2, Köhler K. 3, and Pfleghaar S. 4
1 Justus Liebig University Giessen, Institute of Parasitology, Giessen, Germany; 2 University of Leipzig, Institute of Parasitology, Leipzig, Germany; 3 Justus Liebig University Giessen, Institute of Veterinary Pathology, Giessen, Germany; 4 Practice of Veterinary Pathology, Munich, Germany
- 571 **Anthelmintic baiting to wild foxes by local residents for the control of alveolar echinococcosis in Hokkaido (Koshimizu and Kutchan), Japan**
 Oku Y. 1, Nonaka N. 1, Inoue T. 1, Ganzorig S. 2, Okazaki K. 3, Iwaki T. 4, and Kamiya M. 5
1 Hokkaido University, Graduate School of Veterinary Medicine, Laboratory of Parasitology, Hokkaido, Japan; 2 Forum of Environment and Animals, Hokkaido, Japan; 3 Natural History Museum Kutchan, Hokkaido, Japan; 4 Meguro Parasitological Museum, Tokyo, Japan; 5 Rakuno-gakuen University, Faculty of Environment System, OIE Ref.Lab.for Echinococcosis, Hokkaido, Japan
- 572 ***Echinococcus multilocularis* infection in a Lemurian *Varecia variegata***
 Bourdeau P. 1,2, Risi E. 2, Badie J. 3, and Aguado E. 4
1 Unit of Dermatology - Parasitology - Mycology, Vet. school Nantes, France; 2 Clinics of Zoo and exotic animals, Vet. School Nantes, France; 3 Department of Pathology, Vet. School Nantes, France; 4 Unit of Surgery, Vet. School Nantes, France
- 573 **The carriage of the larval forms of *Echinococcus multilocularis* and other cestodes by the musk rat (*Ondatra zibethicus*) trapped along the river Ourthe and its tributaries (Belgium)**
 Mathy A., Hanosset R., and Losson B.
University of Liege, Laboratory of Veterinary Parasitology, Liege, Belgium
- 574 **Hydatidosis: study of the immune response of a promising new orale vaccine in dogs**
 Petavy A.F., Marchal T., Azzouz S., Hormaeche C., Lahmar S., Ouhelli H., Chabalgoity A., and Sarciron M.E.
1 Université Claude-Bernard Lyon, Faculté de Pharmacie, Laboratory of Parasitology and Medical Mycology, Lyon, France
- 575 **Assessment of microsatellite polymorphism for improving the spatial distribution tracking of *Echinococcus multilocularis***
 Knapp J. 1, Bart J.M. 2, Maillard S. 2, Piarroux R. 2, and Gottstein B. 1
1 Institute of Parasitology, University of Berne, Berne, Switzerland; 2 Parasitology Department, SERF Team, Faculty of Medicine, University of Franche-Comté, Besançon, France
- 576 **Sero-diagnostic potency of purified fractions of hydatid fluid and protoscoleces of both camel and donkey origin**
 Derbala A.A. 1, El-Massry A.A. 2, Mahmoud M.S. 1, and Maarouf O.A. 1
1 Parasitology and Animal Diseases Department, National Research Center, Dokki, Egypt; 2 Parasitology Department, Faculty of Veterinary Medicine, Cairo University, Cairo, Egypt

- 577 **Development of a multiplex PCR to identify carnivore origins of feces for applying to epizootiological surveys of *Echinococcus multilocularis* in Hokkaido, Japan**
Nonaka N. 1, Inoue T. 1, Armua M.T. 1, Sano T. 1, Fukui D. 2, and Oku Y. 1
1 Hokkaido University, Graduate School of Veterinary Medicine, Sapporo, Japan; 2 Asahikawa Municipal Asahiyama Zoological Park & Wildlife Conservation Center, Asahikawa, Japan
- 578 **Diagnosis of infected dogs with *Echinococcus. granulosus* by Dot-blotting test**
Hashemitabar G.R., Razmi G.R., and Keywanloo M.
Department of Pathobiology, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran
- 579 **Evaluation of a coproantigen enzyme linked immunosorbent assay for the diagnosis of canine echinococcosis in Iran**
Siavashi M. 1, and Motamedi Gh. 2
1 Department of Parasitology, Pasteur Institute of Iran, Tehran, Iran; 2 Department of Parasitology, Razi Vaccine and Serum Research Institute of Iran, Karaj, Iran
- 580 **Determination of electrophoretic patterns of proteins of hydatid cyst of two strains of *Echinococcus granulosus* in Iran**
Reza H.S. 1, Eslami A. 2, Shayan P. 2, and Meshki B. 2
1 Sharrekord Azad University, Iran; 2 Tehran University, Iran
- 581 **Survey of appearance of parasite antigen in experimental sheep hydatidosis by coagglutination method**
Razi Jalali M.H., Ghorbanpoor M., Hoghooghi Rad N., Nababvi L., and Esmail Zadeh S.
Veterinary Faculty, Shahid Chamran University, Ahvaz, Iran

Poster group 15: Fasciola and Trematodes

- 582 **The value of pooled sera for the estimation of seroprevalence of liver fluke in a herd of beef cattle**
Meissonnier E. 1, Quillet J.M. 2, and Ogier de Baulny M. 3
1 Janssen Santé Animale, Issy-les-Moulineau, France; 2 Nieul Le Dolent, France; 3 LDA 85, La Roche sur Yon, France
- 583 **Choice of sera in the large Charolais cattle herds for the serological diagnosis of liver fluke - Approach per pasture group**
Courouble R. 1, and Meissonnier E. 2
1 Cabinet Vétérinaire d'Epinac, Saône-et-Loire, France; 2 Janssen Santé Animale, Issy-Les-Moulineaux, France
- 584 **Seroprevalence of bovine fasciolosis in herds of beef breeds in France**
Meissonnier E., and Boutet M.
Janssen Santé Animale, Issy-les-Moulineaux, France
- 585 **Investigations on the role of wildlife in the spread of resistant liver flukes**
Gaasenbeek C., and Borgsteede F.
Animal Sciences Group WUR, Division Infectious Diseases, Lelystad, the Netherlands

- 586 **The prevalence of fluke intermediate stages in *Lymnaea natalensis* collected from streams around Zaria, North Western Nigeria**
 Abdulkareem M. 1, Ulayi B.M. 1, Adamu S. 2, and Ahmed K.H. 1
 1 *Veterinary Parasitology and Entomology*; 2 *Veterinary Pathology and Microbiology*
 Ahmadu Bello University, Zaria, Nigeria
- 587 **Estimating the prevalence of *Fasciola hepatica* infection in dairy cow farms in Schleswig-Holstein, Germany**
 Bolln M., Zahner H., and Bauer C.
 Justus Liebig University Giessen, Institute of Parasitology, Giessen, Germany
- 588 **A bulk milk epidemiological study of *Fasciola hepatica* infection in dairy cattle in Northern Germany**
 Fiedor C. 1, Schunack B. 2, Schnieder T. 1, and von Samson-Himmelstjerna G. 1
 1 *Institute for Parasitology, University of Veterinary Medicine Hannover, Hannover, Germany*; 2 *Novartis Animal Health GmbH, Munich, Germany*
- 589 ***Fasciolasis pestholes* in the North-West of Russia (Novgorod region)**
 Filimonov N.
 St. Petersburg State University, Department of Invertebrate Zoology, St. Petersburg, Russia
- 590 **Prevalence of *Fasciola hepatica* in dairy cattle from an Andean region in Venezuela (Bailadores, Mérida, Venezuela)**
 Pérez A.M. 1, Gauta J.G. 2, Quijada J. 1, García M.E. 1, García H. 1, Armas S. 3, and Leon R. 1
 1 *Central University of Venezuela (UCV), School of Veterinary Sciences, Department of Veterinary Parasitology, Maracay, Aragua, Venezuela*; 2 *Technological Institute of Ejido- Bailadores extension campus, Bailadores, Mérida, Venezuela*; 3 *Central University of Venezuela (UCV), School of Veterinary Sciences, Statistics Department. Maracay, Aragua, Venezuela*
- 591 ***Fasciola hepatica* in Brazil: current situation and perspectives**
 Molento M.
 Universidade Federal do Paraná, Laboratório de Doenças Parasitárias, Curitiba, Brazil
- 592 **Co-infections with the liver fluke, *Fasciola hepatica* and *Salmonella dublin* infections in dairy heifers**
 Karlsen M. 1,2, Nielsen L.R. 2, and Thamsborg S.M. 1
 1 *Danish Centre for Experimental Parasitology, Department of Veterinary Pathobiology, Faculty of Life Sciences, University of Copenhagen, Denmark*; 2 *Population Biology, Department of Large Animal Sciences, Faculty of Life Sciences, University of Copenhagen, Denmark*
- 593 **Survey of *Fasciola gigantica* infection in buffalo and cattle by eggs or/and flukes examination in gall bladders**
 Zhang W.Y., Huang W.Y., and Wang D.Y.
 College of Animal Science and Technology, GuangXi University, Nanning 530004, Guangxi, China
- 594 ***Paramphistomum daubneyi* infection in grazing goats: results from an epidemiological study in Saône and Loire, France**
 Paraud C. 1, Fournier E. 2, Robergeot V. 2, Pors I. 1, Baudry C., and Chartier C. 1
 1 *French Food Safety Agency (AFSSA), Laboratoire d'Etudes et de Recherches Caprines, Niort, France*; 2 *Groupeement départemental de défense sanitaire 71 (GDDS 71), Mâcon, France*

- 595 **An overview of paramphistomosis in ruminants in Punjab, India**
Juyal P.D.
Department of Veterinary Parasitology, College of veterinary science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India
- 596 **Prevalence of dicrocoeliasis in slaughtered animals and its histopathological lesions in liver, in Ardebil, Iran**
Ahadi M.T., Ajdarnejad Sh., Chiniforoush M., and Alavi S.
Islamic Azad University, Ardebil Branch, Ardebil, Iran
- 597 **Comparative analysis of neuropeptide distributions in the larval stages of the trematode *Fasciola hepatica***
Pierson L., Mousley A., Marks N., Brennan G., and Maule A.
Queens University Belfast, School of Biological Sciences, Parasitology Research group, Belfast, Northern Ireland
- 598 **Peroxiredoxins of the liver fluke, *Fasciola hepatica***
Sekiya M., and Mulcahy G.
UCD Dublin, School of Agriculture, Food Science and Veterinary Medicine, Ireland
- 599 **Molecular cloning and bioinformatic analysis of cysteine proteinase (FhPcW1) from adult *Fasciola hepatica***
Jaros S. 1, Januszkiewicz K. 1, and Wedrychowicz H. 1, 2
1 Department of Molecular Biology, Laboratory of Molecular Parasitology, W. Stefanski Institute of Parasitology, Polish Academy of Sciences, Warsaw, Poland; 2 Division of Parasitology, Department of Preclinical Sciences, Warsaw Agricultural University, Poland
- 600 **Molecular cloning and expression of cDNA of a novel gene encoding cathepsin D-like protease from *Fasciola hepatica***
Jaros S. 1, Jarzabkowski M. 2, and Wedrychowicz H. 1, 2
1 Department of Molecular Biology, Laboratory of Molecular Parasitology, W. Stefanski Institute of Parasitology, Warsaw, Poland; 2 Division of Parasitology, Department of Preclinical Sciences, Warsaw Agricultural University, Warsaw, Poland
- 601 **Comparison of ITS-1 and ITS-2 of *Fasciola hepatica* from different hosts and geographical locations in Spain**
Alasaad S. 1, Li Q-Y. 2, Huang C-Q. 2, Granados J.-E. 3, García C. 4, Pérez J.-M. 1, and Zhu X.-Q. 2
1 Departamento de Biología Animal, Biología Vegetal y Ecología, Universidad de Jaén, Campus Las Lagunillas, Jaén, Spain; 2 Parasitology Laboratory, College of Veterinary Medicine, South China Agricultural University, Wushan, Tianhe District, Guangzhou, Guangdong Province, PR China; 3 Parque Nacional de Sierra Nevada, Pinos Genil, Granada, Spain; 4 Laboratorio de Parasitología Animal, S. I. A., Consejería de Agricultura y Medio Ambiente, Toledo, Spain
- 602 **Complete mitochondrial genomes for *Fasciolopsis buski*, *Fasciola gigantica*, *Clonorchis sinensis*, *Opisthorchis viverrini*: highly conserved features**
Le T.H. 1, Sato Y. 2, Nguyen V.D. 3, Nguyen N.B.T. 1, Nguyen N.T.T. 1, Vu T.T. 1, Agatsum T. 4, Blair D. 5, and McManus D.P. 6
1 Institute of Biotechnology, Vietnam; 2 Nihon University, Japan; 3 Hanoi Medical University, Vietnam; 4 Kochi Medical School, Japan; 5 James Cook University, Australia; 6 Queensland Institute of Medical Research, Australia

- 603 **Serotonin and FMRFamid immunoreactivity in *Opisthorchis felineus* (Trematoda, Opisthorchidae) cercaria**
Tolstenkov O. 1, Terenina N. 1, Gustafsson M. 2, Serbina E. 3, Kreshchenko N. 4, and Jashin V. 4
1 Centre of Parasitology of A.N.Severtsov Institute of Ecology and Evolution of Russian Academy of Sciences, Moscow; 2 Department of Biology, Abo Academi University, Abo, Finland; 3 Institute of Animal Systematics and Ecology of Russian Academy of Sciences, Novosibirsk; 4 Institute of Cell Biophysics of Russian Academy of Sciences, Pushino, Moscow region, Russia
- 604 **Role of tryptaminergic agents in regulating the motility of *gastrothylax crumenifer*, a rumen amphistome**
Verma P.K. 1, Kumar D. 2, Tandan S.K. 2, Raina R. 1, and Srivastava A.K. 1
1 Sher-E-Kashmir University of Agricultural Sciences and Technology, R.S.Pura, J&K, India; 2 Indian Veterinary Research Institute, Izatnagar, Bareilly, UP, India
- 605 **Flow cytometric analysis of the early local cellular response in rats infected with *Fasciola hepatica***
Kozak-Cieszczyk M. 1, Wedrychowicz H. 1,2, and Jedlina-Panasiuk L. 1
1 W. Stefanski Institute of Parasitology, PAS, Warsaw, Poland; 2 Warsaw Agricultural University, Poland
- 606 **Serum biomarkers of fasciolosis in sheep: insights into host-parasite interactions**
Rioux M-C. 1, Carmona C. 2, Acosta D. 2, Gibbs B. 3, Bennett H. 3, Ward B. 4, Ndao M. 4, and Spithill T.W. 1
1 McGill University, Institute of Parasitology, Ste-Anne-de-Bellevue, Canada; 2 Instituto de Higiene, Unidad de Biología Parasitaria, Montevideo, Uruguay; 3 McGill University, Sheldon Centre, Montreal, Canada; 4 National Reference Centre for Parasitology, Montreal General Hospital Research Institute, Montreal, Canada
- 607 **Characterization of a 2.9 kDa-*Fasciola hepatica* recombinant protein**
Arias M. 1, Hillyer G.V. 2, Paz-Silva A. 1, Sánchez-Andrade R. 1, Pedreira J. 1, Suárez J.L. 1, Díaz P. 1, and Morrondo P. 1
1 Animal Pathology Department, Epidemiology, Zoonoses and Parasitic diseases, Veterinary Faculty, Santiago de Compostela University, Lugo, Spain; 2 Laboratory of Parasite Immunology and Pathology, School of Medicine, Medical Sciences Campus, University of Puerto Rico, San Juan, Puerto Rico, USA
- 608 **Evaluation of protection induced by recombinant cathepsin L1 in goats challenged with *Fasciola hepatica***
Martinez-Moreno A. 1, Buffoni L. 1, Zafra R. 2, Pérez-Écija A. 2, Mendes R.E. 2, Martínez-Moreno F.J. 1, Acosta-García I. 1, and Pérez J. 2
1 Department of Animal Health (Parasitology), Córdoba, Spain; 2 Department of Anatomy and Comparative Pathology, Córdoba, Spain
- 609 **The effect of adjuvants on the immune response to *Fasciola hepatica* in sheep**
Haçariz O., Sayers G., and Mulcahy G.
University College Dublin, School of Agriculture, Food and Veterinary Medicine, Dublin, Ireland
- 610 **The cellular and humoral response of cattle vaccinated intranasally with a recombined cysteine protease of *Fasciola hepatica* in the form of inclusion bodies**
Kozak-Cieszczyk M. 1, Jedlina-Panasiuk L. 1, Kaliniak M. 1, Kosik M. 2, Pjucienniczak A. 2, and Wedrychowicz H. 1
1 W. Stefanski Institute of Parasitology, PAS, Twarda 51/55, 00-818 Warsaw, Poland
2 Institute of Biotechnology and Antibiotics, Department of Bioengineering, Warsaw, Poland

- 611 **Evaluation of protection induced by recombinant thioredoxin peroxidase in goats challenged with *Fasciola hepatica***
 Pérez J. 1, Zafra R. 1, Pérez-Écija A. 1, Buffoni L. 2, Mendes R.E. 1, Martínez-Moreno F.J. 2, Martínez-Cruz S. 2, and Martínez-Moreno A. 2
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- 612 **Immune response in *Fasciola gigantica* experimentally infected rabbits treated with either carnosine or mirazid**
 Soliman K. 1, Mahmoud M.S. 2, and Abou-EL Dobal S. 2
1 Medical Biochemistry Department, Faculty of Medicine, Cairo University, Cairo, Egypt; 2 Parasitology and Animal Diseases Department, National Research Center, Dokki, Egypt
- 613 **Molecular identification of *Schistosoma bovis* isolated from intermediate snail hosts in Senegal and Uganda**
 Rollinson D. 1, Huyse T. 2, Webster B. 1, Southgate V.R. 1, Vercruysse J. 3, and Stothard J.R. 1
1 Department of Zoology, The Natural History Museum, Cromwell Road, London SW7 5BD; 2 Catholic University of Leuven, Laboratory of Aquatic Ecology, Leuven, Belgium; 3 Faculty Veterinary Medicine, Merelbeke, Gent
- 614 **Proteins expressed on the tegument surface of *Schistosoma bovis*. Which of them play as plasminogen receptors?**
 Oleaga A. 1, Valero M.L. 2, Ramajo-Hernández A. 1, Sánchez-del Pino M.M. 2, Ramajo- Martín V. 1, and Pérez-Sánchez R. 1
1 Unidad de Patología Animal, Instituto de Recursos Naturales y Agrobiología (CSIC), Salamanca, Spain; 2 Unidad de Proteómica, Centro de Investigación Principe Felipe, Valencia, Spain
- 615 **Congenital transmission of *Schistosoma japonicum* and dynamic of serum antibodies**
 Qian B.Z. 1, Sugiyama H. 2, Waikagul J. 3, and Hang Z.Z. 4
1 Institute of Bio-engineering, Zhejiang Academy of Medical Sciences, China; 2 Department of Parasitology, National Institute of Infectious Diseases, Japan; 3 Department of Tropic medicine, Mahidol University, Thailand; 4 Zhejiang, Ninghai, CDC, China
- 616 ***Fasciola gigantica* (Trematoda: Fasciolidae): morphological observations using scanning electron microscopy**
 Naem S.
Department of Pathobiology, College of Veterinary Medicine, Urmia University, Urmia, Iran
- 617 **Modification of dipstick as a field applicable technique for multiple diagnosis of some parasitic infections**
 Hendawy M.A.M. 1, Hasan S.M. 2, and El-Attar G. 3
1 Parasitology Department; 2 Immunology Department; 3 Clinical Department Theodor Bilharz Research Institute, Guiza, Egypt
- 618 **Seroprevalence of fasciolosis and rheumatoid factor in patients with eosinophilia**
 Sánchez-Andrade A. 3, Díez C. 2, Suárez J.L. 1, Romasanta A. 1, Morrondo P. 1, Díez-Baños P. 1, Paz-Silva A. 1, and Sánchez-Andrade R. 1
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- 619 **Paragonimus in Zhrjiang, China: morphology and molecular biology**
 Qian B.Z. 1,2, Sugiyama H. 2, Waikagul J. 3, and Hang Z.Z. 4
1 Institute of Bio-engineering, Zhejiang Academy of Medical Sciences, China; 2 Department of Parasitology, National Institute of Infectious Diseases, Japan; 3 Department of Tropic medicine, Mahidol University, Thailand; 4 Zhejiang, Ninghai, CDC, China

Poster group 16: Arthropods of Large Animals

- 620 **HOBO micro stations and the report of data from Romania in frame of the East BTNET Project**
 Dascalu M.L., and Ionescu A.
Institute for Diagnosis and Naimal Health, Parasitology Laboratory, Bucharest, Romania
- 621 **Study of *Tabanidae* (Diptera) in the Shadegan and Hovayzeh marshland in Khoozestan province - Southwest Iran**
 Navidpour S.H. 1, Jahani Fard E. 2, and Vaziriyan Zadeh B. 1
1 Entomology Research Center, Ahwaz Razi Institute; 2 Department of parasitology, Ahwaz jondishapour Medical University, Iran
- 622 **Epidemiological study of ruminant anaplasmosis: Seroprevalence and associated ticks in North Center of Morocco**
 Rhalem A. 1, Sahibi H. 1, Rahali T. 1, Ait Hamou S. 1, Van Impe G. 2, and Losson B. 3
1 Département de Parasitologie et Maladies Parasitaires, Institut Agronomique et Vétérinaire Hassan II, BP. Rabat, Maroc; 2 Laboratoire de Biogéographie et d'Ecologie, Faculté des Sciences, Université catholique de Louvain, Belgium; 3 Service de Parasitologie Faculté de Médecine Vétérinaire, Université de Liège, Belgium
- 623 ***Amblyomma triste* (Ixodidae) in Uruguay: Distribution, hosts and seasonality**
 Venzal J.M. 1, Castro O. 1, de Souza C.G. 1, Félix M.L. 1, and Estrada-Peña A. 2
1 Veterinary Faculty, University of the Republic, Montevideo, Uruguay; 2 Veterinary Faculty, University of Zaragoza, Spain
- 624 **Ecological variables affecting the tick *Ixodes ricinus* abundance in north-eastern Italy**
 Corrain R. 1, Montarsi F. 2, Cassini R. 1, Drigo M. 1, Danesi P. 2, and Capelli G. 1
1 Faculty of Veterinary Medicine, University of Padua, Italy; 2 Istituto Zooprofilattico sperimentale delle Venezie, Legnaro, Italy
- 625 **Mites as newly emerging disease pathogens in rodents and human beings**
 Beck W. 1,2
1 Pfizer Animal Health, Karlsruhe, Germany, 2 Institute for Comparative Tropical Medicine and Parasitology, LMU Munich, Munich, Germany
- 626 **The prevalence of nymphal stage of *Linguatula serrata* in camels (*Camelus dromedarius*) in Najafabad, Iran**
 Pourjafar M. 1, Azizi H.R. 1, Darabi S. 2, and Khosravi M. 2
1 School of veterinary medicine, Shahrekord University, Shahrekord, Iran; 2 Educated of Shahrekord University, Shahrekord, Iran

- 627 **Population dynamics of the parasitic state of *Boophilus microplus* in bovines of the municipality of Culiacán, Sinaloa, Mexico**
 Gaxiola S. 1, García Z. 2, Cruz C. 3, Quintero T. 4, Portillo J. 1, Vázquez C. 4, Rosario R. 2, and Borbolla J. 1
1 University Autonomous of Sinaloa State, Faculty of Veterinarian Medicine.; 2 Investigation Institute of Morelos, Mexico; 3 Technology Institute of Aguascalientes, Mexico; 4 University National of Mexico. Faculty of Veterinarian Medicine, Mexico
- 628 **Over-wintering of *Lucilia cuprina* in south-eastern Australia**
 De Cat S. 1, Larsen J. 1, and Anderson N. 1
1 The Mackinnon Project, School of Veterinary Science, University of Melbourne, Werribee, VIC, Australia
- 629 **An outbreak of severe chorioptic mange in dairy cows in south-west France**
 Alzieu J.-P. 1, Forbes A.B. 2, Charlier J. 3, and Vercruysse J. 3.
1 Clinique Vétérinaire Les Althéas, Pamier, France; 2 Merial SAS, Lyon, France; 3 Ghent University, Department of Virology, Parasitology & Immunology, Merelbeke, Belgium
- 630 **A report of ovine acariasis due to mixed *Sarcoptes scabii* and *Psoroptes ovis* in a sheep flock**
 Razmi G.R. 1, and Sharifi K. 2
1 Department of Pathobiology, School of Veterinary Medicine, Ferdowsi University of Mashhad, Iran; 2 Department of Clinical science, School of Veterinary Medicine, Ferdowsi University of Mashhad, Iran
- 631 **Generalized demodectic mange in a free-ranging mule deer (*Odocoileus hemionus*) from Colorado, USA**
 Ballweber L.R. 1, Andrews J.J. 2, Baeton L. 3, and Holder Z. 3
1 Colorado State University Veterinary Diagnostic Laboratory, Fort Collins, Colorado, USA; 2 Colorado State University Western Slope Veterinary Diagnostic Laboratory, Grand Junction, Colorado, USA; 3 Colorado Division of Wildlife, Wildlife Research Center, Fort Collins, Colorado, USA
- 632 ***Melophagus ovinus*: Dynamics of sheep ked populations on an infected flock and its effect on weight gains and wool production**
 Olaechea F. 1, Sachero D. 2, Cabrera R. 1, Garramuño J. 3, and Raffo F. 1
1 Grupo Salud Animal, INTA EEA Bariloche, CC 277, Bariloche, Río Negro, Argentina; 2 Laboratorio de Fibras Textiles, INTA EEA Bariloche, CC 277, Bariloche, Río Negro, Argentina; 3 Campo Experimental, INTA EEA Bariloche, CC 277, Bariloche, Río Negro, Argentina
- 633 **Efficacy of moxidectin 2% long acting for sheep against natural infections of nasal bots (*Oestrus ovis*) in sheep in Spain**
 Rojo-Vázquez F.A. 1, Meana A. 2, Valcárcel F. 3, Cordero-Pérez C. 1, Fernández-Pato N. 1, and Álvarez-Sánchez M.A. 1
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- 634 **Evaluation of three solvents as vehicle of crude methanolic plant extracts in the larval immersion test using *Boophilus microplus* ticks**
 Rosado-Aguilar J.A. 1, Aguilar-Caballero A. 1, Rodriguez-Vivas R.I. 1, Borges R. 2, Mendez M. 2, Dorantes A. 2, Caceres M. 2, and Garcia-Vazquez Z. 3
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- 635 **Efficacy of a 20% clorpiriphos + 50% amitraz * formulation against *Rhipicephalus (Boophilus) microplus* in naturally and artificially infested bovines**
Lima R.C.A. 1, Buzzulini C. 1, Paula A.I. 1, Soares V.E. 1, Barufi F. 2, Santos E. 2, Oliveira G.P. 1, and Costa A.J. 1
1 CPPAR/FCAV/UNESP, Center for Research in Animal Health, Jaboticabal, São Paulo, Brazil; 2 Intervet do Brasil Veterinária Ltd, Brazil
- 636 **Efficacy of a long acting moxidectin formulation to protect against ovine *Psoroptic mange* (sheep scab)**
Bates P.G. 1., Rankin M.R. 1., Clifford D. 1, and Parker L.D. 2
1 Veterinary Laboratories Agency (VLA), New Haw, Addlestone, Surrey, UK; 2 Fort Dodge Animal Health, Flanders Road, Hedge End, Southampton, UK
- 637 **Efficacy of a long acting moxidectin formulation to cure ovine *Psoroptic mange* (sheep scab) under field conditions in the UK**
Bates P.G. 1, and Parker L.D. 2
1 Veterinary Laboratories Agency (VLA), New Haw, Addlestone, Surrey, UK; 2 Fort Dodge Animal Health, Flanders Road, Hedge End, Southampton, UK
- 638 **Pathogenicity and thermotolerance of various species and strains of entomopathogenic fungi against *Psoroptes ovis*, the cattle scab mite**
Lekimme M., Focant C., Tombeux S., and Losson B.
University of Liège, Faculty of Veterinary Medicine, Laboratory of Parasitology, Belgium
- 639 **Comparative efficacy of ivermectin and cypermethrin against *Hyalomma* Ticks (Acari: Ixodidae)**
Sajid M.S. 1, Iqbal Z. 1, Khan M.N. 1, Muhammad G. 2, Hussain A. 1, and Saqib M. 2
1 Department of Veterinary Parasitology, University of Agriculture, Faisalabad, Pakistan; 2 Department of Veterinary Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan
- 640 **Does topical insecticides have behavioural effects of lice? An example with imidaclopride and biting lice: *Damalinia (Bovicola) bovis***
Bourdeau P., Bruet V., and Ricklin M.
1 Dermatology - Parasitology - Mycology, Veterinary School, Nantes, France; 2 Dermatology, University of Bern, Switzerland
- 641 **Infestation of horses by flies - Interest of a treatment with deltamethrine diluted in water**
Houffschmitt P. 1, Dussaulx G. 1, Buge S. 2, and Leblond A. 2
1 Intervet, Angers Technopole, Beaucouzé, France; 2 ENVL, avenue Bourgelat, Marcy l'Etoile, France
- 642 **Oestrosis diagnosis in sheep by ELISA test using salivary gland antigens of *Oestrus ovis* (Diptera:Oestridae)**
Angulo C.E. 1, Dorchie P. 2, Cepeda R. 4, Ascencio F. 1, Prevot F. 2, Terefe G. 2, Scala A. 3, and Jacquiet P. 2
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- 643 **Differential study of morphology and Elisa test in diagnosis of Hypoderma of goat of Iran (Khoozestan province)**
Navidpour S.H. 1, Mazaheri Y. 2, Godarzi M.A. 1., and Madani A. 1
1 Razi Vaccine and Serum Research Institute, Iran; 2 University of Shahid Chamran, Veterinary medicine school Ahvaz, Iran.
- 644 **Development and evaluation of an ELISA for diagnosis of *Sarcoptes scabiei*-infection in sheep**
Rodríguez-Cadenas F. 1, Carbajal-González M.T. 1, Fregeneda-Grandes J.M. 1, Aller-Gancedo J.M. 1, Huntley J.F. 2, and Rojo-Vázquez F.A. 1
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- 645 **Comparison of clinical signs, ear swabbing and ELISA for the diagnosis of sub-clinical ovine *Psoroptic otacariasis***
Bates P. 1, Schnyder M. 2, Grimm F. 2, and Deplazes P. 2
1 Veterinary Laboratories Agency (VLA), Addlestone, Surrey, UK; 2Institute for Parasitology, University of Zurich, Zurich, Switzerland
- 646 **Efficacy of an improved ELISA to diagnose sub-clinical ovine *Psoroptic mange* (Sheep Scab)**
Bates P. 1, Schnyder M. 2, Rankin M. 1, Clifford D. 1, Grimm F. 2, and Deplazes P. 2
1 Veterinary Laboratories Agency (VLA), Addlestone, Surrey, United Kingdom; 2Institute for Parasitology, University of Zurich, Zurich, Switzerland
- 647 ***Melophagus ovinus*: Experimental infection of sheep and its survival outside the host**
Olaechea F.V. 1, Larroza M. 1, Cabrera R. 1, and Raffo F. 1
1 Grupo Salud Animal, INTA EEA Bariloche, Río Negro, Argentina
- 648 **Analysis of world strains of *Anaplasma marginale* using major surface protein 1a repeat sequences**
de la Fuente J. 1,2, Ruybal P. 3, Mtshali M.S. 4, Naranjo V. 2, Shuqing L. 5, Mangold A.J. 6, Rodriguez S.D. 7, and Jimenez R. 7
1 Oklahoma State University, Department of Veterinary Pathobiology, Stillwater, USA; 2 Instituto de Investigacion en Recursos Cinegeticos, Ciudad Real, Spain; 3 Instituto Nacional de Tecnologia Agropecuaria, Buenos Aires, Argentina; 4 University of the Free State, Parasitology Research Program, Phuthaditjhaba, South Africa; 5 Shanghai Entry-Exit Inspection and Quarantine Bureau, Shanghai, The Peoples's Republic of China; 6 Instituto Nacional de Tecnologia Agropecuaria, Santa Fe, Argentina; 7 CENID-Parasitologia Veterinaria, Instituto Nacional de Investigaciones Forestales, Morelos, Mexico
- 649 **Effect of hypodermins A, B and C on the secretion of gamma interferon (IFN- γ) by bovine lymphocytes stimulated with two mitogens**
Díez-Baños P., Dacal V., Vázquez L., López C., Cienfuegos S., Morrondo P., and Panadero R.
Animal Pathology Department, Epidemiology, Zoonoses and Parasitic diseases, Veterinary Faculty, Santiago de Compostela University, Lugo, Spain
- 650 **Immunogold localization of serine proteinases in midgut of *Hypoderma lineatum***
Colwell D.D., and Leggett F.
Lethbridge Research Centre, Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada

- 651 **Evaluation of crude and purified gut extract antigens for protection against camel tick *Hyalomma dromedarii* (Acari: Ixodidae)**
Sobhy A., Mahmoud M.S., and Mohamed A.M.
Parasitology and Animal Diseases Department, National Research Center, Dokki, Egypt
- 652 **Novel salivary proteins of the argasid ticks *Ornithodoros erraticus* and *Ornithodoros moubata* identified by a proteomic approach based on de novo sequencing**
Pérez-Sánchez R., Ramajo-Hernández A., Ramajo-Martín V., and Oleaga A.
Patología Animal, Instituto de Recursos Naturales y Agrobiología, CSIC, Salamanca, Spain
- 653 **Molecular and reverse genetic characterization of a hemolytic serine proteinase in the midgut of the Ixodid tick *Haemaphysalis longicornis***
Miyoshi T. 1, Tsuji N. 1, Islam M.K. 1, Huang X. 1, Motobu M. 1, Alim M.A. 1, Hatta T. 1, and Fujisaki K. 2,3
1 Laboratory of Parasitic Diseases, National Institute of Animal Health, National Agriculture and Food Research Organization, Tsukuba, Ibaraki, Japan; 2 National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido, Japan; 3 Department of Frontier Veterinary Medicine, Kagoshima University, Korimoto, Kagoshima, Japan

Poster group 17: Arthropods, miscellaneous

- 654 **Relevant Diptera frequency in public health and *Enterobacteriaceae* associated from Baixada Fluminense area, RJ, Brazil**
Borges- Ribeiro R.S. 1, Khalil E.I. 2, Barreira J.D. 3, Costa A. 3, and Rodrigues M.L.A. 3
1 Veterinary; 2 Med Vet Student, Unigranrio, RJ, Brasil; 3 Vet Med Dep Microbiology and Parasitology, Unigranrio, RJ, Brazil
- 655 **Poultry mite (*Dermanyssus gallinae*) dermatitis in a horse**
Mignon B., and Losson B.
University of Liège, Faculty of Veterinary Medicine, Department of Infectious and Parasitic Diseases, Belgium
- 656 **Finding of *Megninia gynglimura* and *M. crinita* mites as cause of dermatitis and fall of feathers oin egg layer hens of commercial type coming from a farm in Colima State on Mexico**
Quintero M.T. 1, Juarez V.G. 1, Eleno V.A. 1, Grifaldo A.F. 1, and Coronado A. 2
1 Sección de Artropodología Departamento de Parasiología Facultad de Medicina Veterinaria y Zootecnia Universidad Nacional Autónoma de México, México; 2 Private Professional Practic, Mexico
- 657 **Acaricidal activity of *Melaleuca alternifolia* (tea tree) oil and ivermectin in patients with different skin lesions due to *Demodex folliculorum* infestation**
Safia M. A. 1, and Nouran A.A. 2
1 Department of Parasitology, Faculty of Medicine, Alexandria University, Egypt; 2 Department of Dermatology, Faculty of Medicine, Alexandria University, Egypt
- 658 **Effects of 4 different chemical compounds at control and treatment of varroasis in *Apis mellifera*, a comparative study**
Shojaei S.S.R. 1, Nazemnia M. 2, Farshine A. 3, and Najjari H. 4
1 Islamic Azad University, Karaj Branch, Department of Pathobiology, Division of Veterinary Parasitology, Karaj, Iran; 2 I.A.U, Karaj Branch, D.V.M; 3 University of Zanjan, Department of Hygiene & Breeding of Honey Bee, Zanjan ,Iran; 4 Graduate from D.V.M Course,I.A.U, Karaj Branch, Iran

Poster group 18: Coccidia

- 659 **Coccidiosis of calves in Hungarian dairy farms: prevalence and *Eimeria*-species**
Majoros G. 1, Szeidemann Zs. 2, and Farkas R. 1
1 Department of Parasitology and Zoology, Faculty of Veterinary Science, Szent István University, Budapest, Hungary; 2 Bayer Hungaria Ltd., Budapest, Hungary
- 660 **The epidemiology of calve coccidiosis (*Eimeria* spp.) in Poland**
Klockiewicz M. 1, Kaba J. 2, Tomczuk K. 3, Sadzikowski A.B. 3, Studzinska M. 3, Rypula K. 4, Janecka E. 1, and Malecki-Tepicht J. 5
1 Warsaw Agricultural University, Division of Parasitology & Invasiology, Warsaw, Poland; 2 Warsaw Agricultural University, Division of Infectious Diseases & Epidemiology, Warsaw, Poland; 3 Lublin Academy of Agriculture, Division of Parasitology, Lublin, Poland; 4 Wrocław Academy of Agriculture, Department of Epizootiology and Veterinary Administration & Clinic, Wrocław, Poland; 5 Bayer Health Care, Bayer Ltd, Warsaw, Poland
- 661 **Early interactions of peripheral blood mononuclear cells with *Eimeria bovis* infected host cells**
Taubert A., Zahner H., and Hermosilla C.
Justus Liebig University Giessen, Institute of Parasitology, Giessen, Germany
- 662 **Diclazuril for coccidiosis in ruminants: safe for the environment?**
Van Leemput L., and Louineau J.
Janssen Animal Health, Beerse, Belgium
- 663 **Control of coccidiosis due to *Eimeria bovis* and *Eimeria zuernii* in calves with toltrazuril (baycox 5%) under field conditions in comparison with diclazuril and untreated controls**
Mundt H.-C. 1, Rödder F. 2, Mengel H. 3, Bangoura B. 2, Ocak M. 4, and Dauschies A. 2,3
1 Bayer HealthCare AG, Leverkusen, Germany; 2 University of Leipzig, Institute of Parasitology, Leipzig, Germany; 3 Veterinary Clinical Studies (koVet), Leipzig, Germany; 4 Clinical Research and Biometry, Munich, Germany
- 664 **Efficacy of diclazuril against coccidiosis in suckling calves**
Arias M. 1, Vázquez L. 1, Dacal V. 1, Salichs M. 2, Llena J. 3, Homedes J. 2, Morrondo P. 1, Paz-Silva A. 1
Pathology Department, Epidemiology, Zoonoses and Parasitic diseases, Veterinary Faculty, Santiago de Compostela University, Spain; 2 Veterinaria Esteve, Laboratorios Dr. Esteve S.A., Barcelona, Spain; 3 COREN S.A, Polígono Ind. San Ciprián de Viñas, Ourense, Spain
- 665 **The differentiation of *Eimeria* species using PCR-RFLP to analyze the infected caeca of chicken coccidiosis**
Choosaksaengthong S., Chungpivat S., and Viseshakul N.
Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand
- 666 **Immuno-characterization of some *Eimeria* spp. infecting chicken in Egypt**
Abdel-Gawad A., Fahmy M., Mousa W., and El-Behairy A.
University Cairo, Department Veterinary Parasitology, Giza, Egypt
- 667 **Immune enhancement of bacterial CpG DNA on immucox against *Eimeria tenella* infection in chicken**
Xu Q. 1,2, Li P. 1, Ge K. 1, Xu W. 1, and Li G. 2
1 College of Animal Science and Technology, Anhui Agricultural University, Hefei, China; 2 College of Veterinary Medicine, South China Agricultural University, Guangzhou, China

- 668 **The effect of administration of anti-coccidial drugs on oocyst shedding and performance in experimental coccidiosis in broiler chickens**
Pirali-kheirabadi K.H. 1, and Zamani- Moghadam A. 2
1 Dept. of Pathobiology, Faculty of Veterinary Medicine, University of Shahrekord, Shahrekord, Iran; 2 Dept. of clinical science, Faculty of Veterinary Medicine, University of Shahrekord, Shahrekord, Iran
- 669 **A comparative study on effects of experimental coccidial challenge by increasing *E. maxima* oocysts doses on serum carotenoids in chicken broilers**
Hajikhani R. 1, Shojaei S.S.R. 1, Naghash L.A.H. 2, and Azade Ranjbar S. 3
1 Islamic Azad University, Karaj Branch, Dept. of Pathobiology, Karaj, Iran; 2 I.A.U, North-Tehran Branch, Tehran, Iran; 3 I.A.U, Karaj Branch, Iran
- 670 **A field study assessing control of broiler coccidiosis by paracox vaccination or by baycox (toltrazuril) stand-alone treatment**
Claeskens M. 1, Verdonck W. 1, Heesen H. 2, Froyman R. 3, and Torres A. 3
1 Joosen-Luyckx, Turnhout, Belgium; 2 Bayer BV, Netherlands; 3 Bayer HealthCare, Animal Health, Leverkusen, Germany
- 671 **COCCIMORPH: a real-time diagnostic tool based on automatic image recognition of protozoan parasites of the genus *Eimeria***
Castañón C.A.B. 1, Fernandez S. 1, Fraga J.S., Fontoura L.F. 2, and Gruber A. 1
1 Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brazil; 2 Instituto de Física de São Carlos, Universidade de São Paulo, Brazil
- 672 **Immune response to coccidiosis depends on the age of rabbits**
Pakandl M 1, Hlášková L. 1, Salát J. 1, Poplštejn M. 2, Nevečeřalová M. 3, Vodička T. 3, Chromá V. 3
1 Biology Centre of the Academy of Sciences of the Czech Republic, Institute of Parasitology, České Budějovice, Czech Republic; 2 BIOPHARM, Research Institute of Biopharmacy and Veterinary Drugs, a.s., Jílové near Prague, Czech Republic; 3 University of South Bohemia, Faculty of Biological Science, České Budějovice, Czech Republic
- 673 **Prevalence and impact of *Isospora suis* in suckling piglets in Ontario, Canada**
Peregrine A.S. 1, Webster E. 1, Friendship R. 2, Dewey C. 2, Vilaça K. 3, and Aliaga-Leyton A. 2
1 Department of Pathobiology and 2 Department of Population Medicine, Ontario Veterinary College, University of Guelph, Ontario, Canada; 3 Maitland Swine Services, Listowel, Ontario, Canada
- 674 **Control of *Isospora suis* in piglets: toltrazuril versus improved hygiene**
Boes J., and Baadsgaard N.P.
Danish Pig Production, Danish Meat Association, Kjellerup, Denmark
- 675 **Characterisation of mucosal lymphocyte populations during infection with *Isospora suis***
Buggelsheim M. 1, Worliczek H.L. 1,2, Gerner W. 2, Schmidt P. 2, Saalmüller A. 2, and Joachim A. 1
1 University of Veterinary Medicine Vienna, Department of Pathobiology, Vienna, Austria; 2 University of Veterinary Medicine Vienna, Clinical Department for Diagnostic Imaging, Infectious Diseases and Clinical Pathology, Vienna, Austria
- 676 **Anticoccidial efficacy of diclazuril (Clinacox®) in rabbits: three extensive field trials**
Lens S., and Van Lerberghe J.
Janssen Animal Health, Beerse, Belgium

- 677 **Safety of 5% ponazuril (totrazuril sulphone) oral suspension and efficacy against naturally acquired *Isospora ohioensis* infection in puppies**
Charles S.D. 1, Chopade H. 1, Ciszewski D.K. 1, Reinemeyer C.R. 2, and Settje T.L. 1
1 Bayer HealthCare LLC, Animal Health Division, Kansas, USA; 2 East Tennessee Clinical Research, Tennessee, USA
- 678 **A new anticoccidia drug BA3540**
Sung M.
PharmaLipoCore Inc., Raleigh NC, USA

Poster group 19: Toxoplasmosis

- 679 **Occurrence of *Toxoplasma gondii* and *Hammondia hammondi* oocysts in the faeces of cats from Germany and other European countries**
Scharas G. 1, Globokar Vrhovec M. 2, Pantchev N. 2, and Conraths F.J. 1
1 Friedrich-Loeffler-Institut - Federal Research Institute for Animal Health, Institute of Epidemiology, Wusterhausen, Germany; 2 VetMedLabor GmbH, Division of IDEXX Laboratories, Ludwigsburg, Germany
- 680 **Prevalence of *Toxoplasma gondii* in Belgian house cats**
De Craeye S., Van Gucht S., Jongert E., D'Souza S., Francart A., and Le Roux I.
Section of Rabies-Parasitology, Pasteur Institute of Brussels, Scientific Institute of Public Health, Brussels, Belgium
- 681 **Prevalence of anti-*Toxoplasma gondii* antibodies in serum samples from Marsupials In zoological parks in France**
Daudet A. 1, Maillot A. 2, Roman Y. 3, Petit T. 4, Arné P. 1, Aubert D. 5, Villena I. 5, and Guillot J. 1
1 UMR INRA, AFSSA, ENVA, UPVM 956 BIPAR, Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort, France; 2 Parc Zoologique d'Amnéville, France; 3 Parc Zoologique de Clères, France; 4 Parc Zoologique de La Palmyre, France; 5 Laboratoire Parasitologie-Mycologie, Centre National de Référence Toxoplasmosis, Hôpital Maison Blanche, Reims, France
- 682 **Serological survey of *Toxoplasma gondii* in naturally infected cattle in the Netherlands**
Opsteegh M. 1, 2, Langelaar M.F.M. 1, Fonville M.T.M. 1, Koets A.P. 3, and van der Giessen J.W.B. 1
1 National Institute for Public Health and the Environment (RIVM), Microbiological Laboratory for Health Protection, Bilthoven, the Netherlands; 2 Utrecht University, Institute for Risk Assessment Sciences, Utrecht, the Netherlands; 3 Utrecht University, Faculty of Veterinary Medicine, Department of Infectious Diseases and Immunology, Utrecht, the Netherlands
- 683 **The prevalence of serological diagnosed toxoplasmosis cases in animals from Romania between 2006 - 2007 years**
Petriceanu G. 1, Gutu E. 1, Radulescu R.A. 1, Ragalie A. 1, and Tanasuica R. 2
1 Institute for Diagnosis and Animal Health Romania; 2 Sanitary Veterinary and Food Safety Direction Bucharest, Romania
- 684 **Prevalence of *Toxoplasma gondii* in wild game meat from French Guyana**
Halos L. 1,2, de Broucker C.A. 3, Perret C. 2, Thomas M. 2, Marié J.L. 4, Bretagne S. 2, Boireau P. 2, and Davoust B. 4.
1 Unité de Parasitologie- Maladies parasitaires École Nationale Vétérinaire d'Alfort, Maisons Alfort, France; 2 UMR BIPAR INRA/AFSSA/ENVA/UPVM, Maisons Alfort, France ; 3 Direction Inter Armées du Service de Santé, Cayenne, Guyane, France; 4 Direction Régionale du Service de Santé des Armées, Toulon, France

- 685 **Seroprevalence of *Toxoplasma* infection in food animals of Fars Province, Southern Iran**
 Asgari Q. 1, Mohajeri F.A. 2, Motazedian M.H. 1, Kalantari M. 1, Moazeni M. 2, and Esmaeilzadeh B. 1
 1 Department of Parasitology and Mycology, Faculty of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran; 2 Department of Parasitology, Faculty of Veterinary Medicine, Shiraz University, Shiraz, Iran
- 686 **Survey on seroprevalence of anti-*Toxoplasma gondii* in cattle from Tabriz (Iran) by IFAT**
 Nematollahi A.
 University Tabriz, Faculty of Veterinary, Department of Parasitology, Tabriz, Iran
- 687 **Seroepidemiology of sheep toxoplasmosis in Babol northern Iran 2004**
 Youssefi M.R., Sefidgar S.A.A., and Norozian S.M.B.
 Parasitology & Mycology Department, Babol Medical Sciences, Iran
- 688 **Epidemic population structure of *Toxoplasma gondii* in Brazil revealed by multilocus pcr-rflp genotyping**
 Pena H.F.J. 1, Gennari S.M. 1, Dubey J.P. 2, and Su C. 3
 1 University of Sao Paulo, Department of Preventive Veterinary Medicine and Animal Health, Faculdade de Medicina Veterinaria e Zootecnia, Sao Paulo, Brazil; 2 United States Department of Agriculture, Agricultural Research Service, Animal and Natural Resources Institute, animal Parasitic Diseases Laboratory, Beltsville, USA; 3 The University of Tennessee, Department of Microbiology, Knoxville, USA
- 689 **Prevalence and risk factor studies on *Toxoplasma gondii* in ruminants of Punjab (India)**
 Sharma S., Bal M.S., Kumar H., Verma S., and Sandhu K.S.
 Department of Epidemiology and Preventive Veterinary Medicine, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India
- 690 **Occurrence of *Toxoplasma gondii* in urban rodents (*Rattus rattus*, *Rattus norvegicus* and *Mus musculus*) captured in São Paulo, SP, Brazil. Preliminary results**
 Muradian V., Oliveira L.N., and Gennari S.M.
 University of Sao Paulo, Veterinary Preventive Medicine and Animal Health, Sao Paulo, Brazil
- 691 **Seroepidemiological study of toxoplasmosis infection among high school girls Jolfa city using indirect immunofluorescence of assay**
 Mahdipourzareh N., Fallah E., Navazesh R., Kushavar H., and Nokhahi I.
 Tabriz University of Medical Sciences, Department of Medical – surgical Nursing, Tabriz, Iran
- 692 ***Toxoplasma gondii* in healthy lambs: which lambs are infected and why?**
 Mason S. 1, Quinnell R.J. 1, Dubey J.P. 2, and Smith J.E. 1
 1 University of Leeds, United Kingdom; 2 United States Department of Agriculture, Beltsville, Maryland, USA
- 693 **Development of an ELISA for the detection of *Toxoplasma gondii* antibodies in macropod marsupials**
 O’Handley R. 1, and Thompson R.C.A. 1
 1 Murdoch University, Department of Veterinary Biology & Biomedical Science, Perth, Western Australia
- 694 **Comparison of commercial elisa and modified direct agglutination tests for detection of *Toxoplasma gondii* antibodies in aborted and non-aborted ewes in Ahvaz, Iran**
 Hamidinejat H. 1, Ghorbanpour M. 2, Goraninezhad S. 3, Nabavi L. 1, and Akbarnezhad F. 4
 1 University Shaid Chamran, Department Veterinary Parasitology, Ahvaz, Iran; 2 University Shaid Chamran, Department Veterinary Microbiology, Ahvaz, Iran; 3 University Shaid Chamran, Department Veterinary Obstetrics, Ahvaz, Iran; 4 University Shaid Chamran, Ahvaz, Iran

- 695 **Clinical, obstetric and immunological observations on pregnant queens experimentally infected with different isolates of *Toxoplasma gondii* (Nicolle & Manceaux, 1909)**
Sakamoto C.A.M. 1,2, Costa A.J. 1, Gennari S.M. 3, Pena H.F.J. 3, Bichuette M.A. 2, Bettini C.M. 2, Amarante A.T. 4, and Bresciani K.D.S. 2
1 CPPAR/FCAV/UNESP, Center for Research in Animal Health, Via de acesso Prof. Paulo Donato Castellane, Jaboticabal, São Paulo, Brazil; 2 Departamento de Apoio, Produção e Saúde Animal, UNESP, Araçatuba, São Paulo, Brazil; 3 Departamento de Medicina Veterinária Preventiva e Saúde Animal, USP, São Paulo, Brazil; 5 Departamento de Parasitologia do Instituto de Biociências, UNESP, Botucatu, São Paulo, Brazil
- 696 **Development of a sensitive specific PCR assay for the detection of *Toxoplasma gondii* using the repetitive 529bp DNA fragment as genetic marker**
Song H.Q. 1, Zhang D.L. 2, Liao S.Q. 1, Weng Y.B. 1, Lin R.Q. 1, and Zhu X.Q. 1
1 College of Veterinary Medicine, South China Agricultural University, Guangzhou, PRChina; 2 Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Science, Lanzhou, PR China
- 697 **A GRA1-GRA7 cocktail DNA vaccine primes anti-*Toxoplasma* immune responses in mice and pigs**
Jongert E. 1, Melkebeek V. 2, De Craeye S. 1, and Cox E. 2
1 Laboratory for Toxoplasmosis, section of Rabies-Parasitology, Pasteur Institute of Brussels, Scientific Institute of Public Health, Brussels, Belgium; 2 Laboratory of Immunology, Departement of Virology, Parasitology and Immunology, Faculty of Veterinary Medicine, Ghent University, Belgium
- 698 **Isolation of *Toxoplasma gondii* from goat semen**
Santana L.F. 1, Pistoni C.D.N. 1, Lima R.C.A. 1, Santos T.R. 1, Mendonça R.P. 1, Lopes W.D.Z. 1, Oliveira G.P. 1, and Costa A.J. 1
1 CPPAR/FCAV/UNESP, Center for Research in Animal Health, Via de acesso Prof. Paulo Donato Castellane, Jaboticabal, São Paulo, Brazil
- 699 **Aspects of toxoplasmatic infection in reproductive organs of artificially infected male ovines (*Ovis aries*)**
Lopes W.D.Z. 1, Rodrigues J.D.F. 1, Souza F.A. 1, Rossanese W.M. 1, Santos R.S. 1, Santana L.F. 1, and Costa A.J. 1
1 CPPAR/FCAV/UNESP, Center for Research in Animal Health, Via de acesso Prof. Paulo Donato Castellane, Jaboticabal, São Paulo, Brazil
- 700 **Histopathologic evaluation of reproductive organs from male dogs inoculated with *Toxoplasma gondii***
Arantes T.P. 1, Machado R.F. 2, Lopes W.D.Z. 2, Pieroni J.S.P. 2, Pinto V.M.R. 2, Costa A.J. 2, and Oliveira G.P. 2
1 Intervet do Brasil Veterinária Ltda.; 2 CPPAR/FCAV/UNESP, Center for Research in Animal Health, Jaboticabal, São Paulo, Brazil

Poster group 20: *Cryptosporidium* and *Giardia*

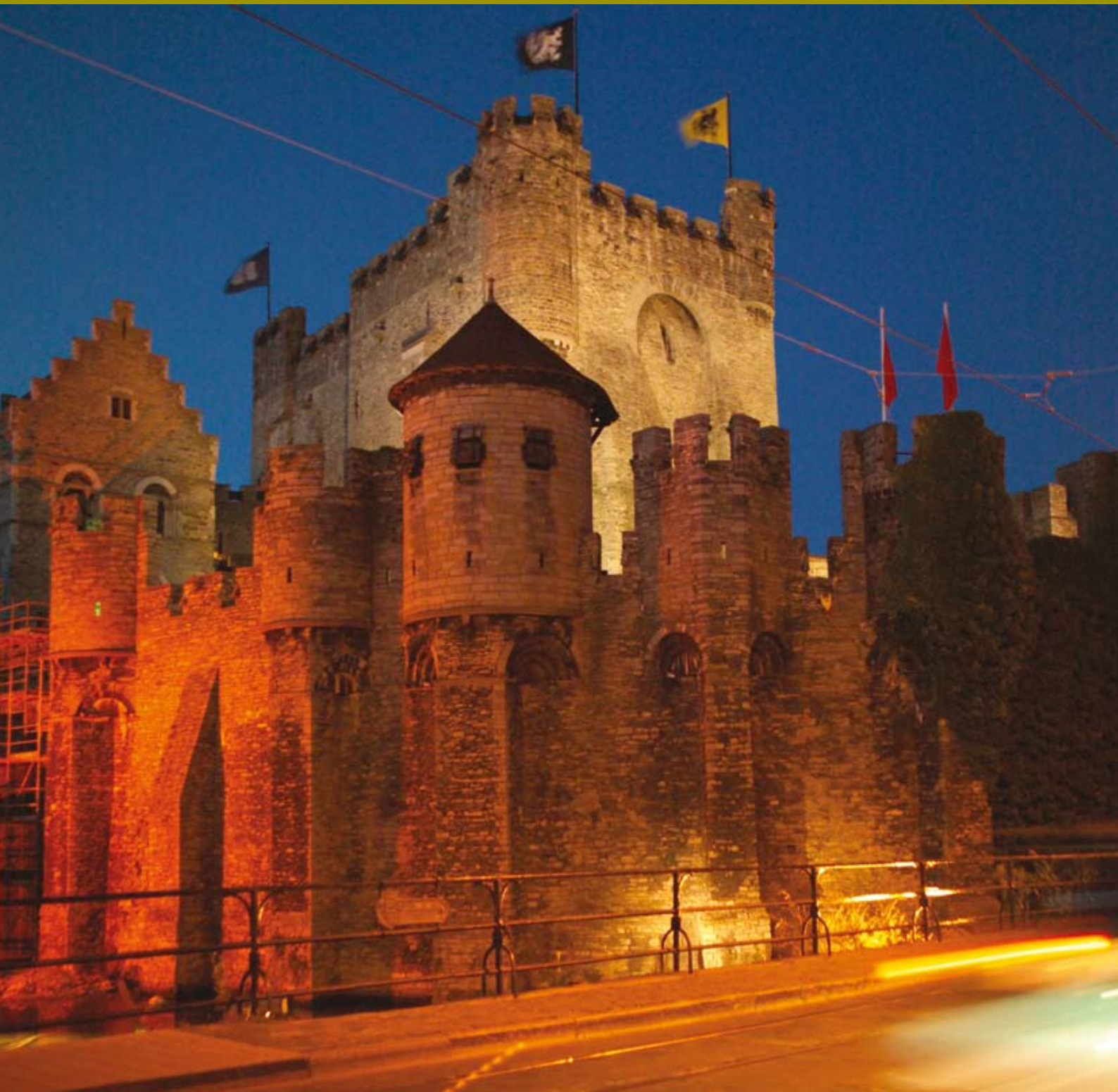
- 701 **Survey for the incidence of *Cryptosporidium* spp. oocysts from pet samples submitted to the laboratory**
Marshall J.A. 1, and Marshall R.N. 1
Veterinary Laboratories Agency (Weybridge), Addlestone, UK
- 702 ***Cryptosporidium* infection in Swedish dairy herds**
Silverlås C. 1, Emanuelson U. 1, and Björkman C. 1
Swedish University of Agricultural Sciences, Dept. of Clinical Sciences, div. of Ruminant Medicine and Epidemiology, Uppsala, Sweden

- 703 **Efficacy of halofuginone lactate on the prevention of cryptosporidiosis in Portugal dairy C-calves**
Martins S. 1, Sousa S. 1, Madeira de Carvalho L.M. 3, Bacelar J. 2, and Cannas da Silva J. 1,3
1 Laboratório de Medicina Veterinária, Ltd., Santarém, Portugal; 2 Escola Universitária Vasco da Gama, Laboratório de Parasitologia, Coimbra, Portugal; 3 Centro de Investigação Interdisciplinar Sanidade Animal, Faculdade Medicina Veterinária, Universidade Técnica Lisboa, Portugal; 4 Cooperativa Agrícola do Bunheiro, Murtosa, Portugal; 5 Intervet Portugal, Lda.
- 704 **Prevalence of bovine cryptosporidiosis in Sardinia (Italy)**
Scala A. 1, Basciu M. 1, Tanda B. 1, Arias M. 2, Cortiñas J. 2, Díaz P. 2, Sánchez-Andrade R. 2, and Paz-Silva A. 2
1 Dipartimento di Biologia Animale, Sezione di Parassitologia e Malattie Parassitarie, Facoltà di Medicina Veterinaria, Sassari, Italy; 2 Animal Pathology Department, Faculty of Veterinary Medicine, University of Santiago de Compostela, Lugo, Spain
- 705 **Genetic identity of *Cryptosporidium* species in captive Palaearctic European tortoise of the genus *Testudo***
Iorio R. 1, Slapeta J. 2, Modry D. 3, and Traversa D. 1
1 Department of Comparative Biomedical Sciences, Faculty of Veterinary Medicine, Teramo, Italy; 2 Faculty of Veterinary Science, University of Sydney, Sydney, Australia; 3 Department of Parasitology, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic
- 706 **Epidemiology and incidence of *Cryptosporidium* in dogs in Iran**
Aldavood S.J. 1, Haddadzadeh H.R. 1, and Akbarein H. 1
1 Veterinary Faculty of University of Tehran, Iran
- 707 **Infection by *Cryptosporidium* spp. in dogs from Brazil: Comparison between diagnostic methods and clinical and epidemiological analysis**
Bresciani K.D.S. 1, Amarante A.F.T. 2, Lima V.M.F. 3, Serrano A.C.M. 1, Ishizaki M.N. 1, Tome R.O. 1, Táparo C.V. 1, and Meireles M.V. 3
1 UNESP - Universidade Estadual Paulista, Departamento de Apoio, Produção e Saúde Animal, Faculdade de Odontologia, Araçatuba, SP, Brazil; 2 UNESP, Instituto de Biociências, Departamento de Parasitologia, Botucatu, SP, Brazil; 3 UNESP, Departamento de Clínica, Cirurgia e Reprodução Animal Araçatuba, SP, Brazil
- 708 **Ocurrence of *Cryptosporidium* spp. in domestic and captive wild birds in Brazil**
Meireles M. 1, Simões D. 1, Nakamura A. 2, Antunes R. 1, Soares R. 2, and Gennari S. 2
1 Curso de Medicina Veterinária, Universidade Estadual Paulista, Araçatuba, Brazil; 2 Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, Brazil
- 709 **Risk assessment of *Cryptosporidium* sp. in watershed associated with wildlife**
Ziegler P. 1, Wade S.1, and Mohammed H. 1
College of Veterinary Medicine, Cornell University, Department of Population Medicine and Diagnostic Sciences, Ithaca, New York, USA
- 710 **Natural infection with zoonotic subtype of *Cryptosporidium parvum* in Capybara (*Hydrochoerus hydrochaeris*) from Brazil**
Meireles M. 1, Soares R. 2, Bonello F. 3, and Gennari S. 2
1 Curso de Medicina Veterinária, Universidade Estadual Paulista, Araçatuba, Brazil; 2 Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, Brazil; 3 Faculdade de Ciências Agrárias e Veterinárias, Fundação Educacional de Andradina, Andradina, Brazil

- 711 **Molecular identification of *Cryptosporidium* spp. isolates from fecal samples of felines, canines and bovines in the State of São Paulo, Brazil**
 Thomaz A. 1, Meireles M.V. 2, Soares R.M. 1, Pena H.F.J. 1, and Gennari S.M. 1
1 University of Sao Paulo, Department of Preventive Veterinary Medicine and Animal Health, Faculdade de Medicina Veterinária e Zootecnia, Sao Paulo, Brazil; 2 Curso de Medicina Veterinária, Universidade Estadual Paulista, Araçatuba, Brazil
- 712 **Molecular characterisation of *Cryptosporidium* isolates from pet reptiles**
 Pedraza-Díaz S. 1, Gómez-Bautista M. 1, Álvarez Carrión B. 2, Navarro V. 1, López-Pérez I.C. 1, and Ortega-Mora L.M. 1
1 SALUVET, Animal Health Department, Faculty of Veterinary Sciences, Complutense University of Madrid, Ciudad Universitaria s/n, 28040 Madrid, Spain; 2 Clínica Camaleón, Madrid, Spain
- 713 **Neonatal foal cryptosporidiosis in New Zealand**
 Grinberg A. 1, Pomroy W.E. 1, Learmonth J.J. 1, Kwan E. 1, Carslake H. 1, and Widmer G. 2
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- 714 **Comparison of three halofuginone treatment regimens in the control of ovine cryptosporidiosis**
 Naciri N. 1, Bareille S. 2, and Fournier R. 2
1 INRA, UR1282, IASP213, Nouzilly, France; 2 Intervet SA, Angers Technopole, Beaucouzé, France
- 715 **Control of cryptosporidiosis in neonatal calves: the use of halofuginone lactate**
 de Waele V. 1, Murphy T. 1, and Mulcahy G. 2
1 Central Veterinary Laboratory, Baskweston, Celbridge, Co. Kildare, Ireland; 2 School of Agriculture, Food Science and Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland
- 716 **Prophylactic and therapeutic use of nitazoxanide against cryptosporidiosis in calves**
 Schnyder M. 1, Kohler L. 1., Hemphill A. 2, and Deplazes P. 1
1 Institute of Parasitology, Vetsuisse Faculty, University of Zurich, Switzerland; 2 Institute of Parasitology, Vetsuisse Faculty, University of Berne, Berne, Switzerland
- 717 **Efficacy of nitazoxanide against experimental cryptosporidiosis in goat neonates**
 Viel H. 1, Rocques H. 1, Martin J. 1, and Chartier C. 1
1 French Food Safety Agency (AFSSA), Laboratoire d'Etudes et de Recherches Caprines, Niort Cedex, France
- 718 **Stability of *Cryptosporidium parvum* oocysts against chlorine for treatment of water**
 Shemshadi B. 1, and Hosseini H. 2
1 Islamic azad university, Garmsar branch, Garmsar, Iran; 2 Food & Drug control Lab, Ministry of Health and National Nutrition & Food Technology Institute, Shahid Beheshti Medical University, Tehran, Iran
- 719 **Avoiding codon usage bias for better expression of the *Cryptosporidium parvum* protein P23 in prokaryote systems**
 Strubbe B.M., de Graaf D.C., and Jacobs F.J.
Ghent University, Laboratory of Zoophysiology, Ghent, Belgium
- 720 ***Giardia duodenalis*: novel proteins from an ancient eukaryote**
 Steuart R. 1, O'Handley R. 1, Lipscombe R. 2, and Thompson R.C.A. 1
1 Environmental Biotechnology Co-operative Research Centre, School of Veterinary and Biomedical Science, Murdoch University, Western Australia; 2 Proteomics International, Perth, Western Australia

- 721 **Molecular identification of *Giardia duodenalis* isolates from humans, dogs, cats and cattle from São Paulo state, Brazil by sequence analysis of fragments of glutamate dehydrogenase coding gene**
Souza S.L.P. 1, Gennari S.M. 1, Richtzenhain L.J. 1, Pena H.F.J. 1, Funada M.R. 1, and Soares R.M. 1
1 University of São Paulo, Department of Preventive Veterinary Medicine and Animal Health, São Paulo, Brazil
- 722 **Prevalence, and molecular biologic characterization of *Giardia* in household dogs in Hungary**
Szénási Z. 1, Marton S. 1, Kucsera I. 1, Tánczos B. 1, Orosz E. 1, Lukács Z. 2, and Szeidemann Z. 2
1 National Center of Epidemiology, Department of Parasitology, Budapest, Hungary; 2 Bayer Hungaria Ltd., Budapest, Hungary
- 723 ***Giardia* spp. in dogs: Genotyping of 75 samples from randomly selected dogs presented at local veterinary clinics**
Barutzki D. 1, Thompson R.C.A. 2, Wielinga C. 2, and Schaper R. 3
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- 724 ***Giardia* prevalence in household dogs and cats and validation of a commercial ELISA test**
Brianti E. 1, Otranto D. 2, Ferlazzo M. 1, Gaglio G. 1, and Giannetto S. 1
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- 725 **Prevalence of *Giardia intestinalis* infection in Punjab (India) and its association with diarrhea in dairy calves**
Singh B.B. 1, Sharma R. 1, Kumar H. 2, Banga H.S. 3, Gill J.P.S. 1, Aulakh R.S. 1, and Sharma J.K. 1
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- 726 **Prevalence and genotyping characterization of *Giardia duodenalis* infecting water buffalo calves (*Bubalus bubalis*) from Egypt**
Hilali M. 1, Nassar A. 1, Salem G. 2, and Gurgis S. 2
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ABSTRACTS ORAL COMMUNICATIONS



001 **African trypanosomiasis: from immune escape and immunopathology to immune intervention**

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African trypanosomes can cause prolonged chronic infections through a mechanism of antigen variation whereby they manipulate the humoral immune system of their hosts. However, besides antigenic variation these extra-cellular parasites exert other immunoregulatory activities mainly mediated by innate cells in particular macrophage-like (M) cells. In this review the modulation of M cells through parasite factors and host cytokines as well as their role in parasite control and immunopathology will be examined. The concept of M cell polarization into distinct activation states (M1, M2) that may contribute to trypanosusceptibility or resistance will be discussed. Finally, the possibility to interfere with such activation states hereby providing new therapeutical modalities in the treatment of this infectious disease will be illustrated.

002 **The effect of nematode infections on vaccination to protozoal and bacterial infection**

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T helper (Th) cells produce signature cytokine patterns, induced largely by intracellular versus extra cellular pathogens, that provide the cellular and molecular basis for counter regulatory expression of protective immunity during concurrent infections. The production of IL-12 and IFN- γ , for example, resulting from exposure to many bacterial, viral, and protozoan pathogens is responsible for Th1-derived protective responses that also can inhibit development of Th2-cells expressing IL-4-dependent immunity to extra cellular helminth parasites and vice versa. In a similar manner, concurrent helminth infection alters optimal vaccine-induced responses in humans and livestock; however, the consequences of this condition have not been adequately studied especially in the context of a challenge infection following vaccination. Demands for new and effective vaccines to control chronic and emerging diseases, and the need for rapid deployment of vaccines for bio security concerns requires a systematic evaluation of confounding factors that limit vaccine efficacy. One common albeit overlooked confounder is the presence of gastrointestinal nematode parasites in populations of humans and livestock targeted for vaccination. This is particularly important in areas of the world where helminth infections are prevalent, but the interplay between parasites and emerging diseases that can be transmitted worldwide make this a global issue. In addition, it is not clear if the epidemic in allergic disease in industrialized countries substitutes for geohelminth infection to interfere with effective vaccination regimens. This presentation will focus on recent vaccination studies in mice experimentally infected with *Heligmosomoides polygyrus* to model the condition of gastrointestinal parasite infestation in mammalian populations targeted for vaccination. In addition, a large animal vaccination and challenge model against *Mycoplasma hyopneumonia* in swine exposed to *Ascaris suum* will provide a specific example of the need for further work in this area, and for controlled field studies to assess the impact of other similar scenarios.

003 **Interactions between helminths and tuberculosis in man**

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Tuberculosis has become a major public health threat again due to the combined effect of the deteriorating socio-economic situations and the emergence of HIV/AIDS pandemic. The only vaccine available against TB, although good at reducing the burden of childhood TB, shows enormous variability in its efficacy against pulmonary TB which is the most common form of the disease in adults. Most areas of high TB incidence and poor TB efficacy were reported to have high prevalence of intestinal helminth infections. Such infections have been shown to cause a range of immunomodulation characterized by enhanced Th2 type cytokine profile, high IgE levels, up-regulated regulatory T cell activity as well as chronic immune activation. Altered background immune profile could have adverse effects on the outcome of subsequent infections as well as vaccinations. In support of this hypothesis, studies conducted in animals as well as humans living in worm endemic areas have shown that helminths impair resistance against a number of infections of major public health importance including tuberculosis, malaria as well as HIV/AIDS. Understanding such interaction could assist in the design of vaccines against these diseases.

004 **Helminths and malaria - interactions in man**

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Schistosoma mansoni and malaria infections are often endemic in the same communities in sub-Saharan Africa, and both have pathological effects on the liver and the spleen. Hepatosplenomegaly associated with *S. mansoni* is exacerbated in children with relatively high exposure to malaria. Treatment with praziquantel reduces the degree of hepatosplenomegaly, but the condition does not completely resolve in some cases. We have found co-infection with schistosomiasis and malaria a major risk factor in this form of childhood morbidity, and that T-helper 1 anti-malaria cytokines may be skewing children's responses to schistosome eggs, that lodge in the microvasculature of their livers, suppressing the usual T-helper 2 anti-inflammatory, while promoting more damaging proinflammatory, responses. We have found co-infection with schistosomiasis and malaria a major risk factor in this form of childhood morbidity, and that T-helper 1 anti-malaria cytokines may be skewing children's responses to schistosome eggs, that lodge in the microvasculature of their livers, suppressing the usual T-helper 2 anti-inflammatory, while promoting more damaging proinflammatory, responses. The present analysis focused on the possibility that exposure to malaria infection may have limited the resolution of hepatosplenomegaly in a cohort of Kenyan schoolchildren. exposure to malaria infection may be a significant factor affecting the outcome of praziquantel treatment to reduce the level of hepatosplenic morbidity.

005 **The immunoepidemiology of nematode infections in humans**

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Our immunoepidemiological studies on nematode infections in different Cameroonian cohorts (at Ayéné and Nyomo) suggest that resistance to *Ascaris lumbricoides* and *Trichuris trichiura* is positively associated with Th2 responses (reflected by levels of IL-5 and IL-13 expression in parasite-specific whole blood recall assays). At Nyomo, a longitudinal reinfection study further indicated that IL-5 recall responses were associated with resistance to reinfection with *T. trichiura* but not *A. lumbricoides*. Also, Th2 mediators measured before treatment were significantly negatively associated with contemporaneous *A. lumbricoides* infection, but then with *T. trichiura* infection 8-9 months post-treatment. This suggests a successional interaction between Th2-driven immune responses and species infection over time. The existence of complex immune-mediated interspecific interactions was also indicated by a longitudinal study in another Cameroonian cohort (at Mbangassina) co-exposed to *Onchocerca volvulus*, *A. lumbricoides* and *T. trichiura*. In this case, Th2 mediators (IL-5 and IL-3 expression in parasite-specific whole blood recall assays; specific IgE serum levels) were found to correlate negatively with *O. volvulus* microfilaraemia, but unusually not to *A. lumbricoides* and *T. trichiura* fecundities. This suggests a trade off in allocating immune resources to the more pathogenic filarial tissue infection. The levels of regulatory responses, exemplified by constitutive levels of IL-10 and TGF β , were positively associated with GI nematode worm burden and negatively associated with responses to bystander antigens. The role of regulatory responses induced by chronic long-lived helminth parasites to the outcome of other infections will be discussed.

006 **Immunomodulation in *Fasciola hepatica* infection; implications for animal health**Flynn R.J., and Mulcahy G.*School of Agriculture Food Science and Veterinary Medicine, University College Dublin, Delfield, Dublin 4 Ireland*

The helminth *Fasciola hepatica* can persist in a bovine host for extended periods of time by evading the host's immune system and subverting effector mechanisms aimed at parasite destruction. We have studied the effects that infection and parasite derived molecules have on macrophage activation, in vivo and in vitro respectively. Macrophages derived from *F. hepatica* infected animals and *F. hepatica*/*Mycobacterium bovis* BCG co-infected animals were examined. Monocyte derived macrophages were also alternatively-activated by parasite molecules and their subsequent responses to TLR2 and TLR 4 antagonists were examined. In vitro, alternative activation of both ovine and bovine macrophages was achieved by parasite derived molecules. In vivo, macrophages isolated from infected and co-infected animals were also found to be alternatively activated. These co-infected animals were found to have altered *M. bovis* BCG specific immune responses, including poor IFN – gamma production, higher IL – 4 levels and reduced DTH responses. These effects have implications for the control of bovine tuberculosis. In vitro, negative effects of parasite-derived molecules were seen when co-stimulation occurred with TLR 2 and TLR 4 antagonists. These included reduced nitric oxide and IFN – gamma release and higher levels of the regulatory cytokine IL – 10. *Fasciola hepatica* infection, therefore, may have an impact on the ability of cattle to deal with other pathogens, and on their response to diagnostic tests.

007 ***Dirofilaria immitis* - clinically relevant immunoregulation in dogs?**

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Dirofilaria immitis causes canine and feline heartworm disease worldwide. Adult worms survive over 5 years in the immune-competent host and the immune response during infection is still under study. Like many pathogenic filariae (*Onchocerca* sp, *Brugia* sp, *Wuchereria bancrofti*), *D. immitis* harbours the intracellular bacteria Wolbachia. It is now widely accepted that the endosymbiotic relationship between Wolbachia and the filarial host is essential for both worm fecundity and survival. More recent research, however, has been aimed at defining what role Wolbachia may play in the immune response in the filarial-infected host and it has been shown that Wolbachia interacts specifically with the innate and acquired immune systems. Interestingly, evidence suggests that Wolbachia is also in part responsible for both inflammatory pathology and immunotolerance during filarial infection. Indeed, Wolbachia-derived proteins act as pathogen-associated molecular patterns (PAMPs) through contact with Toll-like receptors (TLRs), thus stimulating innate immune responses. The immunomodulatory activity of Wolbachia includes for example the stimulation of iNOS and NO production by macrophages and production of interleukin (IL)-8 by neutrophils. It has also been reported that monocytes from filarial-infected humans incubated for prolonged periods with a major surface protein of Wolbachia (WSP) produce high levels of IL-10, suggesting that Wolbachia may contribute to the down-regulation of pro-inflammatory mediators, thus establishing the necessary homeostasis for chronic infection. More recently it has been reported that inflammatory responses to Wolbachia is TLR2 and TLR6 – dependent. What potential influence could *D. immitis* infection, and in particular interaction with Wolbachia, have on innate immune responses to other pathogens and on immune function in general will be the topic of this presentation.

008 **Egg excretion by gastrointestinal strongyles in dairy goats: circadian rhythm and relation with worm burden**

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Gastrointestinal (GI) strongyle infections remain one of the main constraints to goat production worldwide. Even if several indicators of GI strongyle infections have been proposed, Faecal egg count (FEC) techniques remain the most common approach for the estimation of their prevalence and intensity. However, FEC may be subjected to a within-individual variation due to factors like weather, season, random day-to-day variation, phase of the parasitic infection and hour of sampling. A study on GI strongyle FEC in dairy goats was conducted to evaluate the circadian rhythm of the GI strongyle egg excretion and the relationship between FEC and worm burden. The study was performed on a total of 63 female Siriana goats, 1.5 years old, at their second pasturing season, naturally infected by *Teladorsagia circumcincta*, *Haemonchus contortus*, *Trichostrongylus colubriformis* and *Oesophagostomum venulosum*. The circadian rhythm of egg excretion by GI strongyles was studied each 3 weeks for 21 weeks, utilizing 3 individually caged female Siriana goats. Faecal samples were collected from each cage every 2 h, and were analyzed by the FLOTAC technique. The results of the General Linear model did not revealed any significant effect of the hour ($P = 0.449$), whereas a significant effect of the week (seasonality) was found ($P = 0.000$). The same 3 goats used for the experiment 1 were then slaughtered. Thus, the relationship between worm burden (at species level) and EPG was evaluated using Pearson correlation. Positive relationship between EPG and worm counts ($P = 0.000$) was found, in particular regarding *H. contortus*.

009 **Sequential patterns of serum and milk antibodies following experimental infection with *Dictyocaulus viviparus* in lactating dairy cows**

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During the development of a bulk milk tank ELISA for *Dictyocaulus viviparus*, the course of infection and the immune response were determined in adult lactating dairy cows. Twenty housed dairy cows were each infected with 2000 infective *D. viviparus* larvae. Larvae were first detected in the faeces 22-26 days post infection (pi) and shedding continued for 49 days. Antibody titres in sera and milk were evaluated using a recombinant antigen derived from a Major Sperm Protein (MSP), present in sexually mature *D. viviparus* only. In the sera, MSP antibodies above the predefined cut-off value were detected in all cows by 30 days pi and peaked shortly afterwards, following which they gradually declined, remaining above the cut-off value for 125 days. Antibody titres in milk were lower than those in sera, but the evolution was similar in that antibodies exceeded the cut-off value 29 days pi and they remained above this value for 68 days. In another study using the same infection protocol, treatment with eprinomectin on day 19 pi, prior to patency, resulted in an absence of larval shedding and no antibody response, confirming the specificity of the ELISA to adult worms only. Treatment on day 50 pi (25 days after larvae were first detected) eliminated larval shedding immediately, but had limited effect on the evolution of antibody titres. Serum and milk antibodies in the treated cows were detected above the cut-off value at day 29 pi remaining for 134 days in serum and for 98 days in milk.

010 **A survey to determine the range of bulk tank milk antibody titres to *Ostertagia ostertagi* in West European countries**

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Quantitative measurements of antibody titres to *Ostertagia ostertagi* in bulk tank milk (BTM) have value as diagnostic indicators for potential production losses and anthelmintic treatment responses in dairy herds. Most of the initial studies in Europe have been undertaken in Belgium; the purpose of this study was to determine the distribution of *O. ostertagi* titres in other European countries. BTM samples were collected during the autumn of 2005 and 2006 from a total of 2206 dairy herds from important dairy regions in Denmark, France, Germany, Italy, the Netherlands, Portugal, the Republic of Ireland, Spain and the UK. Antibody titres to *O. ostertagi* were determined by indirect ELISA and expressed as optical density ratios (ODR). In addition, relationships between ODR and management practices were investigated. For each country the mean ODR and the 25th to 75th percentile values were determined. Mean ODR values ranged from a low of 0.20 in Italy to a high of 0.60 in France, with an overall range of -0.21 to 1.57. Variations between countries appeared to reflect either different husbandry practices - particularly those related to access to pasture - or seasonality. The ODR values obtained from those countries where husbandry practices and the timing of sample collection were similar to the studies undertaken in Belgium were of the same order as the ODR values from Belgium and, therefore, it should be reasonable to extrapolate from the Belgian results and association analyses to other European countries.

011 **An allergen protein for *Fasciola hepatica* diagnosis**

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Fasciolosis is an important economic disease in ruminants and is present in several parts of the world and also in some regions of Portugal (Vagos and Baixo Mondego regions). The development of a high sensitivity and specificity diagnostic test is very important to support the fasciolicide application, avoiding unnecessary treatments and contributing towards the prevention of anthelmintic resistances.

Recombinant antigens homologous to ESP (excretion/secretion) proteins were expressed in *E. coli* M15 (pREP4) using expression vector pQE (Qiagen). A recombinant antigen, designated as *Fasciola hepatica* allergen protein (rFhAP) was identified and produced. This rFhAP showed characteristics of an allergen, namely the induction of IgE when injected in BALB/c mice and the induction of a hypersensitivity response when used in skin test in infected ruminants. It was applied as an intradermoreaction in experimentally infected sheep and naturally infected cattle. In sheep, the site of application, dose, reading time and wheal dimensions were defined. In this animal species the inflammatory reaction is observed 45 minutes after inoculation. This reaction consisted of an increased skin thickness of 3 mm and a visible red wheal of 2cm diameter. In cattle, the reading time is shorter (15 minutes) and oedema and swollen lymph nodes are frequently evident signs. In these animal species no anaphylactic or discomfort reaction were observed. The rFhAP will provide a reliable, cheap, quick and easy to apply tool for *F. hepatica* diagnosis under field conditions, though as an individual diagnostic tool. This protein has already been registered as a patent.

012 **Towards a quantitative interpretation of the existing coprological and serological techniques for the diagnosis of fasciolosis in cattle**

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The diagnosis of fasciolosis in cattle relies to a large extent on the detection of *Fasciola hepatica* eggs in the faeces or *F. hepatica* specific antibodies in serum. However, these tests are only qualitative and do not estimate the intensity of infection or the extent of associated morbidity. Moreover, the effect of season on their performance has insufficiently been investigated. Therefore, our objectives were (1) to investigate the relationships between the outcomes of the currently used coprological and serological tests and intensity of infection and morbidity parameters and (2) to assess if seasonality influences these relationships. In the spring and the autumn of 2006, 100 livers were collected each time from adult cows in the abattoir, together with a corresponding blood and faecal sample. Within each sampling period the test outcomes of 2 sedimentation-flotation techniques and 2 indirect ELISAs were related to the number of flukes present in the liver, a liver score indicating the extent of visible fibrosis and the level of gamma glutamyl transferase (GGT) in serum. Forty-nine % of the inspected livers was infected with liver fluke. Morbidity (liver score and increased GGT-levels) was associated with levels of infection. Animals with a high worm burden (> 30 flukes) could be identified using a sedimentation-flotation test using 4 g of faeces (Se 93%, Sp 90%). Serological test outcomes were associated with intensity of infection and morbidity and could be used for a quantitative diagnosis of fasciolosis in the future. No substantial differences were observed between seasons in the infestation level or diagnostic test performances.

013 **Estimating the prevalence of *Fasciola hepatica* in cattle: which method is the best diagnostic test?**

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Introduction. Prevalence of *Fasciola hepatica* seems to be decreasing in most countries. Nevertheless, fasciolosis induces severe financial losses mainly in dairy herds. Many diagnostic tests are available and it is necessary to choose the best adapted to local conditions. It is the reason why a comparison has been done between several validated methods. Material and methods. Three hundred and ten heifers close to their first delivery have been chosen randomly in 31 herds in France. ELISA tests for specific antibody detection in serum either with ES antigen or with f2 fraction, and coproscopy either with routine sedimentation test on 5 g of faeces or improved sieving method on 50 g of faeces have been performed. Results. Antibody ELISA test revealed that 79% of cattle were positive with ES Ag but only 25,6% with F2 fraction. Comparison of the two coproscopy methods showed that examination of 5 g indicated that only 11,3% were eggs excreting but improved sieving method on 50 g detected 31,6% of infected cattle excreting eggs. In the same survey, 33 % of cattle were excreting paramphistomids eggs with the 5 g method and 67% with the 50 g. Conclusion: We confirm the results of Rapsch *et al* 2006 estimating that the prevalence of bovine fasciolosis is higher than previously thought due to low sensitivity of routine methods. It appears that increasing the amount of faeces examined in a simple sieving method detects more cattle excreting eggs.

014 **Tanniferous forage plants with anthelmintic properties: the example of sainfoin (*Onobrychis viciifolia*)**

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Introduction: Sainfoin is a high-quality condensed tannin containing forage plant. The knowledge about its anthelmintic effects against sheep nematodes and about the influence of plant preservation (e.g. ensiling) on the anthelmintic activity is limited.

Method: 36 parasite naïve lambs (6 groups of 6 animals each) were infected with *H. contortus* (7,000 L3) and *C. curticei* (15,000 L3). From day 28 until day 44 p.i., groups A1, B1 and C1 received fresh, dried or ensiled sainfoin. Groups A2, B2 and C2 served as respective tannin-free controls. Individual faecal egg counts on a dry matter basis (FECDM) were performed every 3-4 days. On day 44, all animals were slaughtered and adult worm populations were determined.

Results: The consumption of sainfoin was associated with a reduction of adult *H. contortus* (35% (P<0.1), 47% (P<0.05) and 49% (P<0.1) for fresh, dried and ensiled sainfoin, respectively) but had little effect on adult *C. curticei*. Compared to the controls, *H. contortus* specific FECDM were reduced by 62% (P<0.05), 58% (P<0.01) and 48% (P<0.1) for fresh, dried and ensiled sainfoin. FECDM specific to *C. curticei* were decreased by sainfoin hay and silage (hay 81% , silage 74%, both tests P<0.001) but were unchanged when fresh sainfoin was administered.

Conclusion: For *H. contortus* the FECDM decrease seemed to be due to a nematocidal effect towards adult *H. contortus*. In contrast for *C. curticei*, the reduction in FECDM appeared to be a result of a reduced per capita fecundity. The use of conserved tanniferous fodder offers promising perspectives as a complementary control approach against nematodes in sheep.

015 **Effects of sainfoin (*Onobrychis viciifoliae*) extracts on the establishment of abomasal nematode larvae in fundic explants**

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The anthelmintic properties possessed by tannin-rich legume forages offers a possible alternative solution to the current chemicals that are employed to control gastrointestinal nematodes. However, the mode of action of these bioactive plants remains largely unknown. It has been previously shown that tannin-rich extracts interfere with the primary phase of the host invasion process, i.e. the exsheathment of the infective third-stage larvae (L3). In this study, we examined the hypothesis that exposure to tannins would also affect the secondary phase in the processes that lead to parasitic establishment, i.e. the close association of the exsheathed larvae with the mucosal surface and entry of the larvae into the digestive mucosae. An in vitro direct challenge technique using fundic tissue explants (6 replicates per treatment) was used in the study. Following 3 hours of contact with sainfoin extracts (1200 µg/ml) the association of exsheathed L3 of *Haemonchus contortus* and *Teladorsagia circumcincta* with the fundic mucosae was significantly reduced. With *Haemonchus contortus*, a significant dose-response relationship was demonstrated by comparing the effects of 1200, 600 and 300 µg extracts/ml on mucosal association/tissue penetration. For both nematode species, the effect was totally alleviated after PVPP, an inhibitor of tannins, was added to the sainfoin extracts (1200 µg/ml). This last result suggests that condensed tannins may play a major role in the observed effects. Together with the delay of inhibition of larval exsheathment previously demonstrated, these effects could explain how tanniniferous plant might serve to reduce the establishment of infective larvae in small ruminants.

016 **Influence of inulin on *Trichuris suis* infection in pigs: elimination by metabolic products**

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Our previous investigations have shown that the highly degradable carbohydrate inulin, widely recognized for its probiotic and bifidogenic properties, can significantly reduce *Oesophagostomum dentatum* (nodular worm) infection in the pig. The objective of this experiment was to investigate the influence of inulin on *Trichuris suis* infection in growing pigs. Two experimental diets were formulated based on barley flour with added either insoluble fibre from oat husk (Diet 1) or a pure inulin (16%) supplementation (Diet 2). Twenty-eight 10-week old pigs were divided randomly into 4 groups (Groups 1-4) each of 7 pigs. All pigs were infected with a single dose of 2,000 infective *T. suis* eggs. *Trichuris suis* worm burdens were determined for all pigs. Preventive use of inulin reduced the EPG by 87% compared to the pigs on standard diet ($P < 0.0001$), and the number of worms recovered from pigs on the inulin diet was significantly reduced by 71% compared to the pigs on standard diet ($P < 0.01$). Therapeutic use of inulin resulted in 47% reduction of the worm burden compared to the control pigs ($P < 0.01$). Further, the inulin-fed pigs exhibited a significant reduction in female worm fecundity and worm large intestine location was more distal compared to those from pigs on standard diet. Thus, our findings suggest that the biochemical changes associated with fermentation of carbohydrates of which the major part derived from the mix of oligo- and polysaccharides from inulin in the large intestine can have significant antiparasitic effects and underscores the role that the intestinal microflora can have on the host-parasite relationship.

017 **The effect of dietary fructans on *Oesophagostomum dentatum* and *Ascaris suum* infections in pigs**

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Introduction: Dietary fructans that are easily fermented in the large intestine can influence aspects of porcine helminth infections such as worm burden and fecundity. To utilise this as a natural alternative to anthelmintics is of particular interest in the organic pig production.

Method: Fifty outdoor pigs were trickle infected in the diet (control diet) with *Oesophagostomum dentatum* (weeks 2-7) and *Ascaris suum* (weeks 0-7). Week 7, 5 groups of 10 pigs were established. One group continued on the control diet while 3 groups were given a diet including fructan rich dried chicory roots (CR) and 1 group was given a diet containing purified CR fructans (Inulin/Raftiline®ST, Orafit, Belgium). Week 8, after 1 week on the CR diet 1 group was returned to the control diet. This was repeated week 9 for a second CR group. Egg counts and weight gain were monitored until slaughter week 11 for worm recovery.

Materials: The pigs were helminth free Danish Landrace/Yorkshire/Duroc crossbreeds.

Results and conclusion: Within 2 days *O. dentatum* egg counts were reduced by 94-98% by the experimental diets. After removal of the CR diet egg counts returned within 1-2 weeks. *Oesophagostomum dentatum* worm burdens were reduced by 68-94%. Two and 4 weeks feeding with CR were more effective than 1 week. Four weeks feeding with CR and Inulin was statistically comparable. Weight gain was not statistically affected by the diets. As there was no effect on the *A. suum* infections it appears that dried CR may only be used to help control *O. dentatum* infections.

018 **An increasing level of maize supplementation provides better control of gastrointestinal nematodes in browsing Criollo goats**

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The objective was to explore the effect of different levels of maize (M) supplementation on the resilience and resistance of browsing kids against gastrointestinal nematodes (GIN). 42 five months-old Criollo kids (c. 15 kg BW) were allocated into five groups: infected, not supplemented (I-NS) (n=10), infected and supplemented (I-S) with 108 g/d M (I-S108), I-S at 1% (I-S1%) and 1.5% BW (I-S1.5%) and a non infected group (moxidectin 0.2 mg/kg BW s.c. every 28 d) supplemented at 1.5% BW (NI-S1.5%) (in all four group n=8). Animals were weighed every 7 days to adjust supplementation levels. Blood and faecal samples were collected every 14 days to determine PCV, Hb and eggs per gram (EPG) of faeces. At the end of the trial (day 112), four kids from each group were slaughtered to determine the adult worm burdens, female worm lengths and number of eggs in utero. Groups I-S108, I-S1% and I-S1.5% had higher weight gain, PCV and Hb than I-NS group (P<0.05). Resilience was improved in all the supplemented groups compared to the I-NS group (P<0.05). Growth rate of the I-S1.5% reached that of the NI-S1.5% (P>0.05). Furthermore, the I-S1.5% group had 55% lower EPG than I-NS group (P<0.05). The I-S1.5% group showed a tendency to reduce its worm burdens compared to I-NS but was only significant for *Trichostrongylus colubriformis* (P<0.05). An increasing quantity of M supplement, relative to animal's BW, was more effective than a fixed quantity for improving resilience and resistance of browsing kids against GIN infections.

019 **Effect of *Sericea lespedeza* hay feeding on growth rate of goats infected with gastrointestinal nematodes**

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Infection with gastrointestinal nematodes (GIN) is a major constraint to economic production of goats in the USA. Widespread anthelmintic resistance in goat GIN populations world-wide has led to development of alternative control strategies, including use of forages containing condensed tannins (CT). *Sericea lespedeza* [SL, *Lespedeza cuneata* (Dum-Cours.) G. Don], a high CT forage, has been reported to reduce GIN levels in goats when fed as hay, but little is known about its effect on growth rate in young goats. An experiment was designed using infected and non-infected male kids (Kiko X Spanish, 6 mo. old) fed diets consisting of 75% hay [SL or bermudagrass (BG; *Cynodon dactylon* (L.) Pers.)] and 25% concentrate (n = 10 per treatment). The kids were weighed every two weeks, and fecal and blood samples taken weekly for fecal egg count (FEC) and packed cell volume (PCV) determination, respectively. Animals were slaughtered at the end of the trial and GIN in the abomasum and small intestines recovered and counted. Parasite infection levels were relatively low in both infected groups by the end of the trial, but SL infected kids had consistently lower (P<0.05) FEC and higher (P<0.05) PCV than the BG infected goats. Adult worm numbers were not different. The SL infected and non-infected kids gained 101 and 109 g/d, respectively, compared with 72 and 79 g/d in BG-fed kids. Feeding SL hay reduced effects of GIN infection and increased animal performance compared with BG hay, suggesting that the CT in this plant had beneficial nutritional and anti-parasitic properties.

020 **Chemical genetic approach to novel neurological receptor discovery in *Caenorhabditis elegans***

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The free living nematode *C. elegans* is a useful model for parasitic nematode species and has previously been used to define the mode of action of anthelmintic drugs in common use. The strategy has been to use mutagenesis and classical genetic mapping to identify the responsible mutations. Compounds with novel anthelmintic activities might be useful to identify novel neurological receptors using a similar strategy. This project involves the production and screening of mutant *C. elegans* strains for resistance to a compound with novel anthelmintic activity. Phenotypic and genetic analysis suggests that this compound acts by a different mode-of-action to anthelmintic compounds in current use. Resistant mutants have been isolated and characterised and are being mapped using snip-SNP mapping.

021 **Modulation of nematode and arthropod reproductive systems by anthelmintics, with special focus on ivermectin**

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The control of parasites of livestock and companion animals relies solely on a few frontline drugs and a variety of generics. The most successful class of anthelmintic marketed to date is the macrocyclic lactones comprising the milbemycins and the avermectins. They have broad spectrum activity against both arthropod and nematode parasites and have high potency and persistence. A common feature of the main anthelmintic classes is that they target motor function and compromise neuromuscular control in parasites. Increasing resistance to anthelmintics is becoming a serious problem in parasite control and it is important to fully understand the mode of action of anthelmintics to determine how resistance develops. This work aims to discover if anthelmintics modulate reproductive function by testing them on nematode and arthropod reproductive systems, leading to a further understanding of how anthelmintics exert their effects. Four anthelmintics, piperazine, pyrantel, levamisole and ivermectin, were tested on the ovijector of *Ascaris suum* and the oviduct of *Schistocerca gregaria*, using an optoisolator transducer system. All except piperazine had concentration-dependent effects on the ovijector. Ivermectin was the only anthelmintic with concentration-dependent effects on the oviduct. Ivermectin was tested in the presence of Cl⁻ free HBSS to further investigate its mode of action. Further investigations were carried out to investigate its effect in the presence of glutamate, GABA, PF4 (KPNFIRFamide) and Cl⁻ channel blockers on the ovijector. Results show that anthelmintics inhibit reproductive function. Preliminary results suggest that ivermectin may not be acting on GluCl channels on the ovijector.

022 **The nicotinic acetylcholine receptors of *Ascaris suum***

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Nicotinic acetylcholine receptors (nAChRs) are targets of the anthelmintic drugs levamisole and pyrantel, but most studies of the molecular biology of nematode nAChRs have been conducted on the free-living nematode *C. elegans* rather than on parasites. Pharmacological studies of nAChR responses to drugs have previously been conducted on the large roundworm of pigs *Ascaris suum*.

To enable such pharmacological observations to be interpreted in terms of the molecular biology of the nAChR subunits, we have used the *Ascaris suum* EST database, and the genome of another Clade III nematode, *Brugia malayi*, to predict which nAChR subunits are present in these parasitic nematodes. Primers were then designed on these sequences to amplify the nAChR subunits of *Ascaris suum* by PCR.

Homologues to the *C. elegans* nAChR subunits unc-38, unc-29, and unc-63 have been successfully amplified from *Ascaris suum*; these subunits are believed to be components of the levamisole-sensitive neuromuscular nAChR. Another novel subunit most similar to a nAChR subunit found in the snail *Lymnaea stagnalis* has also been amplified from *Ascaris suum*.

We conclude that although certain *C. elegans* nAChR subunits are conserved between clades, Clade III parasitic nematodes have many fewer nAChR subunits than Clade V nematodes, including one not present in *C. elegans*.

023 **Temporal synergism renews efficacy of ivermectin in resistant roundworms**

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The drug resistance of livestock gastrointestinal nematodes is an increasing problem that is unlikely to be overcome by development of new anthelmintics. A limited choice of effective nematicides on the market, and the shortage of potential new compounds means that existing drugs must be used in ways to maximise their impact on the parasite population, whilst delaying, or if possible avoiding, the emergence of resistance. In arthropods resistance is often due to metabolic factors, that can be overcome by Temporal Synergy. Such a resistance has also been implicated for resistance in nematodes. Larval Feeding Inhibition Assays on *Trichostrongylus colubriformis*, *Teladorsagia circumcincta*, and *Haemonchus contortus* demonstrated that a 30 minute pre-treatment of synergist gave approximately a 25 to 100-fold increase in IVM efficacy with resistant strains. An adult motility assay was run on a field strain of *T. circumcincta* using adult worms recovered from the abomasum of a freshly killed sheep (naturally infected male). A comparison between a single IVM treatment, a mixture of verapamil and IVM and a 30 minute pre-treatment of verapamil followed by IVM demonstrated that a pre-treatment of verapamil resulted in a significant increase in IVM efficacy on adult worm motility. The MD50 decreased from >50ug/ml to 6ug/ml. A new technology such as this, which targets the resistance mechanism rather than a new site of action, would prove invaluable for controlling resistance in nematodes.

024 **Modulation of P-glycoprotein enhances ivermectin and moxidectin systemic availabilities and their efficacy against resistant nematodes in cattle**

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The involvement of the efflux-transport protein P-glycoprotein on both the pharmacokinetic disposition and resistance mechanisms to macrocyclic lactones has been described. This work aimed to study the effects of loperamide, a P-glycoprotein modulating agent, on ivermectin and moxidectin pharmacokinetics and efficacy against resistant nematodes in cattle. Fifty Aberdeen Angus male calves (180-200 kg) were assigned into five experimental groups. Group A remained as untreated control. Other animals received ivermectin (Group B) or moxidectin (Group C) (200 µg/kg, subcutaneously) either alone or co-administered with loperamide (0.4 mg/kg, 3 times every 24 h) (Groups D and E). Blood samples were collected between up to 30 days post-treatment and drug plasma concentrations were determined by HPLC with fluorescence detection. The pattern of efficacy was estimated by the faecal egg count reduction test (FECRT). Nematode larvae were identified by 4 pooled faecal cultures for each group. *Cooperia* spp. and *Ostertagia* spp. were the predominant nematode larvae in pre-treatment cultures. The FECRT values confirm the presence of resistant nematode populations (largely *Cooperia* spp) to both ivermectin (23%) and moxidectin (69%). Loperamide enhanced the plasma concentrations of ivermectin and moxidectin in co-administered calves. This loperamide-induced pharmacokinetic modification accounted for enhanced egg counts reduction for both ivermectin (50%) and moxidectin (87%). The in vivo modulation of the P-glycoprotein-mediated drug efflux increased drug systemic availability and efficacy against resistant nematodes. Further work to evaluate the clinical relevance of this pharmacokinetic/pharmacodynamic interaction is undergoing in our laboratory.

025 **Canine echinococcosis in Kyrgystan**

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Human case incidence of echinococcosis in Kyrgystan is approximately 4 times higher than when the Soviet Union collapsed in 1991. Dogs are the normal definitive host of *Echinococcus granulosus* but can also be infected with *E. multilocularis*, the causes of human cystic and alveolar echinococcosis respectively. Hence a study of canine echinococcosis in 466 dogs was undertaken in 2005 in Naryn Oblast, Kyrgystan. Helminths were identified following purgation of dogs with arecoline hydrobromide. Taeniid eggs were isolated from the faecal samples, collected at the same time, by a combination of sequential sieving and flotation of the eggs in zinc chloride solution. Eggs were analyzed by multiplex PCR to confirm species identity and some samples were cloned and sequenced. Logistic regression was used to explore the relationship between presence of infection and various risk factors. In addition, a total of 633 rodents were trapped and necropsied for the presence of the larvae of *E. multilocularis*. *E. granulosus* was found in 82 dogs (17%). Both the G1 (sheep strain) and G6 (pig strain) were present. *E. multilocularis* was found in 50 (11%) dogs. Larvae of *E. multilocularis* was found in 42 (6.6%) rodent. These included 14 of 227 (6.2%) house mice (*Mus musculus*). Dogs that are allowed to roam have a significantly increased risk of infection. A high prevalence of infection of *Echinococcus spp* in domestic dogs presents a significant public health risk. The results also indicate the possibility of a domestic cycle of transmission of alveolar echinococcosis between house mice and dogs and a transmission cycle of *E. granulosus* between sheep and perhaps wild pigs and dogs.

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026 **Cross-sectional survey on the prevalence of *Echinococcus multilocularis* and *E. granulosus* infections in domestic dogs in Germany**

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Objective: To evaluate prevalence of *Echinococcus multilocularis* and *E. granulosus* infections in domestic dogs in Germany.

Material&Methods: Faecal samples of dogs from Germany routinely submitted to one private veterinary laboratory were examined using the ZnCl₂-NaCl flotation method between June 2004 and June 2005. Taeniid egg-positive samples were subjected to a simplified DNA preparation method and a modified two step PCR for the detection of *Echinococcus spp.* based on mitochondrial 12S rRNA genes (Dinkel et al., 1998; Stefanic et al., 2004). *Echinococcus*-negative but cestode-positive PCR products were additionally cloned and sequenced.

Results: Taeniid eggs were detected in 53 (0.3%) of 17,391 samples examined. Cestode-specific PCR products were gained in all these cases. *E. multilocularis* or *E. granulosus* DNA was amplified in 43 (0.24%) and 4 (0.02%) of the samples, respectively; in 3 cases DNA of both *E. multilocularis* and *E. granulosus* was amplified. In at least 4 cases of *E. multilocularis* infection a faecal shedding of *Taenia spp.* proglottids was also recorded. *Echinococcus*-negative but cestode-positive PCR products were identified as *Taenia crassiceps* (8), *T. pisiformis* (1) and *T. taeniaeformis* (1).

Conclusions: The results of this first countrywide survey for canine echinococcosis show that dogs are a potential infection risk for humans in Germany.

027 **Prevalence of *Echinococcus multilocularis* in wild and domestic hosts in Lithuania**

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Introduction. Recently the occurrence of *E. multilocularis* was reported in the Baltic and neighboring regions. However limited information is available on the epidemiology of this serious zoonotic agent in Lithuania.

Material and methods. PCR was done with necrotic lesions from pig livers (n=690). The small intestines of red foxes (n=269) and raccoon dogs (n=85) from 17 districts were examined by the sedimentation and counting technique (SCT). Multiplex PCR was done with isolated *Taenia spp.* eggs from 241 dog faeces. From 1997 to June 2006, 80 AE cases have been diagnosed at the Hospital of Tuberculosis and Infectious Diseases.

Results and conclusion. 57.3% of red foxes and 9.7% of raccoon dogs were infected with *E. multilocularis*. Two dogs (0.8%) were found infected with *E. multilocularis*. *E. multilocularis* was identified in 0.4% of pigs. In 26 (33%) of 80 AE patients, metacestodes were found in the bilateral liver lobes, in 20 (25%) in the right lobe. Metacestodes had also spread into extra hepatic tissues and metastasized to distant organs in 18 (23%) of the patients. Twelve (15%) of 80 patients died. Eighty one percent of AE patients were farmers or persons involved in agricultural activities. The high number of human AE cases, high prevalence of *E. multilocularis* in definitive wild hosts as well as prevalence in pigs and dogs document that *E. multilocularis* is of emerging concern in Lithuania suggesting that the whole territory should be considered as an endemic area.

028 **Endogenous development in the countermeasure of multilocular echinococcosis in red foxes: a successful and sustainable approach**

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Introduction: This paper describes successful countermeasures against *Echinococcus multilocularis* in red foxes by endogenous development “auto-generativo” initiatives of local residents’ Nonprofit Organization (NPO) using local resources with full assistance of Forum on Environment and Animals (FEA), Limited Liability Company founded by our laboratory.

Materials and Methods: Koshimizu, Hokkaido (200 km²) was first surveyed in 1997 for baseline coproantigen prevalence rate. Baiting distribution was initiated in 1998 until 2001 using praziquantel fortified fish sausage baits. Annual baiting campaigns were conducted from 2002 until present by local NPO (Okhotsk Sanctuary) using anthelmintic laced baits. Prevalence rates in red fox feces were assessed annually using EmA9 coproantigen ELISA. Kutchan (100 km²), was surveyed in July, September and November of 2005 by examining 268 fox fecal samples. From May to November 2006, around 1,500 anthelmintic laced baits were distributed by local residents’ NPO (WAO) at specific locations guided by a GIS-based map identifying fox foraging areas. Coproantigen ELISA test on fox feces was conducted in October 2006 to assess prevalence rates.

Results and Conclusions: Initial prevalence rates in Koshimizu in 1997 were 51.6-66.7%. Fecal examinations conducted in 2006 revealed a zero coproantigen prevalence rate after annual bait distribution by local residents. In Kutchan, a coproantigen prevalence rate of 21% was registered during baseline surveys. Seven months after continuous baiting, the prevalence rates decreased remarkably to 2%. This novel concept of endogenous development by local resident volunteers through NPO’s is seen as a key to a wider and sustainable control campaigns against echinococcosis in Hokkaido.

029 Evaluation of a serological method for the detection of *Taenia saginata* cysticercosis using serum and meat-juice samples

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Two peptides, HP6-2 and Ts45S-10, were used as antigens for the detection of antibodies against *T. saginata* cysticercosis in serum and meat juice samples using ELISA. Positive control samples were obtained from animals experimentally infected (serum) and from animals naturally infected (meat juice).

The two peptides and a pooled preparation of both peptides were evaluated and their cut-off points using a negative population serum samples (n=100) with both sample categories were calculated. ELISA results from these different peptides were compared. Sensitivity and specificity of the different peptides were calculated from another negative serum population (n=50). The sensitivity and the specificity of HP6-2 and Ts45S-10 peptides using serum were identical, and were calculated as being 100%, showing to be higher than the values of the pooled peptides.

HP6-2 peptide was used to examine serum samples from animals with cysts and random field serum samples. For meat juice samples the pooled peptides showed the highest sensitivity and specificity, as they were 100% and 95% respectively.

The goal of this study was to evaluate and improve a diagnostic method for bovine cysticercosis detection. The use of serological methods will help to get a more accurate estimation of the epidemiological situation of bovine cysticercosis in Germany. From the other side, it was also important to evaluate the reliability of visual diagnosis, molecular biologic and serological methods in correlation with each other for the detection *T. saginata* cysticercosis.

030 Risk factors for bovine cysticercosis in Switzerland

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Bovine cysticercosis caused by *Taenia saginata* represents an unsolved problem worldwide as cysts in meat cause financial losses and pose a steady source for human tapeworm infections. A case-control study on a farm-level was set up to identify risk factors for bovine cysticercosis in Switzerland. The case group (n=119) consisted of farms with infected animals identified at slaughter in 2005 and 2006. The control group (n=66) comprised farms with animals slaughtered in the same period without detectable infection and with a confirmed history of absence of this disease. In a personal interview with the farm owners, information was collected on the local surrounding and farm management. Data were analysed by logistic regression. The following factors were positively associated with the occurrence of bovine cysticercosis: the presence of a railway line or a car parking place close to cattle feeding areas, leisure-activities around these areas, use of purchased roughage and having visitors to a large extent on the farm itself. This information is useful for the authorities to direct control strategies as well as for farmers to take measures that are suited for the local situation.

031 ***Cryptosporidium parvum*: major aetiological agent of neonatal diarrhoea in dairy calves**

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032 **Efficacy of halofuginone lactate for the treatment and prevention of cryptosporidiosis in lambs and kids: An extensive field trial**

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033 **Targeted selective treatments an innovative strategy for the control of nematode infections in sheep and goats**

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Maintaining a population *in refugia* is a prerequisite for slowing the development of anthelmintic resistance. Sustainable, regionally appropriate control strategies that maintain susceptibility require the ability to target therapeutic and prophylactic treatments towards those animals that require them, rather than rely on whole-flock chemoprophylaxis. The key aim of an EU funded project (PARASOL, started in spring 2006) was to develop novel solutions for the sustainable control of nematodes in ruminants. A series of PARASOL field studies, conducted in various participants' countries, have examined a range of pathophysiological and production markers which could be used to apply targeted selective treatments (TST). These studies will also determine the impact of TSTs on productivity and the phenotypic and genotypic expression of resistance. Regional differences in the prevailing parasite species and their epidemiology make it imperative to test different indicators that associate with parasitic effects upon host appetite/liveweight gain, body condition score (BCS), diarrhoea index and anaemia. Initial results suggest that, in regions where *Teladorsagia* is the dominant species, liveweight gain has potential to provide a sensitive marker for TST. However, BCS lacks sensitivity in this respect. A questionnaire has been developed and used by several partners to determine the acceptability to farmers of the TST approach with favourable initial responses. The effect of TST on benzimidazole and macrocyclic lactone resistance has been examined in field studies and initial results show, as expected, that there is a tendency for the phenotypic expression of resistance to increase with the intensive application of anthelmintics

034 **Assessment of the repeatability and reproducibility of a serum-pepsinogen assay and an indirect milk ELISA to monitor gastrointestinal-nematode infections in cattle**

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Anthelmintic treatments are essential for effective nematode control in cattle herds. However, the applied treatment programmes are often too intensive, due to the lack of practical diagnostic measures to monitor gastrointestinal nematode (GIN) infections. Previously, pepsinogen levels in serum and *Ostertagia*-specific antibody levels in milk have shown value for monitoring GIN infections in first-season grazing and adult dairy cattle, respectively. One of the objectives of PARASOL, a European Framework 6 funded project, is to develop standardized tools to monitor GIN infections in cattle and is aimed at reducing the use of anthelmintics. Standardized procedures for a serum pepsinogen assay *Ostertagia* ELISA were developed and ring tests were performed using 104 serum samples and 80 milk samples, respectively. All samples were tested during 3 different days in 4 laboratories. The ring-test of the pepsinogen assay revealed that considerable and unacceptable differences exist between the results obtained by the 4 laboratories. The within-laboratory repeatability and between-laboratory reproducibility of the *Ostertagia* ELISA were good with mean concordance correlation coefficients ranging between 81 to 96% and 90 to 99%, respectively. The results indicate that further standardization of the pepsinogen is needed to obtain similar results in different laboratories. In contrast, the *Ostertagia* ELISA seems suitable for use in different laboratories. In a next phase, the evaluated methods will be used in field surveys in Belgium, Germany, Sweden and the UK to investigate the distribution of gastrointestinal-nematode infection and the associations with management factors.

035 **The development and standardisation of methods for detecting anthelmintic resistance in gastrointestinal nematodes of ruminants**

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PARASOL is a Framework 6 project evaluating the use of Targeted Selective Treatments for the sustainable control of gastrointestinal parasitic nematodes of ruminants. The success of this approach requires accurate information on anthelmintic efficacy and the extent of resistance in the target nematode population. We therefore need standardised reliable and sensitive resistance tests. We are producing a standard operating procedure for the Faecal Egg Count Reduction Test, developing and evaluating novel statistical tools for its interpretation. We have ring-tested egg hatch and larval development tests for benzimidazole resistance of cattle and sheep nematodes: the EHT worked reproducibly in different laboratories, but some variation was found in the LDT. The LDT is being assessed for ML-resistance in *C. oncophora*: EC₅₀s of 0.09nM and 0.37nM were obtained for susceptible and resistant isolates, respectively. Results with *O. ostertagi* were more variable. We developed larval migration inhibition tests for ML- and levamisole-resistance. These provided reliable reproducible results with sheep and cattle nematodes; the range of drug concentrations requires optimisation for cattle parasites and further standardisation is ongoing. We are developing PCR-based tests for resistance alleles. A Taqman assay has been developed for the BZ-resistance F200Y polymorphism of *H. contortus*, *O. ostertagi* and *C. oncophora*, and a Pyrosequencing assay developed for the polymorphisms at positions 167, 198 and 200. We assessed the reported polymorphism at position 256 of a glutamate-gated chloride channel subunit for use in the detection of ML-resistance alleles, but were unable to detect this sequence change in any ML-resistant nematode examined.

036 **The epidemiology fasciolosis in Europe**

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Our understanding of the epidemiology of fasciolosis is being increased by determining the farm specific risk factors for infection and the role of wild life in transmission, improving the sensitivity of diagnosis of patent infection and improving our understanding of how genetic variation in populations of fluke affect their interaction with the intermediate snail host and their infectivity and pathogenicity for farmed livestock. This should lead to web based predictive models which farmers can use to assess the seasonal and year on year risk of infection and disease in their herds and flocks and assist in deciding whether treatment is necessary, when to treat and which drug to use. The significance of fluke infection in humans within the EU will also be determined. Antibodies in over 6,000 samples of milk and serum from the UK, Spain and Ireland have been measured to determine the prevalence of infection in livestock. The large scale survey in the UK has provided a complete coverage of the country and enabled maps to be constructed showing the spatial distribution of *F. hepatica*. A risk analysis is underway. RFLP analysis of mitochondrial DNA from flukes has provided markers that are being used to assess the genetic diversity of flukes of known provenance, isolated from different regions of the EU. Initial results suggest that there is extensive diversity between isolates that is not geographically restricted. This raises questions about potential differences in the virulence and infectivity between different isolates and also the potential for the emergence and spread of drug resistance throughout Europe.

037 **Fasciolicide resistance in liver fluke**

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The uptake and metabolism of triclabendazole (TCBZ) by the fluke is now well documented so its effect on flukes in the definitive sheep host is under examination. The relative activity of TCBZ and its metabolites are being determined. By proteomic analysis a number of potential target sites for TCBZ have been identified and production of recombinant forms of these proteins has begun. This will assist the production of antibodies to these proteins in order to determine tissue sites of drug action and to generate three-dimensional models of the proteins for future drug binding studies. Sequencing of α - and β -tubulin isotypes in one TCBZ-susceptible isolate and one TCBZ-resistant isolate has been completed. The sequences are identical at the proposed benzimidazole (BZ) binding site, suggesting that mutations in the β -tubulin molecule are not involved in the mechanism of resistance, in contrast to the situation in BZ-resistant nematodes. Results from pharmacological and morphological studies point to changes in drug uptake and metabolism being involved and these ideas will be explored further. The proteomic studies may reveal additional information on other molecules. Assays are being developed for the detection of triclabendazole resistance,

038 **Development of vaccines against *Fasciola hepatica***

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The objectives are to characterise protective elements of innate and adaptive immune responses to *F. hepatica*., to identify additional candidate vaccine antigens from juvenile and adult flukes and synthesise them by recombinant technology. Immunoregulatory molecules and mechanisms in *F. hepatica* infection are being studied and the methods of administration optimised, including DNA vaccination, dose, and finding adjuvants for vaccine antigens with the best protective profile. Several vaccine trials in sheep, goats and cattle have been carried out or are ongoing.

Experimental work on the mechanisms that may be involved in immunomodulation has concentrated on the activation of macrophages in fluke-infected animals and in ruminant macrophages exposed to fluke antigens *in vitro*. We have shown that liver fluke infection and exposure to fluke excretory/secretory proteins and to recombinant PRx, induces alternative-activation of macrophages. The alternatively-activated state is characterised by production of arginase and by chitinase/chitinase-like molecules, and by lack of NO production. Macrophages in this state are known to be associated with chronic infections, with fibrosis and with tissue repair processes generally. We hypothesise that this macrophage phenotype may also be involved in increased susceptibility of fluke-infected animals to bacterial infections.

039 ***Neospora caninum* – lack of evidence of human exposure in an English population**

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Since *Neospora caninum* is so closely related to *Toxoplasma gondii* and parasite stages exist both in the tissues of meat animals and as resistant oocysts in the environment the possibility that *N. caninum* is zoonotic is of concern. We sought evidence of human exposure to *N. caninum* infection by specific antibody detection in two populations. A cross-sectional sample was selected from English regions using stratified random sampling techniques for sera collected in the year 2000 and a sample size estimate of 3430 calculated to detect an overall prevalence of 2.4% with a precision of 0.5% in adults. Sera were tested by inhibition ELISA using whole tachyzoite coated plates (Mastazyme ELISA). After initial screening 21.4% of the samples had a per cent inhibition $\leq 20\%$. Because the test has not been validated in humans the frequency distribution of actual percentage inhibitions was plotted, the logged data showed a single log normal distribution with a mean close to 0% inhibition indicating that all samples conformed to a single population with no evidence of a positively exposed population. Furthermore when all samples with a PI $\leq 20\%$ were tested with an IFAT all failed to give positive fluorescence at 1:50 dilution. Similarly, the testing of 518 samples from a high risk group (farm-workers) provided no evidence of exposure. This is the most extensive serological examination of a human population for evidence of exposure to *N. caninum* and failure to find evidence suggests that, in Britain at least, *N. caninum* is rarely, if ever, zoonotic.

040 **Investigations into the involvement of *Neospora caninum* in unexplained sheep abortions**

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Introduction: Experimental infection of pregnant ewes with *Neospora caninum* tachyzoites has been shown to induce abortion or congenital infection. Naturally occurring ovine neosporosis has been reported but has not been regarded as a significant cause of abortion in sheep. Three million hoggets are mated in New Zealand each year and as many as 20% of a flock can report unexplained abortions. There has been little research and no prevalence data on ovine neosporosis in New Zealand.

Methods: Whole blood and serum from up to ten aborting and non-aborting ewes plus aborted tissues, together with a farm history, were collected from twenty farms throughout New Zealand with an abortion problem during the 2006 lambing session. PCR was used to detect the presence of *Neospora* DNA and IFAT serology was used to detect *N. caninum* antibodies.

Results: Initial examination of the data from this study revealed similar numbers of sero-positive animals in both aborting (41%, range 0/10-7/10) and pregnant (42%, range 0/9-6/10) ewes. Additionally, *Neospora* DNA was amplified in the blood of both aborting (10%, range 0/10-3/9) and pregnant (10%, range 0/10-2/9) ewes. *Neospora* DNA was also isolated from the brain (14%, 14/99) and uterine tissue (31%, 8/26) of aborted foetuses. Two of the foetal brains positive for *Neospora* DNA also had observable lesions, although no protozoal life stages were observed.

Conclusion: This study suggests that *Neospora* infection is widespread throughout the New Zealand sheep population and is likely to be playing some role as a cause of ovine abortion.

041 **No evidence of transplacental transmission in pregnant heifers inoculated with *Neospora caninum* tachyzoites by conjunctival route**

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The aim of this study was to evaluate whether *Neospora caninum* tachyzoites inoculated by conjunctival route in pregnant cattle were able to infect the foetus. A pathogenic strain of *N. caninum* (Nc-1) cultured under standardized conditions was used. Eight cows separated in four experimental groups were studied: Group 1 were naturally infected cows (n=2); Group 2 were cows inoculated with 2.5×10^8 tachyzoites by intravenous route (n=2), Group 3 were cows inoculated with 2.5×10^8 tachyzoites by conjunctival route (n=2), Group 4 were negative control cows (n=2). All 4 inoculated cows were challenged at fifth month of gestation. An indirect fluorescent antibody test was used to analyze the humoral immune response in dams and their fetuses. Routine microscopic evaluation of H&E stained fetal tissues was made. The tissues of fetuses and placentas with lesions of non-suppurative multifocal necrotizing meningoencephalitis, myocarditis, hepatitis, and placentitis were processed by immunohistochemistry (IHC). Although all dams from Groups 1, 2 and 3 were seropositive, only fetuses from Groups 1 and 2 developed *N. caninum* specific antibodies. Similarly, histopathological lesions compatible with those caused by *N. caninum* were observed in fetuses from Groups 1 and 2. More over, *N. caninum* cysts and tachyzoites were observed by IHC on tissues from fetuses from Group 1 and 2, respectively. Fetal tissues are currently being processed by PCR. Further work is needed in order to clarify the consequences of this novel experimental route of infection not only on the fetus but also on the dam.

042 **Usefulness of the recombinant proteins rNcSAG4 and rNcGRA7-based ELISA tests to distinguish primo-infection, recrudescence and chronic bovine neosporosis**

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The main challenge of currently employed diagnostic tests in bovine neosporosis is to differentiate the different phases of infection for control measures. Serological avidity tests are known to discriminate between acute and chronic infections. However avidity tests do not allow differentiating recrudescence from chronic *Neospora caninum* infection in cattle. In addition several studies have suggested that avidity tests should be mainly employed at herd level. In the present study two recombinant antigen-based ELISA assays have been tested to solve these limitations. To achieve this the recombinant version of a dense granule *N. caninum* antigen –NcGRA7– and a bradyzoite stage specific antigen –NcSAG4– were employed in ELISA tests. The usefulness of both proteins was evaluated by testing a well characterized panel of sera. Sera from experimentally infected cattle belonged to different groups regarded as representative of primo-infection, re-infection/ recrudescence and chronic infection. Sera from naturally infected cattle belonged to non-aborting and aborting dams with an endemic pattern of abortions. In addition sera from farms with a previous history of epidemic abortions confirmed by avidity tests were also analysed. Absence of background and cross-reaction with closely related parasites was obtained. Data from experimentally and naturally infected cattle show that high levels of antibodies directed against rNcGRA7 protein are detected after primo-infection. On the other hand detection of rNcSAG4 may be considered a good marker of chronic infection. In addition, high levels of antibodies directed against both recombinant proteins are associated with a re-infection or recrudescence of a previous chronic infection. At herd level the recognition of rNcGRA7 and the absence of recognition of rNcSAG4 are indicative of a recent infection.

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043 **The magnitude of cytokine expression in the placenta of cattle in naturally and experimentally infected cattle and its association with foetal death**

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Neospora caninum is acknowledged as a major cause of bovine abortion worldwide. Foetal infection results from reactivation of an existing persistent infection in the mother or from the dam ingesting oocysts from the environment during pregnancy. As a result the foetus may be killed or born infected but apparently healthy. The time during gestation when foetal infection occurs appears to determine whether or not the foetus survives since experimentally cattle abort following infection with the parasite in early gestation but the foetus survives infection in late gestation. We have therefore compared, using real-time quantitative RT-PCR, expression of cytokines at the foetomaternal interface of cattle experimentally infected with *N. caninum* in early and late gestation. In early gestation, following foetal death, expression of IL-gamma, IFN- α , IL-12, TNF- α , IL-10 and IL-4 was increased by up to 500-fold. A much smaller increase in cytokine expression occurred in late gestation when the foetuses survived. We have also examined cytokine expression in the placenta of naturally infected cattle in which parasite recrudescence occurred during the second half of pregnancy. Moderate levels of regulatory and pro-inflammatory cytokines were detected despite the fact the foetuses were not killed by the infection. This suggests that abortion due to *N. caninum* is multifactorial and that high levels of pro-inflammatory cytokines alone do not account for foetal death

044 **A molecular in vitro assay to assess the parasitocidal activity of toltrazuril against *Neospora caninum***

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Neospora caninum is an intracellular parasite inducing abortion in cattle. No efficient treatment strategy has been elaborated for cattle so far. Conversely to the in vivo situation, a wide range of compounds have been investigated in cell culture-based assays. In this work, we present a molecular assay that allows to distinguish between live and dead parasites. This method is anticipated to be used for in vitro drug treatment experiments, i.e. to determine the efficiency of a drug and also for the detection of live parasites in vivo. Live parasites can be detected by measuring the mRNA level of specific genes, making use of the specific mRNA available in live cells. Recently, the dense granule NcGra2 gene was identified with a 56% sequence similarity to the related parasite *Toxoplasma gondii* TgGra2 gene. The NcGRA2 gene was used to establish a reverse transcription (RT) PCR, based on mRNA isolation and cDNA synthesis. We could show that this RT-PCR is highly sensitive and specific. The PCR can also be used for real-time RT-PCR to quantify parasites in a sample. To test the system, an already established in vitro drug treatment experiment with Nitazoxanide (NTZ) was repeated. *Neospora* infected cells were treated for three or five days with 10 μ g/ml NTZ and then grown in absence of the drug. The same approach is presently being carried out with toltrazuril. We are using 30 μ g Toltrazuril per ml medium, as this concentration was shown (by others) to cause severe destruction that could be lethal for the parasite. Until now, effects of Toltrazuril on *N. caninum* were only studied by light and electron microscopy by others. With both in vitro- and DNA-PCR-assay, we could now find out that a 10-day-treatment exerts a parasitostatic activity, whereas a 14-day-treatment was able to kill all parasites. Thus, no proliferation could be detected anymore in infected and 14-day-treated monolayers when maintained in the absence of the drug for up to 4 subsequent weeks. These results could also be backed up with the new RT-PCR.

045 **A descriptive epidemiological study on canine babesiosis in the Lake Geneva region**Porchet M.-J., Sager H., Muggli L., Oppliger A., Mueller N., Frey C., and Gottstein B.*Institute of Parasitology, University of Berne, Berne, Switzerland*

Canine babesiosis appears to become emerging in some parts of Switzerland. A respective study was carried out in the district of the Lake Geneva between March 2005 and August 2006 to assess the incidence and prevalence of *Babesia*-species, and to address the potential of autochthonous transmission. This included a data assessment on the different tick-populations occurring in the area and on the prevalence of *Babesia*-DNA in these ticks. A total of 56 veterinary practices participated in the study. By blood smear and PCR, *Babesia canis canis* was found in 12 out of 21 cases with suspected babesiosis. In an additional 13th case, the parasite could only be detected by PCR. All autochthonous cases originated from the Western part of the Lake Geneva region. Clinical signs in affected dogs included inappetence, apathy, anemia, fever, hemoglobinuria and thrombocytopenia. There were no risk factors with regard to age, sex and breed. Most cases were diagnosed in the spring periods of 2005 and 2006 (11 cases) and two cases in autumn 2005, coinciding with the main activity period of *Dermacentor reticulatus*. A total of 495 ticks were collected on patients by the veterinarians, 473 were *Ixodes* sp., 7 *Rhipicephalus sanguineus* and 15 *Dermacentor reticulatus*. While *Ixodes* sp. was found in the whole study area, *D. reticulatus* and *R. sanguineus* occurred only in the Western part till Lausanne. PCR and sequencing yielded *B. canis canis* positivity in three *D. reticulatus* specimen. All *R. sanguineus* were negative by *Babesia*-PCR. Global warming, ecological changes in the potential habitat of ticks, increasing host- and vector-populations and increasing mobility of dog owners may be responsible for an emergence situation of infection risk for *Babesia* spp. by time. E.g., *Dermacentor reticulatus* has become autochthonously prevalent in larger parts of the Lake Geneva region, and further surveillance is suggested to tackle this emerging problem.

046 **Molecular characterization of a *Babesia* sp. from sable antelope**Oosthuizen M.C. 1, Collins N.E. 1, Zwegarth E. 2, and Penzhorn B.L. 1*1 Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; 2 Onderstepoort Veterinary Institute, Onderstepoort, South Africa*

Introduction: Sable antelope (*Hippotragus niger*) is listed as “conservation dependent” on the IUCN Red List. Declining numbers could lead to a “threatened” listing in the near future. Babesiosis (due to *Babesia irvinesmithi* infection) has caused mortality in sable antelopes. This piroplasm has not been characterized molecularly.

Materials & methods: Specimens from a sable antelope that presented a sudden onset of disease and that subsequently died were submitted for molecular characterization. Microscopic examination of thin blood smears revealed small piroplasms. DNA was extracted; the V4 variable region of the 18S rRNA gene amplified and analyzed using the Reverse Line Blot (RLB) assay. The PCR products hybridized only with the *Babesia* / *Theileria* genus-specific probe and not with any of the species-specific probes present, indicating the presence of a novel species or variant. Full-length 18S rDNA was amplified, cloned and the recombinants were analysed by sequencing analysis. Sequencing data were analysed with the Staden package, aligned with published sequences of related genera using ClustalX and phylogenetic trees were constructed using neighbor-joining in combination with the bootstrap method.

Results: Sequence similarity analysis indicated that a *Babesia* species infection was present with the highest similarity with *B. orientalis*, and an unnamed *Babesia* species isolated from a bovine. Furthermore, a species-specific RLB oligonucleotide probe was deduced from the hypervariable V4 region of the 18S rRNA gene, synthesized and used to screen sable samples to determine the prevalence of the parasites in sable antelope in South Africa.

047 **Molecular diagnostic method for Babesiosis; Direct detection of *Babesia microti* DNA from heat-treated blood by loop-mediated isothermal amplification**

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Babesia microti, intraerythrocytic protozoa, is frequently seen in small wild rodents and also a causative agent of human babesiosis. The aim of our study was to develop an effective molecular diagnostic method based on a loop-mediated isothermal amplification (LAMP) technique with a DNA sample which was prepared from heat-treated blood infected *B. microti*. The LAMP technique has been used as a novel DNA detection method, whereby the target DNA can be amplified with high specificity and sensitivity under an isothermal condition using a set of four specific primers. A set of the LAMP primers was designed, targeting the small subunit ribosomal RNA gene of *B. microti*, in which a restriction enzyme cleavage site was inserted into one pair of the species-specific inner primers to confirm the amplified DNA fragments in the following electrophoresis analysis. Standard PCR assay was used as the control method for the LAMP assay. Four of the designed LAMP primers specifically amplified the positive LAMP amplicons of typical ladder patterns from the heat-treated blood of the infected mice, but not from the control samples of normal murine and human blood, and also other protozoan species. The sensitivity and specificity of the LAMP assay were 100 times higher than those of classical PCR method. In conclusion, these findings indicate that our constructed LAMP method is a novel tool for the rapid detection of *B. microti* from the infected blood.

048 **Epidemiological and molecular genetic investigations of *Babesia divergens*/*B. capreoli*-like parasite findings in free-ranging wild ruminants in eastern Switzerland**

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In 2005 and 2006, three adult female chamois were found dead in the eastern Swiss Alps. PCR on DNA extracted from blood or spleen of the infected animals revealed sequence identity of the amplified part of the 18S rRNA gene with GenBank entries attributed to *B. divergens* of cattle origin or *B. capreoli* of wild ruminant origin which have never been described before in this region. Examination of 430 blood samples from 312 head of cattle from this area by IFAT, microscopy and PCR provided no evidence for babesial infection. Six of 887 ticks collected from cattle were PCR-positive, and sequencing revealed *Babesia* sp. genotype EU1 in five and *B. divergens*/*B. capreoli* in one of them. A *Babesia* isolate of chamois and one from a roe deer from the same region were genetically compared with two Swiss *B. divergens* isolates of cattle origin by analysing genomic rDNA. Whereas the full length sequence of the 18S rRNA gene was virtually identical among all four isolates (2 polymorphic sites), distinct differences between the two isolates from cattle isolates on one hand and the two isolates from wild ruminant on the other hand were observed in the sequences of the internal transcribed spacers 1 and 2 (ITS1, ITS2) and in part of the 28S rRNA gene. These results indicate that, albeit genetically very closely related, these *Babesia* from cattle and from free-ranging wild ruminants indeed are distinguishable organisms with different host specificities.

049 **Correlation between *Babesia rossi* genotypes and clinical canine babesiosis**

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Babesia rossi, an intra-erythrocytic protozoan, causes a severe often threatening disease of domestic dogs. Dogs treated early for *B. rossi* infection usually recover from the infection, but dogs left untreated or treated at a later stage of infection seldom survive the infection. Dogs infected with *B. rossi* have varied clinical manifestations that can be categorised as uncomplicated (with a good prognosis) and complicated (with a poor prognosis). Different *B. rossi* isolates have previously been identified by typing of a polymorphic repetitive region from the gene *Babesia rossi* Erythrocyte Membrane Antigen (*BrEMA1*). This gene encodes for a phosphoprotein localised on the cytoplasmic surface of the infected erythrocyte. One hundred and twenty two (122) blood samples were collected from dogs with babesiosis presented to the Onderstepoort Veterinary Academic Hospital (OVAH). An additional twenty three (23) sample were obtained from private clinics. DNA was extracted from 200 µl of venous blood collected in EDTA from each dog. The samples were screened for the presence of *B. rossi* DNA using PCR and the Reverse Line Blot (RLB). Positive samples were re-amplified using primers specific for the *BrEMA1* gene and different gene profiles were visualised by gel electrophoresis. Amplicons were then sequenced. Analysis of the *BrEMA1* amino acid sequence revealed that there could be antigenic diversity. Eleven different genotypes were identified and named *Babesia rossi* types 1-11. The genotypes were compared to the case data. *Babesia rossi* genotypes 7 (35%), 9 (22%), 10 (18%) and 2 (13%) were the most prevalent genotypes and also the genotypes associated with poor prognosis and fatalities. Our preliminary findings indicate that there might be a clinically important difference between the various *B. rossi* genotypes identified.

050 **Effects of protein kinase inhibitors on the in vitro growth of *Babesia bovis***

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The intra-erythrocytic protozoan, *Babesia bovis*, is one of the major causative agents of babesiosis in cattle and responsible for significant losses in cattle industry worldwide. A better understanding of the biological processes involved in the growth at the erythrocytic stage is required for the development of effective therapeutic tools against bovine babesiosis. Protein kinases play essential roles in various signalling pathways of eukaryotic cells by catalysing the transfer of phosphate from ATP to an amino acid side-chain of a protein. They are vital for cell growth and survival, and any irregularity in their enzymatic activities may result in serious diseases, such as cancer. Since protein phosphorylations are also essential in protozoa, the protein kinase inhibitors, staurosporine, Ro-31-7549, and KN-93 were tested for their effects on the in vitro growth of *B. bovis*. Staurosporine was the most effective inhibitor, completely destroying the parasites as early as the first day of exposure at a concentration of 0.1 mM. Moreover, staurosporine caused a significant increase in the percentage of extracellular merozoites, most likely due to the inhibition of erythrocyte invasion by the parasite. In contrast, 5 mM Ro-31-7549 and KN-93 acted suppressive, but not destructive to the parasite. Interestingly, concentrations of 0.5 to 5 mM KN-93 influenced the parasitic development within the infected erythrocytes. The present study suggests that *B. bovis* requires, to a certain extent, the phosphorylations mediated by parasite- or host erythrocyte-protein kinases, in particular for the processes of successful invasion of erythrocytes and intra-erythrocytic development.

051 **Molecular-based prediction of the geographical spread of *Angiostrongylus vasorum* in dog and fox populations**

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The heartworm *Angiostrongylus vasorum* is an important disease of dogs and other members of the Canidae causing complicated chronic pathogenesis and often a high mortality. Infection with this nematode has previously been suggested to be confined to small endemic foci in Europe however recent reports indicate an increasing distribution. Various gene loci including both ribosomal and mitochondrial genes were amplified and sequenced for *A. vasorum* isolates obtained from different geographical locations worldwide. For the first reported time, *A. vasorum* from the UK, Ireland, Portugal, Denmark, the Netherlands and Canada were molecularly characterised. Levels of genetic heterogeneity were also assessed phylogeographically to determine the movement of *A. vasorum* across multiple continents. Limited genetic variation was observed in the conserved ribosomal genes, suggesting a recent spread of this nematode throughout Europe and eastern Canada, while mitochondrial genes exhibited a higher level of population dependent variation. Population dynamics of *A. vasorum* between dog and fox hosts and between different countries are discussed.

052 **Predicting the spread of the canid heartworm *Angiostrongylus vasorum***

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Angiostrongylus vasorum is a metastrongylid nematode that is found in the heart and pulmonary arteries of canids, and causes severe disease in dogs. In recent years, the disease has increasingly been reported outside known endemic foci in Europe, and there is growing concern that expansion of the parasite's range could put a large number of dogs at risk, for example in North America. Predictions of parasite spread are undermined by poor basic knowledge of the factors that influence transmission and persistence. We present a simple climate-based model that successfully predicts existing global distribution of *A. vasorum* in foxes, and use it to investigate the likely effects of movement of untreated dogs, and changes in climate, on parasite distribution. Sensitivity analysis of model parameters provides some guidance concerning the aspects of transmission biology that should be prioritised for further empirical study.

053 Prevalence of haemotrophic *Mycoplasma* and *Bartonella* spp. in fleas of companion animals from Germany and France

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Fleas of companion animals are vectors for various pathogens of medical and veterinary importance but their role in the biology of some of these pathogens is not well understood. In terms of a risk factor analysis, we report prevalences of feline and canine haemotrophic *Mycoplasma* spp. as well as *Bartonella* spp. in natural populations of fleas from pets in Germany and France.

952 fleas of six species (*Ctenocephalides felis*, *C. canis*, *Archaeopsylla erinacei*, *Pulex irritans*, *Ceratophyllus gallinae*, *Spilopsyllus cuniculi*) were collected from 148 cats and 133 dogs at 18 widely distributed geographic locations in Germany and France and tested for the presence of pathogens by PCR.

In 91 fleas (9.6 %) DNA of one or more *Mycoplasma* spp. could be detected. 7.9 % of the samples were positive for “large” haemoplasmas (*M. haemofelis* and *M. haemocanis*), 2.7 % for “small” haemoplasmas (“*Cand. M. haemominutum*” and “*Cand. M. haematoparvum*”), 0.4 % for “*Cand. M. turicensis*”. Fleas from France had higher prevalences than fleas from Germany (12.2 % vs. 4.4 %) and fleas from dogs were more frequently infected than fleas from cats (11.7 % vs. 7.6 %). 35 fleas (3.7 %) were positive for *Bartonella* spp. DNA. Out of these, 21 were *B. clarridgeiae* (2.2 %) and 14 (1.5 %) were *B. henselae*. DNA of other *Bartonella* spp. could not be detected. *B. clarridgeiae* was the dominating species in samples from France (19 cases), whereas *B. henselae* was more frequent in fleas from Germany (11 cases).

Compared to data from other European countries, these prevalences are rather low and the epidemiological importance of haemoplasma transmission to pets by fleas needs further analysis. The molecular evidence of *Bartonella* infections reveals, that agents of zoonotic potential are established in flea populations and that the spectrum of species can vary significantly from country to country.

054 Development of a challenge model and efficacy of 5% ponazuril (toltrazuril sulfone) oral suspension against experimental *Isospora canis* infection in puppies

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In a pilot study, three pairs of Beagle pups were inoculated with 25, 37.5 or 50 x 10³ sporulated oocysts of *Isospora canis*. All puppies exhibited patent infections from Day 10 to Day 18, and oocyst counts peaked on Day 13 at 17.9 to 73.8 x 10³ oocysts per gram (OPG) of feces. This model was adopted for a dose determination study in which 30 Beagle puppies were infected with 5 x 10⁴ sporulated oocysts of *I. canis*. Puppies were ranked by oocyst counts, and randomly allocated to receive 20, 30, 40, or 50 mg/kg Ponazuril on Day 0, or 30 mg/kg Ponazuril on Days 0 and 7. Daily health observations, clinical signs and oocyst shedding were monitored for 14 days post treatment. All dosages significantly decreased oocyst counts >97% (P<0.05) by Day 4. In an attempt to prolong patency during a second dose determination study, 40 Beagle pups were inoculated with 104 sporulated *I. canis* oocysts on Days -10, -8, and -6. Puppies were randomly allocated to receive 30 or 50 mg/kg Ponazuril on Day 0, or 30 mg/kg ponazuril or placebo on Days 0 and 7. All dosages significantly decreased fecal oocyst counts (P<0.05) by Day 2. Analysis in both studies indicated that greater oocyst reduction was achieved by higher dosages of Ponazuril, and by a second dose on Day 7. No significant differences were observed in clinical signs between the various treatment groups in both studies. No adverse effects were associated with any dosages of Ponazuril.

055 **Autochthonous transmission of *Thelazia callipaeda* in southern Switzerland**

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Thelazia callipaeda (Spirurida, Thelaziidae) is commonly known as the “oriental eye worm”. This species affects dogs, cats, wild carnivores and occasionally humans. In Europe, *T. callipaeda* has been reported in Italy, France and Germany in dogs, cats and foxes. In southern Switzerland (Ticino), the first case of *T. callipaeda* was detected in 2000. Because of an increasing number of dog thelaziosis, a retrospective survey in veterinary practices was carried out, and in 2005 all veterinarians in Ticino reported their cases using a questionnaire. Collected nematodes were morphologically identified as *T. callipaeda* and molecularly processed to amplify and analyse a partial sequence of the mitochondrial cytochrome c oxidase subunit 1 gene (cox1). Since 2000, 129 *Thelazia*-cases were reported out of approximately 20'000 dogs living in Ticino, and 4 in cats. 72 of these animals (59.5%) had never crossed the Swiss border. From July to September 2006, 529 randomly chosen dogs, to which anaesthesia was given, were additionally checked for the presence of adult specimens of *Thelazia*: 28 dogs were found positive (prevalence of 5.3%). *Thelazia*-infection was furthermore diagnosed in 7 of 126 foxes (5.6%) shot in Ticino from December 2005 to February 2006. Affected foxes and dogs originated from the same regions. The results indicate that thelaziosis is endemic in that area and thus the insect acting as vector is present in southern Switzerland. Considering the identity of the haplotype target sequence identified, it is arguable that the infection originated from endemic areas of northern Italy.

056 ***Dirofilaria immitis* and *Wolbachia*: Effects of treatment of dogs with ivermectin, doxycycline and/or melarsomine**

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This study evaluated ivermectin and doxycycline, alone or together (with or without melarsomine) against *Dirofilaria immitis* infection and monitored and assessed the response to treatment. Thirty beagles were infected with 16 (7 M, 9 F) adult HW by IV transplantation. Six wks later, dogs were allocated to 6 gps of 5 each (Day 0). Gp 1 received IVM (6 mcg/kg) weekly for 36 wks. Gp 2 received DOXY (10 mcg/kg/day) orally during wks 1-6, 10-11, 16-17, 22-25, and 28-33. Gps 3 and 5 received both IVM and DOXY. At Wk 24, Gps 3 and 4 received an IM injection of MEL (2.5 mg/kg), followed 1 month later by 2 injections. Gp 6 was not treated. All 10 IVM+DOXY dogs were amicrofilaremic after Wk 9. Mf gradually decreased in dogs treated with IVM or DOXY, but most had a few at necropsy. Ag scores gradually decreased with IVM+DOXY and after MEL (1.8-2.2); scores for IVM or DOXY alone were similar to controls (4.0-4.2 at necropsy). At Wk 36, percent reduction of adults for IVM, DOXY, IVM+DOXY+MEL, MEL, and IVM+DOXY treatments were 20.3, 8.7, 92.8, 100, and 78.3, respectively. Clinical findings included eosinophilia in most dogs treated with IVM or DOXY alone at 12 wks, only dogs that received IVM did not have elevations in serum ALT and MEL had no apparent effect on serum chemistries. Gross pathological lesions were most severe in MEL-only dogs; dogs given IVM+DOXY+MEL had the fewest lung lesions. Mosquitoes fed on DOXY dogs had L3 that were normal in appearance, but not infective.

057 **Genetic and phenotypic divergence in australian *Haemonchus contortus***Hunt P.W., Le Jambre L.F., McNally J., Anderson L., and Knox M.R.*Moredun Research Institute, Penicuik, UK*

Introduction - Gastrointestinal nematode parasites of livestock, such as *H. contortus* were introduced into Australia via their hosts between 1788 and 1885. The sheep and goat hosts came from Europe, North America, Africa and Asia. Australian authorities have since restricted the importation of livestock and diseases. Populations of livestock nematodes in Australia therefore began as a mixture of genotypes from varied sources, and have been isolated from overseas populations for 55-98 years. Given divergent environmental selection, limited gene-flow, and genetic drift, genetic divergence within Australian parasite populations may have occurred.

Materials and Methods - Using microsatellite markers, and isolates separated both geographically and temporally, we have studied genetic divergence in Australia.

Results - We find a large amount of intra-population genetic diversity, however some isolates are genetically distinguishable. The 6 isolates in our study were grouped into 4 populations, implying some genetic structure in Australian *H. contortus*. This structure better distinguishes isolates separated geographically rather than temporally. Phenotypic characteristics (host infectivity and vulval morphology) are also divergent between isolates.

Conclusion - These phenotypic and genetic differences imply issues for the control of drug resistant genotypes and suggest population genetics will be an important consideration for future *H. contortus* control strategies.

058 **Genetic characterisation of *Haemonchus contortus* laboratory isolates and a rapid method of genetic fingerprinting using microsatellites**Redman L. 1, Packard E. 1, Grillo T. 1, Smith J. 1, Jackson F. 2, and Gilleard J. 1*1 Division of Infection and Immunity, Inst. Comparative Medicine, Faculty of Veterinary medicine, University of Glasgow, UK; 2 Moredun Research Institute, Edinburgh, UK*

Experimental work is conducted on a variety of different laboratory-maintained and field isolates of *Haemonchus contortus* worldwide. Recently it has become apparent that *H. contortus* is a genetically diverse organism and consequently there is a pressing need to be able to genetically define and monitor both laboratory and field isolates. We have developed a panel of microsatellite markers to undertake a detailed analysis of a number of commonly used *H. contortus* laboratory isolates. The genetic differentiation between these isolates is very high; AMOVA analysis based on 8 loci shows that 19.27% of the total genetic variation partitions between the populations. We also show that it is possible to estimate microsatellite allele frequencies from bulk DNA preparations of both adult and L3 worms. This can be exploited as a simple and rapid method of genetic fingerprinting for routine identification and monitoring of laboratory and field isolates of this parasite.

059 **A practical and cost effective mutation scanning approach for investigating the population genetics and epidemiology of *Cryptosporidium* from humans**

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Extending previous studies, we used a single strand conformation polymorphism (SSCP) – targeted sequencing approach to assess the genetic make-up of *Cryptosporidium* from humans in Victoria, Australia. Faecal samples (n = 98) from humans with cryptosporidiosis were collected between 1999 and 2006. Using two nuclear ribosomal loci, *Cryptosporidium hominis* (n = 74), *C. parvum* (n = 23) and *C. meleagridis* (n = 1) were identified. Using the ‘variable’ glycoprotein 60 gene (gp60), nine distinct genotypes were defined for the three species. *C. hominis* samples were classified as Ib A10G2R2, Id A15G1R2, and a new genotype, designated Ib2, was subgenotypically classified as A18G1R2. *C. parvum* samples were classified as IIa A18G3R1, IIa A20G3R1, IIa A22G3R1, IIa A23G3R1 and IIc A5G3R2. The richness of genotypes was compared between *C. hominis* and *C. parvum* and interpreted in relation to *Cryptosporidium* metapopulations and transmission patterns. The unique features of the present approach as a population genetic and epidemiological tool will be discussed.

060 **Diversity in mitochondrial lineages of liver fluke (*Fasciola hepatica*) populations**

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The sequencing of informative regions of the mitochondrial genome can provide data relating to the diversity of infrapopulations and their biogeography. In this study we have investigated the level of diversity amongst mtDNA sequence data over the first 2000 bps of the mitochondrial genome of *Fasciola hepatica*, thereby covering genes for coxIII, tRNA-His, cytB, nd4L and nd4. Samples of liver fluke infrapopulations from cattle and sheep were obtained from three geographically distant regions in Europe, from Australia and New Zealand, South America and sub-Saharan Africa. Seventeen haplotypes were present in a total population of 62 individual flukes. Some infrapopulations contained seven or more haplotypes, indicating that cattle and sheep are hosts to genetically diverse infrapopulations. This implies that selection for phenotypic characteristics such as drug resistance may be frequent. Although some haplotypes appeared to be confined to specific regions, others were distributed over three continents, suggesting that the different intermediate hosts encountered do not pose a barrier to the transmission of these genotypes. Diversity between haplotypes ranged from 0.086% to 2.84% with the flukes from Africa showing the greatest divergence. These results provide an insight into the phylogeography and transmission biology of fluke populations.

061 **Molecular characterization and phylogenetic studies of *Steganinae* (Diptera, Drosophilidae) inferred by the mitochondrial cytochrome C oxidase subunit I**
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Introduction: *Steganinae* subfamily ranks flies displaying zoophilic feeding attitudes at larval and/or adult stages, some of them acting as vectors of *Spirurida* eyeworms infecting carnivores and humans. Little is known about their taxonomy and phylogeny and several aspects of their systematics are lacking. The aim of the present work was to provide a data set for the molecular identification and phylogenesis of genera ranked into *Steganinae* Subfamily based on a partial mitochondrial cytochrome c oxidase subunit 1 (cox1) sequence. Materials and methods: One hundred thirty-four flies belonging to 13 species and 8 genera of *Steganinae* were morphologically identified. DNA was then isolated from each specimen and the most variable part of the cox1 gene was amplified by PCR. All sequences were subjected to molecular analysis and phylogenetic relationships among taxa were determined using the maximum-likelihood approach. Results: The mean nucleotide variation within *Steganinae* subfamily was 8.1%, with a variation within genera ranging from 1.6% (in *Phortica spp.*) to 21.8% (in *Amiota spp.*). Interspecific pairwise divergence ranged from 1.6 to 24.8% while intraspecific divergence from 0 to 1%. Seventy of the 233 amino acids were variable, including 26 parsimony informative sites and 44 singleton sites. The maximum-likelihood-based phylogenetic analysis provided strong support for genus *Phortica* as a monophyletic group, independent from genus *Amiota*. *Gitona distigma* was differentiated from all other *Steganinae* species examined and was placed in an unresolved position adjacent to the out-group taxon, *Drosophila yakuba*. Conclusion: Molecular data here reported represent the first dataset for *Steganinae* flies and may contribute a basement for further investigations about the molecular evolutionary relationships among this subfamily and other parasitic arthropods displaying zoophilic feeding habits.

062 **Molecular identification and geographic distribution of food and water- borne parasitic zoonoses in Vietnam focusing on (hybrid) *Fasciola gigantica*; *Opisthorchis viverrini*; *Clonorchis sinensis*; *Fasciolopsis buski*; *Haplorchis pumilio*; *H. taichui* and *Paragonimus heterotremus***

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Sequences of mitochondrial DNA (cox1; nad1), nuclear internal transcribed spacer 2 (ITS-2) and ribosomal 18S rDNA were obtained and used as genetic markers for the molecular identification and geographic distribution of the food and water-borne zoonotic parasites, *Fasciola gigantica*, *Fasciolopsis buski*, *Opisthorchis viverrini*, *Clonorchis sinensis*, *Haplorchis* spp and *Paragonimus heterotremus* in Vietnam. For *Fasciola* spp, of 21 Vietnamese isolates, 12 samples were of human origin of which 2 were miracidia hatched from eggs of infected patients and 2 were unusual migratory adults obtained from the skin and breast. For *F. buski*, 6 samples were collected from humans and pigs. For *O. viverrini*, *C. sinensis* and *Haplorchis* spp, eggs, metacercariae and adult samples originated from humans and fish from 18 provinces. For *P. heterotremus*, samples of different life-stages (eggs, miracidia, metacercariae, adults from natural and experimental hosts) and host species (crabs, dogs, cats and humans) were collected. Molecular analysis revealed that the Vietnamese *Fasciola* population belonged to two genotypes: a pure *Fasciola gigantica* and a hybrid form of *Fasciola gigantica* and *F. hepatica*; both are distributed throughout Vietnam. Molecular analysis confirmed that opisthorchids in Vietnam comprise *O. viverrini* which occurs in the South and *C. sinensis* in the Northern provinces. *Fasciolopsis* spp of human and pig origin were molecularly identified as *F. buski*. *P. heterotremus*, collected from different life-stages and hosts in Vietnam, was unique and found only in hosts from the mountainous provinces of North Vietnam. The intestinal flukes were identified as a mixed population of *H. pumilio* and *H. taichui*, with a possible intermediate genotype based on the variability of mini-satellites in the ITS-2 marker. The *Haplorchis* complex is distributed in 13 provinces throughout Vietnam. An increase in the number of human reports of fascioliasis/fasciolopsiasis and newly identified clonorchiasis/opisthorchiasis, paragonimiasis and haplorchiasis cases emphasize the urgent need to undertake additional molecular epidemiological surveys in Vietnam and neighbouring countries. We thank the ICGEB and FIBOZOPA (grant to Le TH) for financial support to this investigation.

063 **Zoonotic nematode parasites in wild caught African green monkeys (*Chlorocebus sabaues*) on St Kitts, West Indies**

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During the 17th century, African green monkeys (*Chlorocebus sabaues*) were brought to the island of St Kitts, West Indies from Africa and are now considered an evolutionarily separate species. The population on this island is estimated to be in the tens of thousands, although no recent formal population studies are available. They flourish in the high density residential areas and rural, agricultural lands making contamination of the water and food supply a major human health concern. The parasites of wild caught monkeys on St Kitts have not been investigated so this pilot study was to determine the parasites in both fecal and blood samples. A total of 45 monkeys from 12 different locations were trapped. Feces were examined by both the bench top fecal flotation and McMaster's egg counting method. Adhesive tape method was also used to identify Oxyurida and tapeworm eggs. A modified Knotts test was performed for microfilariae and a visual ectoparasite examination was performed on each monkey. Four nematode parasites were determined including *Strongyloides* (98%), most likely *S. fuelleborni*, *Trichuris* (84%), hookworms (16%) and *Capillaria spp* (11%). The McMaster egg count method was used in 22 of 45 fecal samples with a range of 1,400-23,500 eggs/gram. This is the first study to investigate parasites in these monkeys on this island and their zoonotic potential as pets or through contamination of water supply or local agriculture. This study shows all monkeys in all areas have parasites and may be a reservoir of infection to humans on the island.

064 **Ocular oxyspirosis of lower primates in zoos: intermediate host, worm morphology, and probable origin of the infection in Moscow zoo**

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Ocular oxyspirosis in lower primates is rare, only reported in zoos. Two species were described: in Berlin, *Oxyspirura* (O.) *conjunctivalis* from *Microcebus murinus* (lemurid), later also identified in *Loris gracilis* (lorisid); in Jacksonville, O. (O.) *youngi* from *Erythrocebus patas* (cercopithecid). The present Moscow case was among Old World lemuriforms and lorisiforms, and South American monkeys. (i) The intermediary host was elucidated. As for O. (O.) *mansoni*, from poultry, it was a cockroach (*Nauphoete cinerea*). Complete similarity of ITS1 rDNA sequences from adult and larval worms confirmed the identification. (ii) The Moscow material from *Nycticebus c. coucang* was compared to the three materials previously studied. The gubernaculum, which was used as a distinct character, varied in length and shape. However other characters (vulvar bosses, extremity of left spicule) showed that the samples were not exactly similar and that each seemed characteristic of a zoo. (iii) The infective stage, alike the adult, presented longitudinal cuticular crests. This together with several other morphological and biological characters (long tail and oesophagus, vector) emphasize that the ocular parasites *Oxyspirura* and *Thelazia* are unrelated. (iv) The disease in Moscow zoo seemed to start with *Nycticebus pygmeus*, imported from Vietnam and it is suggested that Asiatic lorisids are at the origin of the Moscow case. Natural hosts for the other cases remain unknown but are very unlikely those found infected in zoos. Consequently the notion of type hosts here is artificial and the three agents of oxyspirosis are provisionally placed in the taxon O. (O.) *conjunctivalis*.

065 **Mixed *Giardia* infections in captive non-human primates in Belgium and the Netherlands**

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Giardia duodenalis and *G. enterica* are frequently diagnosed in the stool of human patients with diarrhoea. Non-human primates (NHP) are also host to both *Giardia spp.*, but prevalence data in wild and captive NHP are scarce and the possible reservoir function of NHP for zoonotic transmission of this parasite is unclear. The objective of the present study was to estimate the occurrence of *Giardia spp.* in captive NHP and to study the zoonotic reservoir function of NHP. To this end, a cross sectional study was conducted at 4 Belgian and 3 Dutch zoological gardens between August 2004 and September 2006. A total of 1396 faecal samples from 58 groups of NHP were examined by immunofluorescence assay for the detection of *Giardia spp.* A novel PCR targeting the triose phosphate isomerase gene was used for the species-specific detection of *Giardia* isolates. A subset of the samples was also identified using a previously described PCR based on the b-giardin gene. *Giardia* was found in 34% of the examined groups. Between these groups the number of infected samples varied from 2 to 93%. Based on the b-giardin gene the majority of the samples were identified as *G. enterica* (94 %), but mixed *G. enterica* and *G. duodenalis* infections were also frequently diagnosed (35 %). These findings suggest that *G. enterica* is highly prevalent in captive NHP and that these animals are a potential reservoir for zoonotic transmission. They also demonstrate that mixed *Giardia* infections commonly occur in NHP.

066 **Molecular characterisation of *Blastocystis* isolates found in animals and zoo-keepers at the Perth Zoo**

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Introduction: Routine microscopy analysis detected *Blastocystis* in the faecal samples from primates at the Perth Zoo. Previous studies have shown that it is common for primates in the zoo environment, and animal workers to harbour *Blastocystis*. **Materials and methods:** Faecal samples were collected from a variety of animal hosts from the Perth Zoo, including eastern grey kangaroos, southern hairy nosed wombats, various birds species, pythons, various carnivores, and all primate species, as well as volunteers from the staff at the Zoo. Information regarding the general health and lifestyle of the volunteers were also collected. A previously published nested PCR was used to detect and amplify *Blastocystis* from the faecal samples prior to dideoxysequencing. Faecal samples from the staff at the Zoo were also screened for other enteric parasites by microscopy. **Results:** This is the first report of *Blastocystis* found in the southern hairy nosed wombat. Most of the primate species and their respective zoo-keepers at the Perth Zoo are harbouring *Blastocystis*. Ten isolates from seven different primate hosts belonged to Subtype 1, which were genetically similar to some of the isolates from the primate zoo-keepers. Two of these primate hosts harboured mixed infections of Subtypes 1 and 5. **Conclusion:** This study has demonstrated that *Blastocystis* is widespread particularly in primates and their keepers with evidence of a zoonotic cycle of transmission. *Blastocystis* may be of clinical significance particularly when present with other enteric protozoan species.

067 **Molecular evidence of two species of *Oesophagostomum* in fecal samples from wild chimpanzees in Uganda**

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Nodular worms (*Oesophagostomum* spp.) are commonly found as nematode parasites of pigs, ruminants and primates. Several investigations demonstrated that the prevalence of infection by *O. bifurcum* may be high in some human communities from Africa. In wild chimpanzees, the prevalence of strongylid eggs (including *Oesophagostomum* spp.) may be high in fecal samples and clinical cases of oesophagostomosis have been recently described. However, there are still many gaps to be filled in our understanding of the basic population dynamics and transmission characteristics of primate-related nodular worms. The objective of the present study was to define the importance of *Oesophagostomum* infection in a group of free range chimpanzees in Kanyawara area, Kibale National Park, Uganda. From December 2005 to March 2006, fresh fecal samples were collected and subjected to direct examination and flotation for the detection of parasite eggs, oocysts or cysts. A total number of 302 samples (representing 33 different animals) were examined. *Strongylid* eggs were detected (at least once) from fecal samples of all the animals. Coproculture was performed from 20 samples (representing 17 chimpanzees). Characteristic L3 *Oesophagostomum* larvae were observed in 15 samples (representing 14 chimpanzees). Larval DNA was extracted and regions of rRNA were amplified using the PCR assay described by Gasser et al. (1993, 1999). Only 3 samples yielded a PCR product compatible with *Oesophagostomum* (310 bp). Further endonuclease analysis with Nla III and direct sequencing demonstrated that two chimpanzees were infected by *O. stephanostomum* and one chimpanzee was infected by *O. bifurcum*.

068 **Emergence and vector borne diseases in Livestock - a bright future for veterinary entomologists**
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In recent years, several vector-borne, parasitic, zoonotic diseases have (re)-emerged and spread across Europe. These events have a profound impact on human health, ecology, socio-economics and disease management. Most of these outbreaks can be linked to global and/or local changes resulting from climate change, human-induced landscape changes or the changing activities of human populations. These changes have led to the invasion of new insect disease vectors, the improved vectorial capacity of autochthonous vectors to new or established pathogens, or to the introduction and establishment of new pathogens.

Arguably the best documented example of veterinary importance is the recent twofold invasion of bluetongue in Europe. During the late nineties the tropical midge, *Culicoides imicola* started invading the Mediterranean basin from Northern Africa and the Near East taking in its wake a series of bluetongue virus serotypes - BTV 1, 2, 4, 9 and 16 - which are now endemic in several parts of the Mediterranean region. Most of the bluetongue outbreaks have been attributed to this invasive tropical midge. Nevertheless at the fringes of its still expanding distribution area (e.g. the Balkan), local midges apparently have taken over the role of the tropical midge, by carrying and spreading the virus. What is of even more concern is the recent successful northern outbreak of the BTV8 serotype, north of latitude 51°. This serotype is unknown around the Mediterranean basin and thus the outbreaks are clearly independent of the previously recorded southern outbreaks. Within this northern zone only native midges are present and disease spread can only be attributed to these native species. Whilst these outbreaks may be related to the extreme weather conditions prevailing during the summer of 06, they may anticipate the siege of fortress Europe by a whole set of new pathogens in the coming decade since more than 50 viruses have been associated to *Culicoides* midges. The fact that viral infectious diseases of livestock in Europe are no longer restricted to diseases transmitted through direct contact, but now also include vector-borne diseases may have a significant impact on research and disease management. In the past decades research focus has moved from the 'adventurous field' to 'the safety of the bench' and the word 'entomology' almost disappeared from the veterinary curricula. This was due to very diverse reasons, one of which is the current 'publish or perish' culture forcing researchers to allocate their limited resources towards more rapidly rewarding bio-molecular work as compared to boots-on field work, and another which is the withdrawal of research programs in the tropics. To prepare for future threats, this trend should be reversed and institutions should invest again in training field entomologists and parasitologists as essential partners in multidisciplinary research and disease management.

teams. To make significant progress towards improving our understanding of changing disease patterns such a winning new-borne parasitologists cocktail should include:

1. Field knowledge of changing biotopes and habitats at various scales;
2. Improved molecular tools to accurately identify pathogen and vector species;
3. Remote sensing data and spatial modeling approaches to predict presence and spread.

Given examples include not only Bluetongue in Europe, but also *Fasciola* in Belgium and Tsetse transmitted trypanosomiasis in Southern Africa.

069 **Emergence of vector borne zoonosis, focus on granulocytic anaplasmosis (*A. phagocytophilum*), tick borne encephalitis and flea transmitted rickettsiosis (*R. Felis*)**

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070 ***Toxocara canis* in Swiss dogs: temporal aspects of different anthelmintic treatment procedures**

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Coproscopic examination of 505 dogs of Switzerland revealed an overall prevalence of 7.1% for *Toxocara canis*, as compared to hookworms (6.9%), *Trichuris vulpis* (5.5%), *Toxascaris leonina* (1.3%), *Taeniidae* (1.3%), *Capillaria sp.* (0.8%), and *Diphyllobothrium latum* (0.4%). Potential risk factors for infection were identified by a questionnaire, including dogs from rural versus urban areas, access to small rodents, offal and carrion and feeding of fresh and uncooked meat. In order to study the incidence upon different treatment strategies, a group of 111 dogs was treated every 3 months with a combined medication of pyrantel embonate, praziquantel and febantel, and another group of 112 dogs with a monthly treatment based upon milbemycin and praziquantel. Fecal samples were collected for coproscopy in monthly intervals. The yearly incidence of *T. canis*, hookworms, *T. vulpis*, *Capillaria sp.* and *Taeniidae* was assessed, and findings indicated that an infection risk with potential zoonotic pathogens cannot be completely ruled out for the dog owner, this, however, in dependence of the different deworming strategies used.

071 **Induction of patent *Toxocara canis* infections in previously exposed and in helminth free, regularly treated or untreated young dogs after low dose inoculations with embryonated eggs**

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The onset of patent infection with *Toxocara canis* in the dog depends on the migratory pathway of parasite larvae (somatic or tracheal) which is considered to be largely related to the host's age and its immune status. Therefore, adult canids are not regarded as main shedder of eggs of this zoonotic parasite. However, epidemiological surveys show age independent prevalences of patent *T. canis* infections in healthy adult animals. In a study with 18 dogs (8-9 months of age, 10 females, 8 males), the controlled induction of patent infections after repeated egg inoculations in treated and untreated dogs was investigated in 3 groups of 6 animals over a period of 7 months. Animals of group nos. 1 and 3 were inoculated twice with 100 infective *T. canis* eggs followed 10 days later by anthelmintic treatment with Milbemax® in group 1 (group 3 remained untreated). The animals of the non-inoculated control group 2 were treated as in group 1. We succeeded in inducing patent infections in all animals of group 3. Following a three-month observation period, all 18 animals were treated with piperazine citrate to prove or exclude intestinal worm infestation and all were administered 100 infectious eggs. Patent infections were detected in all groups (group 1, 1 dog; group 2, 5 dogs; group 3, 4 dogs). Observed prepatency periods were 40–56 days. The findings are significant regarding veterinary public health aspects. Based on the prepatency, anthelmintic treatments of adult dogs in 6 weeks intervals could prevent patent infection and thus minimize the zoonotic hazard.

072 **Geographical information systems in health application: experience on filariosis and other vector-borne diseases**

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Climatic changes, together with an increase in the movement of cats and dogs across Europe, have caused an increase in the geographical range of several vector-borne parasites, e.g. *Dirofilaria*, and in the risk of infection for animals and humans. Geographical Information Systems (GIS) are increasingly used to study the epidemiology of parasitic diseases, particularly arthropod-borne diseases, and to spatial and temporal predictions based on the climatic and/or environmental characteristics of a certain area and the climatic and/or environmental requirement(s) of a certain species. Climate-based forecast systems, employing the concept of growing degree days (GDD), have been developed for different diseases of parasitological importance. Regarding heartworm (HW) disease caused by *Dirofilaria immitis*, a GIS-based model has been developed using temperature records from 2,256 meteorological stations in 58 European countries, spanning a 15-year period. This model was based on the fact that: (i) the *D. immitis* rate of maturation to infective L3 in the mosquitoes depends mainly on temperature, (ii) there is a threshold of 14°C below which development will not proceed, (iii) there is a requirement of 130 GDD for larvae to reach infectivity and a maximum life expectancy of 30 days for a vector mosquito. As result of this model, predictive maps have been drawn, assessing the duration of the HW transmission risk period and the efficient timing of HW chemoprophylaxis. The meteorological data used for the HW model, can also be extrapolated to study spatial and temporal transmission risk of other climate-based vector-borne parasites of companion animals.

073 **Infection biology of *Angiostrongylus vasorum* in foxes (*Vulpes vulpes*) with emphasis on the impact of host age, infection dose and exposure**

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Wild foxes are considered to form an important reservoir for *A. vasorum* to dogs but extremely little is known about the infection dynamics in foxes, or in dogs. Two experimental studies in foxes were performed in order to determine the impact of host age and infection dose (study 1) as well as that of repeated infections on the establishment and persistence of adult *A. vasorum* (study 2). In the first study, 14 farm bred female adult foxes (1m -3 years old) and 14 juvenile foxes (4 months of age) were inoculated orally with either 50 (low dose) or 200 (high dose) *A. vasorum* third stage larvae. Six uninfected foxes served as controls. Ten weeks post infection (p.i.) the foxes were euthanized and the adult worm burdens (WB) were assessed. Patency was reached from week 6 p.i. in the juvenile groups compared to week 7 p.i. in the adult groups. Mean worm burdens differed significantly between the two juvenile groups (mean worm burden of 69 in the high dose and 30 in the low dose group). This difference was not present between the two adult fox groups with mean worm burdens of 16 and 20 respectively. Thus, the onset of patency and establishment of adult worms appeared to be dependent on host age. In the second study foxes were inoculated with either a single, two or three doses of 100 larvae and euthanized at different times. The experiment will end in May 2007, and the results will be presented at the conference.

074 **The ESCCAP guideline for worm control in dogs and cats**

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European Scientific Counsel Companion Animal Parasites (ESCCAP) was formed in 2005 when it was perceived that there was a gap in Europe for a consistent approach to the diagnosis and treatment of companion animal parasites. ESCCAP's objective is to develop guidelines for the control of major parasitic infections in companion animals in order to protect the health of pets, enhance the safety of the public and preserve the bond between pets and people. The guidelines will be subject to regular revision in dialogue with practitioners and researchers across Europe, as the great diversity in parasites and their importance is recognised. Guidelines and other news for vets and pet owners will be presented on the website (www.esccap.org) and will also be disseminated by other means. Guideline No.1: Worm control in dogs and cats, was launched on the website December 2006. The guideline deals with geographical distribution and prevention by environmental control of parasite transmission, animal management and treatment. It identifies three "key" parasite groups that cause severe disease and/or pose a zoonotic risk and have high prevalence in some or all areas of Europe. ESCCAP is an independent, non-profit making organisation registered in UK, expecting to get charitable status in 2007. It is presently sponsored by a number of pharmaceutical companies. ESCCAP has 8 members (and 3 trustees) with a solid background in parasitological research and/or clinical practice. Members are responsible for a region of Europe and all are rotated within 7 years.

075 **P-glycoproteins in *Haemonchus contortus*: Ivermectin and moxidectin select for constitutive overexpression and induce further overexpression of P-glycoproteins**
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Previous work has indicated ivermectin (IVM) and moxidectin (MOX) selection on some P-glycoprotein genes in parasitic nematodes. We have analyzed different P-glycoprotein cDNAs from *Haemonchus contortus* and compared them with P-glycoprotein sequences in the *Caenorhabditis elegans* genome. Using realtime PCR on the linker region, we have measured the levels of mRNA for six P-glycoproteins in four strains/isolates of *H. contortus*. These isolates were an unselected strain (PF), two laboratory strains, derived from the PF strain, selected with IVF or MOF, and a field multi-drug, including macrocyclic lactone, resistant strain (Wallangra). IVM selection/resistance resulted in an increase in constitutive expression of several of the Pgps, while MOX selection also resulted in an increase in constitutive expression of some of the Pgps; but generally to a lesser extent than IVM. The induction of overexpression, by these macrocyclic lactones, was also examined in these Pgps in the field strain that was strongly IVM resistant and moderately resistant to MOX. IVM induced further overexpression, 24 hours after treatment of infected sheep, in four of the *H. contortus* Pgps, while MOX induced overexpression in one of the same Pgps, and another Pgp not induced by ivermectin treatment. These results show that constitutive and induced overexpression of some P-glycoproteins is associated with IVM or MOX selection and resistances. However, the constitutive and induced P-glycoprotein profiles are somewhat different for MOX compared with IVM selection.

076 **Investigations into the effects of p-glycoprotein and/or cytochrome P450 inhibitors on the phenotypic responses of various developmental stages of ovine nematodes**

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The reported prevalence of multiple drug resistant (MDR) isolates of nematodes in sheep has increased worldwide, with over 20 countries reporting the presence of such nematodes in their flocks. Works on specific mechanisms of anthelmintic resistance has focused on genetic mutations at target sites, alterations of the pharmacokinetics and overexpression of target genes to name a few. Work on non-specific mechanisms of resistance have confirmed the importance of the ATP binding cassette (ABC) proteins e.g. P-glycoproteins (PgP) and drug metabolising enzymes e.g. cytochrome P450 (CYP450).

Studies have shown that the use of PgP inhibitors e.g. Ketoconazole and valspodar or the CYP450 inhibitors e.g. piperonyl butoxide can alter the efficacy of anthelmintics. These inhibitors were used in combination with thiabendazole (TBZ) and macrocyclic lactones (ML) in egg hatch assays, larval feeding inhibition assays and motility assays to determine the effect on egg development, first stage larval feeding and L4 and L5 motility.

Both PgP and CYP450 inhibitors reduced the concentration of ML required to prevent 50% of IVM resistant and susceptible first stage larvae feeding by greater than 0.5 - 6 fold compared to drug alone treated controls. The effects of the inhibitors on hatching were around 0.1-4 fold lower. The results from the motility assay showed similar trends to those seen in the above two assays, but were not as marked.

The findings from these in vitro assays suggest that the phenotypic expressions of resistance against TBZ and ML are also influenced by mechanisms that affect xenobiotic transport and metabolism.

077 **Pyrosequencing of two loci in the beta-tubulin gene, codons 167 and 200, in *Cyathostomin* populations of known benzimidazole resistance status**

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Cyathostomins are currently the most important equine parasite. High levels of infection can result in mortality whilst sub-clinical levels may be responsible for poor condition. Over 50 cyathostomin species exist, however up to 90% of all infections are composed of 10 common species. Using anthelmintic drugs as the primary method to control these parasites has led to widespread resistance to several drug classes including the benzimidazoles (BZs). Drug resistance in related ruminant intestinal nematodes is associated with T/A polymorphisms at codons 167 and 200 (F_Y amino acid change) of the BZ molecular target, β -tubulin isotype 1. However the mechanism for BZ resistance in cyathostomins is still unclear. We previously reported genotyping cDNA from 3 populations of parasites of known fenbendazole resistance status by pyrosequencing, revealing there are a statistically greater number of resistant parasites with the F167Y and F200Y mutations. Here we report the design of assays to genotype genomic DNA from adults and L3. Target sequence was generated from 95 individuals from 6 geographical locations representing 13 cyathostomin species. Degenerate primers were designed using a consensus sequence and their ability to amplify the target region from multiple species was confirmed. A high through-put pyrosequencing approach was applied to individual L3 from field populations of known BZ resistance status. A minimum of 100 L3 were genotyped, giving 1% sensitivity. For the first time we report the application of a high through-put method to compare the genotype, at 2 loci, and phenotype of individual cyathostomin larvae from field populations.

078 **Flow cytometry in anthelmintic resistance research**

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In vitro bioassays are an essential tool in the study of anthelmintic resistance, but extensive microscopy makes these assays time-consuming. The use of a large particle flow cytometer to identify nematode eggs and larvae from egg hatch and larval feeding assays and as a tool for speciation of nematode eggs was studied in order to provide a rapid alternative to microscopy. The studies used a Cytosense benchtop flow cytometer with a 1mm aperture developed by Cytobuoy bv. originally for use in situ to analyse marine plankton populations but subsequently adapted for laboratory use. Data was analysed using Cytobuoy developed software designed for identification of multicellular organisms

Eggs and larvae from the egg hatch assay were distinguished based on particle size while fed larvae from the feeding assay were identified by fluorescence from FITC labelled *E. coli* in the intestinal lumen. Data from the cytometer proved to correlate at a level of 0.99 with microscopy derived data when counted at a concentration of 1000 parasites per ml, although some under-estimation of larval numbers was noted. The use of direct fluorescence values as a measure of feeding was also investigated.

Further studies into the viability of using flow cytometry to identify small numbers of *Haemonchus contortus* eggs used fluorescent peanut agglutinin which has previously been shown to specifically bind to these eggs showed that the cytosome was capable of identifying fluorescent eggs.

079 **Single or multiple appearance of benzimidazole resistance: a microsatellite investigation of goat nematode in two French regions**

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Phe200Tyr in isotype 1 β -tubulin gene is the main mutation that confers benzimidazole (BZ) resistance in natural populations of *Teladorsagia circumcincta*, *Haemonchus contortus* and *Trichostrongylus colubriformis* (Trichostrongylid nematodes). After sequencing of isotype 1 β -tubulin gene from 21 BZ-resistant populations from two French regions, we found only one allele in 11 *T. colubriformis* populations, supporting the “diffusion hypothesis”: mutation Phe200Tyr appeared once and spread to populations through introduction of parasitized goat. For *T. circumcincta* and *H. contortus*, we found some polymorphism (SNP and indels): some alleles were shared by several populations. To test whether if those alleles, shared by several populations, have a common origin (“diffusion hypothesis”) or if the same mutation occurred in different geographical locations (“local mutation and then selection hypothesis”), we investigated neutral genetic markers to characterise the genetic diversity of these BZ-resistant populations. We used 7 microsatellites loci from *T. circumcincta* and *H. contortus*. Under the “diffusion hypothesis”, gene flow between populations should be relatively important and no genetic differentiation between populations is expected (index of differentiation, $F_{st} < 0.1$). Conversely, under the “selection hypothesis”, the same BZ-resistant allele may appear in diverse populations, even within populations genetically differentiated ($F_{st} > 0.1$). Results indicate that gene flow is strongly reduced between regions, with higher F_{st} between regions (F_{st} range from 0.20 to 0.29) than among regions (F_{st} range from 0.02 to 0.15). The range of F_{st} values between populations from the same region (0.02 to 0.15) is consistent with both hypotheses. Among populations from a region, intensity of gene flows is highly variable. However, the lower F_{st} are not found for populations that share BZ-resistance allele, supporting the “diffusion hypothesis”.

Part of this research was undertaken within the frame of Parasol EU program.

080 **Macrocyclic lactones selection on beta-tubulin in *Haemonchus contortus*: Implications for cross-resistance selection to benzimidazole and macrocyclic lactone anthelmintics**

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Macrocyclic lactone (ML) endectocides, such as ivermectin (IVM) and moxidectin (MOX), are used to control nematode parasites in humans and animals. Unfortunately, ML resistance has developed in many parasites. Changes in the ligand-gated chloride channel (target receptor of MLs) genes may be associated with resistance to these drugs. Alternatively, IVM has been shown to be an excellent substrate for P-glycoprotein transport. It has been reported that amphids are also altered in ML-resistant *Haemonchus contortus*. We have analyzed alpha- and beta-tubulin isotypes 1, 2 and 3 genes from different field and laboratory *H. contortus* strains. Here we report that repeated IVM or MOX use changes the frequency of beta-tubulin isotype 1 alleles and selects for specific SNPs. ML treatments select for three amino acid changes in beta-tubulin. Two of these SNPs have been described to confer benzimidazole resistance. This has major implications for parasite control because ML use may predispose nematodes to benzimidazole resistance and it could have implications for the sustained use of IVM/benzimidazole combinations against livestock and human parasites. The role of beta-tubulin in ML resistance requires further investigation, but mutations in this gene could affect the uptake of MLs via the amphids, or affect ligand-gated chloride channel function and transmission of neuromuscular signals, since microtubules play a role in anchoring neuro-receptors at synapses. Understanding ML resistance is an important step in reducing selection pressure for resistance, and for developing molecular markers which can be used to monitor the development of resistance and its spread.

081 **Horn fly (*Haematobia irritans*) olfactometric responses to cattle host odour in a Y-tube**
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The horn fly, *H. irritans* (L. 1758) (Diptera: Muscidae), is a widespread ectoparasite which causes substantial losses to the animal industry as high as US 24.5 million per year, in Chile. The aim of this work is to find natural semiochemicals (behaviour-modifying chemicals) emitted from cattle previously classified as high- and low-fly carriers, with the identified compounds being developed for use as attractants or repellents in a future integrated pest management strategy. Preliminary behavioural bioassays were carried out in a Y-tube olfactometer (0.9 mm d; 110 mm stem length; 90 mm arms length). A number of compounds identified in body odour extracts collected from cattle hosts were tested in doses from 0.01 μg to 1 μg (N=30). Data were analysed by Chi square test (Stat Direct software). Flies were significantly ($P < 0.05$) attracted by higher doses (1 μg) of three compounds and were repelled by one of them. This is the first report on the use of Y-tube bioassays to study *H. irritans* behaviour, in response to semiochemicals identified from host cattle.

082 **Effect of cypermethrin selection pressure and substitution with amitraz on phenotypic and genotypic measures of cypermethrin resistance in field populations of *Boophilus microplus* (Acari: Ixodidae) in the Mexican Tropics**

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There have been few studies of arthropod field populations quantifying the kinetics of phenotypic and genotypic evolution of resistance to chemical intervention in response to presence or absence of selection pressure. In a prospective controlled intervention field study we measured the resistance phenotype (by bioassay in a larval packet test expressed as Resistance Factor, RF, derived from probit analysis of dose mortality regressions) and genotype (by PCR to determine the frequency of a sodium channel mutation – 4Phe-Ile amino acid substitution - associated with pyrethroid resistance), in field populations of *Boophilus microplus* on 11 farms where cypermethrin (CY) had been regularly used and where varying degrees of resistance were present. On five farms CY continued to be used and on six it was substituted with amitraz used every 30-45 days. After 24 months of continued selection pressure with CY the RFs had increased 2-125 fold. The frequency of the resistance allele increased on all five farms from a starting range of 6-47% to a range of 66-95% after 24 months. On six farms treated with amitraz neither the RFs nor the frequency of the resistance allele changed. It is concluded that cypermethrin selection pressure on field population of *B. microplus* produce a rapid development of resistance with increases of RF which correlate with increased frequencies of the resistance allele. In populations in which CY was substituted with amitraz RFs and frequencies of the resistance allele remained stable but did not decline over 24 months.

083 Effectiveness of a community-based strategic anthelmintic treatment programme in the control of gastrointestinal nematodes and *Fasciola gigantica* in cattle in Kilolo district, Tanzania

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A longitudinal study was carried to determine the effect of strategic community-based worm control (CBWC) programme in the control of gastrointestinal (GI) nematodes and *Fasciola gigantica* in cattle. Two villages namely, a CBWC village and a village without CBWC were selected in Kilolo district, Tanzania. Animals in both villages were under traditional management and used the village communal grazing area. At the village with CBWC, 60 zebu cattle (6-18 months old) were selected and ear tagged for monthly sampling and weighing. A strategic CBWC was instituted whereby all animals in the village were treated with albendazole 10% at 10 mg/kg four times a year e.g. February, May, September and December. In the village without CBWC, 68 zebu cattle (6-18 months old) were selected and tagged for monthly sampling and weighing. Farmers in the village without CBWC were allowed to continue with normal management practices including anthelmintic treatments. Faecal and blood samples from tagged animals and pasture samples from communal grazing areas were collected on monthly basis for 13 months. Results showed that strategic CBWC programme was highly effective in reducing faecal egg counts (FEC) and the proportion of animals passing *Fasciola* eggs in faeces. Strategic CBWC reduced FEC of animals by 60% compared to animals at the village without CBWC ($P < 0.001$). Four strategic CBWC treatments per year reduced the proportion of animals passing *Fasciola* eggs in faeces by 82.5% compared to animals at the village without CBWC ($P < 0.001$). From commencement to the end of the trial, animals at the village with CBWC outgained animals at the village without CBWC by 13.9kg ($P < 0.05$). It is concluded that strategic CBWC programme was highly effective in reducing FEC, reducing the proportion of animals passing *Fasciola* eggs in faeces and significantly improved weight gain in animals.

084 A case-control study on outbreaks of lungworm disease in dairy herds in The Netherlands

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A case-control study was conducted to investigate some risk factors of lungworm outbreaks in adult dairy cattle in The Netherlands. A questionnaire was used to inquire about aspects of the lungworm outbreaks, and grazing and rearing management of the dairy herds. In total 32 case and 32 control farms were included. Most lungworm outbreaks were observed in August and occurred 4 to 5 months after turnout.

The a priori expectation was that lungworm outbreaks particularly occur on farms where the rearing management hinders or prevents the build-up of immunity against lungworm infection. Though difficult to prove due to limitations in number of farms that could be investigated, a few risk factors were identified supporting that hypothesis. Zero-grazing of young stock only occurred on case farms. Preventive anthelmintic treatment of the yearlings prior or during the grazing season was applied on twice as many case than control farms (OR 3.23, $p < 0.05$). If heifers were introduced into the dairy herd during the grazing season, most of them had been introduced slightly over one month before an outbreak occurred with a smaller introduction peak at about two months prior to an outbreak.

Finally, all 4 farms that had turned out their whole dairy herd after two or more years of zero-grazing subsequently experienced an outbreak within one or two years. Apparently, lungworm was rapidly (re)introduced into a free and susceptible herd, but the source of infection could not be determined.

085 **Original systematic combination of moxidectin pour-on and long action in beef cattle for control of gastro intestinal strongylosis. Control of the efficacy of the protocol by dosing blood pepsinogen**

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In France, beef cattle breeders usually sell the male calves at weaning. So, these one have to reach a maximal weight, expecting a perfect control of gastro-intestinal strongylosis. This can be done by using long-acting antiparasitic treatments. Conversely, for female calves intended for replenishing the herd, more short-acting antiparasitic agents should be used to achieve controlled parasitic contact, which will confer immunity without compromising growth.

We chose to test a new anthelmintic protocol in a Charolais herd with 46 pairs calf-mother, over two groups from different fields. During the turnout to grass period, the male calves were treated with an injectable solution of long-acting moxidectin (active for 120 days against *Ostertagia*) and the females were treated with a pour-on solution of moxidectin (active for 35 days against *Ostertagia*). Among the calves born after the turnout to grass period, only males were treated with long-acting moxidectin in July. This use of two different galenic formulations meets the objectives mentioned above.

Visually, the growth of the animals was perfect, higher for the males than in previous years (estimated at 1.150 g/day for the first part of the grass season). Results from the coproscopies performed in July reported excretion that varied from 0 to 210 OPG. The serum pepsinogen dosed in October gave results between 313 and 1211 mU of tyrosine (dosed with the French method) for females and between 914 and 2454 for males. An episode of dictyocaulosis was recorded at the beginning of September in one of the batches.

086 **Characterization of South African *Theileria equi* and *Babesia caballi* isolates based on 18S rRNA gene sequences**

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Genetic diversity amongst the protozoal parasites that cause equine piroplasmosis was revealed during development of real-time PCR assays for the detection of *Babesia caballi* and *Theileria equi*. SimpleProbes were designed and used in real-time PCR assays to detect *B. caballi* and *T. equi* infections from two experimentally infected ponies. *T. equi* infections could be detected from day 5, post infection (p.i.) and *B. caballi* from day 8 p.i. The assays were further tested on samples that were positive in culture for either *B. caballi* or *T. equi*. The probes were however, not able to detect target DNA in all of these samples. Parasite 18S rRNA genes were therefore amplified and sequence analysis revealed variation in the sequences, explaining the failure of the real-time PCR assay to detect all samples. The sequence diversity of the 18S rRNA gene was further assessed using samples representative of different geographical locations around South Africa. Whole blood samples were obtained and tested using the reverse line blot hybridization assay. Samples that hybridized only to the *Theileria/Babesia*-genus specific probe and not to the *B. caballi* or *T. equi* species-specific probes were selected for further sequence analysis. Results indicated that extensive sequence variation occurs in the 18S gene of *T. equi* and *B. caballi* isolates. It is also evident that it will not be possible to design a real-time PCR assay to detect all *T. equi* and *B. caballi* isolates based on the 18S rRNA gene and that a different gene will need to be identified for that purpose.

087 **Genetic diversity of South African *Theileria parva* isolates**

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Corridor disease is the most important form of cattle theileriosis in South Africa today after the eradication of East Coast fever (ECF) in the early 1950s. However, there is a concern that *Theileria parva* parasites that could cause ECF may still exist in the African buffalo reservoir host in South Africa.

To determine the genetic diversity of *T. parva* isolates, cattle and buffalo blood samples were collected from different areas in South Africa. The variable region of the p67 gene was amplified by PCR. The sizes of the p67 amplicons were determined by agarose gel electrophoresis. Selected amplicons from different isolates were purified, cloned into a plasmid vector and sequenced.

In this study, up to four PCR products (ranging in size between 850 and 1200 bp) were obtained from isolates investigated. Four p67 profiles were identified including a “single”, “double”, “three” and “four” band profile. Sequence analysis revealed p67 gene sequences with a 130 bp insert and sequences without the insert, similar to the buffalo- and cattle-associated sequences, respectively, as already reported from East Africa. Additional two novel p67 sequence variants were also identified. Phylogenetic analysis of p67 gene sequences revealed three major groups. Sequence variations within the insert were noticed between the three major groups.

The p67 gene profiles appear to be more complex than previously thought and probably cannot be used to distinguish between cattle- and buffalo-associated isolates in South Africa. The significance of the different genotypes in the epidemiology of *T. parva* in South Africa still needs to be established.

088 **The epidemiology of theileriosis in Rwanda and the significance of the polymorphic immunodominant molecule (PIM) of *Theileria parva* in the vaccine development approach**

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Current breed improvement strategies adopted to overcome the high population pressure on land in Rwanda are severely hampered by the tick-borne diseases, especially *Theileriosis* caused by *Theileria parva* protozoan parasite. Control of *Theileriosis* has proven to be difficult principally because of the lack of epidemiological information. The antigenic polymorphism reported in *T. parva* has hindered efforts towards the development of immunisation programmes and to date there is no master strain to be used as vaccine on a wider geographic scale. A VLIR funded research project has been initiated to assess the baseline epidemiological situation in the field and to investigate into the possibility of developing an immunisation programme. A series of country wide epidemiological surveys revealed the coexistence of 4 *Theileria spp.* reflecting the presence of various tick species in the field. *T. parva* infection was the most prevalent species correlating with data obtained during a similar tick-survey where its main vector, *Rhipicephalus appendiculatus* was the most dominant tick species (91.84%) amongst the 6 species collected. Results from a parallel sero-survey revealed higher antibody prevalence (>50%) in all age categories indicating that an intensive host-tick interaction prevails in the field. A particular epidemiological situation was found in high land zones marginal for tick survival where the majority of animals did not seroconvert. A PCR-RFLP based multi-locus analysis of alleles obtained from three different loci (PIM, MS321 and P104) was used to characterise the *T. parva* field isolates. The PIM gene showed a higher resolution and subdivided the Rwandan *T. parva* populations into 2 dominant allele families. Further cross-immunity studies to correlate the genomic with the immunological differences of the identified PIM populations are in progress.

089 **Impact of tsetse-transmitted trypanosomiasis: variations on a theme**

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Tsetse-transmitted trypanosomiasis is a major constraint to animal production in sub-Saharan Africa. Huge benefits may accrue from the removal of the disease from the African continent. However, many of the studies quantifying the benefits of trypanosomiasis control suffer from the inevitable generalization of its impact on livestock production. A range of factors contribute to the impact of an infection on livestock production. They relate to the vector, the host and/or the parasite. Host-related factors such as breed are well-known. Recent studies in southern Africa have shown that human interference can also affect the epidemiology of the disease with important consequences for its impact. Man-induced changes in the natural habitat of tsetse flies have important repercussions for the distribution, density and infection rate of tsetse flies resulting in localized epidemiological hot spots where challenge is high and the gradual disappearance of tsetse from other areas. Moreover, the encroachment of people and their livestock into tsetse-infested zones has ramifications for the distribution and density of wild reservoirs of trypanosomes. The change from a mainly sylvatic transmission cycle with trypanotolerant game animals serving as the most important host/reservoir to a domestic transmission cycle, where trypanosusceptible livestock take over the role as host/reservoir, has consequences for the virulence of the circulating trypanosome strains and thus impact of the disease in extensive cultivated areas. It thus seems that through a better understanding of the processes currently affecting the epidemiology of livestock trypanosomiasis the quantification of the impact and benefits of control can be improved substantially.

090 **Epimastigote morphogenesis and the cell cycle of *Trypanosoma brucei* in the tsetse vector revisited**

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The cell division cycle and development of *Trypanosoma brucei* is characterised by certain morphotypes, specific for the establishment of infection and replication in the mammalian host and insect vector. These include the cryptic stages undergoing nuclear and kinetoplast repositioning and asymmetric cellular division in the *Glossina* vector. We studied the cell cycle and ontogenesis of *T. brucei* in *Glossina morsitans morsitans* by using the mitotic chromatid cohesion subunit TbSCC1 as a molecular marker, and a nucleic acid stain (Hoechst 33342) to visualize the nucleus and kinetoplast. The expression profile of TbSCC1 in the proventricular long trypomastigotes and pre-division epimastigotes indicate that these cells are in late S or G2 phases of the cell cycle. The nucleus takes on a unique elongated form only observed in these cell stages. The onset of the asymmetric cellular division is characterised by the anterior-lateral repositioning of the kinetoplast, the pooling of cytoplasm towards the posterior pole of the cell, and degradation of TbSCC1 from the chromatins. The nucleus and kinetoplast segregates almost concurrently at this stage. The whole process of asymmetric division can be completed in the proventriculus with the production of long and short epimastigotes. The long post-division epimastigotes degenerate and the nucleus fragments. The short post-division epimastigotes migrate to the salivary glands and reinitiate DNA synthesis. Asymmetric division in *T. brucei* is a necessary facet of its life cycle as this generates short epimastigotes that transform to infective metacyclics in the salivary glands.

091 **Tsetse habitat fragmentation dynamics and bovine trypanosomosis epidemiology in the Oti river basin of Northern Togo**

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A tsetse control project was conducted in Togo during ten years, from 1989 to 1999. During this period, because of socio-politic events, some of the protected areas were partially or fully resettled by human populations who had been expelled from these areas in the past. Sixteen years after this resettlement and seven years after the end of project, two cross sectional surveys to measure the presence and abundance of tsetse flies and the prevalence of bovine trypanosomosis were conducted at the beginning and end of the dry season, respectively in December and April 2006. The obtained field data were related to data on agriculture, land use / cover, demographic data and landscapes derived from a Landsat image. The obtained information was compiled in a spatial data base and included in a Geographical Information System (GIS) to produce a set of thematic maps. The preliminary results showed that Oti river basin is now fragmented into three main parts. The fragmentation of natural riverine vegetation, the preferred tsetse habitat, decreases following a North to South axis. The tsetse flies apparently disappeared from the protected areas of the north-western part of the former Oti game reserve where wild animals now are rare. *Glossina tachinoides* represented 99% of the tsetse flies captured, while *Trypanosoma vivax* was detected as the main parasite (9/10) responsible of bovine trypanosomosis. Future surveys will further clarify these preliminary results and the relationship between habitat fragmentation and trypanosomosis epidemiology.

092 **Immune cell kinetics in sheep abomasal tissues following infection with *Haemonchus contortus* L3 larvae**

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H. contortus infection in sheep can result in a range of problems from ill thrift to death, causing large losses to farmers. Repeated exposure in some sheep to *H. contortus* larvae can result in the development of effective immunity and the expulsion of the parasites from the gastrointestinal tract (GIT).

The cellular processes leading to the development of this immunity are poorly understood. Our studies were designed to elucidate the cellular kinetics of resistance. Eight month old sheep were given multiple sensitizing infections before having abomasal cannulae implanted and then challenged with *H. contortus* L3 larvae. Following the challenge, cellular changes in sequential abomasal biopsies were investigated.

Sheep determined to be resistant by adult worm counts had increases in abomasal mucosal eosinophils, globule leucocytes (GL), mast cells, CD45R+ B-cells, CD4+ T-cells and gamma-delta T-lymphocytes, but not CD8 T-lymphocytes at the time of putative parasite rejection. Of the cellular increases, mucosal eosinophils and CD45R+ B-cells showed biphasic responses at the time of both putative larval rejection and the appearance of adult worms. We propose that these increased local levels of eosinophils, GL and lymphocytes (CD4, CD45R and B- cells) may be associated with the ability of sheep to expel *H. contortus* larvae and adults from the GIT.

093 **Immune responses to *Haemonchus contortus* primary infection in susceptible (INRA 401) and resistant (Barbados Black belly) breeds of lambs**

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Introduction. Inter-breed variations in the resistance to *Haemonchus contortus* infection in sheep have been demonstrated in a number of studies. However, little is known about the immune mechanisms underlying such differences. The objectives of this study was to compare cytokine gene expressions and effector cells (eosinophils, mast cells...) and antibody responses between a resistant (Barbados Black Belly, BBB) and a susceptible (INRA 401) breed of sheep upon experimental primary infection with *H. contortus*. Material and methods. Ten six month-old male lambs of both breeds were infected with 10 000 infective larvae, half of them being slaughtered 4 days and the other half 30 days post infection. In addition, 8 INRA 401 and 7 BBB were kept uninfected. Results. As expected, the BBB demonstrated an overwhelmingly lower faecal egg count, longer prepatent period, lower worm counts and female fecundity compared to the INRA lambs. The cytokine patterns following infection indicate a Th2-biased response in both breeds. However, a more persistent and elevated IL-5 mRNA expression and blood eosinophilia were noted in infected BBB lambs. IgG antibody response developed earlier and stronger in the BBB lambs than in the INRA 401. Abomasal recruitment of eosinophils, mast cells and globule leucocytes were similar between the two breeds when evaluated at days 4 and 30 post-infection. Conclusion. The BBB lambs proved their strong resistance to *H. contortus* and the regulation of parasite populations seems to occur very quickly.

094 **Vaccination against *Haemonchus contortus*: performance of native parasite gut membrane glycoproteins in Merino lambs grazing contaminated pasture**

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In a replicated trial, parasitological and antibody responses of grazing weaner Merino sheep were assessed following vaccination with gut membrane proteins prepared from adult worms of *Haemonchus contortus*. Each vaccinated animal received 100 micro g native H11 and 100 micro g native H-gal-GP combined together in 5mg Quil A administered intramuscularly on days 0, 34, 80 and 127. Control animals received 5 mg Quil A alone on the same days. Vaccinated and unvaccinated control animals grazed pastures contaminated with the parasite from day 34 of the trial, and levels of parasitism were monitored by worm-egg counts in faeces (WEC) and packed cell volumes (PCV) in blood. The level of larval contamination on pasture was estimated from the worm counts of tracer sheep introduced monthly to the paddocks. WECs and anaemia were significantly reduced in vaccinated animals, and, in contrast to vaccinates, all control sheep required salvage treatment with anthelmintic. By the last two months of the trial, pastures grazed by vaccinated animals had significantly lower contamination with *H. contortus* larvae. Vaccinated animals had high levels of vaccine antigen-specific IgG1 and IgG2 antibodies in plasma, whereas those responses in the control sheep were very low. IgG1 titres in the vaccinated group, but not IgG2 titres, were inversely correlated with worm-egg counts. The results showed that H11 and H-gal-GP behaved like "hidden" antigens producing high levels of protection that were probably mediated through mechanisms involving antibodies, and, in particular, IgG1. It was concluded that if similar protective effects could be obtained with recombinant versions of the proteins present in either H11 or H-gal-GP, then the prospects for a commercial *Haemonchus* vaccine were real.

095 **Effect of vaccination of goats with H-gal-GP and H11 antigens from intestinal membrane cells of *Haemonchus contortus***

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Extracts of adult *Haemonchus contortus* were purified and used as a vaccine against the blood feeding parasite in goats as previously studied in sheep. The proteins used were H11 and H-gal-GP, hidden gut antigens from the microvillar membrane of the gut of the worm and combined with Quil A as adjuvant. The antigens were then administered to a group of goats kept on concrete then artificially infected with *H. contortus*. The control group received Quil A injections and was also infected. This study was performed to analyze the effects of the H11/H-gal-GP vaccine when given to goats as compared to sheep. The trial showed that IgG levels peaked three weeks after the first vaccine and remained high throughout the remaining booster series but began to wane after artificial infection. However, the IgG levels remained significantly higher than in the controls throughout the entire study. Overall mean fecal egg counts (FEC) were significantly higher in the controls and packed cell volume levels were significantly higher in vaccinated goats compared to controls from Week 3 post infection to the end of the study. A booster vaccine given Week 7 pi caused a sharp increase in IgG levels, elimination of worm burdens and decrease in FEC in the vaccinated group. Ninety-six percent fewer *H. contortus* adults were recovered at necropsy in vaccinated group compared to controls and >96% reduction in FEC after booster vaccine given during established infection. This study shows that the H11/H-gal-GP vaccine was sufficient in protecting goats after challenge infections but is shorter lived than when given under the same conditions in sheep as shown in previous trials. Booster vaccine given when infection levels are rising are effective in eliminating infections, reducing FEC and therefore may be used in place of an anthelmintic to control haemonchosis in goats as in sheep.

096 **Characterization of the intestinal contortin structure in *Haemonchus contortus***

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Contortin was the first intestinal antigen of the sheep parasite *Haemonchus contortus* which induced significant levels of protection when used to vaccinate lambs. This antigen is present in large amounts in the intestine of L4 and adult worms as a helical polymeric structure attached to the luminal surface of the intestinal cells. However, the protein itself and its function were never investigated. In the present study contortin was isolated and analysed by peptide mass fingerprint and LC/MS-MS. These analyses indicated that contortin comprises two proteins, Hc-PCP1 and Hc-PCP2, with homology to prolyl carboxypeptidases. The two proteins show 64 % amino acid identity to each other and both are comprised of two prolyl carboxypeptidase S28 type domains organized in a tandem repeat. The transcription of both genes is switched on from the L4 stage onwards, coinciding with the onset of blood feeding. Addition of contortin to complete blood plasma significantly inhibited coagulation in a dose dependent manner. Fibrinogen was identified as the main target since similar results were obtained with a purified fibrinogen monomer solution. SDS-PAGE analysis and mass spectrometry indicated that contortin specifically degraded the C-terminal end of the fibrinogen alpha chain, which has been shown previously to be essential for clot formation. The process happens within seconds after addition and can be partially inhibited by the peptidase inhibitor DiprotinA, an inhibitor of prolyl carboxypeptidases such as dipeptidyl peptidase IV. The data suggests the prolyl carboxypeptidases are intestinal anticoagulants used by *H. contortus* to interfere with blood coagulation.

097 **Speciation of ovine gastric nematodes using two molecular based techniques, SSCP and Pyrosequencing**

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Nematode parasites have classically been identified morphologically either by examining infective larvae or adult males. Modern molecular biological techniques offer the potential to identify all of the life cycle stages at the species level. However, many of these approaches involve species-specific primers which would mean in turn that individual lysates would potentially have to be used several times to reach identification. Molecular-based methods that have been investigated for this paper were SSCP and Pyrosequencing. Both methods use the same set of primers to amplify a 230-240bp region of the ITS-2 ribosomal RNA gene (taken from a paper published by Heise et al). With SSCP, a unique banding pattern is obtained for each species on a 15% polyacrylamide gel. The technique is relatively simple and inexpensive but does involve some subjective interpretation of banding patterns. Pyrosequencing offers conclusive results through a short sequencing run which takes around 3 hours to analyse 96 samples. The assay has been designed to identify 5 common UK ovine species (viz. *Haemonchus contortus*, *Teladorsagia circumcincta*, *Trichostrongylus axei*, *Trichostrongylus vitrinus* and *Trichostrongylus colubriformis*). The pyrosequencer has been successfully used to speciate individual eggs, L1s and L3s. Further development of the pyrosequencing technique is required in order to incorporate more species and to develop a means of obtaining quantitative data from pooled samples, with the assay working in SNP mode.

098 **The biological significance and practical implication of antigenic variability in protective T cell responses against *Theileria parva***

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The evolution of antigenically distinct pathogen strains that fail to cross-protect is well documented for pathogens controlled primarily by humoral immune responses. Unlike antibodies, which recognise native proteins, protective T cells can potentially recognise epitopes in a variety of proteins that are not necessarily displayed on the pathogen surface. Moreover, individual hosts of different MHC genotypes generally respond to different sets of epitopes. It is therefore less easy to envisage how strain restricted immunity can arise for pathogens controlled by T cell responses, particularly antigenically complex parasites. Nevertheless, strain restricted immunity is clearly a feature of a number of parasitic infections, where immunity is known to be mediated by T cell responses. One such parasite is *Theileria parva* which induces potent CD8 T cell responses that play an important role in immunity. CD8 T cells specific for parasitized lymphoblasts exhibit strain specificity, which appears to correlate with the ability of parasite strains to cross-protect. Studies using recently identified *T. parva* antigens recognised by CD8 T cells have shown that the strain restricted nature of immunity is a consequence of the CD8 T cell response in individual animals being focused on a limited number of dominant polymorphic antigenic determinants. Responses in animals of different MHC genotypes are often directed to different parasite antigens, indicating that, at the host population level, a larger number of parasite proteins can serve as targets for the protective T cell response. Nevertheless, the finding that parasite strains show overlapping antigenic profiles, probably as a consequence of sexual recombination, suggests that induction of responses to an extended but limited set of antigens in individual animals may overcome the strain restricted nature of immunity.

099 From parasite genome to parasite control

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The diseases caused by parasitic nematodes in domestic and companion animals are major factors that decrease production and quality of the agricultural products. Methods available for the control of the parasitic nematode infections are mainly based on chemical treatment, non-chemical management practices, immune modulation and biological control. However, even with integrated pest management that frequently combines these approaches, the effective and long-lasting control strategies are hampered by the persistent exposure of host animals to environmental stages of parasites, the incomplete protective response of the host and acquisition of anthelmintic resistance by an increasing number of parasitic nematodes. Therefore, the challenges to improve control of parasitic nematode infections are multi-fold and no single category of information will meet them all. However, new information, such as nematode genomics, functional genomics and proteomics, can strengthen basic and applied biological research aimed to develop improvements.

In this review we will, summarize existing control strategies of nematode infections and discuss ongoing developments in nematode genomics. Genomics approaches offer a growing and fundamental base of information, which when coupled with downstream functional genomics and proteomics can accelerate progress towards developing more efficient and sustainable control programs.

100 New insights into the application of geographical information systems and remote sensing in veterinary parasitology

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Disease mapping and environmental risk assessment using geographical information systems (GIS) and remote sensing (RS) technologies are now established as basic tools in the analysis of both human and veterinary health. However, epidemiologists have needed time to become convinced that the prospects offered by GIS and RS could be useful in their own discipline. The last 15-20 years have seen an ever increasing reliance on cartographic representation of infectious diseases, particularly parasitic infections and their vectors. The publication of thematic international peer-reviewed journals - including the current launch of *Geospatial Health* - pertaining GIS and RS attest to the increased interest in these new technologies. In veterinary sciences, particularly in veterinary parasitology, GIS and RS offer powerful means for disease mapping, ecological analysis and epidemiological surveillance and have become indispensable tools for processing, analysing and visualising spatial data. They can also significantly assist with the assessment of the distribution of health-relevant environmental factors via interpolation and modelling. The most important applications of these tools in veterinary parasitology, including recent advances in territorial sampling, will be discussed. Disease mapping, spatial statistics, ecological analyses, including updates dealing with the photo-interpretation of pasturing areas using digital orthophotos, and epidemiological surveillance will be also addressed.

101 **Climate, satellites and the ecology of vector-borne parasites**Malone J. 1, Nieto P. 1, and Bavia M. 2*1 Louisiana State University, Pathobiological Sciences, Baton Rouge, LA USA; 2 Federal University of Bahia, Preventive Medicine, Salvador, Bahia, Brasil*

Temperature and moisture regime are fundamental determinants of the distribution and abundance of parasites and vectors. The unique thermal-hydrological preferences and limits of tolerance of individual parasite-vector systems, if not known from the literature, can be defined at known sites of occurrence by geospatial analysis methods and then extrapolated within a GIS to create maps of areas compatible with the environmental niche of disease agents in the landscape. Time series data can be used to map development rate, time of first possible transmission, and relative risk based on the number of potential life cycle generations completed in a given year or transmission season. Relevant sources of climate and environmental satellite data via the internet data is increasingly available at no cost. Examples will be presented on application of climate station parameters, climate grids, growing degree day-water budget analysis, climate surrogate data from earth-observing environmental satellites to develop regional risk maps for schistosomiasis and leishmaniasis in Brazil, using both iterative and automated statistical ecological niche modeling methods.

102 **Spatial modeling tools in parasitology: ready for consumption?**Hendrickx G., and Ducheyne E.*Avia-GIS, Zoersel, Belgium*

In recent years the use of spatial tools is becoming increasingly popular in many scientific fields dealing with understanding and controlling diseases including parasitology. These include the use of both geographical and remote sensing data within geographical information systems. In the first part of this paper the authors review published results over the past five years and compare these with an assessment made in 2000. In the second part, the various inputs needed to achieve scientifically sound results are discussed: (a) spatial field sampling strategies, (b) remote sensing needs and (c) computing requirements. In the last part, different types of statistical spatial modeling techniques using both presence/ absence or presence only data are reviewed. These modeling techniques include logistic regression, discriminant analysis, artificial neural networks, random regression forests and ecological niche factor analysis. Pro's and con's are documented using practical examples both in a tropical and temperate climate setting. Each of these models is documented in view of the modeling objective, the level of expertise needed to run them, how they can be biologically interpreted and what their automation potential is. It is concluded that many tools are now available, but that they should be consumed with both the necessary care and expertise to avoid pitfalls.

103 **Predictive mapping of the habitat suitability for ticks**

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Climate and vegetation are the major factors affecting the distribution of ticks. Consequently, spatial distributions can be analysed by statistical methods that look for correlations between abiotic factors and known data about tick presence/absence. Remote sensing features can be obtained from a wide database of sensors with different characteristics, and then applied to the problem of mapping models of tick distribution. Remotely sensed information is available at different resolutions and at different temporal frames, thus allowing the possibility to understand the patterns of tick distribution at different scales. Remote sensing data can assist in mapping parasite, vector, and hosts distributions, monitoring changes in habitats, and predicting associated changes in tick distribution. Some studies had demonstrated that the use of these abiotic variables from satellite imagery has biological significance; therefore, statistical accuracy of these distribution models can be interpreted under an ecological framework. Furthermore, models can be linked to these predictive maps, enabling the forecast of spatial and temporal dynamics of ticks, looking for seasonal patterns of activity and accurate use of acaricide treatments. This presentation focuses on recent advances on the use of models to map the expected distribution of ticks and to recognize the climate niche of each tick species, as an additional tool to further understand the ecological relationships between ticks and environment.

104 **PARAGIS - Assessing eco-climatic factors affecting the spatial distribution of helminth parasite burdens in dairy herds**Charlier J. 1, Ducheyne E. 2, Claerebout E. 1, Williams D.J.L. 3, Hendrickx G. 2, and Vercruysse J. 1

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Climatic and environmental factors have a large impact on the epidemiology of many parasites. Establishing relationships between these factors and parasite distribution are an essential step towards developing spatial distribution models. PARAGIS is an ongoing research project which aims at modelling the spatial distribution and impact on animal health of the most important helminth infections in dairy cattle in Europe. In a first stage the project focusses on infections with *Fasciola hepatica* and *Ostertagia ostertagi*. A sample of thousand seven-hundred dairy herds was selected, representative for the prevailing eco-settings in Flanders (Belgium). The exposure of each herd to *F. hepatica* and *O. ostertagi* infections was determined by use of bulk-tank milk ELISAs. A wide range of predictor variables was selected, including geographical datalayers (e.g. soil type, digital terrain model), satellite-derived data (e.g. Land Surface Temperature, Vegetation Indices) and management data obtained by a questionnaire (e.g. stocking rate, length of grazing season). Eco-climatic factors affecting parasite distribution were investigated using regression forest and logistic regression analysis. Preliminary results are shown. In a second phase, spatial distribution models will be developed to identify zones with high risk for increased exposure to helminth infections and to monitor the infections over time.

105 **Treatment of *Neospora caninum* and other apicomplexan parasites with nitazoxanide and other thiazolides interferes in protein disulfide isomerase enzyme activity**

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The thiazolide nitazoxanide (2-acetolyloxy-N-(5-nitro 2-thiazolyl) benzamide; NTZ) exhibits a broad spectrum of activities against a wide variety of intestinal and tissue-dwelling helminths, protozoa, and enteric bacteria infecting animals and humans. The efficacies of several modified versions of the drug including bromo- chloro- and nitro-derivatives and collectively named thiazolides, were demonstrated against infective stages of the apicomplexan parasites *Neospora caninum*, *Toxoplasma gondii*, *Cryptosporidium parvum* and *Besnoitia besnoiti* in vitro. We show that the thiazole-associated nitro group is not necessarily required for the action of thiazolides against these intracellular pathogens, and that methylation of the salicylate-ring at selected positions resulted in reduced anti-parasitic activity. QSAR employed with the findings on 29 different thiazolides obtained on *N. caninum* suggested that modification of the amide-bond connecting benzene- and thiazole-ring could result in improvement of the efficacy.

With respect to *N. caninum*, bromo- and nitro-thiazolides induced rapid egress out of the host cells. Drug-induced egress was Ca²⁺-dependent. In addition, the bromo-derivative Rm4847 but not the nitro-thiazolide tizoxanide (TIZ), the primary NTZ-metabolite, inhibited host cell invasion by *N. caninum* tachyzoites. This demonstrated that bromo- and nitro-thiazolides exhibit differential efficacies against *N. caninum*, and possibly also the other apicomplexans.

In order to identify potential thiazolide-targets, affinity chromatography of *N. caninum* extracts on epoxy-agarose-coupled TIZ was performed. The major protein retained on the column and eluted with increased drug concentrations was *N. caninum* protein disulfide isomerase (NcPDI). Protein disulfide isomerases maintain the correct three-dimensional conformation of proteins by either reduction, oxidation or isomerization of disulfide linkages during protein synthesis. NcPDI was functionally expressed in *E. coli*, and indeed those thiazolides that were active against *N. caninum* also impaired the enzymatic activity of NcPDI in vitro, while compounds that did not impair parasite proliferation in vitro had no effect on the enzymatic activity of NcPDI. This indicates that in intracellular apicomplexans NTZ interferes in protein disulfide function.

106 **Inoculation of balb/c mice with live attenuated tachyzoites protects against a lethal challenge with *Neospora caninum***

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MoreDun Research Institute, Edinburgh, UK

Neospora caninum tachyzoites attenuated through prolonged passage in tissue-culture (Bartley et al., 2006) were tested for their ability to induce protective immunity against a lethal challenge dose of parasites. Mice were inoculated with either 1x10⁶ attenuated (n=10) or 1x10⁶ virulent tachyzoites (n=10), control mice received an inoculation of Vero cells (n=10). All mice were challenged 28 days later with 5x10⁶ virulent parasites. Animals inoculated with either attenuated or virulent parasites showed only mild transient clinical symptoms following both inoculation and challenge. At post-mortem on day 28 post-challenge, 56% (n=9) of the animals initially inoculated with the attenuated parasites showed both neuropathology and parasite DNA in brain tissue, and in three of these mice immunohistochemistry (IHC) demonstrated very low numbers of *Neospora*-positive infiltrating mononuclear cells, not associated with lesions. Following inoculation and challenge with the virulent parasites, 71% (n=7) of mice had demonstrable neuropathology and parasite DNA in the brain; tachyzoites and *Neospora*-antigen associated with moderate brain lesions were also found in one mouse from this group. Following challenge the control mice showed severe morbidity and 100% mortality, parasites were detected by IHC in brain and lung tissues in 62% of mice (n=8), parasite DNA was detected in all brain samples and when tested by qPCR was found in significantly (P<0.001) greater quantities compared to animals in the other groups.

This study shows that it is possible to protect against a lethal challenge of *N. caninum* and help limit the establishment of the parasite through inoculation with tissue-culture attenuated *N. caninum* tachyzoites.

107 **Eimeriidosis in camelids of Miandoab region, Iran**Yakhchali M., and Cheraghi E.*Faculty of Veterinary medicine, Nazlu campus, Urmia University, Urmia city, West Azerbaijan province, Iran*

An investigation into eimeriidosis of camels was carried out in two camel-raising areas of Miandoab region, Iran, to determine frequency and diversity of *Eimeria* species. Eighty five bacterian camels (*Camelus bactrianus*) and 40 dromedary camels (*Camelus dromedarius*) which were from one to four year-olds were subjected to examination. Fecal samples were collected and subjected to flotation technique using for demonstrating the presence of oocysts and sporulation of oocysts were performed, with an overall prevalence 12.8%. Five *Eimeria* species were identified in both camelids: the highest rate belonged to the *E. bactriani* (42.2%), followed by *E. rajasthani* (only in the dromedary camels, 26.7%), *E. pellerdyi* (only in the bacterian camels, 15.6%), *E. cameli* (11.1%) and *E. dromedarii* (4.4%). All 12.8% of infected camels had mixed infections at least with three species. Consistency and intensity had significantly correlation with age ($P<0.01$). The sex and age of the camels had significant effect on prevalence ($P<0.01$). These findings may be useful to evaluate infection potential when considering control programs, especially for young camels in this area.

108 **Innate immune reactions against *Eimeria bovis***Hermosilla C., Behrendt J.H., Zahner H., and Taubert A.*Justus Liebig University Giessen, Institute of Parasitology, Giessen, Germany*

The apicomplexan parasite *Eimeria bovis* is an enteropathogen of cattle causing clinical disease especially in calves. *E. bovis* sporozoites have to traverse several cell layers to reach their host cells, endothelial cells of the lymph capillaries, thereby representing potential targets for leukocytes of the host. We confronted bovine PMN and monocytes with sporozoites of *E. bovis* to mimick this situation in vitro and found that PMN seem to eliminate sporozoites. These reactions were clearly enhanced by immune serum supplementation. Heat inactivation or IgG depletion of immune serum eliminated this effect. Transmission and scanning electron microscopy showed PMN extending filopodia towards the sporozoites and engulfing the parasites. PMN reacted by increased transcription of the IL-6, IL-8, IP-10, MCP-1, GRO- α , TNF- α , IL-12p40 and iNOS genes upon confrontation with sporozoites or merozoite-antigen. To clarify the potential role of PMN and monocytes in vivo during cattle coccidiosis, we further analyzed the phagocytic and oxidative burst activities of PMN and monocytes obtained from primary *E. bovis* infected calves. Enhanced levels of both activities in PMN were found early after infection (1-5 days p. i.) and towards the end of first schizogony (13-22 days p. i.) confirming the in vitro data. These results suggest that PMN-mediated, innate immune reactions play a role in the early immune response of primary *E. bovis* infections in calves.

109 **Cellular immune response to *Isospora suis* infections in piglets**

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Isospora suis is the causative agent of neonatal coccidiosis. Infection leads to damage of the mucosal surface in the jejunum and ileum and to non-haemorrhagic diarrhoea, resulting in reduced weight gain of piglets and increased mortality due to secondary infections with other enteric pathogens. Despite its economic and veterinary importance, interactions between the parasite and the innate and the antigen-specific immune system of the host are still poorly understood. Immune responses in vivo are examined by phenotypical characterisation with multicolour flow cytometry (FCM) of leukocyte populations isolated from blood (PBMNC), mesenteric lymph nodes, spleen and the mucosa of the small intestine. *I. suis* specific immune response is characterised by proliferation assays, ELISA and ELISPOT assays for cytokines in vitro after re-stimulation of lymphocytes from infected swine with complete *I. suis* antigen. FCM analyses showed a strong increase of CD3+ cells, CD4+ T-helper cells and natural killer cells during infection and a slight increase of cytotoxic T-lymphocytes (CTL). In the lamina epithelialis, a strong increase of CD3+ cells and CTL was observed. Production of interferon-gamma after in vitro re-stimulation was shown for piglets between 6-10 weeks. Splenocytes but not PBMNC and MLN-derived lymphocytes reacted with a dose dependent interferon-gamma-production. We could show infection-dependent changes in leukocyte populations during *I. suis* infections which reflect involvement of both innate and adaptive components of the porcine immune system. Moreover, the results from antigen-specific restimulation in vitro strongly suggest an adaptive immune response to *I. suis*.

110 **Metaphylactic efficacy of diclazuril (vecoxan) against naturally acquired *Eimeria alabamensis* infection in calves**

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Introduction: The field studies were designed to demonstrate the efficacy against the species *E. zuernii*, *E. bovis* and *E. alabamensis* in housed calves, which had never been turned out. It is recognized that severe infections with *E. alabamensis* may cause clinical outbreaks. Anyhow, the prevalence of this species in stabled animals is less common compared with calves on pasture.

Materials and methods: On four sites in total 76 calves received orally a single dose of diclazuril (Vecoxan, 1 mg/kg BW) and 74 calves remained untreated. The product was given as a metaphylactic treatment when the calves were on average 1.5 months of age, before clinical outbreaks were expected. For three weeks after the treatment, oocyst counts (OPG) and differentiation were noted every other day.

Results On the four sites *E. alabamensis* was regularly found during the entire observation period. The day of treatment an average *E. alabamensis* of 410 and 443 OPG was counted in the treated and control group respectively. Within 5 days after treatment the average oocyst count had dropped to nearly zero (arithmetic mean: 0.6) in the treated animals, while the oocyst count remained at the same level (arithmetic mean: 363) in the control group. Based on the area under the curve (d1-d21) there was a statistically significant difference present between both groups (< 0.0001) with a reduction of 95,8 % of the infection with *E. alabamensis* in the treated calves.

Conclusion : Diclazuril dosed at 1 mg/kg is as effective against *E. alabamensis* as against the more pathogenic species *E. bovis* and *E. zuernii*.

111 ***Taenia solium* cysticercosis in western Kenya**

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Objective: To assess the burden of cysticercosis in selected areas of western Kenya in conjunction with collection of information on pig management practices and other potential risk factors.

Methods: A cross-sectional sample of 250 small-scale pig farmers in Western Province was conducted. Questionnaire, mapping and diagnostic tools were utilized to provide quantitative and qualitative data concerning pig farming practices and *T. solium* infection status of pigs as well as other relevant information relating to risk factors and linkages with epilepsy and poverty. The location of study households was enabled through the use of collected GPS data mapped using ArcGIS Geographical Information Systems software.

Results: In total 320 pigs were examined for lingual cysts of *T. solium* with 16 (5%) found positive. Ten percent of households had positive pigs. Half the farmers reported allowing their pigs to freely range in spite of regulations requiring their confinement. Most households (86%) reported consuming pork and preparing it by frying. Most households (94%) also reported having a latrine. Cases of porcine cysticercosis were then mapped at the district level based on GPS data.

Discussion: *Taenia solium* cysticercosis is endemic in western Kenya. Frying pork may not kill all *T. solium* cysts. The presence of porcine cysticercosis suggests that although most households reported having a latrine they are apparently not being used properly. The evidence gathered is being shared with stakeholders to serve as a basis for formulating a national plan of action to address the emerging problem of cysticercosis in Kenya.

112 **Prevalence of porcine cysticercosis and human taeniasis/cysticercosis in Nepal**

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The problem of cysticercosis is now considered arising after introducing intensive pig farming system in the rural and peri urban settings of farming during past decade under poverty elevation programme of the government. Previous baseline epidemiological study has detected the high evidences of porcine cysticercosis and human taeniasis/cysticercosis. Based on the study results a new 4 years project involving the Flemis Interuniversity Council, Ghent University, National Zoonoses and Food Hygiene research Centre and Christian Medical College, Vellore, India was initiated in January 2006. One of the project aims to provide the evidence base on the presence and impact of *T. solium* infection.

A cross- disciplinary approach method was used to tackle the problem. Surveys conducted simultaneously in humans and animals from the same environment to correlate data on an epidemiological level would enable a comprehensive and accurate approach to study the transmission and the burden. So far 121 pigs serum samples and 50 human serum samples were collected for testing by Western immunoblot techniques. About 52 pig carcass were examined for *Taenia* cysts of which 2 (3.85%) pigs had found positive. Out of 55 human serums tested 5 (9.09%) have shown immunoblot positive bands for taeniasis. This study is still in progress at first phase of the project.

113 **Taeniasis/cysticercosis in Vietnam: more questions than answers**Somers R. 1, Dorny P. 1,2, Lan Anh N.T. 3, Linh B.K. 3, and Vercruysse J. 1

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Several studies in humans and animals collected pieces of the taeniasis/cysticercosis puzzle in Vietnam. It is generally accepted that only cysticerci of *Taenia solium* may establish in man and cause symptoms, including severe headache, epilepsy and death. Seroprevalence studies (Ag detection) in man in North Vietnam indicated regional differences (0.0% - 5.7%), probably related to general living and hygienic conditions. Hospital-based studies indicated that an uncommonly high proportion of cysticercosis patients had subcutaneous cysts (73%) and that a surprisingly high proportion of adult tapeworms were *T. saginata asiatica* (55.4%) while only 6.2% were *T. solium*. However, there is no evidence to support the hypothesis that *T.s.asiatica* might cause human cysticercosis. Slaughterhouse-based studies in pigs showed a high proportion of cysts in the viscera and lesions in the pigs' livers, which were indicative for cestode infections. Most cysts and lesions were due to metacestodes of *Taenia hydatigena*, a common tapeworm of dogs; however, some liver lesions resembled cysticerci of *T. s. asiatica*, which are now being examined with molecular techniques. Infected organs or carcasses are usually not condemned, and consequently these findings are not officially reported. This may explain why cysts of *T. s. asiatica* have never been reported in Vietnam. Poorly documented eating habits form a final missing piece in the puzzle. Some traditional dishes contain raw pork but livers are usually fried. Future studies should include investigation of eating habits and the effect of Vietnamese cooking methods on the viability of cysts.

114 **Porcine antibody isotype responses in oxfendazole treated and non treated pigs naturally infected with *Taenia solium* cysticercosis**Sikasunge C.S. 1, Johansen M.V. 2, Phiri I.K. 1, Willingham III A.L. 3, and Ørnbjerg N. 2

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The livestock and public health significance of *T. solium* porcine cysticercosis has led to consideration of several different potential intervention measures for controlling the disease. One such proven intervention tool is treatment of pigs with oxfendazole. Studies have shown that once treated, pigs are refractory to re-infection for at least three months after therapy. To determine the type of immunoglobulin (Ig) isotype responses in pigs treated for *T. solium* porcine cysticercosis, an Ab-ELISA was utilized to determine total Ig, IgG1, IgG2 and IgA to *T. solium* crude cyst fluid antigen. Ten (10) pigs naturally infected with *T. solium* cysticercosis were randomly allocated into 2 groups: Treatment group (n = 5) was treated with an oral dose of 30mg/kg Oxfendazole while the Treatment control group (n = 5) was infected but not treated. A third group (n = 5) comprised healthy negative control pigs. Blood was collected from pigs fortnightly until 14 weeks post treatment (wpt). Treated pigs showed higher total Ig, IgG1, IgG2 and IgA antibody responses 2 wpt. There was a significant difference in total Ig and IgG1 responses between treated and untreated pigs 8 wpt (p = 0.031) and 12 wpt (p = 0.038), respectively. At 10 wpt, IgG2 responses were more dominant than IgG1 suggesting a shift towards IgG2 responses. The low but persistent levels of IgG2 in treated pigs may suggest an associated Th1 cellular response to *T. solium* infection and may explain why pigs are resistant to re-infection with *T. solium* after successful treatment.

- 115 **Nanobodies® as a tool for specific diagnosis of porcine cysticercosis (*Taenia solium*)**
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Taenia solium cysticercosis is a major helminth zoonosis in developing countries. The pig is the most important intermediate host mediating transmission of infection. Accurate diagnosis of infected pigs requires sensitive and specific assays. Unfortunately, no such tests are currently available: the immunoblot (EITB) detects antibodies and as such only reflects exposure to the disease, not necessarily infection; the monoclonal based antigen detection ELISA only detects living cysts. Furthermore, the use of this test in pigs is hampered by the occurrence of cross reactions with *Taenia hydatigena*, a common cestode infection in pigs in developing countries.

The objective of this study was to produce recombinant single domain antibodies specific for *T. solium* cysticercosis. Three animals (2 dromedaries and 1 llama) were immunized with either *T. solium* cyst fluid or excretion secretion antigens and their peripheral blood lymphocytes were employed to clone three gene banks of the variable domain of the heavy chain antibodies (i.e. the Nanobodies®). Several Nanobodies® were retrieved after panning of the phage displayed libraries: *T. solium* specific, *T. hydatigena* specific and cross reactive nanobodies, depending on the panning method. Their specificity was checked in ELISA and Western blot. The Nanobodies® were further characterized by BIAcore affinity measurements and competition ELISA. They will be adapted for use in antigen ELISA for the detection of active *T. solium* infections in pigs.

- 116 **Optimisation of the cysticercosis diagnosis in pigs through Bayesian modelling**
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Cysticercosis is a common cause of preventable neurological disorders in man in many areas of the developing world. Paradoxically, the World Health Organisation classifies it as a “Neglected Tropical Disease (NTD)” because of the general lack of interest to control the parasite affecting mainly poor people. A good diagnosis of cysticercosis in pigs is the backbone of an optimal control strategy. A recent published epidemiological study using a Bayesian approach carried out on Zambian village pigs estimated the true prevalence of the disease and diagnostic test characteristics in this pig population. The four tests used in this model were tongue palpation, carcass inspection and two serological tests (ELISA) for the detection of specific antibodies and circulating antigens of the parasite. The aim of the present study is the optimisation of the cysticercosis diagnosis in pigs evaluating the possibility of a reduction of the number of tests required, hence the diagnosis costs, to three (tongue palpation and carcass inspection and antigen ELISA) and further to two (tongue palpation or carcass inspection and antigen ELISA) while maintaining the diagnostic properties of the set of the initial four tests.

117 **Interaction of macrocyclic lactones (MLs) with multidrug resistance transporters**

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The P-glycoprotein, an active drug transporter involved in the multidrug resistance (MDR), appears as a major key target for the modulation of ML pharmacokinetics in host and in parasites. By using in vitro and in vivo approaches, we compared the interaction of MLs with the MDR-transporters and studied their contribution to the ivermectin disposition and efficacy in host and in parasites.

By following the rhodamine123 accumulation in cells overexpressing P-gp, the ability of all MLs tested to inhibit P-gp function was demonstrated, with a 10-fold lower potency of moxidectin when compared to avermectins. In membrane vesicles overexpressing P-gp or MRP1, ivermectin modulated stimulated-ATPase activity that revealed a rather good affinity for MRP1 ($K_i=0.5\mu\text{M}$), although it was lower than that of P-gp ($K_i= 0.05\mu\text{M}$). The in vivo contribution of these transporters was confirmed in dog and in sheep where the co-administration of ketoconazole, used as P-gp inhibitor, increased the plasma concentration of ivermectin. Finally, by using optimised larvae feeding inhibition assay (LFIA), concomitant addition of ketoconazole with ivermectin was able to increase the susceptibility to ivermectin in resistant nematodes isolates.

A clear structure-affinity relationship was established for interaction of MLs with P-gp which could be related to the pharmacokinetics properties of the drugs. Beside P-gp, MRP1 provided complementary functionality as ivermectin efflux pump. Because MDR-interfering agents improved ivermectin bioavailability in host and efficacy in parasite, their use in ML-based antiparasitic treatments represents a potent tool for maintaining MLs clinical efficacy.

118 **Latrophilin-like proteins in parasitic nematodes**

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Introduction: The new anthelmintic compound emodepside belongs to the class of cyclooctadepsipeptides. In *Haemonchus contortus* the G-protein coupled receptor (GPCR) Hc110-R has been identified previously as a putative target. It binds emodepside and alpha-latrotoxin, a neurotoxic component of the Black Widow spider venom. In *Caenorhabditis elegans* the orthologous protein latrophilin-like protein 1 has been identified as well as some of the key components of the signaling cascade. This receptor also binds alpha-latrotoxin and emodepside. A second receptor, latrophilin-like protein 2, is discussed as an additional receptor for emodepside in *C. elegans*.

Materials and Methods: EST databases were searched for fragments of orthologous genes in the canine hookworm *Ancylostoma caninum* and the cattle trichostrongylids *Cooperia oncophora* and *Ostertagia ostertagi*. PCR and RACE (Rapid Amplification of cDNA ends) experiments led to the full-length coding sequences. The sequences were analyzed.

Results and Conclusion: In the present study orthologous genes of Hc110-R were identified in *A. caninum*, *C. oncophora* and *O. ostertagi* and were named dephosphilins. Specific antibodies were developed and tested on recombinant protein. Orthologous genes of latrophilin-like protein 2 of *C. elegans* have been identified in *A. caninum*, *H. contortus*, and *C. oncophora*. All identified receptors show structural similarities with GPCRs of the secretin family, including receptors for alpha latrotoxin in mammals, latrophilins, although the overall sequence identities were low (up to 26 %). Furthermore, in *A. caninum*, *H. contortus*, and *C. oncophora* the potassium channel SLO-1 was identified, which is discussed to be involved in the signaling cascade. The binding affinities of the newly identified putative targets remain to be examined.

119 **In vitro and in vivo activity of the anti-cancer agent 2-methoxyestradiol (2ME), either alone or in combination with albendazole, against *Echinococcus multilocularis* metacestodes**

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The metacestode (larval) stage of the tapeworm *Echinococcus multilocularis* causes alveolar echinococcosis (AE), a mainly hepatic disease characterized by continuous asexual proliferation of metacestodes by exogenous budding, resulting in the tumor-like, infiltrative growth of the parasite lesion. Current chemotherapeutical treatment of AE relies on the use of benzimidazoles (albendazole, mebendazole), but these drugs act parasitostatic rather than parasitocidal, and in case of side effects such as liver toxicity, patients are left without valuable alternatives. 2-ME is a natural metabolite of estradiol, with a documented anti-angiogenic and anti-tumour activity, most notably against breast and prostate cancer. Treatments of in vitro cultured *E. multilocularis* metacestodes with 2-ME (2-10 μ M) showed that the drug has an adverse effect on parasite viability. First, addition of 2-ME (5 and 10 μ M) resulted in increased alkaline phosphatase activity in medium supernatants within 10 days of treatment. Second, scanning and transmission electron microscopy showed that the germinal layer of *E. multilocularis* metacestodes was dramatically damaged following 2-ME treatment, and the effect was dose dependent. Third, 2-ME in vitro treatment downscaled the 14-3-3-pro-tumorigenic zeta-isoform expression in *E. multilocularis* metacestodes. Bioassays were performed in mice injected with 2-ME-treated and albendazole-treated metacestodes, or parasites treated with both 2-ME and albendazole. These assays indicated that, in vitro, a parasitocidal effect was achieved by treatment with a combination of both compounds, while both albendazole and 2-ME alone do not kill the parasite completely. Currently, in vivo treatment experiments in *E. multilocularis*-infected mice are being performed. Mice are applied either 2-ME, albendazole, or a 2-ME/albendazole combination by daily intra-gastric inoculation for a period of 6 weeks. These investigations will show, whether either 2-ME, or a 2-ME/albendazole based treatment, is also effective in vivo.

120 **In vitro analysis of glutathione transferases from the mite *Sarcoptes scabiei***
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The mite *Sarcoptes scabiei* causes the disease scabies or sarcoptic mange, which affects both animals and humans worldwide. The infection is immunopathological and arises because of the mite and the tunnels it digs in the skin of its host. The ectoparasite not only causes suffering in the host but also financial losses in e.g. pig herds. Recently, descriptions of acaricide resistance in *S. scabiei* infected hosts were reported, but very little is known about the mechanisms of resistance in *S. scabiei*. We have characterised glutathione transferases (GSTs), which are multifunctional enzymes linked to resistance against many insecticides. To date six different *S. scabiei* GSTs have been identified, three from the delta-class and three from the mu-class. We have cloned and analysed these enzymes using affinity chromatography, steady-state kinetics, immunoblot, HPLC and mass spectrometry. One delta-class GST has also been successfully crystallised. In both kinetic studies and immunoblot analysis the recombinant GSTs showed varying characteristics independent of class. Furthermore, inhibition studies with acaricides demonstrated differences in sensitivity between GSTs. On-going experiments using steady-state kinetics together with HPLC and mass spectrometry will elucidate how the recombinant enzymes interact with various acaricides. In conclusion, these results show that GSTs are likely to be essential for the metabolism of acaricides in *S. scabiei*, and a detailed understanding of the kinetic mechanisms is an important factor for future control of this and similar parasites.

- 121 **Relative activity of triclabendazole metabolites against the liver fluke, *Fasciola hepatica***
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Triclabendazole (TCBZ) is metabolised into a number of metabolites by the host: sulphoxide (TCBZ.SO), sulphone (TCBZ.SO₂) and hydroxy metabolites. TCBZ.SO is considered to be the main active metabolite, although TCBZ.SO₂ has been shown to display some activity in vivo. This investigation set out to determine the relative activity of TCBZ, TCBZ.SO and TCBZ.SO₂ against adult TCBZ-susceptible flukes following incubation in vitro for 24h in each compound at a concentration of 15 µg/ml. Morphological changes to the tegument were assessed by SEM and TEM and alterations in tubulin distribution by immunocytochemistry. Both TCBZ and TCBZ.SO₂ induced swelling and blebbing of the tegument, but the level of severity was greater with TCBZ.SO₂. Neither drug caused spine loss and tegumental sloughing, as was observed with TCBZ.SO. Internally, accumulations of secretory bodies were observed at the apex of the tegumental syncytium and in the cell bodies following treatment with TCBZ and (more particularly) TCBZ.SO₂. The secretory activity of the cells was reduced, as evidenced by the disruption of the Golgi complexes. More severe inhibition of secretory activity was observed after TCBZ.SO treatment and the syncytium showed signs of vacuolation at the base which ultimately led to its sloughing. Treatment with TCBZ.SO led to a total loss of tubulin immunoreactivity (IR) in the tegument. TCBZ.SO₂ caused a less marked reduction in IR, whilst TCBZ itself produced the smallest decrease of the three treatments. From this study, the order of activity of the three compounds is TCBZ.SO>TCBZ.SO₂>TCBZ. The results confirm TCBZ.SO as the most active form of TCBZ, but suggest that other metabolites may contribute to drug action in vivo.

- 122 **Local cellular response of sheep to infection with *Teladorsagia circumcincta***
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Teladorsagia circumcincta is an important nematode parasite of sheep in temperate regions, both in terms of economic loss and animal welfare. Protective immunity can be acquired to this parasite. An experimental model was used to study naïve and previously infected sheep challenged with 50 000 infective larvae of *Teladorsagia circumcincta*. Efferent gastric lymph was collected on a daily basis via an in-dwelling cannula, and the lymphocytes analysed by FACS to monitor the kinetics of the local cellular response. In previously infected sheep, an increase in both total cell and lymphoblast output was observed from day 3, compared to 8 days post challenge in the naïve sheep. Increases in CD4+, CD25+ and CD8+ T cells, B cells, IgA positive cells and IgE positive cells were observed in both groups of sheep, with the previously infected animals responding more rapidly.

123 **Proteomic characterisation of mucus derived from gastric mucosa during the immune exclusion of *Teladorsagia circumcincta***

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The study used an in vitro model to identify molecules that limit or prevent *Teladorsagia circumcincta* larvae from associating with abomasal tissue. Abomasal tissue was collected at slaughter from previously naïve and immune sheep and was either challenged in vitro with 2,500 exsheathed L3 of *T.circumcincta* or remained the unchallenged control. Following 3h incubation, mucus was collected in PBS from tissue explants for shotgun proteomic analysis. Larvae counts confirmed that immune tissue excluded infective *T.circumcincta* at a higher rate than naïve tissue. Approximately 150 proteins were identified in the mucosal washes, following SDS-PAGE, tandem mass spectrometry and database searching algorithms. Cytoskeletal proteins such as actin, and gelsolin and plasma derived proteins such as acute phase proteins, A2 macroglobulin and complement components were identified in abundance in all samples. Certain indicators of innate immunity, such as lysozymes were identified in washes from naïve and immune explants, following the parasite challenge. Indicators of adaptive immunity, such as sheep mast cell proteinase-1, galectins 11 and 14 and IgA were identified in the washes from immune challenged or unchallenged explants. Intelectin, a soluble lectin associated with innate immune responses was only identified in samples deriving from challenged immune tissue. In conclusion, the phenomenon of *T.circumcincta* exclusion from the gastric mucosa seems to be associated with a variety of molecules related to innate and adaptive immune responses. The in vitro organ culture model used in this study offers a relatively simple means of detecting changes to the mucosal proteome resulting from parasite challenge.

124 **Local cellular changes in serial abomasal biopsy samples and their association with the cost of immunity in *Teladorsagia circumcincta* infected sheep**

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Cellular proliferation associated with the acquisition of immunity is believed to contribute to the nutritional cost to the animal during gastrointestinal nematode infections by diverting nutrients away from productive functions. Here we examined cellular profiles in serial abomasal tissue biopsies to obtain an insight into the immunological changes associated with the nutritionally disruptive phase of the immune response. Twelve parasite-naïve 6-month-old ewe lambs that had been surgically fitted with an abomasal cannula were infected with 4000 *Teladorsagia circumcincta* L3 larvae d-1 for 91d. Six lambs received the infection only (group IF) while the remaining six lambs were concurrently treated weekly with 1.3mg per kg liveweight (LW) of the corticosteroid methylprednisolone acetate in order to suppress immune function (group ISIF). Compared with IF animals, corticosteroid treatment reduced mean mast cell and eosinophil numbers in abomasal tissue by proportionate 0.37 ($p=0.021$) and 0.66 ($p<0.01$), respectively, and abrogated the rise in globule leukocytes that was observed from day 42 in IF animals ($p<0.01$). Abomasal pH was elevated in IF animals only from day 49. Compared with the ISIF group, relative feed intake (gDM consumed per kgLW) of IF animals was reduced by 0.26 between days 7 and 56 ($p<0.01$), however, feed conversion efficiency (g LWG per kg feed consumed) was reduced in IF animals between days 21 to 28 only ($p<0.03$). The timing of the events indicates there is no direct causal relationship between sequestration of nutrients for the production of mast cells, eosinophils or globule leukocytes and reduced productivity in nematode-infected lambs.

125 **Vaccination of calves against *Ostertagia ostertagi* with subfractions of the protective ES-thiol**

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Previous vaccination trials against *Ostertagia ostertagi* in cattle have demonstrated the protective capacity of a protein fraction termed ES-thiol, which is enriched for activation-associated proteins (ASPs) and cysteine proteases. In this study, ES-thiol was subfractionated through Q-Sepharose anion exchange chromatography to determine whether ASPs and/or cysteine proteases are responsible for the induced protection. Calves (seven/group) were immunized three times intramuscularly with 100 microgram of ES-thiol or equivalent amounts of an ASP fraction, a cysteine protease fraction or a rest fraction, with QuilA adjuvant. A control group only received QuilA. After the final immunization the animals were challenged with a trickle infection of 25,000 infectious L3 larvae (1,000 L3/day; 5 days/week). During a two-month period the geometric mean cumulative faecal egg count of the ES-thiol group was reduced by 62% compared to the QuilA control group ($P < 0.05$). Groups injected with the ASPs, cysteine proteases and the rest fraction demonstrated a reduction in cumulative faecal egg count of 74%, 80% and 70% respectively ($P < 0.01$). Although no significant reductions in worm burdens were observed, adult male and female worms were significantly smaller in all vaccinated groups ($P < 0.05$), except for male worms from the ES-thiol group. In conclusion, the protective capacity of the ES-thiol fraction was confirmed and it was demonstrated that all three sub-fractions contain protective proteins. The protein composition of the different fractions and the importance of antigen conformation and glycosylation in antibody recognition of the different antigens is currently being analyzed.

126 **A comparative analysis of immunogenic excretory/secretory proteins from *Teladorsagia circumcincta* harvested at 1, 3 and 5 days post infection**

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Introduction: *Teladorsagia circumcincta* is the most important parasitic nematode of sheep in the UK. Unfortunately, there are increasing levels of anthelmintic resistance in this species and multi-drug resistant populations have been identified. Alternative methods of control are urgently required for this parasite. As sheep develop protective immunity, vaccination is a valid alternative option. For this reason, we are investigating *T. circumcincta* excretory/secretory products (ES) for possible vaccine candidates

Materials and Methods: Larvae from sheep sacrificed 1, 3 and 5 days post infection were washed carefully to remove host proteins and then cultured for 24hrs in an RPMI based medium to obtain ES products. Culture supernatants were concentrated at least 100 fold then buffer exchanged into PBS prior to analysis using one and two dimensional electrophoresis combined with western blotting and mass spectrometry

Results and Conclusion: We have identified a number of immunogenic proteins released by *T. circumcincta*, some of which are common or unique to each development stage. Results will be presented, highlighting the major differences found between the stages and with respect to any known or predicted functions of each protein.

127 **A calcium-dependent, structural homologue of a novel salivary apyrase from blood-feeding arthropods has been identified in *Ostertagia* third stage larvae**

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Apyrases (ATP diphosphohydrolase) comprise a ubiquitous class of glycosylated nucleotidases that hydrolyze extracellular ATP and ADP to orthophosphate and AMP. Most apyrases have been structurally linked to the heat shock70/sugar kinase/actin superfamily which is associated with motility, adhesion, secretion, regulation of hemostasis, non-synaptic information transfer and platelet formation. A second class of newly-described, calcium-dependent, salivary apyrases known to counteract blood-clotting, has been identified in several hematophagous arthropods. Herein, we have cloned an apyrase (OoAp) gene encoding a protein that structurally conforms to the calcium-activated apyrase from the bed bug, *Cimex lectularius*, by immunologically screening an *Ostertagia* L4 cDNA expression library with hyperimmune bovine serum. The full-length sequence was subcloned and the protein expressed (rOoAP). Hydrolysis of various dNTPs by rOoAP was monitored through the release of orthophosphate. Our results showed that the rOoAP is a Ca²⁺-dependent protein with greatest activity on ATP, ADP, UTP and UDP, and a pH maximum between 6.5 and 7.5. Hydrolysis was not observed on CTP or AMP. Host antibodies to OoAp appeared 14 dpi and increased through 30 dpi. Immunohistochemical and Western blot analyses demonstrated that OoAp is produced or collects within the muscle layer below the hypodermis, and is developmentally-regulated. Peak protein expression likely preceded L3 development given the absence of significant mRNA transcription in this stage. Mouse anti-rOoAP recognized conserved epitopes in protein extracts from *Haemonchus*, *Cooperia*, and *Oesophagostomum*. Currently, work is focusing on the relationship between apyrase activity and maintenance of the L3 and/or their arrested state of development.

128 **The three Rs: setting the context**

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This use of animals in research often causes them pain, suffering, distress and lasting harm and this raises concern for the public as well as for scientists who use them. The Three Rs, first proposed by Russell & Burch in 1959, have become the international ethical framework regulating the experimental use of vertebrate animals, and comprises a series of questions helping ensure that animals are only used when there is no alternative. It adopts the following sequence of questions. Is it possible to REPLACE the proposed use of animals using non-sentient alternatives such as computer modelling, physico-chemical tests, cell cultures? If the scientific question is really worth asking and there are no replacements, then can the numbers of animals be REDUCED through e.g. improved statistical analysis or experimental design? The third R questions whether the research can be REFINED so that animal suffering can be minimised (e.g. humane endpoints) and whether the wellbeing of the animals can be improved e.g. through enriching their cages, training programmes. The practical application of the Three Rs will be presented in this session with worked examples in the field of parasitology.

129 **Statistical support for the three Rs**

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The paradox is that bringing about reduction by reducing the number of animals used in a study, perhaps by reducing group size, may risk wasting all the animals in the study unless well-justified and statistically valid. International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) anthelmintic guidelines have recommended six as a minimum group size for anthelmintic dose determination and confirmation studies. Any decrease further decrease in group size would need to be well justified and discussion with the appropriate regulatory authorities would be advisable.

In some cases, for instance where parasite establishment may be variable, it may be necessary to increase the group size in order to achieve a statistical difference following treatment, and thus not to waste the study and the animals used. It is important to give thought to analysis at the design stage, together with interim analysis if recruitment is staggered to ensure that the original assumptions are borne out.

Other opportunities for reduction in animal numbers may be achieved through refining study design, or by combining two studies into one where this can be achieved without compromising animal welfare.

Alternatives and innovation in analysis may allow reduction. For example, in cases where relevant data are distributed amongst a number of studies, it may be valid to analyse the combined data using techniques such as meta-analysis if the study designs

130 **Regulatory process**

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3Rs has been discussed and accepted in many regulatory forums as a factor for developing regulatory guidelines. Debate should not be limited to efficacy studies as regulation extends more widely.

Regulation takes account of the current state of knowledge where the scientific justification is robust and can sustain the protection of public and animal health and the environment. Guidance is not law but offers a scientific standard of work and therefore assists interpretation of law and facilitates a predictable process. Guidelines are developed given the state of current knowledge and are flexible where this is sustainable. It is recognised that specific guidance cannot be applied to all situations and study designs may need adaptation going beyond guidance and this produces a challenge for research and regulation. In such circumstances the study design is best agreed in advance to ensure the outcomes will be acceptable for both sides.

A validated 3Rs driven modification should be considered sympathetically and, logically, progress should be reflected in a renewed guidance but changes take time as discussion involves all affected parties and international harmony needs to be maintained. Even where change is made, adoption can be slow due to increased costs involved.

Although, innovation takes time to be reflected formally this is not necessarily a barrier to progress. Overarching scientific committees exist to agree on the application of novel developments where guidelines are not appropriate. Such scientific advice is part of the regulatory process and should be used to ensure novel science can be accepted.

131 **The perspective from industry**

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It is the goal of this symposium to encourage a discussion of possibilities for the implementation of the 3Rs into the field of veterinary parasitology. The VICH and World Association for the Advancement of Veterinary Parasitology (WAAVP) Guidelines have helped to clarify and define the scope of studies necessary for a marketing authorization of a new veterinary product and are a useful reference for data evaluation. These guidelines should reduce repetitive studies and ensure meaningful data are produced. International acceptance of guidelines have helped harmonization of requirements and thus contributed to implementation and progress of the 3Rs in the development of parasiticides across international boundaries.

It is a logical consequence that an industry that is committed to the health and welfare of animals considers the welfare of the animals used during drug development. Seeking alternative methods and thus deviating from the guideline framework may increase the risks of acceptability of the data which may lead to an increase in time and cost for the approval of a new drug. However, exploration and acceptance of alternative study designs, selection of appropriate statistical methods and the use of sound parasitological principles to predict and evaluate data should be explored to allow the application of the 3Rs to be more fully adopted in drug development.

132 **The CRO perspective**

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Most endo- and ecto-parasite studies conducted at CROs must be conducted in the target species. Therefore, replacement is not feasible. However, there are several opportunities for reducing or refining the use of animals. The number of animals used in a study can be reduced through a better understanding of the statistical requirements, basic knowledge on establishing infections, and the use of combination studies. A key area of refinement for dog and cat studies would be changes in the housing conditions especially in regards to group and single housing requirements. The barriers to adopting these methods include, but are not limited to:

- the uncertainty of the effectiveness of some alternative methods;
- lack of basic research to support some alternative data gathering methods;
- perceived or actual acceptance of these methods by regulatory officials;
- a hesitancy to stray from a strict interpretation of the guidelines; and
- perceived or actual costs.

Currently there are few if any incentives to adopt methods for reducing or refining the use of animals in endo- and ecto-parasite studies. A better understanding of the advantages and application of these methods could increase their adoption and result in improved study designs and cost and time savings.

133 **Resistance of *Parascaris equorum* against ivermectin due to frequent anthelmintic treatment of foals in The Netherlands**

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Resistance of *Parascaris equorum* against macrocyclic lactones has been reported from the Netherlands (Boersema et al, 2002), the U.S.(Hearn and Peregrine, 2003), Canada (Slocombe et al, 2003) and the U.K.(Stoneham and Coles, 2006).

Between September and December 2005 the occurrence of resistance of *P. equorum* against ivermectin was studied on 43 farms using 332 foals. Faecal egg counts were performed using a modified McMaster-technique. Foals positive for *P. equorum* were treated with ivermectin. Fourteen days after treatment faecal examination was repeated.

P. equorum was found in 27% (88/332) of the animals on 26 of the 43 farms and 54 foals were treated with ivermectin. Mean efficacy of ivermectin against *P. equorum* was 54% and in 34 foals (63%) efficacy was less than 90%. Virtually no effect of ivermectin against *Parascaris* was observed in 22 out of 27 foals from farms that treated foals very frequently (at least once in the first two weeks of life, once at the age of 1 month, and at least every 8 weeks) In contrast a 100% effect was seen in all 9 foals from farms treating less frequently. Efficacy was also poor in 12 out of 18 foals from farms with unknown history.

It is concluded that resistance of *P. equorum* against ivermectin is common in the Netherlands and is associated with very frequent deworming of foals.

134 **Experimentally induced ivermectin resistance in *Ostertagia ostertagi* in cattle**

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An anthelmintic susceptible *Ostertagia ostertagi* population was selected for ivermectin resistance by repeatedly exposing the population to subtherapeutic and therapeutic levels of ivermectin over 10 generations. In each selection round, a group of calves was infected with the progeny of the previous ivermectin-selected *O. ostertagi* population. In the last selection round a therapeutic ivermectin dose (0.2 mg/kg BW) only reduced the faecal egg counts by 57 and 65 % on days 7 and 14 after treatment, respectively. A significant linear relationship was observed between the administered ivermectin doses and the ivermectin plasma concentrations ($P < 0.05$) in the 10 passages, confirming that each calf had received the appropriate treatment. These results demonstrate that *O. ostertagi* can develop resistance against ivermectin, but in comparison with other parasites the development is slow. The ivermectin-resistant *O. ostertagi* population is currently being used to study the molecular mechanisms of ivermectin resistance. Two genes, p-glycoprotein A (PgpA) and glutamate gated chloride Channel $\alpha 3$ (GluCl $\alpha 3$), associated with ivermectin resistance in related nematodes, are under investigation. The mRNA transcriptional level of PgpA was compared between the ivermectin-susceptible and ivermectin-resistant female and male adult *O. ostertagi*. A 2.5-fold increase of the PgpA transcription could only be observed in the ivermectin-resistant compared to ivermectin-susceptible male worms. Pyrosequencing and direct sequencing techniques will be used to investigate the polymorphisms of the *O. ostertagi* PgpA and GluCl $\alpha 3$ in the susceptible and resistant *O. ostertagi* isolates to assess their association with ivermectin resistance.

135 **The identification of cattle nematode parasites resistant to multiple classes of anthelmintics in a commercial cattle population in the US**

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Introduction: Resistance to anthelmintics by ruminant nematode parasites has largely been reported in parasites from small ruminants. There are increasing reports of such resistance in cattle, but there have been no such reports of resistant parasites in North America. Recently a producer in the upper midwest of the US who intensively manages young cattle acquired from the southeastern US reported that his usually highly effective deworming program was resulting in animal productivity less than expected.

Methods: Over a 2 year period the operation was monitored for the presence of drug resistant nematodes by assessing the effectiveness of drug treatment in reducing fecal egg counts (EPG) and by slaughter of animals after treatment. Treatments used over the 2 years consisted of : ivermectin injectable, moxidectin pour-on, doramectin injectable, eprinomectin pour-on, albendazole oral, fenbendazole , levamisole and 2 combination treatments of eprinomectin plus levamisole and eprinomectin plus fenbendazole .

Results and Discussion: The only treatments that resulted in at least 80% reduction in EPG included levamisole either singly or in combination with a second drug. The results for both years were very similar and show the following pattern. *Haemonchus contortus* was resistant to both macrocyclic lactones and benzimidazoles. In contrast *H. placei* was still sensitive to the benzimidazoles. Few *Ostertagia ostertagi* were seen in the studies, and they were mostly restricted to the levamisole treated animals. In the small intestine the predominant species encountered was *Cooperia punctata*, with smaller numbers of *C. oncophora*, and *C. spatulata* which were resistant to the macrocyclic lactones

136 **Statistical approach to measure efficacy of anthelmintic treatment on horse farms**

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Resistance to anthelmintics in gastrointestinal nematodes of livestock is a serious problem and appropriate methods are required to identify and quantify resistance. However, quantification and assessment of resistance depend on an accurate measure of treatment efficacy, and current methodologies fail to properly address the issue. The fecal egg count reduction test (FECRT) is the practical gold standard for measuring anthelmintic efficacy on horse farms, but these types of data are fraught with high variability that greatly impacts the accuracy of inference on efficacy. To address this issue we have developed a statistical model to measure, assess, and evaluate the efficacy of anthelmintic treatment in horses as determined by FECRT that is free of distributional assumptions on the egg count distributions. Novel robust bootstrap methods are developed to assess efficacy and are compared to other suggested methods in the literature (such as logarithmic and arcsine transformations) in terms of Type I error and power. In addition, data from multiple farms are studied and statistical models developed that take into account between farm variability. Our results demonstrate that the bootstrap methods have optimal Type I error rate and high power to detect differences between the presumed and true efficacy without the need to know the true distribution of pre-treatment egg counts. Our analyses also establish that if inter-farm variability is not taken into account, misleading conclusions about resistance can be made.

- 137 **Evidence of macrocyclic lactone-resistant *Parascaris equorum* on a Turkish horse farm**
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On a horse farm treatment failure was observed when numerous eggs of *Parascaris equorum* were detected in some horses after ivermectin administration. In order to find the possible reason of this treatment failure two clinical trials were carried out. In January 2006, faecal samples of 16 horses were taken rectally and examined using the McMaster technique. Eight horses were found to be infected with *P. equorum* and three of them were also infected with strongyles. All these horses were treated with ivermectin oral paste and *P. equorum* + strongyle egg counts were determined 9-13 days post-treatment. A Faecal Egg Count Reduction Test was used to evaluate the results. Ivermectin decreased *P. equorum* and strongyle egg output by 24.6% and 100%, respectively. In January 2007, a second trial on the same farm was performed with 28 horses naturally infected with *P. equorum* and/or strongyles. Treatments were ivermectin, moxidectin, pyrantel pamoate, fenbendazole and some horses left as untreated. Ivermectin and moxidectin treatments reduced *P. equorum* egg counts by only 39.6% and 58.6%, respectively, but resulted in complete elimination of strongyle eggs. Both pyrantel pamoate and fenbendazole showed 100% efficacy against *P. equorum*. In conclusion, there appeared to be *Parascaris* resistance to macrocyclic lactones. Pyrantel pamoate and fenbendazole were highly effective for *P. equorum*.

- 138 **Gene silencing of the tick protective antigens, Bm86, Bm91 and subolesin, in the one-host tick *Boophilus microplus* by RNA interference**
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The use of RNA interference (RNAi) to assess gene function has been demonstrated in several three-host tick species, but adaptation of RNAi to the one-host tick, *B. microplus*, has not been reported. We evaluated the application of RNAi in *B. microplus* and the effect of gene silencing on three tick-protective antigens: Bm86, Bm91 and subolesin. Gene specific dsRNA was injected into two tick stages, freshly molted unfed and engorged females, and specific gene silencing was confirmed by real time PCR. Gene silencing occurred in injected unfed females after they were allowed to feed. Injection of dsRNA into engorged females caused gene silencing in the subsequently oviposited eggs and larvae that hatched from these eggs, but not in adults which developed from these larvae. dsRNA injected into engorged females could be detected by quantitative real-time RT-PCR in eggs 14 days from the beginning of oviposition, demonstrating that unprocessed dsRNA was incorporated in the eggs. Eggs produced by engorged females injected with subolesin dsRNA were abnormal, suggesting that subolesin may play a role in embryonic development. The injection of dsRNA into engorged females to obtain gene specific silencing in eggs and larvae is a novel method which can be used to study gene function in tick embryogenesis.

- 139 **Immunization studies in rabbits using gut membrane-bound proteins derived from *Rhipicephalus appendiculatus*, *R. evertsi evertsi* and *Amblyomma variegatum* female ticks**
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The objective of this study was to immunize rabbits with Gut Membrane-Bound Proteins (GMBP) derived from partially engorged *Rhipicephalus appendiculatus*, *R. evertsi evertsi* and *Amblyomma variegatum* female ticks and to assess whether the elicited immunity was protective against both homologous and heterologous tick instars and to isolate and identify the protective antigens.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of the GMBP antigens demonstrated protein bands with molecular weights ranging from 16 to 140 KDa. Thirty-seven, 45 and 39 protein bands were fractionated from *R. appendiculatus*, *R. evertsi evertsi* and *A. variegatum* GMBP antigens, respectively, all possessing molecular weights ranging from 14 to 140 KDa. Twenty-two of the isolated proteins were shared among the three tick species. Immunized rabbits acquired resistance to challenge infestation by all instars of the three tick species. Resistance was manifested by prolonged feeding, reduction in engorgement weights, egg mass weights, moulting and percentage hatchability and increased mortality. Immunization of rabbits with GMBP antigens generated protection and cross-protection against challenge infestation with homologous and heterologous instars, respectively. Cross-protection was more pronounced in the homologous than heterologous systems. Enzyme-Linked Immunosorbent Assay (ELISA) technique detected circulating antibodies in the anti-sera to GMBP from homologous and heterologous systems one week after the primary dose. Ouchterlony double immunodiffusion reactions with anti-tick GMBP sera formed 2 to 3 precipitin lines with homologous GMBP antigens and one to two precipitin line(s) with each heterologous GMBP antigens. A line of complete identity was observed when anti-sera to GMBP antigens reacted with GMBP from homologous and heterologous tick species, suggesting common antigenic epitopes. Western blot analysis on GMBP of *R. appendiculatus*, *R. evertsi evertsi* and *A. variegatum* with sera from immunized rabbits detected protein bands specific to the homologous GMBP antigens, and revealed considerable cross-reactions in the heterologous systems. These results suggested further the presence of common antigens. The presence of cross-reacting antigens conferred cross-protection.

- 140 **Identification of novel antigens from the sheep scab mite, *Psoroptes ovis***
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Sheep scab is an important welfare condition in the UK and other countries across the world, caused by the ectoparasitic mite, *Psoroptes ovis*. Current control methods (dipping in acaricides) are failing and alternative methods need to be developed quickly. One possible alternative is a vaccine to prevent the disease. This approach is feasible as sheep have been shown to develop immunity to the disease.

In this project, a combination of protein analysis and molecular biology was used to identify and characterise novel antigens from *P. ovis*. An antioxidant enzyme, thioredoxin peroxidase, was identified by EST analysis, and a full length gene sequence was amplified and expressed in bacteria. The resulting recombinant protein was used to raise antibodies for immunolocalisation studies. A subtractive hybridisation experiment was performed to identify genes upregulated during feeding, which may encode proteins involved in digestion. These could be utilised as hidden (concealed) antigens in the future.

Our results show that the thioredoxin peroxidase is highly homologous to a secreted enzyme from a tick, and appears to be localised around the pharynx in *P. ovis*. Several interesting genes were shown to be upregulated during feeding, including homologues of house dust mite allergens and salivary gland derived proteins. These results suggest that thioredoxin peroxidase may be secreted by *P. ovis* and could interact with the host immune response; and that the genes identified by subtractive hybridisation may encode proteins involved in digestion which could be pursued as hidden antigens in the future.

141 **Epidemiology of *Dermanyssus gallinae* and acaricidal efficacy of phoxim 50% (ByeMite®) in alternative housing systems during the laying period of hens**

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Dermanyssus gallinae the poultry red mite represent the major ectoparasite problem in poultry. In laying hens this bloodsucking parasite may cause a decrease in egg production and death of birds. The aim of this study was to follow up the epidemiological pattern of a *D. gallinae* population in two alternative housing systems, an aviary and a one-floor plastic slat system and to assess the effect of an acaricidal treatment on the epidemiological pattern. Both layer houses had history of *D. gallinae* problems. Cardboard traps to quantify the mite infestation were used during the whole laying period of the hens. The efficacy of phoxim (ByeMite®, Bayer HealthCare AG) which proved to be efficacious against *D. gallinae* in caged layers was evaluated in both layer houses. After re-stocking, mites were detected in the monitoring traps for the first time in both sheds at day 26 and day 39, respectively. The number of mites steadily increased from the first detection of mites until the treatment with phoxim. The highest numbers of mites were trapped at the supply and resting areas in both housing systems. The 2,000 ppm phoxim spray solution was administered twice with a 7-day interval on days 227/234 and 278/285, respectively. After treatment till the end of the laying period at day 390 and day 382 respectively, the reduction in the number of mites was 99.3 % to 90.0 % in the aviary system and 97 % to 94.5 % in the plastic slat system.

142 **Genetic differences in *Dermanyssus gallinae* from wild birds and layers**

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Dermanyssus gallinae, also known as the poultry red mite, is a blood-feeding parasite distributed worldwide. The parasites spend most of their life aggregated in cracks and crevices in the poultry facility only visiting the host for blood meals needed for development and reproduction. In optimal conditions the life cycle can be completed within one week, causing populations to establish rapidly in the facilities. Affected birds show decreased production, irritation and, in severe infection, anemia, which may even cause death. The routes of infection of *D. gallinae* are not fully understood, and it has been suggested that wild birds could transfer mites into poultry facilities. To assess the population genetic structure in *D. gallinae* found in poultry facilities and mites parasitising wild birds, we sampled *D. gallinae* from wild bird nests and from commercial layers in three geographical regions of Sweden. DNA was amplified and the SSU, ITS1, 5.8S and ITS2 regions were analysed. Preliminary results, based on fixed differences in the ITS1 sequences suggests that *D. gallinae* from wild birds can be genetically separated from mites infecting the layers.

143 **Candidate endoparasitic fungi for use in the biological control of ovine parasites**

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Endoparasitic nematodes are a major constraint to production in ruminants. Conventional methods of control are anthelmintics. However an alternative method is required, one option may be a biological control. *Nematophagous fungi* are natural enemies of the nematode. They can be divided into two major groups; predacious and endoparasitic fungi. Previous work has concerned predacious fungi that trap nematodes. In contrast, endoparasitic fungi infect nematodes by producing spores that attach to the nematode's cuticle/egg or are eaten by the nematode. The objective of this study was to evaluate the effect of 20 isolates of endoparasitic nematophagous fungi on larval development.

Twenty fungal isolates (native to Ireland and or UK) were obtained from the Centraalbureau voor Schimmelcultures, Netherlands. The ability of the fungi to reduce *Teladorsagia circumcincta* larval numbers in coprocultures was assessed over 4 time periods (1, 2, 7, 14 days) and 2 temperatures (16 & 24 °C). Fungi which demonstrated the greatest endoparasitic potential and produced chlamydospores were subsequently evaluated for their ability to survive the gastrointestinal tract in 9 month old ram housed lambs.

At 24 °C, 15 isolates demonstrated an ability to affect development of *T. circumcincta* in coprocultures, 5 of which were effective on the egg stage. At 16°C, 4 of the 20 isolates demonstrated ability to affect development of *T. circumcincta*. Four isolates produced chlamydospores and passage through the GI tract was observed.

Four fungi with ovicidal ability in vitro are potential candidates for biological control of nematode parasites.

144 **Control of the sheep scab mite *Psoroptes ovis* in vivo and in vitro using fungal pathogens**

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As part of a programme to identify biological control agents for sheep scab, the pathogenicity of the fungus *Metarhizium anisopliae* to *Psoroptes* mites placed on sheep skin and wool, was examined in vitro. No inhibitory effects of skin and wool were observed and high levels of infection were recorded. Subsequently the pathogenicity of formulations of both *M. anisopliae* and *Beauveria bassiana* to *Psoroptes ovis* was studied in vivo. For this, 36 batches of 20 adult female *Psoroptes* mites were confined in 25 mm diameter chambers attached to the backs of 6 scab-naïve sheep. In some treatments, mites were exposed to the fungal pathogens for 48 h in vitro before being placed on the host, while other treatments involved mites with no prior exposure placed directly onto the skin of the treated host. After 48 h on the host, mites were removed, incubated and all infections were recorded. Fungal infection was observed in all treatments, except untreated controls. However, *B. bassiana* infected greater numbers of mites than *M. anisopliae* with the formulations examined. Infection rates were highest when mites were exposed to dry conidia (>90%) and lowest with *M. anisopliae*, in diatomaceous earth. Overall, the infection rate was not affected by whether or not the mites were exposed to the conidia before being placed on the sheep. The results demonstrate that *Psoroptes* mites can become infected by entomopathogenic fungi on a living sheep, providing a first demonstration of the potential of this technology for the control of sheep scab.

145 **The acaricidal effect of the essential oil of *Artemisia siberi* and *Zataria multiflora* extract against adult stage of *Rhipicephalus (Boophilus) annulatus* in Iran**

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The acaricidal effect of the essential oil of *Zataria multiflora* and *Artemisia siberi* extract on *Rhipicephalus (Boophilus) annulatus* was tested in the Laboratory condition. 5 serial dilutions (5, 10, 20, 40, and 80 micro Liter/cm²) of *Artemisia siberi* extract (provided by Barij Essence Co., Kashan, Iran) and 8 serial dilutions of *Zataria multiflora* (1.25, 2.5, 5, 10, 20, 40 and 80 micro Liter/cm² in three replicate were used against engorged female ticks *Rhipicephalus (B) annulatus* using immersing method in vitro. The engorged ticks were immersed in different dilutions (five ticks in each triplicate) for one minute and then were immediately incubated at 26°C with 80% relative humidity. Mortality rate for each treatment were recorded over 6 days post incubation. The most mortality was observed one day post inoculation and the mortality rate was 100% in dilutions more than, 0.01 micro Liter/cm² in *Zataria multiflora*. Also all of essential oil dilutions except 0.0625 and 0.0125 micro Liter/cm² caused 100% failure in egg laying in engorged female ticks whereas no mortality was observed in the control groups. In case *Artemisia siberi* the mortality rate in dilutions of 0.04 and 0.08 micro Liter/cm² were demonstrated 100% which happened after first day post inoculation. Also failure in egg production was recorded 100% in essential oil dilutions more than 0.005 micro Liter/cm². In both bio-acaricides with increase in dilutions mortality rate was increased. LD₅₀ was calculated in different dilutions of both tested extract and essential oil with Probit software which it was 0.007 for *Zataria* and 0.002 for *Artemisia*. It can be concluded that *Zataria* and *Artemisia* have high potential acaricidal effects on engorged stage of *Rhipicephalus (Boophilus) annulatus* in vitro and the virulence of *Zataria* was revealed more than *Artemisia*. Anyway further more in vivo research needs to verify this conclusion.

146 **Genetic resistance to *Heterakis gallinarum* in two chicken layer lines following a single dose infection**

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Groups of White Leghorn (WL) and New Hampshire (NH) chicks were divided into two groups at an age of eight weeks. One group was artificially infected with 100 embryonated *Heterakis gallinarum* eggs. After eight weeks animals were slaughtered and worm burden was counted. Worm lengths were measured. Body weights were recorded. Total worm burden was significantly ($p < 0.0001$) higher in infected males than in infected females. Furthermore WL showed a significantly ($p = 0.011$) higher number of worms when compared with NH animals. Average worm lengths were not significantly different between the breeds ($p = 0.155$) and sexes ($p = 0.062$). Correlations between worm burden and worm length was 0.08 ($p = 0.140$). The daily weight gains between 8 and 16 weeks of age of the infected animals were significantly ($p = 0.038$) lower when compared with the control group. Estimated correlation between daily weight gain and worm burden was - 0.013 ($p = 0.793$). The estimated heritabilities for loge worm burden were 0.41 (SE: 0.086) in WL and 0.31 (SE: 0.126) in NH, respectively.

147 **Length of *Ascaris suum* larvae is affected by parental genotype and location in the intestine in pigs**

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The effect of parasite genetics on establishment of *Ascaris suum* in pigs was investigated. A PCR-RFLP method was used to discriminate between 4 *Ascaris* females (Types A, B, C, and D). Each of 26 pigs was then inoculated with 500 eggs from each female and 6, 6, and 14 pigs were slaughtered at days 14 (before expulsion), 17 (during expulsion), and 28 pi (after expulsion), respectively. At slaughter the small intestine was divided into 8 sections of similar length and the larvae were isolated. The length and genotypes of the larvae was determined. At the three slaughtering days, 6 (100%), 5 (80%) and 5 (37%) pigs were positive for *Ascaris* and totals of 1908, 1616, and 675 larvae were analysed, respectively. Random effect models with pig as a random variable were used to analyse the response variable length on each day. Both at days 14 pi and 17 pi the length of the larvae was significant affected by the interaction between type and section ($P < 0.0001$) as well as the total number of larvae in the sections ($P < 0.001$). At day 28 pi the interaction between section and type was significant at $P=0.03$. In conclusion we have found preliminary evidence for a parental effect on the size of *Ascaris* larvae in addition to a location-size effect, both possibly with a consequence on larvae fitness and therefore survival in the host.

148 **Epidemiology and control of gastrointestinal nematodes of horses in Ukraine**

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During last three years we have made the research of parasitological situation concerning gastrointestinal nematodes of the horses on the equine farms of Kyiv region and on Kyiv State Race Track, where the horses from many regions of Ukraine are represented. It should be mentioned that almost all best existing chemicals are represented for more than five years on the market of anthelmintics of Ukraine (Moxidectin, Abamectin, Ivermectin).

But the rapid improvement of parasitological situation is not observed because of the absence of control strategy. After completion of more than 4000 coprological research from almost 2000 horses we found almost 100% contamination with Strongilidae if the last deworming was made not earlier than 3-4 months before the research. Maximum contamination (up to 2000-2500 EPG) was detected in the 2-4-years aged horses and in the 15-years aged dams. Contamination of horses older than 5-6 years didn't exceed 100-250 EPG.

On the equine farms, where the foals are bred, it was found that during first 4-8 months almost 100% of foals are contaminated with parascariosis with the EPG up to 3000-3800 and the disease is expressed clinically. In the 4-years aged horses only sporadic cases of parascariosis are registered.

Also we have made hematological and biochemical research of blood of horses, naturally contaminated with cestodoses. Biochemical research was made on the automatic analyzer BTS-370 (BioSystems, Spain) and semiautomatic analyzer BTS-330 (BioSystems, Spain). We have detected presence of eosinophilia, monocytosis, haemoglobinemia, proteinemia, hypoalbuminemia and hyper- β -globulinemia.

After the chemicals were used (Moxidectin, 0,4 mg/kg BW and Abamectin, 0,2 mg/kg BW) in oral form biochemical indexes of blood stabilized to physiological norms during the first 7-28 days. But in the researched group (Abamectin) the level of eosinophiles and β -globulins began to increase again on the 30-th day after deworming, proteinemia and other biochemical changes were observed, which shows the renewal of toxic action of Cyathostomes. To our mind this is connected with start of intensive development of hypobiotic stages of nematodes. In the groups, dewormed with Equest, improvement of biochemical and hematological indexes of horses' blood remained stable for 2-3 months after deworming.

149 **Evaluation of the association between *Anoplocephala perfoliata* infection and colic in horses in Ontario, Canada**

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Infection with *Anoplocephala perfoliata* has been associated with spasmodic and ileal-impaction colic in horses in the United Kingdom. However, this association has not been evaluated elsewhere. The objectives for this study were to determine if infection with *A. perfoliata* is associated with risk of colic in horses in Ontario, Canada, and to identify risk factors for exposure to *A. perfoliata*. Using a case-control study design, horses in southern Ontario diagnosed with colic by veterinarians, and control horses from the same stable, were matched by age, breed and sex where possible. Data collected from 117 pairs of horses over a 16-month period were examined for evidence of an association between the risk of colic and *A. perfoliata* infection. Management data were similarly evaluated for evidence of association with *A. perfoliata* infection. Tapeworm infection status was defined on the basis of results on coprological examination using sucrose centrifugation or examination of blood samples with an antibody-ELISA. Fifty-six percent of 234 horses were seropositive for *A. perfoliata*; eggs of *A. perfoliata* were found in samples from 6% of horses. Using logistic regression with a random effect for matched pair, horses dependent on pasture for a large part of their diet were significantly more likely to have ELISA optical density levels above 0.600 compared to other horses (odds ratio = 6.02; $p = 0.024$). However, using conditional logistic regression models, we found no statistical evidence for an association between tapeworm infection (defined either serologically or coprologically) and colic.

150 **Age clustered pooled faecal samples as monitoring tool in cyathostominosis in horses**

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Faecal egg output of cyathostomines in horses is age dependent, but also may differ considerably between animals of the same age. This implies that worm control should ideally be based on monitoring infections through faecal examination. However, a serious constraint is that costs for faecal examination by far exceed costs for deworming. Costs could be reduced by initially using pooled age clustered faecal samples. The reliability of such pooled samples for monitoring was studied.

In total 112 horses from two farms ($n = 58$ and 54 , respectively) were treated with 0.2 mg/kg ivermectin in July 2006. Faecal samples were collected from all horses at treatment and weekly from 6-14 weeks after treatment. Samples were examined individually and as age (yearlings, 2 year-old, 3-year old, adults) clustered pooled samples with a maximum of 10 samples per pool, using a modified McMaster method.

Correlation between EPG in pooled samples and the mean of individual samples was extremely high ($R^2 = 0.943$). Eight weeks after treatment $\text{EPG} > 200$ was observed in 5% and 4% of horses on both farms and after 14 weeks this was 28% and 22%. Only in 9 out of 82 adult horses $\text{EPG} > 200$ was reached within 14 weeks.

Using pooled faecal samples for monitoring appears to be appropriate. Considering the age dependency of EPG's pools should be age clustered.

151 **Isolation and characterization of stage-specific antigens of *Gasterophilus intestinalis***

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Infestations with the bot fly *Gasterophilus intestinalis* are very common in horses, but the immunological processes in this host-parasite system remain largely unknown. The objective of this study was to isolate and characterize stage specific antigens of *G. intestinalis* which can possibly also be used for diagnostic purposes and/or epidemiological studies. Using a total larval extract of *G. intestinalis* in an ELISA - test, neither the specificity nor the sensitivity of a test using total larval extract provided satisfactory results. Consequently, larvae 2 (L2) and larvae 3 (L3) were collected in a slaughter-house as a basis for further immunochemical analysis. Initially, an extract of L3 was separated by chromatography and the collected fractions were used to localise possible antigens. Their identification was performed by 1 D and 2 D electrophoresis, western-blot as well as by indirect ELISA - tests. Thereafter, similar reactions were successfully performed using L2. For a further differentiation of the detectable immune responses and the elimination of possible cross reactions, the detectable and promising antigens were used to immunise mice. The transfer into the mouse model allowed the identification of stage-specific antigens of L2 and L3. These antigens have been characterized by proteomic and sequence analyses (Maldi-Tof).

152 **Larval viability and serological response in horses with long-term *Trichinella spiralis* infection**

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The horse is considered an aberrant host for the nematode parasite *Trichinella spiralis*. It has been reported that experimentally-infected horses produce a transient serological response to infection and that muscle larvae are cleared more rapidly than in parasite-adapted hosts such as the pig and humans. However, limited numbers of animals have been studied, and both the longevity of larvae in horse musculature and the immune response to *Trichinella* larvae remain unclear. In this study, we infected 35 horses with 1000, 5000, or 10,000 *T. spiralis* muscle larvae and followed the course of infection for 1 year, assessing larval burdens in selected muscles, then condition and infectivity of recovered larvae, and the serological response of infected horses. The results demonstrated that *T. spiralis* establishes infection in horses in a dose dependent manner. Anti-*Trichinella* IgG antibodies peaked between weeks 6–10 post-inoculation. Viable, infective larvae persisted in horse musculature for the duration of the study (12 months), and exhibited no apparent reduction in muscle burdens over this period. Encapsulated larvae showed no obvious signs of degeneration in histological sections. Larval capsules were surrounded by infiltrates consisting of mature plasma cells and eosinophils. Macrophages were notably absent. Given the lack of a detectable serological response by 26 weeks p.i. and the persistence of infective muscle larvae for at least 1 year, parasite recovery methods are currently the only suitable detection assays for both meat inspection and epidemiological studies of *Trichinella* infection in the horse.

153 ***Bartonella* spp. – new pathogens, old diseases**

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The genus *Bartonella* comprises a unique group of emerging, Gram-negative, facultative intracellular bacteria which can cause long-lasting intraerythrocytic bacteremia and employ hematotropism as a likely parasitic strategy. *Bartonella* infections are widely underestimated: in fact, the seroprevalence in humans is ~ 6-12% and 13% of all unclear tumors in the head-neck region are caused by *Bartonella* spp. Only 1990, the human pathogenic bacterium *B. henselae* was firstly described and since this time it became evident that *B. henselae*, which is transmitted by cats, cat fleas and most likely by ticks, is the most common cause of cat-scratch disease and the vasculoproliferative disorders bacillary angiomatosis in humans. The closely related species *B. quintana*, which is transmitted via body lice, causes “trench fever” (also called “five-day fever”) characterized by periodic feverish relapses due to intraerythrocytic bacteremia. It became known during World War I when more than one million soldiers suffered from this disease. Today, *B. quintana* is a well known cause of fever, bacteremia, and endocarditis in HIV-seronegative, homeless, inner-city patients with chronic alcoholism. The laboratory diagnosis of *Bartonella*-infections is mainly made by immunofluorescence testing, however, this method is limited due to cross-reactivity and antigen variability. *Bartonella* spp. employ several remarkable pathogenicity strategies to survive in their hosts, e.g., (a) activation of angiogenic signalling pathways resulting in the formation of new blood vessels which represent the presumptive ecological niche, (b) inhibition of apoptosis in endothelial cells and monocytes and (c) persistence of pathogens in human hematopoietic and endothelial progenitor cells.

154 **Efficacy of a combination of 10% imidacloprid / 50% permethrin for the prevention of Leishmaniosis in kennelled dogs in endemic area**

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Introduction: Canine leishmaniosis (canL) due to *Leishmania infantum* is among the most important protozoal diseases of dogs worldwide. In Italy the prevalence of the disease ranges from 1.7 to 48.4% of dogs and new foci have been also reported in north-eastern regions. The efficacy of imidacloprid 10% and permethrin 50% (Im/Per; Advantix®; Bayer AG, Germany) in a spot-on formulation was evaluated in the field as a control measure to prevent canL in an endemic area of southern Italy. Materials and methods: In February 2005, 845 asymptomatic dogs were enrolled into a field study in two kennels. All dogs were initially tested for canL and 631 dogs were negative at serological and parasitological examination (lymph-node cytology and PCR on skin samples) while 24.6% (208 dogs) were positive. Negative dogs were allocated to one of three groups: Group A - treated with Im/Per once a month; Group B - treated every two weeks; and Group C – as untreated control. Within the groups, the dogs were evenly distributed ($P < 0.05$) for individual characteristics (sex, age, weight, coat length). All the dogs were examined serologically and parasitologically for canL prior to the start and the end of the sand fly season, and again prior to the following sand fly season. The sand fly population density was monitored in both kennels from April to November. Results: In the kennel of Bari *Leishmania* infection, inferred by positivity in at least one of the three tests performed at the interim or final follow-up, was found in one dog from Group A and in 9 from Group C. No positive dogs were detected in Group B, thus giving a final protection efficacy of 88.9% in Group A and 100% in Group B. In the second kennel (Ginosa) *Leishmania* infection was identified in one dog from Group A, one from Group B and 11 from Group C (protection efficacy of 90.36% in Group A and 90.73% in Group B). Sand flies first appeared in June and disappeared by mid-October in both kennels, and *Phlebotomus perniciosus* was the most common sand fly species identified (58.6%). Conclusion: The results clearly show that a combination of Im/Per, by virtue of its repellent activity against sand flies, was effective to prevent canine leishmaniosis.

155 **Spread of companion animal vector-borne parasitic disease in US and lessons to be learned**

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Three vector-borne animal parasitic diseases occur in the United States and appear to be spreading and becoming more common: canine heartworm, canine visceral leishmaniasis, and feline cytauxzoonosis. Heartworm is now endemic throughout the United States, having taken only about 50 years to engulf the entire 50 states of this country. Unfortunately, the United States has a wildlife reservoir host, the coyote, that has probably facilitated the spread of this parasite on the North American continent. Visceral leishmaniasis seems to be making inroads into the United States, but it remains unclear why infections are disproportionately represented in foxhounds and how the disease is transmitted between animals in this country. Finally, *Cytauxzoon felis*, is a parasite of the domestic wild felid, the bobcat (*Lynx rufus*), that causes acute and fatal disease in domestic cats that share the tick vector. It seems that more and more cats are becoming infected in a wider geographic area than ever before. It is important to be aware how and where these infections are spreading, but we also must be committed to preventing their movement into areas where they are currently not found. We should be proactive in our protection of pets and parasite-free geographic areas. Europe should consider the history of heartworm in the United States and consider acting now to insure that spread does not occur. In fact, Europe should consider perhaps even a policy of heartworm eradication.

156 **Genetic diversity and infection levels of anisakid nematode parasites of aquatic organisms of Boreal and Austral regions**

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Anisakid nematodes have complex life-cycles, that include invertebrate and vertebrate hosts at various levels of the marine food chain. Different types of habitat disturbances of the marine ecosystem (pollution, overfishing, by-catch) could impoverish the host population size, resulting in concomitant and detrimental effects on parasitic nematode populations. This in turn would lead to the loss of genetic diversity of these parasites at both the species and population levels. In order to test for a correlation existing between the genetic diversity of anisakid nematodes and habitat disturbance, the genetic variability, estimated by nuclear markers (19 allozyme loci), was evaluated among several anisakid populations from fish and marine mammals in various areas of the Boreal and Austral regions. Antarctic and subantarctic populations showed significantly ($P < 0.001$) higher levels of genetic diversity (on average, $He = 0.23$) than those from the Arctic and sub-Arctic populations and species (on average, $He = 0.07$). Correlations between the degree of genetic variability and the levels of parasitic infections within their hosts were considered. Data revealed higher intensities in anisakid infections in Antarctic and sub-Antarctic hosts, presumably resulting from a lower degree of habitat disturbance in less stressed areas. The absence of disturbance presumably allowed anisakid species to reach a larger population size, with a reduced probability of genetic drift in their gene pools. This suggests that anisakid nematodes, and their levels of genetic diversity may be suitable indicators of the integrity of marine food webs and of the general biodiversity of a marine ecosystem.

157 **Veterinary epidemiology and Bayes: Commensalism, not parasitism**Lesaffre E., *Catholic University of Leuven, Belgium*

Interpretation of the result of a diagnostic test depends not only on the actual test result(s) but also on information external to this result, namely the test's sensitivity and specificity. This external information (also called prior information) must be combined with the data to yield the so-called updated, posterior estimates of the true prevalence and the test characteristics. The Bayesian approach offers a natural, intuitive framework in which to carry out this estimation process. The influence of the prior information on the final result may not be ignored. Guidance for the choice of prior information not in conflict with the data can be obtained from a set of statistics and indices (DIC, pD , Bayes-p).

158 **A web-based MSc course in veterinary tropical diseases: an impression of the first two years**Madder M. 1, Venter L. 2, and Coetzer J.A.W. 2*1 ITMA, Belgium; 2 University of Pretoria, Department of Veterinary Tropical Diseases, Pretoria, South Africa*

The web-based Master Course in Veterinary Tropical Diseases and the Certificated Online Modules (CPD) in Tropical Animal Health developed by the Department of Veterinary Tropical Diseases in Pretoria, South Africa and the Institute of Tropical Medicine in Antwerp, Belgium has been a great success in 2005 and 2006! In 2006 thirteen students registered for the complete Master Course and in total 138 subscriptions were recorded for any of the modules for MSc or CPD purpose of which 116 completed their module successfully. The countries represented were the Democratic Republic of Congo, Lesotho, Mozambique, Namibia, New Zealand, Saudi Arabia, South Africa, the United States of America, Zimbabwe and Zambia. The experiences and impressions of developers, lecturers and students are presented to give the complete picture of this new initiative, which has proven to be very much appreciated by all stakeholders. A glimpse of future developments and ideas to further fulfill the needs of the target group will be presented.

159 **The present and coming mobile learning**de Waard I. 1, Correwyn S. 1, Iluyemi A. 2, Kiyan C. 1, and Zolfo M. 1*1 Institute of Tropical Medicine, Antwerp Belgium; 2 Centre for Healthcare Modelling & Informatics, UK*

During the last decades, the evolution of different technologies (portable computers, multimedia, telecommunication technology, web ...) has changed the way we can use communication. Everything can be accessed in a mobile way. This opens up scientific possibilities that were not there 10 years ago.

At this moment the exchange of data and files between peers, learners and teachers is possible but the continued search for a direct and fast relationship between them has increased the use of different kinds of Information and Communication Technology (ICT). The possibilities for using ICT mobile technology and improving the teaching-learning process in a different relationship with the citizen are now opening up.

But how can you keep track as a scientist when technology is changing so fast, even on a daily basis?

Material and Methods

We will analyze and review the evolution of different technologies, the conditions that could enhance scientific use of mobile technology and that allow us to have an overview beyond to the future.

Conclusion

Because veterinary science is deeply rooted with education, grass root level farmers and of course the scientific community, it gives an extra dimension to mobile technology.

Mobile devices will change our life style view related with science, entertainment and communication giving a personal space to every citizen adding a new quality of life.

Focusing on the future of this technology will allow us to make the right choices and reassure a long-term use of the mobile technology in all our lives.

160 **Radically new approach required for countering progressively failing information transfer in the field of parasite management**

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Introduction: As regards wide adoption and application by farmers, any but the most elementary routine drenching plans for worm management have failed, leading to severe anthelmintic resistance (AR) globally. Hence there is an urgent need for implementing new measures for worm management, which have been developed in the interim.

Present measures for countering AR are much more complex than conventional drenching programmes, at a time when institutional outlay on extension services is drastically being reduced globally, with persons with the necessary expert knowledge not being replaced. In developing countries the problem is exacerbated by a lack of effective landline infrastructure.

Methods and conclusions: It is suggested that the full potential of new methods of communication, such as wireless (mobile 'phone, satellite) technology, is not being utilised for information transfer in parasitology. Even in resource-poor communities with poor infrastructure this should bring relief by communication with existing networks of advisors and transfer of information by mobile 'phone, the per capita uptake of which thus far is considerably higher in developing than in developed countries. It is suggested further that decision support software can be developed for wireless transfer, which incorporates optimum methods of integrated parasite management (IPM) and is so specific as, through integrative modelling of climatic and parasitological factors (e.g. the history of drug resistance on a given farm), to generate precise recommendations such as, in systems of targeted selective treatment, categories of animals to examine and/or deworm at time T on Farm X and drugs to use/not to use.

161 **Canine Vector-Borne Diseases: from the dog to the parasite. A new teaching approach to clinical parasitology**

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Introduction: It is well known that canine vector borne diseases (CVBDs) are characterized by a wide range of clinical symptoms and the final diagnosis has to be reached throughout several diagnostic steps. Nowadays, multimedia technologies may support and improve teaching parasitology being an easy tool to present clinical cases of CVBDs to practitioners. The aim of this work was to propose a new method to teach clinical parasitology presenting clinical cases, step by step, by means of a film. **Materials and methods:** Six cases of CVBDs in dogs from southern Italy are presented including 3 cases of canine leishmaniasis, one of babesiosis and two of ehrlichiosis in acute and chronic form. The film has been orally commented and added with some written sentences focusing on some relevant clinical issues. Final general statements on the disease have also been included. **Results:** This teaching method has permitted to focus on specific clinical signs (i.e. ocular abnormalities) to visualize and evaluate laboratory abnormalities alterations (i.e. ipergammaglobulinaemia in serum protein electrophoresis) and to show diagnostical techniques (i.e. lymphnodal sampling, buffy-coat smear preparation). **Conclusion:** The implications of this approach to teach clinical parasitology include the attraction of the student attention in different ways (i.e. watching, reading, listening), making them closer to the clinical presentation of CVBDs.

162 **The tick game: using an interactive board game for teaching tick biology and chemical resistance counter-strategies**

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Strategic Bovine Services

The cattle tick *Boophilus (Rhipicephalus) microplus* is a common external parasite of cattle in Northern Australia, costing producers over A\$200m per year. Across the tropical areas of the world it is associated with lowered production and mortality due to the blood-sucking habit of the tick and the fact that it acts as a vector for several species of intra-erythrocytic protozoan species. Resistance to common acaricides has resulted in farmers in some areas unable to economically farm cattle. The problem develops rapidly with the introduction of each new acaricide, and is compounded by a lack of appreciation by farmers and chemical retailers for the mechanisms of the development of resistance. The Tick Game was developed to teach farmers and chemical retailers the fundamental aspects of tick biology, and how various management practices can either contribute to or prevent the onset of acaricide resistance. The game board illustrates the life cycle of the tick. Up to twelve people can play at a time. Players advance their ticks around the game board using dice. Conditions favourable to tick development are depicted by a spinner that speeds or slows progress as appropriate. Acaricide treatments are given at random times, slowing progress. However, ticks can acquire a resistance gene when treated, allowing them to ignore acaricide treatments. The first players to finish the game by taking their ticks around the complete life cycle are the winners. The Tick Game has been used to educate rural store staff in Northern Australia, but can be adapted to any country using different cards to reflect available chemicals and local conditions. It is also suitable for teaching farmers or university students.

163 **Why is anthelmintic resistance more common in *Teladorsagia (Ostertagia) circumcincta* than *Trichostrongylus colubriformis*?**

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Reports of drug resistance is rare in *T. colubriformis* (*Trich.*) by comparison with *T. circumcincta* (*Ost.*). In Western Australia confirmed cases of ivermectin resistance on tested sheep farms are <1% and 75% for *Trich.* and *Ost.* respectively despite both species being prevalent. If resistance were recessive in *Trich.* and dominant in *Ost.* this could explain the difference. Another explanation for this observation is available from examining the difference in the population biology of the two species. A simulation model was used to examine the potential role of host regulation and mode of inheritance in determining rate of selection for anthelmintic resistance. Some key assumptions were: In susceptible lambs approximate establishment rates are 35% & 60% for *Ost.* and *Trich.* respectively so it is more difficult for *Ost. larvae* on pasture to establish in a susceptible sheep (all else being equal). The cascade of events for acquired immunity that determines the dilution rate of selected worms in the host is the same for both species: (1) reduced fecundity, (2) reduced establishment rate, and finally (3) adult rejection. However, the rate at which these events happen is different for the species. A 5-fold increase in refugia gave a 2-fold delay in selection for resistance for both species. Using a combination treatment provided a 3 and 13-fold delay in selection for resistance in *Ost.* and *Trich.* respectively. In *Trich.* we assumed the drug can select either more or less resistance-alleles than in *Ost.* Despite this, selection still was delayed 2-6 fold by comparison with *Ost.*

164 **Development of genetic crossing methods to identify genes associated with macrocyclic lactone resistance in *Haemonchus contortus***

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There is a pressing need to develop strategies to slow the emergence of macrocyclic lactone resistance in sheep flocks. Management practices aimed at maintaining anthelmintic susceptible nematodes in refugia, while achieving a satisfactory level of production may prove to be useful. However sensitive, molecular tests are required to monitor the subtle effects of these practices on the frequency of resistance alleles within nematode populations. To-date, studies of candidate genes coding for the known methods of action of macrocyclic lactone anthelmintics have produced a complex picture, highlighting the relevance of different approaches to the identification of resistance markers.

This paper describes the development of genetic crossing methods to identify novel genes, for example coding for drug regulatory mechanisms, or to verify candidate gene markers should they be found. The development of a single nematode mating method and its verification using a panel of molecular markers is described. Potential problems associated with the phenotypic analysis of a second filial generation that will be required to identify polymorphisms in microsatellite markers putatively linked to resistance genes are discussed. The progress of backcrossing experiments initially between macrocyclic lactone susceptible ISE and resistant White River and CAVR strains of *H. contortus* and then between ivermectin treated first to fourth filial generations and ISE *H. contortus* is described. These experiments should enable comparative genomic analysis to identify resistance genes derived from White River or CAVR *H. contortus* in a fourth filial generation of predominantly ISE genotypes.

165 **In vitro detection of anthelmintic resistance in *Haemonchus contortus* by larval development test**

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Anthelmintic resistance among parasites of sheep and goats has been known to occur at least for four decades. Over this period it has developed in more nematode species to each of the broad spectrum anthelmintic drug groups. A wide variety of tests are available to monitor anthelmintic resistance. The most widely used test to assess anthelmintic efficacy is faecal egg count reduction (FECR) test. Although this standardized test is valuable in detecting of anthelmintic resistance, in vitro tests are cheaper to perform and therefore more suitable for large surveys. A number of versions of the larval development test (LDT) that can detect resistance to all classes of anthelmintics have been developed. The aim of this study was to evaluate potential of two versions (liquid and agar) for definition of resistance to benzimidazole and ivermectin anthelmintics in *Haemonchus contortus* of sheep. Single infection with 5000 L3 of four susceptible and four resistant (benzimidazole/ivermectin) isolate of *Haemonchus contortus* has been carried out. Three different versions of LDT were performed and faecal samples were collected four times during patency. The results of the tests are presented as LC50/LC99 values. The LDT showed high ability to distinguish between benzimidazole susceptible and resistant strains. In order to increase the sensitivity of the test, LC99 or MIC (minimum inhibitory concentration) values is recommended to use. The ivermectin resistant strains were also distinguishable from the susceptible ones by using of eprinomectin, however the resistance factors were lower than those obtained with benzimidazole drug.

166 **The development of diagnostic assays to evaluate triclabendazole resistance in the field**

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Fasciola hepatica is an important parasite of domestic livestock. Triclabendazole (TCBZ) is a fluke-specific compound. The efficacy of TCBZ against the early migratory stage is important as other currently available flukicides are not effective against this stage. Its future use maybe compromised by the development and spread of resistance to it among fluke populations. TCBZ-resistant field isolates of *F. hepatica* have been identified in Australia and several European countries. This work aims to develop a reliable diagnostic assay to test for TCBZ resistance in the field. Preliminary work has shown that a modified sedimentation technique is more sensitive than a flotation method in detecting fluke eggs in faecal samples. A faecal egg count reduction test, using sheep with TCBZ-susceptible fluke infections, revealed a rapid drop in egg counts, over a two-week period after treatment, but occasionally eggs were detected in faeces after this time. A commercial coproantigen test (ELISA) is also being evaluated for sensitivity in detecting fluke-specific antigens. Results from a sheep trial that has been established in order to compare and contrast these two methods will be presented.

167 Anthelmintic resistance survey in French dairy goat nematodes in four main breeding areas

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The occurrence of levamisole (LEV) and ivermectin (IVE) resistance was investigated in 36 randomly selected dairy goat herds located in the four main goat breeding areas in France in 2000-2002 : north-east (10), south-east (10), south-west (7) and west (9). On each farm, 45 adult goats were randomly allocated into three groups of 15 animals : an untreated control group, a levamisole group (12 mg/kg BW per os) and an ivermectin group (0.3 mg/kg BW SC or per os). Individual faecal egg counts and pooled larval cultures were done 10-11 days (control and LEV groups) and 16-17 days (control and IVE groups) after anthelmintic treatment. Faecal egg count reductions (FERC) were calculated for LEV and IVE, and, when less than 95 per100, were considered as indicative of anthelmintic resistance. When suspicion of IVE resistance occurred, 2 naive goats were infected with larvae from control group of the same farm (one lamb per group), were treated with ivermectin (SC) and necropsied for worm recovery. Additionally, an in vitro larval development test (LDT) was conducted with ivermectin on larvae isolated from pooled faeces in these particular farms. FECR indicated the occurrence of levamisole resistance in three out of 36 farms (78%, 65%, 44%), all the farms being located in western part of France. Nematode genera involved were only *Teladorsagia*/*Trichostrongylus*. In six farms out of 36, all located in north-east, FECR indicated ivermectin resistance (84%, 77%, 87%, 88%, 84%, 72%). *Teladorsagia*/*Trichostrongylus* were the main nematode genera involved. However, necropsic exams after IVE treatment did not confirm the suspicion of resistance (efficacies > 99%) whereas LDT values ranged from 11.77 to 40.71 ng/ml.

168 The search for mutations associated with ivermectin resistance in the ligand-gated chloride channel genes of *Teladorsagia circumcincta*

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Ligand-gated chloride channels, operated by their natural ligands glutamate and GABA, respectively, are unique in invertebrates and are believed to be the primary binding site for the macrocyclic lactone anthelmintics. It has been postulated that resistance to the MLs is mediated through genetic changes, i.e. target site mutations, in these ion channels. The GluCl_s are predominately localised on the pharyngeal musculature and the GABA-Cl_s on the somatic musculature of nematodes. We have been using larval feeding and migration assays to evaluate the relative roles of these ion channels in the phenotypic expression of resistance and to identify the most resistant sub-fractions of our *Teladorsagia circumcincta* populations for subsequent genetic analysis. Previous studies have shown that the GluCl alpha-subunit gene is under selection, with a loss of polymorphism and a build-up of a nominal allele "E" in L1 larvae capable of feeding in high concentrations of IVM (>0.09ng/ml) and in adults able to survive a therapeutic dose (0.2mg/kg). We are in the process of attempting to identify the genetic changes associated with this allele by sequencing directly from pooled cDNA generated from these phenotypically resistant parasites. However, the analysis is complicated by the subunit nature of these genes and the presence of multiple splice variants. What we can say at this stage is that IVM resistance in *Teladorsagia* is not associated with the L256F mutation in the GluCl gene that has been reported in the cattle parasite, *Cooperia oncophora*.

169 **Discovery of new molecular targets for drug & vaccine design against liver fluke**

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Liver fluke infections are caused by the helminth parasite, *Fasciola spp.*, and are amongst the most important global pathogenic organisms infecting both animals and humans. Since 1995, resistance against the only effective anthelmintic drug, triclabendazole, has been increasingly reported worldwide, providing increasing impetus for discovering new targets for liver fluke control. Research in this area has however been hampered by the lack of acquired resistance in most host species.

We have recently performed extensive comparative infection studies that demonstrated conclusively that Indonesian thin-tail (ITT) sheep were able to develop acquired resistance to *F. gigantica*, but not *F. hepatica*. Subsequent studies revealed critical differences in the susceptibility of *Fasciola* species to host resistance mechanisms, both in vivo and in vitro. In particular, the juvenile stage of *F. hepatica* is highly resistant to antibody-dependent cell mediated cytotoxicity (ADCC) by activated sheep macrophages, while juvenile *F. gigantica* are susceptible to ADCC. These results indicate that resistance to *Fasciola* infection is determined in part by biochemical differences between species of *Fasciola*, controlling susceptibility to ADCC killing. It is anticipated that molecules critical to the parasite's defense against host attack will provide excellent targets for designing new drugs or developing novel vaccines. We are currently using genomic and proteomic approaches to reveal critical molecules differentially expressed by these closely related parasites.

170 **The alternative way of vaccine targeting, phosphoglycerate kinase (FhPGK) from the adult *Fasciola hepatica* – evaluation of the cDNA and recombinant protein vaccine protectivity**

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The best way of prophylactics against fasciolosis may be vaccination. In our research we focused on the new vaccine candidate antigen which we cloned from the adult fluke, and simultaneously, new vaccination strategy, which is blocking of parasite energy metabolism.

Preliminary vaccination trial conferred protection on the level of 52% in male rats vaccinated intramuscularly. These results indicate that FhPGK can be an effective vaccine antigen. Because outcome of vaccination depends very much on the route of the vaccine delivery as well as on the form of the antigen and physiological status of the host we decided to conduct experiments using cDNA or protein form of FhPGK and parallel groups of male and female rats in order to establish vaccination scheme conferring the highest possible level of protection.

In the second vaccination trials which presently are being conducted, each experimental rat will receive two doses of recombinant eukaryotic expression vector (FhPGK/pCMV): first 25 ug and second 50 ug, intramuscularly or intranasally in four week interval. A month later an oral challenge of 35 metacercariae will be given to vaccinated and challenge control rats. Parallel experiment is running using recombinant FhPGK protein as a vaccine. Experimental rats receive two doses of 100 ug of the purified FhPGK. All rats will be dissected at week 9 after the infection, their livers will be isolated, the flukes collected and counted in order to estimate the level of protection.

171 **Molecular discrimination of *Fascioloides magna* and *Fasciola hepatica* based on the species-specific ITS regions**

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The discrimination of adults of liver flukes *Fascioloides magna* and *Fasciola hepatica*, parasitizing wild and domestic ruminants, is easy because of significant differences in size, shape and morphology. On the other hand, their intramolluscan larval stages and eggs are morphologically almost indistinguishable. Consequently, fecal examinations and search of infected molluscs using only morphological examination could not serve for undisputable differentiation of both species. Therefore, we decided to sequence complete small subunit (SSU) and internal transcribed spacers (ITS1 and ITS2) of the ribosomal DNA of *F. magna* and compared them with already published data of *F. hepatica*. As a result of their comparison, fixed interspecific genetic differences were determined and suitable method for species delineation was proposed. A comparison of SSU rDNA sequences of the two flukes revealed only a little sequence divergence (0.9%). The number and distribution of polymorphic sites did not permit design of species-specific markers. On the other hand, sequence polymorphism in ITS1 and ITS2 enabled selection of short sequence motifs with rather high degree of interspecific variation and suitable for design of species-specific primers. The *F. magna*-specific primers amplified 127 bp ITS1 and 152 bp ITS2 fragments in *F. magna* DNA, and did not anneal in *F. hepatica*. The 223 bp ITS1 and 122 bp ITS2 fragments were amplified with *F. hepatica*-specific primers in *F. hepatica* DNA, with no amplification products in *F. magna*. Thus, PCR amplification of species-specific ITS regions allows molecular differentiation of *F. magna* and *F. hepatica* in all developmental stages.

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172 **SEM observations on the impact of a methimazole on triclabendazole (TCBZ) action against TCBZ-resistant and -susceptible isolates of *Fasciola hepatica***

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Recent studies have shown that triclabendazole (TCBZ)-resistant isolates of *Fasciola hepatica* have an increased capacity to oxidise TCBZ and triclabendazole sulphoxide (TCBZ.SO) to inert forms, which affects the ability of the drug to reach its target within the fluke and exert its fasciolicidal effect. In this in vitro study methimazole, a metabolic inhibitor of the flavin monooxygenase (FMO) system, was used to assess the effect of metabolic inhibition on TCBZ-resistant and -susceptible flukes. The FMO system was inhibited by a 2-h pre-incubation in methimazole (100 μ M). The flukes were then incubated for 22h in NCTC medium containing either methimazole; methimazole+NADPH (1nM); methimazole+NADPH+TCBZ (15 μ g/ml); or methimazole+NADPH+TCBZ.SO (15 μ g/ml). Flukes were then processed for scanning electron microscopy. Preliminary results show surface disruption to both isolates of fluke. Treatment of flukes with TCBZ in combination with methimazole and NADPH caused swelling and localised blebbing of the tegument in both isolates. Combination of TCBZ.SO with methimazole and NADPH caused tegumental blebbing and swelling in the TCBZ-susceptible isolate, while the -resistant isolate showed greatest surface disruption in the form of swelling, blebbing and sloughing. Results suggest that an inhibition of drug metabolism can lead to greater surface disruption to TCBZ-resistant than -susceptible flukes following TCBZ.SO treatment. The morphological observations support previous pharmacological information indicating that an altered metabolic capacity within TCBZ-resistant flukes may contribute to the development of TCBZ resistance.

173 **Uptake of triclabendazole by the liver fluke: trans-tegumental or oral?**

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For the liver fluke, *Fasciola hepatica*, two routes of drug uptake are available: trans-tegumental diffusion and oral ingestion. The adult fluke lives in the bile duct and is primarily a blood-feeder. The two main metabolites of triclabendazole (TCBZ), triclabendazole sulfoxide (TCBZ.SO) and triclabendazole sulphone (TCBZ.SO₂) bind strongly (>99%) to plasma proteins. This would point to oral uptake being their major route of entry into the fluke. However, ~45% of the administered dose of TCBZ is excreted in the bile, so the fluke surface would be exposed to the metabolites. Experiments have been carried out in vitro to determine the relative susceptibility of the tegument and gut to drug action. They involved the use of ligatured flukes, to block the oral route of entry of drug. This was achieved by tying a ligature of surgical thread around the apical cone region of the fluke. Ligatured and non-ligatured flukes were incubated in TCBZ.SO at a concentration of 15 µg/ml for 24h. Morphological changes to the tegument and gut were assessed by scanning and transmission electron microscopy. Considerable disruption to the tegument of both ligatured and non-ligatured flukes was observed following treatment with TCBZ.SO. In contrast, there was no noticeable disruption to the gut of either ligatured or non-ligatured flukes following treatment. The results indicate that trans-tegumental diffusion is the main route of uptake of TCBZ metabolites into the fluke. They support those of a previous pharmacological study, which demonstrated that trans-tegumental diffusion is the predominant means by which TCBZ metabolites enter the fluke.

174 **Molecular identification and genotypic characterization of *Fasciola* spp first isolated in goats in Vietnam**

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A portion of mitochondrial *cox1* and *nad1*, and the entire nuclear internal transcribed spacer 2 (ITS-2) were obtained and used as genetic markers for the molecular identification and genotypic characterization of *Fasciola* spp isolates first collected in goats in Vietnam. These sequences were also comparatively analyzed with the Vietnamese and global *Fasciola* isolates, including samples of different developed stage from buffalo, cattle, sheep, goat and human origin. Molecular analysis revealed that the Vietnamese *Fasciola* goat-isolates belonged to two genotypes: a pure *Fasciola gigantica* and a hybrid form between *F. gigantica* and *F. hepatica*. Morphologically, the isolates are divided into 2 kinds of shapes: a long, *F. gigantica*-typical and a short, *F. hepatica*-like shape. Together with the previous findings, plus aberrantly migrated *Fasciola* in human (Xuan et al., 2005; Le et al., 2007), our morphological and molecular analysis confirmed that there are two genotypes of large liver flukes, pure *F. gigantica* and an intermediate form of hybridization (*F. hepatica* (male) x *F. gigantica* (female)) existing in Vietnam in all the host population. Genomic change, mixed ploidy and hybrid/introgressed forms of *Fasciola* spp as an evolutionary pressure may be reminded. We thank the financial support (VLIR to Nguyen, TGT; ICGEB to Le, TH).

175 **Epidemiology of blowfly myiasis in domestic rabbits in the UK – a nationwide study**

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Blowfly strike in rabbits is widespread in the UK and frequently results in the death of infested animals. A nationwide study was conducted during 2006 designed to (1) establish whether the green bottle fly, *Lucilia sericata*, was of equal importance across the entire country and throughout the blowfly season; and (2) assess the relative importance of different risk factors likely to predispose for the condition. A total of 3,022 RCVS-registered veterinary practices were sent (1) a sample bottle, for the collection of < 5 blowfly maggots from each case of myiasis seen in domestic rabbits (these bottles contained 80% ethanol that enabled subsequent morphological and molecular identification of the larvae); and (2) a questionnaire to be completed in consultation with the owner. The latter was designed to provide information on the rabbit, its husbandry, the owner's awareness of blowfly strike, whether preventative treatment had been given, clinical details of the case and subsequent treatment, and eventual outcome. By the end of December 2006, maggots and completed questionnaires relating to 387 individual cases of blowfly strike had been submitted for analysis from 289 veterinary practices from all over the UK. Almost all of the maggots recovered were *Lucilia* spp. Epidemiological factors that exerted a significant effect on host susceptibility or survival included environmental temperature, rabbit location (in- or out-of-doors), urinary incontinence, extent of maggot-associated injury and veterinary treatment. Financial support provided by Novartis Animal Health UK Ltd, Vetoquinol UK Ltd., Bayer HealthCare, Rabbit Welfare Fund and Nelson Veterinary is gratefully acknowledged.

176 **Prevalence of *Anaplasma (A.) phagocytophilum* infection in dogs in Germany**

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The objective of this study was to evaluate whether the prevalence of tick-transmitted *A. phagocytophilum* infection in sick dogs in an area with very high *Ixodes ricinus* populations was different from clinically healthy dogs. Between June 2005 and December 2006, a total of 522 dogs (264 healthy dogs = group 1 and 258 with clinical or laboratory abnormalities suspicious for canine granulocytic anaplasmosis - CGA = group 2) were tested. Blood samples collected from the dogs were evaluated by complete blood count (CBC), Indirect Fluorescent Antibody Test (IFAT) and Real-time PCR for *A. phagocytophilum*, and blood smear examination. The overall seroprevalence for *A. phagocytophilum* was 45%, being 41% in group 1 and 49 % in group 2. Twelve seropositive and 12 seronegative clinically healthy dogs had laboratory abnormalities such as leukopenia, leukocytosis, thrombocytopenia, and mild anemia. Ten healthy dogs showing positive PCR results had normal CBC values. Based on clinical signs and positive PCR results, CGA was diagnosed in 20 sick dogs, from which 9 had morulae in blood smears and 6 were seronegative. Consequently, in high *I. ricinus* endemic areas, the prevalence of *A. phagocytophilum* infections in dogs can be high. A diagnosis of CGA cannot be based only on serology, since dogs might be seronegative and clinically ill or vice versa. Similarly, PCR- and morulae positive dogs can be clinically and haematologically normal.

177 **Immunological and serological study on the infectivity of host animals and ticks (Ixodidae, Argasidae) to CCHF virus in west Azerbaijan, Iran**

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West Azerbaijan province is located in North western Iran bordered with Turkey and Azerbaijan. The main activities of people in this region is agriculture and cattle rearing. A comprehensive study was carried out in year 2004-2005 for determination of presence of virus in ticks and antibody against CCHF in their hosts. The villages selected randomly and ticks with their host sampled according to standard method. Results showed that the majority of ticks host were sheep and goat. At the same time all the collected ticks were searched for the presence of CCHF virus using RT-PCR method. Among 47 sera of sheep it is found that 50% of them was found IgG positive. One out of 20 shepherd alsoe was positive. Different species of soft and hard ticks including *Rhipicephalus*, *Hyalomma*, *Dermacentor* *Haemaphysalis* and *Ornithodoros* was collected from various types of houses, stables and animal shelters comprises 340 specimens. Results of RT-PCR revealed that 33. % of *Hyalomma* collected was positive to virus. The figures for *Dermacentor marginatum* and *Ornithodoros lahorensis* was 27% and 40% respectively. The main species of infected ticks to CCHF was: *Rhipicephalus bursa*, *Haylomma dermoderii*, *Hyalomma asiaticum*, *Hylaomma detritum*, *Hyalomma marginatum*, *Hyalomma anatolicum*. *Rhipicephalus bursa*, *Rh.sanguineus* *Dermacentor marginatus*, *Haemaphysalis.sulcata*, *Hae.concinna*, *Hae.punctata* and *Ornithodoros lahorensis*

Keywords: tick, CCHF, Iran

178 **Seasonal and geographic variation in the epidemiology of *Anaplasma Phagocytophilum* and *Rickettsia spp.* in the hard tick *Ixodes ricinus* in Bavaria, Germany**

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The presence of tick-borne pathogens like *Borrelia spp.* and *Babesia spp.* in Germany has long been known. In recent years, also *A. phagocytophilum* and *Rickettsia spp.* have been detected in *I. ricinus* in Germany and thereby, a focal distribution has been suggested for *A. phagocytophilum*. This study investigates the prevalence of *A. phagocytophilum* and *Rickettsia spp.* in DNA-Extracts of 2862 unfed ticks (adults and nymphs) from 8 sites in Munich, collected regularly over 5 months, by PCR and Real-Time PCR.

The overall prevalence was 3.60% and 6.34% for *A. phagocytophilum* and *Rickettsia spp.* respectively, 0.35% were coinfectd. The prevalence varied between 0 % and 11.77%, regarding the different pathogens, areas and months under investigation. The prevalence of *A. phagocytophilum* in a recreational park in the centre of Munich, highly frequented by people and dogs, was higher than in a natural forest outside of Munich, whereas the prevalence of *Rickettsia spp.* was opposite. For both pathogens, the prevalence in adults was higher than in nymphs. 16S rDNA nested-PCR products of *A. phagocytophilum* and gltA/ompA PCR products of *Rickettsia spp.* were sequenced. The majority belonged to *Ehrlichia sp.* “Frankonia 2” and *R. helvetica*, the presence of other species or variants of *A. phagocytophilum* and *Rickettsia spp.* was also detected.

This study manifests clearly a strong seasonal and geographic variation of *A. phagocytophilum* and *Rickettsia spp.* and shows that a big city park is an area of accumulation for *A. phagocytophilum*. Further studies will be needed to evaluate risk areas of these pathogens.

- 179 **Distribution and phylogeny of intramitochondrial bacteria in Ixodid ticks**
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'*Candidatus Midichloria mitochondrii*' is a recently described intramitochondrial bacterium observed in the ovarian cells of the hard tick *Ixodes ricinus* (Ixodidae). The biological role of this symbiont is unknown. Information regarding the presence of related bacteria in other tick species is limited to few nucleotide sequences in the databases. We have performed a PCR screening for *Midichloria*-related bacteria in DNA extracts from ticks of the five subfamilies of the Ixodidae. PCR was performed on ticks collected in Italy, representing five tick genera. A total of six species were found positive for bacteria closely related to *M. mitochondrii*. Automated sequencing was performed on a ~1100 bp fragment of the bacterial 16S rRNA gene of positive specimens. Phylogenetic analyses on the obtained sequences revealed that the bacteria form a monophyletic group within the order Rickettsiales, and likely represent a single species. No correspondence between the phylogenies of the tick bacteria and their hosts was found; this result suggests the possibility that horizontal transmission of bacteria may occur. Ovarian samples of *Rhipicephalus* sp. ticks, that were found positive in the PCR screening, were then analyzed by transmission electron microscopy (TEM). TEM examination of the ovaries revealed the presence of bacteria both in the cytoplasm and in the mitochondria of the oocytes. Whether all of the bacteria in the *Midichloria* genus share the intramitochondrial niche is yet to be revealed, but the fact that both tick species thus far examined by TEM show the presence of bacteria in the mitochondria supports this hypothesis

- 180 **Detection of *Borrelia* spp. in natural vector *Ixodes ricinus* in the Hannover region with genus-specific and species specific PCR – a representative sampling approach of public greens**
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The main objectives of this study were to monitor the tick population density in recreational areas in the city of Hannover, Germany, and to investigate the prevalence of *Borrelia burgdorferi* s.l. in these ticks as an example of local epidemiological investigations in *Borrelia* prevalence in public Greens in Central Europe. The occurrence of different *B. burgdorferi* genospecies was studied using two different PCR detection methods. 8802 ticks were collected between March and October 2005 in 12 places of five defined areas of the city of Hannover with flagging. The collecting sites were characterized by the following parameters: easy access, different types of vegetation (meadow, bush areas and woodland) and use by the public for leisure. The collected ticks were identified by species and developmental stage as *Ixodes ricinus* (95%) and *I. hexagonus* (5%) and analyzed for the presence of *Borrelia* by dark-field microscopy in a randomly selected subpopulation. In bushes and greens 35% and 19%, respectively, were collected, 46% of the ticks were collected in woodlands. More than 50% of these ticks were nymphs, 31% were adults and only 17% were larvae. Subsequently, genus-specific ITS2-PCR as qPCR was performed. 915 positive ITS2-PCR results were differentiated using an species-specific rPOB-PCR for the identification of *Borrelia* genospecies.

3962 ticks were evaluated by DFM and qPCR: 11.4% of these ticks were positive by DFM and 23.09% by qPCR. The highest prevalence was found with 36.01% and the lowest with less than 6%.

181 **Is toltrazuril-based prevention of piglet coccidiosis always economically justifiable?**

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Toltrazuril-based prevention protocols against *Isospora suis*, one of the most prevalent parasites in intensive pig production causing clinical diarrhoea in piglets, are efficient and well documented. In this study we aimed to estimate the differences in terms of time to: 1) oocyst excretion and 2) abnormal faecal consistency between toltrazuril-treated and non-treated piglets in a farm where *I. suis* was present but no anti-coccidial compounds were used. The trial included 13 litters (totaling 128 piglets) which were treated with toltrazuril (at the recommended dose rate) on the 3rd day after birth (group A), and 12 untreated litters (totaling 118 piglets) (group B). Faecal consistency scores and oocyst excretion (after examination with a modified McMaster technique and counted under a fluorescence light source) were recorded daily from all piglets starting at day 6 after farrowing until day 13 and subsequently every second day until weaning. Survival-curves for each piglet were estimated as days from birth until initiation of a) oocyst excretion, b) yellow diarrhoea, or c) pasty or yellow diarrhoea. The Kaplan-Meier survival curves were derived using PROC LIFETEST in SAS. The survival curves differed between the two groups for each outcome (all $P < 0.01$). The median time to initiation of oocyst excretion for group B was 21 days (17-25) and >29 days for group A piglets. For yellow diarrhoea the median time for both groups was >29 days. The median time to pasty or yellow diarrhoea was 29 days (19-29) for group B and >29 days for group A piglets. In this *Isospora*-infected herd, the median time to oocyst-excretion or to abnormal faecal consistency of the non-treated piglets was over 20 days. There is an obvious need for further investigation into the factors that contribute to this and make toltrazuril-based prevention economically unjustifiable.

182 **Revitalisation of anticoccidial sensitivity in poultry flocks by the use of a live attenuated vaccine**

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Coccidiosis is a highly damaging disease of poultry which has traditionally been controlled by the addition of in-feed anticoccidial drugs. However, there is increasing concern about rising levels of drug resistance. The degree and impact of this resistance varies between poultry units, but is having a major impact on operations worldwide.

In the UK, in-feed medication is still the major method of coccidiosis control, although there is a licenced live-attenuated vaccine available (Paracox-5) which is successfully being used in broiler birds.

Commercial trials were conducted at 2 poultry farms to investigate if anticoccidial efficacy can be revitalised by introducing Paracox-5 vaccination into poultry rearing operations. On both farms, house 1 received in-feed anticoccidials nicarbazin/narasin followed by monensin, and house 2 was vaccinated at day-old with Paracox-5.

Coccidial oocysts were extracted from faeces samples from the individual houses and Anticoccidial Sensitivity Tests (AST) performed to investigate the degree of resistance to anticoccidial drugs.

The AST involved groups of poultry medicated with anticoccidial drugs, challenged with oocysts from the individual houses and measuring weight gain and coccidial lesions. An infected unmedicated control group of chickens was also monitored. By comparing both parameters from medicated and unmedicated groups it is possible to measure the degree of anticoccidial resistance within the poultry house.

Results from both farms showed that there was a clear difference in weight gain responses and lesion scores between groups of birds infected with oocysts from medicated (house 1) and vaccinated houses (house 2).

Vaccinating birds with a live coccidiosis vaccine resulted in coccidiostat-resistant *Eimeria* being replaced by coccidiostat-sensitive ones – “Revitalisation”.

183 **Immunostimulating complexes incorporated coccidia antigens and low toxicity plant saponins as a novel delivery system for *Coccidia* vaccine immunization**

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Coccidiosis is an intestinal disease causing significant economic losses to the poultry industry. At present the main emphasis in control of poultry coccidiosis has centered on elicitation of protective immune response to parasite infection by development, delivery and effective use of various kinds of parasite vaccines. Immunostimulating complexes (ISCOMs) are self-assembling structures containing antigens, lipids and adjuvant active triterpene saponins as a key component for ISCOMs formation. ISCOMs are one of the most effective delivery systems for vaccine preparations stimulating high levels of antibody, cell-mediated and mucosal immune responses. The adjuvant active low toxicity triterpene saponins GG-6 and AH-6 have been isolated from *G. glabra* and *A. hippocastanum*, plants indigenous to Kazakhstan, by ethanol extraction and purified using HPLC fractionation. ISCOMs were assembled with natural *coccidia* antigens isolated from developing stages of parasite by non-ionic detergent MESK treatment or with the recombinant *coccidia* antigen SO7. The results of this study have shown that ISCOMs containing *coccidia* antigens and the saponins GG-6 and AH-6 stimulated high levels of humoral immune response induced production of cytokines and provided protection against challenge with live parasites. Activity of immune responses and protection against challenge was much higher when *coccidia* antigens were assembled with ISCOMs in comparison with pure *coccidia* antigens. It was concluded that ISCOMs delivery system prepared with triterpene saponins GG-6 and AH-6 could be used for production of a highly immunogenic *coccidia* vaccine with low toxicity.

184 **Molecular-based identification of sarcocysts in bovine meat**

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Introduction: Cattle are common intermediate hosts of *Sarcocystis cruzi*, *S. hirsuta* and *S. hominis*, with canines, felids and primates respectively as definitive hosts. Today, morphology of the cyst wall is used for routine identification. *S. cruzi*, a thin walled species, can be distinguished from *S. hominis* and *S. hirsuta*, both thick walled sarcocysts, by light microscopy. The distinction between the latter two however, is only possible by transmission electron microscopy (TEM). Therefore the objective was to develop a reliable molecular based identification technique. This technique was used to determine the presence of *S. hominis* in raw minced beef in Belgium.

Materials and methods: A PCR assay with genus-specific primers based on the 18S rRNA gene in combination with sequence analysis was developed and applied on DNA extracts from half parts of microdissected sarcocysts. The other half-cysts were examined by TEM, as gold standard. Subsequently, 67 random samples of raw minced meat were obtained from 25 retail stores and thick walled cysts were examined by the developed molecular technique.

Results: The primer set allows detection of the three targeted species and produced a fragment of the expected size range. Sequence analysis showed to be in accordance with the TEM examination. Of the examined minced beef samples, 91% were infected with thick walled cysts of which 97% were identified as *S. hominis*.

Conclusion: The molecular assay can be the method of choice for the identification of bovine sarcocysts. The findings of the study suggest an efficient epidemiological cycle of the zoonotic *S. hominis*.

185 The development of conventional and real-time PCR for the detection of *Besnoitia besnoiti*

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Besnoitia besnoiti, an apicomplexan protozoan parasite, is the causative agent of bovine besnoitiosis. This infection may dramatically affects body condition, and, in males, lead to infertility. The definitive host of this parasite is unknown, and no treatment has been assessed so far that may either cure from disease or eliminate the parasite. Therefore, to practically handle this infection, only vectors' control and identification of infected animals is of crucial relevance to base control measures. While identification of clinical cases and their histopathological confirmation is relatively simple to carry out, finding subclinical forms of infection is more difficult, thus a more sensitive test for the identification of the etiological agent may be an appropriate diagnostic tool. In the present paper, we describe the development and subsequent application of ITS1-sequence-based conventional and real-time PCR tests for diagnosis of *B. besnoiti* infections in cattle. In order to optimise these PCRs in view of their practicability for routine diagnostic application, a recombinant internal positive control was introduced to assess possible sample-related inhibitory effects during the amplification reaction. In order to prevent false-positive results, a pre-PCR treatment of potentially contaminating dU-containing PCR product with uracyl-DNA-deglycosylase (UDG) was followed. Our study revealed that the sensitivity of both tests allowed consistent detection of 1 parasite. As a quantitative assay, real-time PCR may also substantially contribute to those approaches that explore pharmacological and immunotherapeutical aspects of *B. besnoiti* infections.

186 Re-emerging cattle besnoitiosis (*Besnoitia besnoiti*) in France: update on clinical and epidemiological aspects

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Besnoitiosis life cycle is heteroxenous : the primary cycle from cat to cattle seems less frequent than a secondary cycle from cattle to cattle, with appears to be the main route of infection, with mechanic transmission of bradyzoites in skin cysts by insect bites or by infected injection needles.

Cattle besnoitiosis was in 1970 still endemic only in a small area of East Pyrenees mountains in South-Western France. Since 1990 years, besnoitiosis first extended widely around Pyrenees mountains and in some places of Massif Central.

Since 2001, wide areas of French Alpes and several serious outbreaks appears in West of France, sometimes with 10 % of prevalence.

Clinical aspects of besnoitiosis are unchanged, with successively febrile stage, oedema stage and finally depilatory and scleroderma stage.

If diagnosis and treatment are late, besnoitiosis may dramatically affect body condition and fertility (in bulls), resulting in important economic losses.

Recent epidemiological investigations showed the summer clinical expression on mainly young cattle from 2 to 4 years with no racial predisposition and a sensibility closely related to sex.

Summering on pastures with mixed herds and high prevalence of biting insects are the greatest risks factors. The increasing geographical distribution towards northern France imply that besnoitiosis must be more precociously diagnosed by vets and breeders and certainly that further investigations on life cycle must be considered (importance of wildlife intermediate hosts?).

Identification of asymptomatic carriers, serologically positive with new reliable methods (ELISA, Western blot), is of major relevance to elaborate appropriate measures of control.

187 Ticks of dogs and cats and their importance as disease vectors in Europe

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Many species of ticks affect dogs and cats in Europe, the most important species are *Ixodes ricinus*, *Dermacentor reticulatus* and *Rhipicephalus sanguineus*. The geographic distribution of these species and their role as vectors for disease agents is being discussed based on available information. However, the absence of reports does not indicate the absence of any specific tick.

Further, a number of tick-transmitted diseases reported in Europe will be addressed according to a recently published overview by the EFSA and their importance and geographic distribution will be shown. Epidemiological implications and quality of data available are being discussed.

For most zoonotic infections, human cases do not reflect the epidemiological situation in particular countries as it may be due to an increase in travel activity of both humans and pets. Current treatment and prevention options, including manual removal of ticks found on dogs and cats, and a non-chemical tick control including habitat control or change to reduce the tick population, and preventive application of acaricides are highlighted. The major classes of compounds with registration as acaricides are listed and discussed as well as their use. Emphasis is put on regular application for a reason-long prevention, possibilities for this purpose are described and discussed.

188 Delivering a new class of anthelmintics

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Anthelmintic resistance continues to spread in prevalence and severity, affecting pathogenic nematodes of animals. Today, multi-drug resistance against the three major anthelmintic classes, viz. benzimidazoles, imidazothiazoles and the macrocyclic lactones, is a global phenomenon in gastro-intestinal nematodes particularly of sheep and cattle.

In this symposium, Novartis Animal Health discloses the discovery of a novel chemical class of anthelmintic, and describes potential drug development candidates that are efficacious against various parasitic nematode species. These new drug candidates are also efficacious against nematode isolates resistant to the currently available anthelmintics due to a novel mode of action. No new anthelmintic class (for use in livestock species) has reached the market in the previous 25 years, and there is now an urgent need for an anthelmintic with a new mode of action. This new anthelmintic class provides a potential solution for the long-term control of both resistant and non-resistant nematodes in the future.

189 **Anthelmintic resistance in small ruminants and its diagnosis in the field**

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In the past few years the problem of anthelmintic resistance in nematode parasites of small ruminants has entered a new phase of severity. In many areas of the world multiple drug resistance is now the norm, and reports of resistance to all available anthelmintics are increasingly common. The diagnosis of resistance in the field is most commonly made using the fecal egg count reduction test (FECRT). However, when the FECRT is performed using standard and accepted protocols, resistance is only detected once it has reached relatively high levels. But, in order to give the best advice to a particular farm, or to evaluate the impact of various treatment and control strategies on the development of resistance, it is necessary to know more than just whether resistance is present or not. Since the evolution of resistance typically is a slow process taking several to many years, at any given time point it is extremely valuable to know where on the continuum of susceptibility to resistance that a particular population of parasites lies. This could theoretically be achieved using molecular detection methods; however, we still lack useful genetic markers for all anthelmintics except the benzimidazoles. Given this reality, it is of considerable importance to develop and use novel protocols and data analysis methodologies to extract as much information as is possible from a FECRT. A finer calibration of resistance diagnosis would be extremely valuable – particularly for drugs that currently have a low prevalence of resistance. One example of such an approach will be illustrated

190 **Anthelmintic resistance in cattle - a developing problem?**

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Resistance to anthelmintics is a threat to several animal industries world wide. Nevertheless, the use of effective anthelmintics to control nematode infections in cattle still remains irreplaceable. Anthelmintic resistance in cattle has been reported in New Zealand, North and South America and England but so far not in Europe. To be able to determine the extent of anthelmintic resistance in nematodes of farm animals and to monitor the success of any resistance management requires reliable tests for the detection anthelmintic resistance. One of the objectives of PARASOL, a European Framework 6 funded project, is to produce standard operating procedures (SOP) for the running of a faecal egg count reduction test (FECRT). Standardized procedures for the FECRT have been developed and a survey with injectable ivermectin (IVM) was then performed in Germany, Sweden and Belgium in 2006. In all three countries IVM was efficient only on some of the farms. The main species detected after treatment was *Cooperia oncophora* and also *Ostertagia ostertagii* both in Germany and Sweden. Thus presence of worms that were refractory to IVM was much more common than previously realised. Double resistance (to IVM and benzimidazoles) may have started to develop in Germany. Clearly surveys in other EU countries are urgently required. The survey will be repeated with IVM in 2007 and benzimidazole will also be tested. Furthermore, some of the refractory strains will be isolated to test whether the phenomena observed in the field was due to the evolution of anthelmintic resistance.

191 **Alternatives to anthelmintics in parasite control in sheep**Sayers G., and Sweeney T.*School of Agriculture, Food Science and Veterinary Medicine, University College Dublin, Ireland*

Efforts to curb production losses caused by anthelmintic-resistant parasitic nematodes in sheep have led to the development of a number of alternative control methods, designed to complement or replace anthelmintics. The alternatives can be classed based on their nematode control mechanisms, and include grazing management, diet enrichment, biological control, vaccination, and genetic resistance. Grazing management and mixed stock rotation represents a traditional method for nematode control. While a moderately successful technique, their integration into current intensive single enterprise farming is becoming increasingly difficult. Diet enrichment is an effective, albeit costly, method of controlling parasitism. Enrichment with protein and plant extracts influences the degree of expression of immunity leading to reduced nematode burden in addition to higher weight gain. Biological control mechanisms disrupts the life cycle of the nematode using predacious fungi. Used as a zoo-technical additive in feed, the preying action of the fungus has a nematode reducing capacity in sheep. Vaccination remains the desired alternative to anthelmintics, however, despite considerable research, a viable vaccine has not been developed. Natural resistance to infection is well described, with considerable evidence for within- and between-breed differences in resistance. Adopting resistant breeds or breeding for resistance, represents a reliable and inexpensive complementary control measure to anthelmintics. Genetic loci associated with resistance have been identified, facilitating both selective breeding for resistance and deciphering of the immunological mechanisms of natural resistance. The choice of control measures will depend on the breed in production, presiding climate and the extent of nematode resistance to anthelmintics.

192 **Targeted anthelmintic treatment of sheep for resistance management: early experience in Australia and future directions**

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Anthelmintic resistance is widespread in Australia, and on some farms resistance in *Teladorsagia* now involves all available anthelmintic options. New approaches to the use of anthelmintics for sheep nematode control are hence urgently required. In environments such as in Western Australia, where resistance is due mostly to the heavy selection pressure imposed by strategic treatments during dry seasons, resistance management strategies centre around the provision of “refugia” from anthelmintics for non-resistant worms. A “targeted treatment” strategy, by which only sheep exhibiting the effects of nematodes are drenched, achieves the aims of alleviating parasitism on a flock basis and providing a source of worms in refugia. Trials in Western Australia utilised short-interval weight change as an index of the effects of nematodes on sheep, using electronic ear tag identification linked to an automated weighing unit. No significant production loss compared to traditional strategies occurred in any of 4 trials, while a source of worms not exposed to drenches (in refugia) was maintained. Computer simulation modeling will be used to examine the implications for anthelmintic resistance. However, this approach requires a greater labour input than is usually feasible. A project under the CRC for Sheep Industry Innovation will take the targeted treatment concept further to develop a range of practical protocols for application in different environments and worm control situations.

193 **Parasite control in the face of existing resistance in New Zealand**

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A recent national survey of anthelmintic resistance in cattle and sheep in New Zealand indicated that the prevalence or resistant nematodes has now become disturbingly high. There is a particular problem with multiple resistance to all three action families in *Teladorsagia* in sheep, and to macrocyclic lactones and benzimidazoles in *Cooperia* in cattle. More recently severe multiple resistance in *Trichostrongylus* in goats has been identified. However, the majority of affected farmers do not appreciate the scale of the issue on their farm, possibly because most have not formally tested to determine their farm's resistance status. In the future they need to negotiate a balance between achieving good parasite control and the sustainability of those control options. Most accept that anthelmintics will remain the cornerstone of gastrointestinal nematode control in sheep and cattle for the foreseeable future. To ensure their continued effectiveness farmers need to be constantly aware of the need to maintain an effective refugia to minimise the expansion of the resistant population. High-risk practices in relation to selection of resistance need to be identified and avoided or at least their use limited. These include: treating adult animals where there is no identified need, moving newly-treated animals on to 'clean' pasture and failing to effectively quarantine drench bought-in animals. None of these are new concepts but many have not been adopted or practised. A continuing effort is being made to inform veterinarians and farmers of all the issues around nematode control whilst research endeavours to answer some of the fundamental questions.

194 **Dissemination of best practice advice to producers and advisors**Jackson E., Bartley D., Kenyon F., and Sargison N.*Moredun Research Institute, Edinburgh, Scotland*

The identification and implementation of effective management strategies for restricting the development and transmission of anthelmintic resistant nematodes presents a serious challenge to small ruminant producers. The long term goals of maintaining drug efficacy and parasite susceptibility may clash with the short term goal for many farmers of simply maintaining adequate control. This is particularly the case in temperate climates where the emergence of multiple resistance is mostly only diagnosed following the clinical failure of anthelmintic(s). With a limited range of chemical options, the management of both nematodes and anthelmintic resistance is complicated. Specialist skills are usually required to develop and implement sustainable farm specific solutions. It is now widely accepted that 'generic (blueprint)' control strategies for small ruminant nematodes are not sustainable. The principal aim of current research on the management of anthelmintic resistance is to identify a range of appropriate effective strategies to provide best practice advice.. A full understanding of these best practice strategies and, conversely the risks inherent in bad practice, needs to be transferred to veterinarians, advisors and farmers all of whom are involved in developing, implementing and most importantly monitoring site specific nematode control strategies. The last two decades have seen a decline in funding for 'traditional' advisory services however, resources need to be found for improving veterinary and advisor training and for the provision of electronic transfer of information if the considerable hurdle of effectively distributing best practice advice is to be overcome.

195 **First report on population structure for the *Leishmania* major vector, *Phlebotomus papatasi* sandfly using microsatellite loci**

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Although many methods have been applied so far to characterize populations of *Phlebotomus papatasi* sandfly, still their genetic structures are not well understood. This widely distributed sandfly species is the principal vector of *Leishmania major*, which causes cutaneous leishmaniasis in the Old World. Multilocus microsatellite typing (MLMT) was used to infer population structure of these sandflies and assign individuals to populations. This approach used five microsatellite loci and 188 *P. papatasi* individuals originating from 35 populations distributed in 15 countries. Unique microsatellite signatures were observed for some populations analyzed and 114 different genotypes were found in total. Analysis of the data set showed comparable results using model and distance-based methods. Individual-based analyses split the data set into two distinct genetic clusters; 'A' and 'B' with further sub-structuring among each. Within 'A' group, five sub-groups had geographical correlations with large numbers of alleles. The genetic differentiation (F_{ST}) of the five sub-groups within 'A' cluster ranged from 0.816 to 0.403. The degree of genetic isolation was relatively high and statistically significant ($P < 0.005$). There was no correlation between linearized genetic distance and geographic distance as a whole. Understanding the genetic structure of *P. papatasi* populations is important for the implementation of efficient measures for sandfly control.

196 **Canine leishmaniasis candidate vaccines containing ribosomal and histone antigens could not protect dogs from experimental infection with *Leishmania infantum***

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Studies in mice have shown that histone proteins can deliver protective immunity against leishmaniasis. Protection was also found in dogs vaccinated with a fusion protein containing ribosomal- and histone protein epitopes in combination with live BCG as an adjuvant (Molano et al, 2003). In order to further investigate the vaccine potential of ribosomal- and histone epitopes we created a fusion protein from the JPCM5 strain of *Leishmania infantum* (JPCM5-F) and tested its efficacy in dogs. Recombinant JPCM5-F protein was produced in *E.coli* and baculovirus systems and tested in combination with different adjuvants; AIOH, MDP, Matrix C and killed *Propionibacterium acnes* were used in an attempt to elicit different responses across the whole spectrum of Th1 and Th2 directed immunity. Groups of five or seven dogs were immunized with one of six different preparations (total 36 vaccinates, 8 controls), four weeks after the booster injection all dogs were challenged intravenously with 5×10^7 promastigotes of *L.infantum*. After challenge dogs were subject to regular clinical examination and biopsies in order to monitor disease progress and parasite dissemination. After twelve months of follow-up none of the groups was found to be protected by any of the parameters. Immune responses to the vaccine antigen were detected by ELISA, Lymphocyte proliferation assay and Delayed Type Hypersensitivity testing. It is concluded that these fusion proteins could induce immunological responses, but signs of protection were not detected. The absence of live BCG in our candidate vaccines may well explain the observed lack of protective effect.

197 **Detection of natural *Trypanosoma vivax* infections in pigs with microhaematocrit centrifugation and amplification of ITS1 rDNA**

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Different species of trypanosomes may infect their mammalian hosts both singly or in combination in endemic areas. This study was undertaken to determine the trypanosome species that may be afflicting pigs in Uganda. Blood was collected from pigs of all ages and both sexes from two districts, Kasese in Western and Jinja in Central Uganda. Of the 133 pig blood samples from Kasese that were tested for trypanosomes using microhaematocrit centrifugation technique (MHCT), none was found to be infected. However, of the 253 pigs from Jinja district, 9 were infected with trypanosomes of which 3 had *T. vivax* as determined by MHCT. However application of the ITS1 rDNA PCR test revealed that 8 pigs had *T. vivax* in mixed infections and 1 pig had monolithic *T. vivax* infection. These observations show that under certain circumstances pigs may be important hosts to *T. vivax*. Although some previous reports indicate that pigs are refractory to *T. vivax* infection, this study suggests that pigs may commonly be infected by *T. vivax*.

198 **Estimating the impact of *Trypanosoma evansi* (surra) on buffalo populations in the Philippines using data from cross-sectional surveys**

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There are no published studies on the impact of surra on a host population, probably because of the large financial and time cost involved in performing longitudinal studies. A framework is presented for estimating reduced fertility, increased mortality and immigration associated with *T. evansi* infection in domestic buffalo populations. Estimates of these demographic parameters were required for constructing an infectious disease model and estimating production benefits of surra control/treatment. During 2002-6 a cross-sectional survey of *T. evansi* seroprevalence was conducted in Mindanao. Initial data analysis found all areas were dominated by females (69%). The mean calving rate for areas of high and low prevalence was 20% and 40% respectively. Males were removed at a relatively high annual rate of 27% in all areas. In the main reproductive years (4-10) female mortality was <1% and 10% for low and high risk areas respectively. Older females were removed/died at a rate similar to males regardless of area. In high risk areas there were consistently more two year than one year old females and the reverse was true for the low risk areas. This implies females were imported to the high risk areas for breeding. An age-structured state transition model was fitted to the observed percentage of females per age class in the two risk-areas. Assuming a stable age structure and similar size populations in each area, 30% of female calves needed to be moved from low to high risk areas per annum to maintain the observed age structure and demographic parameters.

199 **Trypanocidal drug resistance in trypanosomes of livestock in sub-Saharan Africa: an update**

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Since available trypanocidal drugs for the treatment of bovine trypanosomosis are on the market for 40 to 50 years, it is not surprising that drug resistance has become a serious problem. Currently, trypanocidal drug resistance has been reported in 16 African countries. In nine of them large-scale surveys did show that area-wide drug resistance was present in at least one region of the country.

As an alternative to the conventional *in vivo* techniques for the detection of drug resistance, molecular tools (PCR-RFLP) have recently been developed which allow the identification of most resistant trypanosome isolates within a few days instead of 2 or 3 months as was the case in the past. Although initial surveys showed a consistent correlation between the mouse test and the *Mbo*II-PCR-RFLP test for resistance to isometamidium in 30 (85.7 %) out of 35 *T. congolense* isolates from different geographical origin, a recent survey in Cameroon found a correlation of only 23.5 % (4/17). Since the mouse test was more sensitive than the PCR-RFLP, this indicates the existence of other resistance mechanisms which are not detected by the molecular tool. The percentage of concordance between the mouse test and the *Bc*II-PCR-RFLP for the detection of resistance to diminazene, however, was much higher and reached 88.5 and 85.7 % in two surveys involving 23 isolates from allover Africa and 14 isolates from Cameroon, respectively. Research is currently going on in order to further validate both tests using a higher number of trypanosome isolates.

200 **Immunization of mice with formalin inactivated *Trypanosoma evansi***

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Trypanosomosis or surra caused by *Trypanosoma evansi* is a disease of considerable economic importance in the entire south-east Asia including India. Fever, anaemia with consequent reduction of milk yield and capacity to work as well as high rate of abortion in pregnant animals is characteristic to surra. No dependable vaccine so far has been possible to develop to confer specific protection against the causative organism because of the notorious variable surface glycoproteins. The present work describes evidence for development of a species-specific acquired immunity against *T. evansi* in mice. A group of thirty albino mice were divided randomly into three groups of ten mice each. Each mice was immunized thrice at weekly interval with 100µl of inoculum containing 1x10⁶ *T. evansi* inactivated with 0.05% formalin in a specific adjuvant either the seppic oil adjuvant or aluminium hydroxide gel. The mice were challenged on day 28 with 1x10³ virulent untreated *T. evansi* through intraperitoneal route. Tail blood from each mouse was observed microscopically daily for the onset of parasitaemia. Two mice from the group I immunized with inactivated trypanosome with seppic oil adjuvant showed the onset of parasitaemia on day 15 and 16 post-challenge and were died on day 18 and 22. The mice in group II were immunized with inactivated *T. evansi* in combination with aluminium hydroxide gel adjuvant. A significantly higher level of protection (90%) was observed in this group. One mouse out of ten in this group died on day 21 post-challenge. None of the remaining nine mice evidenced parasitaemia till day 90 post-challenge. Parasitaemia appeared in the unimmunized control group of mice (Group III) on day 9-11 post-challenge and all of the ten mice died by day 13 of infective inoculation. The immunization trial using inactivated trypanosomes administered with adjuvant either seppic oil adjuvant or aluminum hydroxide gel revealed 80-90% protection against a virulent challenge. The immunized mice, which survived the challenge never showed the appearance of the parasites in the circulation for the observation period of 90 days.

201 **Phylogenetic analysis of filarial worm *Setaria digitata* of Sri Lanka based on CO1 and 12s rDNA genes**

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Setaria digitata is a parasitic filarial nematode (family Onchocercidae and sub family Setariinae), present in the peritoneal cavity of cattle and buffaloes, which is non-pathogenic but pathogenic in aberrant hosts such as sheep, goats and horses when transmitted via mosquito vectors. In the aberrant hosts the larvae migrate along the central nervous system and cause mechanical damage and inflammatory reactions, leading to Cerebro Spinal Nematodiasis (CSN). In Sri Lanka, CSN in goats, resulting in lumbar paralysis and death, is a cause of great economic loss each year and difficult to control because of the mobility of the mosquito vector and the risk of development of resistance to drugs used to treat the disease. The aim of this research for the molecular characterization and evaluation of the phylogenetic position of the *Setaria digitata* of Sri Lanka in the evolutionary tree of filarial worms. It was performed by sequencing and analyses of the mitochondrial genes cytochrome c oxidase subunit 1 (CO1) and small subunit ribosomal RNA gene of the mitochondrion (12S rDNA). Among CO1 sequences obtained from different individuals of *Setaria digitata*, intra-species variations were observed. Intra-species variations were not found in the sequences of the 12s rDNA region. Phylogenetic trees inferred from these sequences showed a similar topology. *Setaria* species form a separate lineage in these trees supported by high bootstrap values. *Setaria digitata* of Sri Lanka and *S. labiatopapillosa* branched out from same point showing a close relationship.

202 **Development of real-time polymerase chain reaction assays (PCR) for detection and quantification of pathogen DNA within vector tissues**

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Interest in zoonotic arthropod-borne diseases and their associated vectors has grown, but our understanding of vector-pathogen interactions remains elusive. We aim to investigate pathogen dynamics in micro-dissected vector tissues by using real-time PCR to provide optimal sensitivity, specificity, quantification of DNA and estimation of pathogen load. Two pathogen-vector models are being investigated: *Anaplasma phagocytophilum* (the agent of tick-borne fever) and *Ixodes ricinus*, and *Bartonella henselae* (the agent of cat scratch disease) and *Ctenocephalides felis*. Adaptation of an *A. phagocytophilum*-specific real-time PCR from a previously published multiplex real-time PCR (msp2 gene target), gave an efficiency of 97% and successfully detected only *A. phagocytophilum* and not the related *A. platys*. Three additional real-time PCRs were designed using gene sequences from Genbank. The *B. henselae* PCR targeted the Alr gene with an efficiency of >90% and successfully detected *B. henselae* samples but not the related *B. clarridgeiae*. Vector-specific PCRs were developed to give baseline values between samples for accurate quantification of pathogen DNA. The *I. ricinus* PCR targeted the cytochrome C gene with an efficiency of 90%. The *C. felis* PCR targeted the serpin gene with an efficiency of 98%, and did not hybridise with non-*C. felis* DNA. Coupling of pathogen and vector PCRs will allow quantification of pathogen DNA in different vector tissues, at different time points during and after blood feeding, thus constructing a picture of migration routes through vector tissue, identifying sites of vector-pathogen interaction and areas of pathogen multiplication.

203 **Detection and quantification of *Strongylus vulgaris* DNA in equine faeces by real-time PCR**

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Strongylus vulgaris is an important nematode with high pathogenic potential infecting horses. Recommendations aimed at reducing the problems of anthelmintic resistance in equine small strongyle (cyathostomin) parasites raises a demand for reliable diagnostic tools detecting important parasitic pathogens. Presently, the only means of differentiation between strongyle species in a faecal sample is by identifying third stage (L3) larvae following a two-week coproculture procedure. The aim of the study was to overcome this diagnostic problem by developing a real-time Polymerase Chain Reaction (PCR) assay capable of identification of *S. vulgaris*. Species-specific primers and TaqMan® probe were designed by alignment of published ribosomal DNA sequences of the second internal transcribed spacer of cyathostomin and *Strongylus spp.* nematodes. The assay was tested for specificity and optimized using genomic DNA extracted from identified male worms of *Strongylus* and cyathostomin species. In addition, eggs were collected from the uteri of adult female worms, and used to evaluate the quantitative potential of the assay. Statistically significant linear relationships were found between egg numbers and PCR results. PCR reactions were unaffected by the presence of cyathostomin DNA in the sample, and there was no indication of PCR inhibition from faecal sources. The present assay can reliably and specifically detect minute quantities of *S. vulgaris* DNA whether alone or mixed with cyathostomin DNA. Though the assay can only be used for qualitative detection of *S. vulgaris* eggs in its current form, our results indicate that with further minor optimization the assay has the potential to also be quantitative.

204 **Specific detection of *Histomonas meleagridis* in turkeys by a PCR assay with an internal amplification control**

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Histomoniasis, a disease of gallinaceous birds, is caused by the protozoon *Histomonas meleagridis* and is characterized by lesions in the liver and caeca. Since traditional diagnostics for histomoniasis are complex and far less sensitive than molecular tools, a PCR would provide a valuable alternative. However, intestinal material and droppings, preferably used in epidemiological studies of histomoniasis, often contain PCR inhibitory substances. To detect these false negative results, the use of an internal amplification control (IAC), which is lacking in recently developed PCR, is essential. Therefore, a new 18S-rRNA based, *H. meleagridis* specific PCR, including an IAC, was developed. Distinction between the wildtype PCR product and the control was made by insertion of a DNA fragment into the cloned 18S rRNA gene. Additionally, the diagnostic value of the PCR assay was evaluated in comparison to two other conventional *H. meleagridis* PCR tests (HIS5 and HM).

The sensitivity of the PCR was established at 20 cultured parasites or 50 parasites per mg liver / droppings. Evaluation of the PCR showed no false positive results. However, 18% of the caecal samples contained inhibitory substances and thus resulted in false negative results. Among the lesion-positive, inhibition-free samples, 95.4% were positive by our PCR, while only 66.7 and 83.3% of the lesion-positive organs tested positive by the HIS5 and the HM PCR respectively. In conclusion, our PCR does not only offer the clearly essential IAC but also an increased sensitivity, and thus could be useful for routine diagnosis of *H. meleagridis*.

205 **First evaluation of a Reverse Line Blot hybridization assay for the speciation of drug resistant equine cyathostomes (Nematoda, Cyathostominae)**

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Drug-resistance is a serious problem in the control of equine cyathostomes (Nematoda, Cyathostominae) and for health management of horses worldwide. Nonetheless, information on the individual role played by each of the most common cyathostome species in this drug-resistance is fragmentary. In this study a Reverse Line Blot (RLB) hybridization assay for the molecular identification of multiple-drug resistant cyathostomes has been evaluated. Sixteen (Farm A) and twenty (Farm B) horses with a history of multiple-drug resistant cyathostome infection were subjected to a Faecal Egg Count Reduction Test (FECRT) with fenbendazole (FBZ), pyrantel pamoate (PYR), ivermectin (IVM) and moxidectin (MOX). Post-treatment faecal samples collected from treatment groups were pooled and eggs in each pool were speciated using the RLB assay. The RLB assay was used to simultaneously test for the presence of 13 common cyathostome species. The FECRT confirmed the presence of cyathostomin resistant to FBZ and PYR, while IVM and MOX remained effective. *Cylicostephanus longibursatus* (Farm A), *Cylicostephanus goldi* (Farm A), *Cylicostephanus minutus* (Farms A and B) and *Cylicostephanus calicatus* (Farms A and B) were detected in animals treated with FBZ while *C. minutus* and *Cylicocyclus ashworthi* (Farms A and B) were found in samples collected from animals treated with PYR. These data report the first evaluation of a RLB assay to genetically speciate drug resistant cyathostomes using eggs collected post-treatment. The RLB assay could be a powerful tool in monitoring the presence of anthelmintic resistant cyathostomes and their distribution at species level. It will also be potentially important in other epidemiologic and biologic studies of the cyathostomes.

206 **A rapid and simple field test to detect *Haemonchus contortus* infections in sheep**

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Haemonchus contortus frequently causes large scale losses of sheep, often with little warning. In response, stock owners often resort to excessively frequent anthelmintic treatment, which has produced widespread resistance to all available chemical options. The costs and time required for both regular worm egg counting and examination for anaemia preclude their use in large flocks. A rapid and simple semi-quantitative test for blood in sheep faeces has been developed which provides a graded colour change enabling differentiation between insignificant egg counts, heavier infections requiring treatment and very heavy infections threatening death. A false negative rate of 2.2% occurred for a detection of 2000 epg. The test is best performed on fresh faeces collected from the ground and takes around 40 minutes to complete. Infection is detected from day 11 of infection, well before eggs are present in faeces. A drench decision guide has been developed to interpret test results according to haemonchosis risk factors, which will enable sheep owners to treat when required to avert either immediate mortalities or dangerous levels of pasture larval contamination.

207 **Molecular characterization, longitudinal prevalence and impact on performance of *Cryptosporidium andersoni* and *Giardia* infections in feedlot cattle of Western Australia and Alberta, Canada**

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Prevalence and impact on feedlot cattle performance of *Giardia* and *Cryptosporidium* are not well understood. Four studies were conducted to better understand these factors and their relationship to each other. The first three studies in Alberta monitored 300 hd, 240 hd and 312 hd of steers collecting individual fecal samples. Prevalence of *Giardia* for these groups was 82, 39 and 44% and *C. andersoni* was 12, 3 and 3.5%. There was no significant difference in average daily gain (ADG) between infected and non-infected steers. Molecular characterization of a subset (10) of *Giardia* positive samples revealed 30% assemblage A and 70% assemblage E. The fourth study conducted in Western Australia involved 502 steers. Prevalence of *Giardia* and *C. andersoni* was 8 and 8%. Performance data indicates no significant difference in ADG between *Giardia* or *C. andersoni* infected and non-infected steers. However, a subset of the group comprised of steers averaging 110.7 kg less than their contemporaries had a prevalence of 17 and 26% for *Giardia* and *C. andersoni* respectively and the *C. andersoni* infected steers had a lower ADG than their contemporaries ($P=0.07$). Molecular characterization of a subset (10) of *Giardia* samples revealed 20% assemblage A, 10% assemblage B, 10% assemblage C, 10% assemblage D, 10% mixed infection assemblage B and E and 40% assemblage E. These results demonstrate the variability in prevalence and impact on performance of *Giardia* and *C. andersoni* infections between groups of cattle and the importance of further research to elucidate the factors involved in this variation.

208 **Molecular epidemiology of *Cryptosporidium* in the Netherlands**

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We studied the zoonotic potential of *Cryptosporidium* in the Netherlands by genotyping human and cattle isolates. Feces was collected over the year and from positive samples DNA was sequenced for the following loci: 18S rDNA, COWP, HSP70, microsatellite ML1 and GP60. Genotyping of the human samples showed 69% *C. hominis*, 21% *C. parvum*, in 9% a mix of both and in 1% *C. felis*. For cattle only *C. parvum* was found. For humans the 18S, COWP, HSP and ML1 loci showed less diversity within the *C. hominis* genotype. However, two variants of *C. parvum* were identified by HSP (*C. parvum* and *C. parvum* NL) and 5 variants by ML1 (C1, C2, C2 NL1, C2 NL2 and C3); the GP60 for *C. hominis* was mainly IbA10G2 and *C. parvum* showed 3 variants but mainly IIaA15G2R1. For cattle we only *C. parvum* was found by 18S, COWP and HSP, and two by ML1 (C1 (8%) C2 (92%)); for GP60 over 15 different genotypes from the IIa family were found but mainly IIaA15G2R1 (65%). In humans, *C. parvum* was found relatively constant throughout the year but a *C. hominis* peak was found in autumn indicating human-human transmission. The same for *C. parvum* NL strain, which was also found only in humans. *C. parvum* variant IIaA15G2R1 occurred both in humans and cattle, indicating this strain may be important for zoonotic transmission. In cattle only IIa strains were found indicating that human IIc/d strains either transmit human-human or originate from another animal reservoir.

209 New genotypes of *Cryptosporidium* species and their impact to the drinking water supplies

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Cryptosporidium spp. and genotypes has been determined in various animals (wild, domestic and zoo animals) from various countries (Bulgaria, China, Germany, Greece, Hungary, Japan, Russia) and/or in their drinking water sources. Genomic DNA was extracted from IFA-positive samples and PCR assays targeting various *Cryptosporidium* species genes (Beta-Tubulin, COWP, 70kDa HSP, SSU rRNA, ITS1, TRAP-C1 and TRAP-C2 gene). The species and genotypes have been determined by PCR-RFLP, sequence and phylogenetic analyses. Distinct genotypes from the *C. parvum* and *C. hominis* genotypes suggested the existence of new strains in brown rats. Analysis of isolates from cattle of the GP60 PCR products showed that the most common *C. parvum* subtype was IIaA16G1R1 and *C. parvum* allele IId in addition to a new *C. parvum* IIa A18G1R1 subgenotype firstly described in the *C. parvum*-complex. Sequencing of SSU rRNA and actin genes enabled a distinct molecular characterization of infecting organism as *C. saurophilum* firstly identified in the reptiles *Elaphe guttata guttata*. Other findings indicated *Cryptosporidium cervine* genotype for the first time in goat *Capra ibex*. Taken together the results illustrates that: a) drinking water supplies are subject to contamination with *Cryptosporidium* spp and genotypes, with potential hazards for the public health; b) same *C. species* and genotypes found in water and in animals which excrete *C. oocysts* pathogenic for humans and animals; c) new unknown genotypes circulating in nature have been determined. Cryptosporidiosis is an illness of a significant health aspect associated with the planning and management of the drinking water resources.

210 Occurrence, viability and genotyping of *Cryptosporidium* and *Giardia* in influent water and harvested shellfish in the Varano Lagoon (Italy)

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In order to investigate the presence, viability and species/genotypes of *Cryptosporidium* and *Giardia* in a clearly-defined environment (Varano Lagoon, Southern Italy), from February 2006 to January 2007, samples of treated wastewater and related watercourses flowing into the Lagoon were monthly collected, together with specimens of *Ruditapes decussatus* and *Mytilus galloprovincialis* from a shellfish-farm located in the same Lagoon. Oocysts were detected by IF and DAPI staining and cysts were also subjected to a β -giardin RT-PCR. Hemolymph from shellfish was examined by IF testing. Thus, wastewater and shellfish samples were undertaken to COWP-PCR for *Cryptosporidium* and 18S-rDNA and tpi-PCR for *Giardia*. PCR and RT-PCR positive isolates were then sequenced. A total of 42 wastewater samples were collected: 21 from treated wastewater and 21 from related watercourses. In treated wastewater *Cryptosporidium* and *Giardia* oocysts were found by IF in 5 and 16 samples, respectively, and from related watercourses in 8 and 7, respectively. Higher concentration was registered for *Giardia* cysts. Two and 9 treated wastewater and 4 and 2 from related watercourses samples resulted DAPI+ for *Cryptosporidium* and *Giardia*, respectively. β -giardin RT-PCR gave positive results in 7 samples. *C. parvum* and *G. duodenalis* Assemblages A and B were identified, although incoherent results were obtained by the analysis of the two genetic loci. In shellfish, IF and molecular tests gave negative results for both protozoa. *Cryptosporidium* and *Giardia* oocysts are discharged into the Lagoon but seemingly they did not affect harvested shellfish. The validity of IF test in shellfish for routine investigation is confirmed, while one gene target typing debated.

211 **Giardiasis in cattle - a zoonothroponosis not a zoonosis**

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Introduction: *Giardia duodenalis* infections are common in cattle. Although cattle are most frequently infected with the livestock specific Assemblage E genotype, the zoonotic Assemblage A genotype can also occur in cattle, raising concern that cattle may serve as a reservoir for *G. duodenalis* infections in humans. **Method:** Fecal samples were collected from 922 adult cattle and 183 calves from 11 herds on Prince Edward Island, Canada. Feces were examined for the presence of *G. duodenalis* cysts by immunofluorescence microscopy, and a subset of positive samples were genotyped by sequencing a portion of the SS-rRNA gene. **Results and Conclusion:** *G. duodenalis* cysts were identified in 39% and 51% of fecal samples collected from adult cattle and calves respectively. Sequencing of the *G. duodenalis* SS-rRNA gene proved difficult due to low concentrations of *G. duodenalis* cysts in the feces. Nevertheless, 29% of *G. duodenalis* isolates from calves corresponded to the zoonotic Assemblage A and 71% to the livestock specific Assemblage E. In adult cattle, 15% of isolates corresponded to the zoonotic Assemblage A while 85% corresponded to Assemblage E. However, in adult cattle, all zoonotic isolates occurred in the same herd, a veterinary college teaching herd, where cows had frequent contact with a large number of humans on a daily basis. Although further study is required, these results indicate that the human infectious Assemblage A genotype is only common in cattle that have frequent contact with humans. Thus Assemblage A-associated giardiasis in cattle may be a zoonothroponosis and not a zoonosis.

212 **Resazurin as a viability indicator for *Giardia duodenalis* trophozoites**

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Introduction: current in vitro susceptibility assays for *Giardia* trophozoites are frequently performed in 'large volume' air-tight tubes. Microtiter plate assays are the better alternative, but are problematic due to the need for standardized low-oxygen environment and the lack of standard methods for quantifying total viable trophozoite burdens.

Materials & methods: a simple in vitro susceptibility assay was developed in a 96-well plate whereby three colorimetric endpoint methods were compared and validated against microscopic counting. Using a standard inoculum of 5x10⁴ trophozoites/well (300 µl), optimal growth was obtained after sealing the plate with an air-tight adhesive tape and 72h incubation at 37°C in modified TYI-S-33 medium. The burdens of viable trophozoites were spectrophotometrically determined after addition of the formazan dyes MTT (100 µg/well) and XTT (20 µg/well) and the fluorescent substrate resazurin (2.5 µg/well), however after prior removal of the culture medium since it caused spontaneous reduction of the substrates.

Results: resazurin proved to be far superior to MTT and XTT with a sensitivity level of about 3x10⁴ trophozoites. Inhibitory concentrations (IC₅₀) of reference drugs were comparable with published values: metronidazole 2.25 µM, tinidazole 1.75 µM, albendazole 0.10 µM, furazolidone 4.60 µM and quinacrine 0.32. To check their suitability for decontamination during excystation experiments, chloramphenicol, rifampicin, penicillin G + streptomycin and gentamicin were also evaluated and none showed any inhibitory action at the highest in-test concentrations (64 µM).

Conclusions: resazurin can be used for quantitative in vitro viability measurement of *Giardia* trophozoites in drug-susceptibility assays. The test is reliable, rapid, sensitive, cost-effective and does not require special equipment.

213 **Biodiversity and systematics of *Haemosporidia* infecting South African tortoises**

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Published research regarding blood Haemosporidia infecting South African tortoises is scanty. To date two species of *Haemogregarina* and four species of *Haemoproteus* have been identified from African tortoises (*Terrestrial chelonians*), however this research was done more than 50 years ago. The present study aims to: 1) isolate apicomplexan parasites infecting indigenous tortoises. 2) provide host and locality records of previously described and new apicomplexan species. 3) describe isolated parasites using morphometric tests and DNA analysis. 4) use the acquired knowledge as a biological tag for the future identification of hosts. To achieve above blood smears were made, stained with Giemsa's stain and screened for parasites under a light microscope. Digital micrographs and measurements of all parasites found were taken. A *Haemoproteus* species was found in 2/15 *Kinixys lobatsiana* (13%), and in 1/3 (33%) *K. natalensis*. A *Haemogregarina* species was isolated from 5/9 (55%) *K. belliana belliana*, 2/3 (66%) *K. natalensis*, 1/15 (6%) *K. lobatsiana*, 3/6 (50%) *Geochelone pardalis pardalis* and 1/16 (6%) *G. p. babcocki*. None of these hosts showed concurrent *Haemoproteus* and *Haemogregarina* infections. Morphometrics of the *Haemogregarina* sp. conforms to those of *Haemogregarina fitzsimonsi* Santos Dias, 1953 described from Mozambique *K. b. belliana*. South Africa is thus a new locality record for this parasite. The *Haemoproteus* sp. do not share characteristics with any of the known tortoise haemoproteids and appears to be new to science. It is hoped that these, and future results, may increase the knowledge of apicomplexans parasitizing South African chelonian hosts.

214 **Prevalence of liver flukes (*Fasciola jacksoni*) in free ranging elephants in Sri Lanka**

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The gastro-intestinal parasitic are one of major disease condition lead mortality and morbidity of elephant calves and adults in Sri Lanka, both in captive and free living. Post mortem examinations of wild elephants revealed that Fasciolosis is the major parasitic problem in wild elephants of the country. Although fasciolosis is reported in many mammals and result in one of the major economic losses in cattle, buffalo, sheep and goat all over the world, studies of elephant fasciolosis is scanty.

There are 63 elephants were examined for liver flukes while conducting the post mortems covering the many parts of the country. Each post mortem was conducted several minutes to 48 hours after the death. Adult Flukes were identified visually and species was identified by the laboratory investigations. The severity of parasitic infestation was determined considering the number of flukes in the liver. Laboratory investigations for eggs, carried out for 124 dung samples from the living elephants.

Age categories of study population were 02 infants (< 1 years), 06 calves (1-5 years), 03 juvenile (5-8 years), 10 sub adults (8-15), 33 adults (15-40 years) and 08 prime adults (>40 years). Sex ratio was 49:14. Causes of their death were gun shot associated septicemia (28), gun shot associated brain or lung tissue damages (09), paralysis due to damage on spinal cord (02), wounds due to land mines (02), electrocution (10), drowned (01), obstruction of airways 01, pneumonia (03), chronic diarrhea (02), fasciola associated parasitism (02), could not ascertained (03). Liver fluke presence in the elephants was identified in laboratory as the *Fasciola jacksoni*, average size 12-14mm x 9-12.5mm. There were 36 animals infected with fasciola covering all the age categories except the infants. The severities of infestation were the severe-06 (>100 flukes), moderate-07 (50-100 flukes), mild-09 (10-50), and low-14 (<10) animals. Fifty seven percent of dung samples were positive for the *Fasciola* eggs. There are around 150 wild elephant deaths per year reported in Sri Lanka. Majority of deaths were due to the direct result of existing human elephant conflict. Many elephant deaths recorded from the areas where human accessibility is available, so possibilities of missing the natural deaths are comparatively high. In this study shown that the fasciolosis is very important, when consider the health of wild elephants. Currently we are investigating the life cycle of *F. jacksoni* and path-biological impacts to the elephant in case of Fasciolosis.

215 **Intestinal nematode distributions in wild and captive populations of giant Galápagos tortoises (*Geochelone nigra*)**

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No quantitative data are available about infection level of giant Galápagos tortoises (*Geochelone nigra*) by intestinal nematode fauna, though these parasites have been suspected to be implicated in instances of mass mortality among tortoises in Santa Cruz island. The objective of the present study was to assess the spatial variability of nematode distributions in five wild and three captive tortoise populations located on four Galápagos islands. Coproscopic examination revealed nematodes to be distributed widely across the archipelago, and more than 80% of tortoises were infected in each population. Distribution patterns were highly fluctuating. Five different nematode types were identified: oxyurids, ascarids, trichurids and two types of strongyles. In all nematode communities, strongyles seemed to be the most abundant. Evolutionary and ecological factors are likely responsible for the variation in nematode distributions. Competitive interaction and density-dependent effects on parasite could also regulate parasitism level and alter nematode community structure. Moreover, repatriation of tortoises from captive breeding programmes could be a factor favouring the introduction of nematodes into populations where they are not endemic. However, other investigations are necessary in order to assess the temporal variability of nematode distributions, to identify nematodes to the species level, to increase the reliability of faecal egg counts, and to assess rigorously the new hypotheses that have been proposed for this system.

216 **Lungworms in porpoises and seals do have vertebrate intermediate hosts: molecular tools determine lifecycle**

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Lungworms infect a wide range of mammals and are usually found in the bronchi, parenchyma or blood vessels associated with the lungs. Most terrestrial metastrongyloids are heteroxenous, using molluscs as intermediate host. The biology of lungworms infecting marine mammals is little known and their transmission in the marine environment has been poorly studied. Lungworm disease and its associated pneumonia are particularly important in the health of wild populations and a common cause of mortality in porpoises and seals. Experimental infections using metastrongyloids infecting marine mammals have indicated that they may use a vertebrate as intermediate host while other studies found evidence for prenatal infections with lungworms in cetaceans. In this study various species of benthic fish from the Wadden Sea were dissected. Larval stages of nematodes from the gastrointestinal tract were removed while still alive. Larval DNA was isolated and primers used to amplify the ITS-2 from their rDNA. The same primers had been designed to sequence ITS-2 regions from adult specimens of all lungworm species infecting *Phoca vitulina* and *Phocoena phocoena* in the North Sea. Histology and in-situ-Hybridisation were performed on intestinal tissue. ITS-2 (18S rDNA) sequences of larvae found in several species of flatfish were identified as belonging to *Pseudalius inflexus* (Pseudaliidae), a lungworm of porpoises and to *Parafilaroides gymnuris* (Filaroididae), a lungworm of seals. This is the first study to determine intermediate hosts for lungworms of marine mammals in the wild. It elucidates the as yet unknown life cycle of two metastrongyloid species parasitising seals and porpoises.

- 217 **Chorioptes ear mites in moose (*Alces alces*): pathological and taxonomic investigations**
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Introduction: During the past decade, Chorioptes mites were commonly found in the outer ear canals of Swedish moose during routine autopsies and have been regarded as responsible for inflammation and ulcerations. Here, the mites and the pathological changes in the moose were characterized.

Materials and methods: External ear canals from 54 necropsied moose were examined for the presence of Chorioptes. Samples of ear canals were taken for histopathological, bacteriological and mycological examinations. Mites were investigated morphologically and genetically by PCR and sequence analysis of the second internal transcribed spacer of the rDNA.

Results: Chorioptes infestations were found in 43 (80%) of the 54 moose examined. Mites were associated with inflammation of the external ear meatus. Epidermal hyperplasia and perivascular to interstitial dermal inflammatory infiltrates with mixed T- and B-lymphocytes, plasma cells and some macrophages occurred. *Staphylococcus aureus* was grown from the ears of five of 14 examined moose and *Staphylococcus spp.* from one; yeasts were cultured from two moose. The mites differed morphologically and genetically from *C. texanus* and *C. bovis*, the two species generally accepted within the genus. Morphology also did not unequivocally argue for a diagnosis as *C. crewei*, *C. mydaus* or *C. panda*.

Conclusion: An apparently undescribed Chorioptes species was frequently detected in the outer ear canals of moose in Sweden. The inflammation might be the same type of hypersensitivity reaction seen in other hosts affected by ectoparasites but complicated by secondary infection, or might be a reaction pattern peculiar to the moose.

- 218 ***Toxoplasma* in Australian Marsupials: analysis of pouch young of naturally infected wild kangaroos for evidence of vertical transmission**
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Introduction: *Toxoplasma gondii* is a common parasite among Australian marsupials. Evidence for vertical transmission in marsupials to date is anecdotal, however is well established in a number of species including sheep, mice, rats, cats and humans. Marsupials are unique in that they are not placental for a significant period of time in their development, and the marsupial mode of reproduction focuses on lactation to nourish the poorly developed young. Previous studies using serology and immunoblotting in Agile wallabies supported the theory of vertical transmission in marsupials. In this study, we analyse the pouch young of 10 *Toxoplasma* seropositive and 10 seronegative wild Western Grey Kangaroos for evidence of congenital *Toxoplasma* infection. Materials and Methods: A range of tissues from the pouch young including the brain, heart and lung were screened for *T. gondii* infection using PCR analysis, immunohistochemistry and comparative immunoblots. Results: Although a number of pouch young were seropositive for *T. gondii*, PCR analysis of tissues to date did not amplify *T. gondii* DNA. Tissue samples from seropositive dams were often also PCR negative for *T. gondii*. Conclusion: PCR is not an ideal diagnostic technique for *T. gondii* infection and has a high rate of false negatives due to the small amount of tissue that is tested in one PCR and the sporadic distribution of *T. gondii* within the body. Subsequent immunoblotting and immunohistochemistry of pouch young and dam samples will provide a more thorough analysis of the incidence of vertical transmission of *T. gondii* in Australian marsupials.

219 **New developments in the field of toxoplasmosis**

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The protozoan parasite *Toxoplasma gondii* is the causative agent of a worldwide zoonosis and it is estimated that one third of the human world population is infected with this parasite. It is able to infect all warm-blooded animals. In humans, control of the infection occurs through the induction of strong and persistent cell mediated immunity that results in the control of *T. gondii*. The most important group of individuals at risk are pregnant women, in whom a primary infection during pregnancy leads to infection of the foetus and congenital toxoplasmosis. An important source of human contamination with *T. gondii* is the consumption of raw or undercooked meat products from infected animals. However, in most animals *Toxoplasma* infections occur unnoticed. During infection, the parasite disseminates through the body and remains present under the form of tissue cysts, which are kept under control - but are not eliminated - by the host's cellular immune response. As there is little antigenic variance between different *T. gondii* strains, a natural infection generally offers cross-protective immunity against heterologous strains. Indeed, in humans a primary infection before pregnancy protects the foetus against congenital toxoplasmosis. This has been the basis for the development of vaccines against *T. gondii*. From the veterinary point-of-view, vaccines may prevent the formation of tissue cysts and reduce the risk of human contamination through meat consumption.

It is possible to induce protective immunity in animals with live, attenuated *T. gondii* strains defective in cyst formation. However, immunity is not lasting, side effects are common, the risk of reversion exists and the vaccine is expensive. Based on serological assays, these vaccinated animals cannot be discriminated from infected animals. Studies in mice have focused on the selection of protective antigens. Antigens originating from the surface of the parasite, or secreted from organelles after parasite internalization in the host cell, have been evaluated with varying degree of success. Most promising experimental vaccines now combine several antigens in a cocktail DNA vaccine and immunization of mice with these vaccines offers a strong prevention of tissue cyst formation. Protection elicited by these vaccines is associated with strong and persistent cellular immune responses. It is of interest to determine whether these DNA vaccine elicited immune responses can lower the parasite burden in large animals.

220 **Discussion Workshop: New developments in the field of toxoplasmosis**

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For the veterinary parasitologist, Toxoplasmosis is a disease that is mainly dealt with on sheep or goat farms and even then the problem is only apparent in certain countries or regions in the world. Other farm animals such as pigs and poultry can also become infected but do not present with clinical problems and as such have not been of great interest to the veterinarian. In this workshop we would like to highlight new developments in the field of Toxoplasmosis and animal husbandry and would like to discuss the future role of the veterinary parasitologist in dealing with this infection.

Toxoplasmosis is the most prevalent parasitic zoonotic disease and was recently shown to be the third cause of death following foodborne illnesses (Mead, 1999). Recent models indicate that Toxoplasmosis causes the highest disease burden among seven evaluated food-borne pathogens, ranking higher than *Salmonella spp.*, *Campylobacter spp.*, *Listeria monocytogenes*, *Escherichia coli* O157, noroviruses and rotaviruses (van Kemmeren, 2006). Pregnant women and immune compromised individuals have been regarded as the main risk groups for Toxoplasmosis, and recently retinochoroiditis is gaining importance in the pathologies caused by *T. gondii*. The disease burden calculation for patients with congenital toxoplasmosis is however similar to the disease burden of acquired cases indicating that prevention should not only be directed towards pregnant women, but that essentially any individual can be at risk and should be aware of the risk factors associated with the infection. The disappointing results concerning the effects of therapy on congenital toxoplasmosis (Thiebaut, 2007) have led to a discussion whether prevention should not be redirected towards a more stringent control of the sources of infection. In Europe the main cause of infection for humans is the consumption of undercooked meat or meat products (Cook, 2000). Due to intensive farming systems with high hygienic standards, the incidence of Toxoplasmosis has rapidly dropped in Europe over the last decades. The recent introduction of animal friendly extensive farming systems in Western countries will however lead to a reemergence of *Toxoplasma* in meat products unless monitoring and interventions are applied (Kijlstra 2004). Prevention of the parasite's presence in meat is thus one of the strategies in the battle against toxoplasmosis. The veterinary parasitologist can play an important role in the near future to guarantee that animal products are free from infectious *T. gondii* parasites. Approaches that will be discussed in the workshop include farm management (cats, rodent control, feeding goat whey) post slaughter decontamination and vaccination.

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221 **Ectoparasites: Future challenges in a changing world**

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Ectoparasites are ubiquitous, often highly damaging and in most cases can not be permanently eradicated; hence they must usually be managed at a local scale with insecticides or endectocides. However, the growth in resistance, the slow rate of development of new actives, coupled with environmental and health concerns associated with the continued use of some of the existing neurotoxic insecticides, suggest that more sophisticated approaches to their management need to be identified. These approaches need to allow ectoparasite populations to be maintained at acceptable levels, while conserving the compounds that remain available. The development of integrated approaches, in which cascades of management tactics are deployed, with parasiticides available as one component to be used in requisite circumstances, may be the most appropriate route to achieving this aim. An essential element of such an approach is the clear articulation of the purpose of intervention and rational justification of the time-point and manner in which it is attempted. However, for this to be possible, considerably better information is required about the effects of ectoparasite abundance on animal welfare and productivity, in addition to the greater availability of effective alternative control tools. To this end, recent work undertaken at the School of Biological Sciences at the University of Bristol, into the use of biological control agents, off-host trapping and the selective treatment of more highly susceptible individuals or classes of host, is reviewed here.

222 **Structural biology in search of new therapeutics for protozoal diseases**

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Structural Biology can be extremely valuable in supporting Drug Development in a variety of ways. Here we will briefly discuss the power of protein crystallography, but also nmr methods can make major contributions to Drug Development. In the future it is expected that also high-resolution electron microscopy of proteins and multi-macromolecular assemblies will make contributions to the development of novel therapeutics.

The use of three-dimensional structures of drug targets can be important in the initial stages of a drug development, in the intermediate stages and also in the later steps of “fine-tuning” the pharmacological properties of a drug-candidate. Also, the combination of HT screening and structure determination can be of great value for lead optimization. A brief summary of possible contributions of three-dimensional structures of drug targets to the drug development process will be discussed.

223 **The mechanism of action of metaflumizone and other veterinary ectoparasiticides**

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Metaflumizone is a novel semicarbazone insecticide, derived chemically from the pyrazoline sodium channel blocker insecticides (SCBIs), but with greatly improved mammalian safety. While metaflumizone is a broad-spectrum insecticide, its first registrations, under the trade names ProMeris and ProMeris Duo, are in the veterinary ectoparasiticide market, where the mechanism of action is completely novel. Electrophysiological studies on neurons from lepidoptera larvae and on *Drosophila* Para/TipE sodium channels heterologously expressed in *Xenopus* oocytes confirm that the insecticidal action of metaflumizone is due to state-dependent block of sodium channels. The mechanisms of action of other veterinary ectoparasiticides will be discussed for comparison.

224 **Safety and efficacy of ProMeris® for cats and ProMeris /ProMeris Duo® for dogs: the development of new products for the control of fleas in cats and fleas and ticks in dogs**Rugg D., Holzmer S., Heaney K., DeLay R., and Rock D.*Fort Dodge Animal Health, New Product Development, Princeton, NJ, USA*

ProMeris® for Cats is a spot on product containing the novel semicarbazone insecticide metaflumizone, for the control of fleas on cats. ProMeris/ ProMeris Duo® for Dogs combines metaflumizone with amitraz in a spot on formulation for the control of fleas and ticks on dogs. The ProMeris line of products is currently in the process of registration or approved for use globally. Here we present an outline of the studies that constituted the US development program demonstrating the safety and efficacy of these products in cats and dogs. Data from additional studies conducted globally to evaluate efficacy against a variety of tick species, and comparisons with existing products will also be presented. Safety of ProMeris products was confirmed at 5X the proposed dose in adult dogs and cats and puppies and kittens at 8 weeks of age. Safety was also confirmed for seven 5X doses repeated at 14-day intervals in both species, and for Chihuahua dogs. Efficacy against a variety of strains of cat flea was evaluated in laboratory and field clinical studies and confirmed control of fleas for up to 6 – 8 weeks in dogs and cats. ProMeris/ ProMeris Duo for Dogs provided excellent control of the common ticks infesting dogs for 3 – 6 weeks after treatment. Laboratory studies showed that ProMeris is effective in preventing and controlling flea allergy dermatitis in cats and dogs.

225 **Efficacy of ProMeris® for cats and ProMeris Duo® for dogs in Europe**

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ProMeris® for Cats and ProMeris Duo® for Dogs are spot on products for flea (*Ctenocephalides* spp.) control in cats and control of fleas and ticks on dogs. ProMeris contains the novel semicarbazone insecticide metaflumizone and ProMeris Duo for Dogs combines metaflumizone with amitraz. These products have recently been approved in Europe. Here we present the studies that comprised the EU development program confirming the safety and efficacy of these products in cats and dogs. Four laboratory studies were conducted in England, Germany and Ireland to confirm efficacy against weekly challenges with fleas in dogs and cats. Efficacy against the ticks *Rhipicephalus sanguineus* (Ireland) and *Ixodes ricinus* (Germany) was evaluated in two laboratory challenge studies. Field clinical studies were conducted in Germany and France to confirm efficacy and safety in dogs and cats. In the field studies, 188 cats and 293 dogs were treated with ProMeris products. The laboratory and field clinical studies confirmed that ProMeris for Cats was safe and effective; the product controls fleas for up to 6 weeks after a single treatment. ProMeris Duo for Dogs was shown to be safe and effective; fleas are controlled for up to 6 weeks after treatment and the product provided at least 4 weeks control of the common ticks infesting dogs in Europe (*I. ricinus*, *I. hexagonus*, *R. sanguineus*, *Dermacentor reticulatus* and *D. variabilis*).

226 **Identification and molecular characterization of a vitellogenin receptor from the hard tick *Haemaphysalis longicornis***

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The vitellogenin receptor (VgR) belongs to the low density lipoprotein receptor (LDLR) superfamily and mediates the uptake of vitellogenin (Vg) into developing oocytes of all oviparous animals including insects. We cloned and characterized a putative VgR from ovaries of the hard tick, *Haemaphysalis longicornis* (*HIVgR*). The full-length cDNA is 5,631 bp, including an intact ORF encoding an expected protein with 1,782 amino acids. Deduced amino acid sequence of the cDNA revealed that it encodes a protein homology of the LDL receptor superfamily. *HIVgR* exhibited two ligand binding domains with three Class A cysteine-rich repeats in the first domain, and eight repeats in the second domain and each repeat contained six cysteine residues. Each ligand-binding domain was followed by epidermal growth factor (EGF) precursor homology domains. The mouse anti-*rHIVgR* serum recognized an endogenous 197 kDa protein in both tick ovary and egg. *HIVgR* transcript was detected in ovarian tissues at high levels throughout ovarian development but not in midgut, salivary glands, synganglion and fat body, indicating that *HIVgR* is ovarian tissue-specific. Immunohistochemical analysis using anti-*rHIVgR* antibody indicated that *HIVgR* is localized on the surface of oocyte basement membrane and mature yolk bodies. Gene silencing of *HIVgR* by RNA interference affects the ticks to be unable to lay the eggs. Furthermore, the transovarial transmission of *Babesia* parasites in tick oocyte was regulated by vitellogenin-receptor mediated endocytosis. These results indicate that the *HIVgR* is essential for Vg uptake, egg development in the *H. longicornis* tick and transovarial transmission of *Babesia* parasites.

227 **Tick-vertebrate host interface in the tick-borne flavivirus transmission**

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Flaviviruses (genus *Flavivirus*, family Flaviviridae) have evolved to cause little damage to the arthropod vectors while benefiting from the induction of disease in the vertebrate host. Rather than relying on high virulence and viraemia, tick borne encephalitis virus (TBEV) transmission from infected to uninfected ticks during their co-feeding was demonstrated to be very efficient mechanism.

Co-feeding of infected and uninfected *Ixodes ricinus* ticks on Balb/c mice was used to study TBEV infection in the tick feeding site. Further Balb/c mice were immunized with tick experimental recombinant vaccine (*Rhipicephalus appendiculatus* 64p cement protein) and also used in the transmission experiments.

Virus transmission during co-feeding of infected and uninfected ticks was accompanied by virus infection in the tick feeding sites of both infected and uninfected ticks. The feeding lesion was an important site for primary virus replication and immunocytochemistry revealed cellular involvement with viral antigen detected in the dendritic (Langerhans) cells. Viraemia levels did not correlate with transmission efficiency. If co-feeding transmission results from the activities associated with tick saliva than it would be reasonable to expect a reduction in tick-borne virus replication and transmission after immunization against tick salivary antigens. Immunization of Balb/c mice with tick derived recombinant proteins protected animals against lethal challenge with TBEV-infected ticks and had a virus transmission – blocking effect.

The concept of targeting vector salivary antigens instead of pathogens is an attractive alternative to conventional anti-pathogen vaccines. To improve this approach further research is needed in order to better understand pathogen – tick – vertebrate interactions.

228 **A tick cysteine protease, longipain, from the babesial parasite vector *Haemaphysalis longicornis*, with possible multifunctional roles during parasite transmission**

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Vector ticks possess a unique system that enables them to digest large amounts of host blood and to transmit various animal and human pathogens, suggesting the existence of evolutionally acquired proteolytic mechanisms. We report here the molecular and reverse genetic characterization of a multifunctional cysteine protease, longipain, from *Haemaphysalis longicornis* a vector tick for babesial parasites. Longipain shares structural similarity with papain-family cysteine proteases obtained from invertebrates and vertebrates. Endogenous longipain was mainly expressed in the midgut epithelium and was specifically localized at lysosomal vacuoles and possibly released into the lumen. Its expression was up-regulated by host blood feeding. Enzymatic functional assays using *in vitro* and *in vivo* substrates revealed that longipain hydrolysis occurs over a broad range of pH and temperature. Haemoparasitocidal assays showed that longipain dose-dependently killed tick-borne *Babesia* parasites, and its babesiacidal effect occurred via specific adherence to the parasite membranes. Disruption of endogenous longipain by RNA interference revealed that longipain is involved in the digestion of the host blood meal. In addition, the knockdown ticks contained an increased number of parasites, suggesting that longipain exerts a killing effect against the midgut-stage *Babesia* parasites in ticks. Our results suggest that longipain is essential for tick survival, and may have a role in controlling the transmission of tick-transmittable *Babesia* parasites.

229 **Approaches to improved understanding of vector-parasite interaction within the genus *Theileria* and their Ixodid tick vectors, using genomics**

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The complete genome sequences of the two most economically important *Theileria* species, *T. parva* and *T. annulata* have recently been determined. Salivary gland-expressed sequence tag (EST) databases have also been generated from two *Theileria* tick vectors, *Rhipicephalus appendiculatus*, the major vector of *Theileria parva*, that causes East Coast fever in cattle, and *Ambylomma variegatum* that transmits both the enigmatic *Theileria mutans* and other bacterial pathogens of livestock. This information opens the possibility of using novel bioinformatics algorithms and functional genomics techniques to provide improved understanding of parasite-vector interaction at the molecular level in the *Theileria*-Ixodid tick system. Data generated from *T. parva* infected and uninfected salivary glands has highlighted a statistically significant increase in the levels of transcription of several glycine-proline rich proteins, which are hypothesized to be components of ixodid tick cement. The EST data also suggests that the expression of additional *R. appendiculatus* proteins may be modulated as a result of parasite infection, but more data is required to confirm this hypothesis. Application of one incarnation of the signal sequence trapping technique has identified several additional genes encoding polypeptides that are candidates for secretion by tick salivary glands into the dermis of the vertebrate host, although there is as yet no published information relating to functionality in the mammalian host. *R. appendiculatus* tick populations have also been shown to differ in vector competence for specific *T. parva* stocks and experiments to investigate the molecular basis of these phenotypes are being initiated.

230 **Discussion Workshop: Giardia in veterinary medicine: to consider or not to consider?**
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Worldwide prevalence studies indicate a high prevalence of *Giardia* in livestock and companion animals. Despite the high prevalence, *Giardia* is often not considered as an important pathogen in veterinary medicine due to the vagueness of the clinical signs caused by giardiasis. Although several clinical studies do indicate an association between *Giardia* infection and clinical signs such as diarrhea and ill thrift, numerous other studies demonstrate no clinical disease associated with infection. The pathogenesis and pathophysiology of giardiasis has been demonstrated in experimental infections, and the potential economic impact of the parasite to livestock has been demonstrated in a lamb model. In addition, several *Giardia* species have been described in animal transmission cycles. In livestock and in companion animals, mono-infections are most often reported, and to a lesser extent mixed infections. Whether there is a difference in clinical outcome when animals are infected with a mono-or mixed infection of zoonotic and host-adapted *Giardia* species has not yet been determined, and warrants further studies.

Numerous questions surround *Giardia* infections in livestock and companion animals. Has the limited awareness regarding the pathogenicity of *Giardia* infection accounted for the limited research into treatment and prevention of giardiasis in veterinary medicine? Is treatment and control of giardiasis in livestock economically justified? From a public health point of view, should chemotherapeutic treatment be advocated to prevent environmental contamination and possible zoonotic transmission? At present, only fenbendazole/febantel is registered for the treatment of *Giardia*, and only in dogs. Several other chemotherapeutics are efficacious in the treatment of giardiasis in different hosts, but none are registered. Whether chemotherapeutic treatment is useful in the prevention of infection is uncertain, and additional hygienic and management measures seem to be needed. Although a vaccine has recently been commercialised in the United States for the prevention and curative treatment of *Giardia* in dogs, further research is needed to optimize the vaccination protocol and to extend the use of vaccination to other animal hosts.

The aim of the present workshop is to discuss the importance of *Giardia* infections in veterinary medicine, and to identify the research needs in the fields of pathogenicity, prevention and curative treatment in different hosts.

231 **Control of parasitic gastroenteritis in sheep: modelling worm control strategies**

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Introduction: Recent UK (SCOPS) recommendations have been made in an attempt to limit the development of anthelmintic resistance (AR) in sheep nematodes. This paper presents the next stage of development of a computer simulation model for PGE in sheep that was presented at WAAVP in 2005.

Methods: Data for initial model validation has been derived from an initial cohort of 8 sentinel farms in different regions within the UK by monitoring parasite numbers in ewes and lambs. Nematode populations were also screened for the presence of AR using either FECRT or LDT tests. Statistical analysis of results showed a positive correlation to observed field and simulated counts. Regression analysis suggested an acceptable fit between the data.

The project has now progressed to monitor the effectiveness of worm control strategies on 30 farms using the same parameters over a 2-year period. Twenty of the study farms have been 'paired' as closely as possible. One of each pair will implement SCOPS recommendations as fully as practicable (test group); their partners will not be offered advice, but simply monitored (control 1). The remaining 10 will be monitored and their level of uptake recorded (control 2). Farms will be visited 4 times throughout the period March October for both 2007 and 2008 calendar years to record management practices, stocking rates and performance data. Additional drench test (DT) and Faecal Egg Count Reduction Test (FECRT) data are also being collected.

Results and Conclusion: Parasite data has been compared with predictions made by the computer model throughout the study period and results to date will be presented. The model is in the process of being iteratively refined as a decision support tool using feedback from veterinarians and advisors using the model in the field. Model structure uncertainty has been assessed by sensitivity analysis (SA) at the start of the project and the issue of uncertainty in the field-collected data has been addressed using statistical analysis.

232 **The past and future of *Nematodirus battus***

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Nematodes of the subfamily Nematodirinae originate in the Holarctic but proved equipped to survive in a wide variety of climatic conditions when their hosts moved to the temperate and sub-tropic regions. The species *Nematodirus battus* has thrived on changes in its field ecology and immunoepidemiology brought about by the climate of the temperate regions. However, in the face of climate change, the question arises how it will cope with a further warming of its environment. An analysis of 30 years of veterinary surveillance data revealed a significant increase in UK *Nematodirus* disease cases in the past five years. However, differences in disease patterns between the UK North and South suggested that, in the future, fewer overall cases will occur but in a far less predictable, more all-year-round, pattern. In order to test this hypothesis climate-driven, full-cycle, stochastic mathematical models were built. Parameterization of the models proceeded through a series of incubator and field experiments focusing on egg vital rates, which had not been described for *N. battus*. Some conclusions of these experiments were: 1. There is not only a lower but also a higher temperature threshold for hatching. 2. Depending on the hatching temperature, up to 70% of the egg population may hatch without any chilling experience. 3. There is considerable variation in this trait between field isolates. 4. The vital rates of *N. battus* are in agreement with an arctic origin. The implications of these findings for the future epidemiology of the parasite were explored in the simulation model.

233 **Metazoan-protozoan parasite co-infections and host fitness in St. Kilda Soay sheep**

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Since the Bronze Age, the unmanaged Soay sheep population on St. Kilda have survived despite enduring presumably deleterious co-infections of helminth, protozoan and arthropod parasites and intermittent periods of starvation. The predominant parasite taxa in young Soay sheep are *Trichostrongylus* (*T. axei* and *T. vitrinus*), *coccidia* (11 *Eimeria* species) and keds (*Melophagus ovinus*) and in older animals, *Teladorsagia circumcincta*. In this research, associations between abundance of different parasite taxa were investigated. Secondly, the intensities of different parasite taxa were tested for associations with variation in host weight, which is itself a determinant of over winter survival in the host population. In lambs, strongyle eggs and *Nematodirus* spp. eggs were positively correlated in abundance, while in yearlings and adults strongyle eggs and *coccidia* oocysts, were positively correlated in abundance. In lambs and yearlings, of the parasite taxa tested, only strongyle eggs were significantly and negatively associated with host weight. However in adult hosts, strongyles and *coccidia* were independently and negatively associated with host weight. Taken together, these results suggest a role for multiple parasite species in shaping the fitness and evolutionary trajectory of Soay sheep. Since *Trichostrongylus* predominates in juveniles and *Teladorsagia* in adults these results imply that these genera along with *Eimeria* are exerting independent selection on the population.

234 **The integration of anthelmintic treatment and grazing management for the provision of clean lambing paddocks in a summer rainfall region of Australia**

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The 'smart grazing' program was devised to increase the reliability of the summer drenching strategy in southern Australia (winter rainfall region) and has been shown to be effective in preparing clean paddocks for weaners. It involves alternation between pasture spelling and short, intensive graze periods following anthelmintic treatment. To test its efficacy in a summer rainfall environment we conducted a replicated field experiment using a modification of the program to prepare clean paddocks for lambing.

Three treatments viz 'smart grazing' (SG), the grazing of set stocked sheep (SS) and the grazing of set stocked cattle (SC) were applied to nine 2 hectare paddocks from January until June 2006. Lambing ewes were introduced to the paddocks in August with lamb marking at 9 weeks and weaning at 17 weeks post-introduction. Two tracer sheep were run in each paddock on three occasions and worm egg counts were conducted on faecal samples collected from ewes and lambs at fortnightly intervals.

Ewes on SG and SC paddocks did not require anthelmintic treatment at marking or weaning and had significantly less weight loss than ewes on SS paddocks, which required anthelmintic treatment at both time-points. Lambs on SG and SC paddocks did not require anthelmintic treatment at marking or weaning and had significantly higher daily weight gain than lambs on SS paddocks, which required anthelmintic treatment at weaning.

The 'smart grazing' system was the equal of cattle for preparing clean lambing paddocks with both providing significant advantages over set stocked sheep.

235 **Animal production performance and treatment frequency using the selective/famacha and suppressive strategy of parasite control in ewes: report of a five-year period**

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The observations of the rapid escalation of parasite resistance worldwide suggest the adoption of new alternatives of parasite control in small ruminants. The Integrated System of Parasite Control (SICOPA) similarly to the Target Selective Treatment (TST) program uses the Famacha method that examines individual animals and has the ability to identify sheep and goats that are resistant/resilient and susceptible to *Haemonchus contortus*. The objective of this work was to determine the animal production performance between three years using the SICOPA program with a period without this strategy. A flock of ewes of Ile de France and Texel breeds was followed during August 2001 to July 2003 under suppressive treatment (45-days interval) and from August 2003 to July 2006 under the SICOPA. Fecal samples were collected for egg counts and larvae identification was performed. Selective evaluations indicated that only 9.5% of the animals had to be treated, representing an economical benefit of 450%/year, compared to the suppressive program. Even assuming that there was a strong variability on the between-years effect, the reproductive indexes of delivery (88.9%), birth rate (119%), lamb weight (4.1k) and mortality (5.2%) were similar under the SICOPA regime compared to the previous suppressive period. The results demonstrate that allowing a large number of naturally infected ewes be raised without treatment did not have a negative impact over the reproductive/production indexes. Thus, it is suggested that there is an important intra-species effect that may regulate the rate of infection as well as the number of available larvae on pasture.

236 **Prevalence and seasonal incidence of helminth parasite infections of sheep and goats in eastern Ethiopia: Nematodes and trematodes**

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A 2-year abattoir survey was carried out to determine the prevalence and seasonal incidence of gastro-intestinal (GI) nematodes and trematodes (flukes) of sheep and goats in semi-arid eastern Ethiopia. During May 2003 to April 2005, viscera including liver, lungs and GI tracts were collected from 655 sheep and 632 goats slaughtered at 4 abattoirs located in the towns of Haramaya, Harar, Dire Dawa and Jijiga in eastern Ethiopia. Results showed that 13 species belonging to 9 genera of GI nematodes (*Haemonchus contortus*, *Trichostrongylus axei*, *T. colubriformis*, *T. vitrinus*, *Nematodirus filicollis*, *N. spathiger*, *Oesophagostomum columbianum*, *O. venulosum*, *Strongyloides papillosus*, *Bunostomum trigonocephalum*, *Trichuris ovis*, *Cooperia curticei* and *Chabertia ovina*), and 4 species belonging to 3 genera of trematodes (*Fasciola hepatica*, *F. gigantica*, *Paramphistomum microbothrium* and *Dicrocoelium dendriticum*) were present in both sheep and goats. All animals in this investigation were infected with multiple species to varying degrees. The mean burdens of nematodes were generally moderate in both sheep and goats and showed patterns of seasonal abundance that corresponded with the bi-modal annual rainfall pattern, with highest burdens around the middle of the rainy season. Significant differences in the mean worm burdens of the different nematode species were observed in both sheep and goats between the four geographic locations, with worm burdens in the Haramaya and Harar areas greater than those observed in the Dire Dawa and Jijiga locations. Similar seasonal variations were also observed in the prevalence of flukes. But there were no significant differences in the prevalence of each fluke species between the four locations. Overall, the results showed that *Haemonchus*, *Trichostrongylus*, *Nematodirus*, *Oesophagostomum*, *Fasciola* and *Paramphistomum* species were the most abundant helminth parasites of sheep and goats in eastern Ethiopia.

237 ***Fasciola hepatica*: a study of the vectorial capacity of four different species of lymnaeids snails commonly found in Belgium**

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A previous study in Belgium revealed that genetic material of *Fasciola sp.* was present in four local snail species: *Galba truncatula*, *Radix peregra*, *Radix ovata* and *Lymnaea stagnalis*. Laboratory cultures of these four species were experimentally infected with *F. hepatica*. The collected metacercariae were fed to rats and the infection in that vertebrate hosts was monitored through several techniques. The mortality rates in the breeding unit was less than 10 % in *G. truncatula*, *R. peregra*, and *L. stagnalis* colonies. Microscopy revealed the presence of larval stages in 78.3 % and 48 % of *G. truncatula* and *R. peregra* snails, respectively. These data were confirmed by a PCR that amplifies a parasite specific sequence. *R. peregra* shed fewer metacercariae and more irregularly than *G. truncatula*. In *R. ovata* and *L. stagnalis* the infection rates were low. Specific DNA was detected in both cases by PCR but no metacercariae were shed. The metacercariae shed by *R. peregra* were as infective to rats as those from *G. truncatula*. The PCR technique was more sensitive than microscopic examination. This study demonstrated that *R. peregra* may act as an accidental intermediate host for *F. hepatica* in Belgium.

238 **The population dynamics of *Lymnaea truncatula* in the west of Ireland and the prevalence of *Fasciola hepatica* within the intermediate host**

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Fasciolosis in cattle and sheep, caused by the problematic trematode *Fasciola hepatica*, is costing the Irish farming industry over €25 million per annum. The parasite requires two hosts to complete its life cycle, the intermediate, molluscan host *Lymnaea truncatula* and a final mammalian host i.e. cattle/sheep. Understanding the population dynamics, bionomics and distribution of *Lymnaea truncatula* is important in developing effective parasite control strategies.

Individual faecal samples were obtained from ewes and lambs grazing lowland and upland areas of the study farm (located in the west of Ireland) on a regular basis and fluke egg counts determined using the sedimentation technique. Mean monthly temperature and total monthly rainfall data were collected. Snail populations were monitored on a fortnightly basis following 10 m transects on four sites. *F. hepatica* infection in snails collected was determined via dissection and PCR based procedures. For the July to October grazing period, the number of lambs with positive FEC was greatest in October and September for the lowland (53 %, n = 17) and hill grazed lambs, respectively. Dry conditions (total rainfall of only 39.1 and 47.9 mm recorded for June and July respectively) and temperatures above 14°C during June and July 2006 affected the abundance of *L. truncatula*. Snail population peaked during October and remained present on the soil surface throughout November 2006 until March 2007. Infection levels in the snails varied from 0 to 50 % with peak infection, 8.8 %, occurring in November. Fluctuations in moisture and temperature affected the microhabitat of *L. truncatula* and subsequent population dynamics observed. Atypical observations included snails appearing later in the summer than expected and remaining on the soil surface during the winter.

239 **The dramatic spread of the giant liver fluke in Croatia**

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The following study records epidemiological experience after the dramatic spread of the giant liver fluke *Fascioloides magna* in Eastern Croatia. The giant liver fluke *Fascioloides magna* is an introduced parasite of cervids especially incriminated for high mortality in fallow deers and roe deers. The presence of the liver fluke (*Fascioloides magna*) in Croatian deers was detected for the first time in January 2000 after its spread from Hungary.

From September 2006 to December 2006, faecal samples and livers of red deers (*Cervus elaphus*) and fallow deers (*Dama dama*) were investigated to determine the current distribution of the fluke in the Eastern part of Croatia. Faecal samples were collected from 30 different areas among four Eastern Croatian Counties (Vukovarsko Srijemska County, Osječko Baranjska County, Brodsko Posavska County, Viroviticko Podravska County). The livers of all hunted deers (totally 42) from all studied areas were also dissected. The prevalence of *F. magna* in examined areas determined by coprological examination varied from 13% up to 90%. Moreover, many new foci of *F. magna* infection were discovered especially among Vukovarsko Srijemska County. Up to 90,4 % of livers harbored *F. magna*. Other species of liver flukes were not found. Twenty nidi of infection were found, encompassing all four Eastern Croatian Counties. As many as 58 flukes were recovered from a single red deer. Since deer serve as migrating reservoirs for *F. magna* infections in domestic livestock, it can be concluded that every precaution should be exercised to prevent introduction of the giant fluke into new areas.

240 **Mechanism of *Centrocestus armatus* (Digenea: Heterophyidae) cercariae attachment and penetration to gills of second intermediate fish hosts, *Zacco temminckii***

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Centrocestus armatus is one of the causes of fish-borne parasitic zoonoses in Asia; however, knowledge is limited about its biology and life cycle. The mechanism of attachment and penetration of *C. armatus cercariae* into gill tissues of fish hosts, *Zacco temminckii*, was investigated using electron microscopy, histology, histochemistry and light microscopy. The anatomy of *C. armatus cercariae* was described and contributed to the understanding of gill attachment and penetration. The behavior patterns of *C. armatus cercariae* differed from those known from *Schistosoma* and *Diplostomum* species. After their attachment to gill filaments the cercariae shed their tail and performed a short period of leech-like creeping behavior before they started penetration movements. All penetration sites were through the surface of gill primary lamellae leading to the central venous sinus. Scanning electron microscopy showed that the body of the tail-less larva was divided into two distinct regions, the non-contractile anterior region levering to gill tissues by its hooklets, and the highly contractile body region creating a thrusting force for penetration. Transmission electron microscopy and histochemistry revealed that mucous in surface tegument of the tail-less larvae were glandular secretions released upon entrance to gill chambers and that penetration process was accomplished by both mechanical and chemical means. The presence of penetrating cercariae also increased respiratory frequency rate and mucous production of the fish hosts. This may simply be a stress-related response by the fish hosts to cercariae but this condition would provide more opportunities for entrance and attachment, resulting in greater infection success.

241 **Fishborne zoonotic trematodes in domestic animals in Nghe An province, Vietnam**

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The study was conducted to determine infection status of fishborne zoonotic trematodes (FZTs) in dogs, cats and pigs from 53 randomly selected fish-farming households in Nghe An province, Vietnam. Faecal samples from 83 dogs, 36 cats and 113 pigs were collected /rectum and examined for FZT eggs by a combined filtration, sedimentation and centrifugation technique. The prevalence of FZTs in cats, dogs and pigs were 50.0%, 37.5% and 14.2%, respectively. 96.9 % of the positive cases had FZT infections < 1000 eggs/gram. Twenty-six dogs, 16 cats and 4 pigs were selected among 66 faecal positive animals and necropsied. Recovered trematodes included only intestinal flukes: *Haplorchis taichui*, *H. pumilio*, *H. yakogawi*, *Echinochasmus japonicus* and *E. perfoliatus*, and *Centrocestus* spp. No fishborne liver flukes (i.e. *Clonorchis sinensis* or *Opisthorchis viverrini*) were found, but *Dicrocoelium dendriticum* was recovered from one dog. From a questionnaire survey it was found that pigs were kept in pens, whereas dogs and cats were allowed to roam thus had free access to raw fish and indiscriminate defecation. Farmers often used untreated pig faeces as fish fodder and fed raw fish to their cats, dogs but not to their pigs. Transmission of FZTs in the fish farming communities of Nghe An province is likely caused by egg-shedding reservoir hosts such as domestic animals, in addition to humans. The prevention of FZT transmission on fish farms will require control of the infections in domestic animals as well as in humans.

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242 **The effect of triclabendazole sulphoxide on egg production and hatch rate in the liver fluke, *Fasciola hepatica***

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Triclabendazole (TCBZ) is the main drug used to treat fasciolosis in both humans and livestock. Resistance to TCBZ was first recorded in Australia in 1995 and has since been observed in various parts of the Europe. Resistance does not appear to have any effect on fitness, nevertheless there is little or no published data regarding the effects of triclabendazole sulphoxide (TCBZ.SO) on the eggs produced by drug-exposed flukes. Previous studies have demonstrated the disruptive effect of TCBZ.SO on the testis and vitelline follicles of *F. hepatica* – such disruption could cause an inhibition or cessation of egg production. This study aims to investigate the effect of TCBZ.SO on production, development and hatchability of eggs produced by TCBZ-susceptible (Fairhurst) and TCBZ-resistant (Sligo) isolates of the liver fluke. Additionally, we will investigate the effect of TCBZ.SO on egg development in vitro. Flukes were incubated in TCBZ.SO for up to 24 hours and the eggs produced were assessed for development and hatchability. Additionally, flukes were processed for light microscopy to observe the effect of TCBZ.SO on the reproductive structures of both fluke isolates. Initial data suggest that there was a marked decrease in egg production from Fairhurst TCBZ.SO- treated flukes as well as an observable disruption to reproductive structures. In contrast, in Sligo TCBZ.SO- treated flukes, minimal disruption and effect on egg production was observed. The data suggest that TCBZ.SO does have an effect on egg production in vitro.

243 **The *Haemonchus contortus* genome project; an update and the issue of genome polymorphism**

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The *H. contortus* genome is currently being sequenced by the Pathogen Sequencing group of the Sanger Institute using a hybrid strategy of shotgun sequencing and physical map construction using BAC and fosmid clones. HAPPY mapping is also being performed to provide linkage information to help with scaffold building and for genetic studies. We estimate approximately 85% of the genome is currently represented in shotgun sequence and a number of BAC clones have been fully finished for pilot genefinding and annotation. Progress on these pilot annotations and the most recent information on genome synteny with *C.elegans* will be presented. The major challenge to genome assembly, and the subsequent production of a fully finished annotated genome, is the high level of polymorphism found even within individual isolates. Although the Hco3(ISE) isolate being used for genome sequencing has been inbred it still contains high levels of sequence polymorphism, details of which will be presented. A number of approaches are being adopted to overcome this including further inbreeding of the isolate and construction of single worm fosmid libraries. The presentation will provide an update of the progress of these.

244 **Stage-specific gene expression in *Teladorsagia circumcincta***

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Introduction: The dramatic physiological changes that take place during the transition from the free-living to the parasitic phase of a nematode's life cycle are initiated, and accompanied by, up- and down-regulation in expression levels of suites of genes associated with changes in the nematode's environment and with nutrition, development, onset of sexual maturation and establishment in the host. In the present study we used suppression subtractive hybridisation ("SSH") to enrich genes expressed in a stage-specific manner in infective third larval stage (iL3) and early parasitic fourth larval stage (L4) of the ovine parasite *Teladorsagia circumcincta* prior to gene expression profiling by microarray.

Materials and Methods: Total RNA was extracted from iL3 and day 8 post infection L4. These RNAs were transcribed to cDNA and SSH was performed using the PCR Select kit (Clontech) according to the manufacturer's instructions. Clones from the two resulting stage-specific libraries were sequenced and bioinformatic analyses performed to establish putative functions. Gene-expression profiling was performed using microarrays of the 833 resulting contigs and singletons.

Results and Conclusion: SSH was highly efficient for identifying stage-specific gene expression in *T. circumcincta*. Of the L3-specific transcripts identified, more than 30% represented a single enzyme, GTP-cyclohydrolase, which is the first and rate-limiting enzyme of the tetrahydrobiopterin synthesis pathway and may be involved in critical elements of larval development. In the L4, proteolytic enzymes were highly upregulated as were collagens and a number of E/S products, reflecting the rapid growth of the larva in the abomasal glands.

245 **Functional genomic analyses of excretory/secretory proteins from the parasitic stages of *Haemonchus contortus***

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Excretory/secretory (E/S) proteins expressed during the parasitic stages of nematodes provide a subset of molecules that may be involved in their establishment and/or avoidance of the host immune response. These molecules are rational targets for use in prevention or treatment of nematode infections.

E/S proteins from cultured larvae, or later stages recovered from sheep infected with *H. contortus*, were isolated from nematodes incubated in culture medium for 4-6 h. These proteins were analysed by 2-dimensional electrophoresis (2-DE) to provide proteome maps and their sequences determined by MALDI-TOF/TOF mass spectrometry. Bioinformatic techniques were used to identify homology to related proteins and the presence of targeting motifs, structural and functional domains.

From the E/S molecules expressed at the different parasitic stages, several potential target candidates were selected and stage-specific mRNA expression profiles determined. Their respective cDNA were cloned to enable functional analysis by RNA interference in the free-living stages of *H. contortus*.

Acknowledgements: Funded by Meat & Livestock Australia, Australian Wool Innovation & Department of Primary Industries Victoria.

246 **Differentially expressed genes in infective larvae of *Ascaris suum* identified by microarray and sequencing analyses of an expressed sequence tag library**

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Differential gene expression in *Ascaris suum* was investigated by constructing infective larvae-specific gene archives using a suppressive-subtractive hybridization approach, and 498 differentially expressed sequence tags (ESTs) in the library were identified by microarray analysis, followed by DNA sequencing. 91 unique clusters were grouped using the BLASTALL 2.2.12 program. From these infective larvae EST clusters, 43 (47%) had homology with genes of *Caenorhabditis elegans* as well as other organisms, 48 had no significant homology to sequences held in the GenBank non-redundant database. Infective larvae-specific protein kinases, protein phosphatases, neuropeptide-like peptides and their precursors and enolase transcripts were abundant in the cDNA archive. Genes involved in a number of cellular processes, such as ubiquitination and proteasome function, gene transcription, protein-protein interactions and function were also differentially expressed in the infective larvae. The potential roles of these genes in development in *A. suum* were discussed in relation to the known roles of their homologues in *C. elegans* and other parasitic nematodes. The results of this study provide insights into molecular aspects governing larval developmental processes in *A. suum* and provide a sound platform for future functional genomics studies. Project support was provided in parts by grants from the National Natural Science Foundation of China (grant No. 36071578), International Foundation for Science (grant No. B/4018-1), and the Science and Technology Programme of Guangdong Province (grant no. 2004B50201020).

247 **Analysis of adult *Fasciola gigantica* expressed sequence tags**Huang W.Y., Zhang W.Y., and Wang D.Y.*Guangxi University, College of Animal Science and Technology, Nanning, PRC*

In the present study, a high-quality *Fasciola gigantica* cDNA library was constructed into TriplEx2 plasmid using the SMART technique. The results showed that the un-amplified library capacity was $1.08 \cdot 10^6$ PFU/ml, the library recombination rate was 96.6 %, the titre of the amplified library was $2.41 \cdot 10^9$ PFU/ml and the average inserted cDNA fragment was about 1000 bp. Then the clones from the BM25.8 plasmids were selected to sequence. 8990 EST raw sequences were obtained, of which 8395 EST clean sequences (after remove the vector sequence from raw data, the length of EST must be ≥ 100 bp, and use these clean ESTs to phrap), 1057 contigs and 2237 singlets (totally 3294 unigene sequences). The redundancy (%) $((1 - (\text{Unigene number} / \text{Clean EST number})) \cdot 100)$ was 61%. 860 predicted proteins and 591 unknown proteins were obtained by analyzing the sequences with Blastx NCBI nr. Further study will focus on the investigation of the functions of the unknown proteins by bioinformatics techniques.

248 **An analysis of differential transcription between adult female and male *Haemonchus contortus* using an oligonucleotide microarray platform**Campbell B.E. 1, Nagaraj S. 2, Hu M. 1, Ong B. 3, Loukas A. 4, Beveridge I. 1, Ranganathan S. 2, and Gasser R.B. 1

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A bioinformatic-microarray approach was employed for the analysis of selected expressed sequence tags (ESTs) from *Haemonchus contortus* (Trichostrongylidae: Nematoda). Following cluster analysis of the ESTs using an automated pipeline, 1885 representative ESTs (rESTs) were selected, to which oligonucleotides were designed and spotted on to a microarray. This microarray, containing appropriate controls and replicates, was hybridized with cyanine-dye labelled cRNA probes synthesized from RNA from adult females or males. Differential hybridization was displayed for 301 of the 1885 rESTs (~16%). Of these, 165 (55%) had statistically greater signal intensities using female cRNA and 136 (45%) using male cRNA. Of the 165 rESTs with higher signal intensities in female *H. contortus*, 135 (82%) were predicted to have orthologues/homologues in *C. elegans*, representing translation factors, splicing factors, cell adhesion molecules and structural proteins. Of the 108 rESTs with statistically higher signal intensities in male *H. contortus*, 79% had homologues in *C. elegans*, which included major sperm protein, molecules involved in proteolysis and in various metabolic pathways. Of the rESTs with no known homologues in *C. elegans*, 24 (~13%) had homologues in other nematodes, 7% had homologues in various other organisms and 52% had no homology to any sequence in current databases. This study provides a foundation for the molecular, biochemical and functional exploration of selected molecules with differential transcription profiles in *H. contortus*, for further microarray analyses of transcription in different developmental stages, and for an extended analysis when the imminent full genome sequence of this nematode is known.

249 **Better living through chemistry: new drugs must be found**

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Parasites and their hosts have diverged biochemically over millions of years. Historically (and still today), botanical preparations have been found to provide profound chemotherapeutic resolution of myriad kinds of parasitic infections and infestations. More recently, more-or-less random screening of chemicals (both natural products and synthetic compounds) against parasites in hosts or in culture has led to the discovery and development of a wide array of antiparasitic drugs. The judicious application of these agents has markedly diminished the burden of parasitoses in animals and humans over the past 60 years or so. Although much has been accomplished, we were late to recognize that selection of parasite populations resistant to these drugs would inevitably threaten their utility. As a result, we now stand at a cross-road in antiparasite chemotherapy: we need new drugs, and when we get them, we need to use them better than we have to prolong their useful lives.

How to discover new antiparasitic drugs is the more immediate task. To date, every antiparasitic drug available (at least the prototype of every class) has been discovered in whole-organism based screens. For many reasons, organism-based screening as a strategy has been replaced in large pharmaceutical companies by mechanism-based high-throughput screens. In this tactic, random compounds are tested for their ability to interact with a particular protein target rather than an organism or cell. In the 20 years or so since it has been applied to antiparasitic drug discovery, it has failed to generate new compounds for use in humans or animals. The paradigm is not scheduled for replacement, so we need to do a better job of adapting it for discovering new drugs for the treatment and prevention of parasitoses.

We have not solved the problem of moving from activity in mechanism-based screens to activity in whole-organism assays. More fundamentally, we are still unable to predict which *in vitro* actives will be active in animal models. More basic parasitological work is necessary to make culture environments more like host environments. However, the advent of access to multiple parasite genomes means that one of the fundamental issues constraining mechanism-based discovery, the ability to predict *a priori* spectrum of action, may soon be resolved. We also need to make sure that we are screening the richest possible sources of new antiparasitic molecules, including natural products, to maximize our chances for finding drugs that may lie outside the normal physical-chemical universe of drugs for chronic human non-infectious diseases.

Chemotherapy remains the mainstay of treatment for disease caused by eukaryotic pathogens. We need to do a better job of finding new drugs for this purpose by more thoughtfully exploiting the new technology platforms available to us. Vaccine-based approaches may eventually contribute to the control of some parasites, but the chemical world will continue to be relied upon for the provision of broad-spectrum cures for most infections and infestations.

250 Evolution backs the vaccinologist: Why vaccines will win the battle of the anti-parasitics

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Parasites and their hosts have been locked in a struggle for survival for millions of years. Hosts attack the invaders with a sophisticated array of potentially destructive molecules and cells. Successful parasites have responded, evolving immune evasion mechanisms. The parasites we see now survive in an equilibrium with their hosts, generally invoking immune reactions which often help to limit the parasite burden, but not eliminate it.

A consequence of this evolutionary battle is that the parasites are constrained in the extent to which they can respond to new immunological assaults. The parasite is constrained in its ability to change in an immunological sense, because it risks immune recognition and invoking new and possibly effective immune responses.

Contrast this with the chemotherapeutic strategy. Identifying a novel, small molecule inhibitor, for a parasite enzyme for example, may provide a temporary attack which has not previously been the subject of selection pressure. We could anticipate however that there may be few constraints, other than biochemical, on selection for enzyme mutants that are not inhibited.

For these reasons, vaccines will win the battle as practical preventative agents against parasitic diseases. Can this be achieved? The answer is unequivocally yes.

It is only a little over 20 years since recombinant DNA technology began being adopted in parasitology. Prior to that time there was little hope of developing commercial vaccines against parasitic diseases because the eukaryotic parasites could not be cultured like other infectious micro-organisms. Contrary to popular opinion, anti-parasite vaccine development since the advent of recombinant DNA and other modern technologies has been extremely successful. Whether the level of success has met the grandiose expectations that may have been promulgated by some in the 1980's reflects more on those making the predictions than it does of any perceived failings of vaccine technology *per se*. Although there have not been, yet, successful commercial vaccines developed against the most important human or commercial parasitic diseases (eg malaria, gastrointestinal helminths) there have been many successes in antiparasitic vaccine development, a growing number of which have been released commercially.

Identifying and mass production of effective vaccine antigens remains a major challenge for vaccinologists. Increasingly powerful and adaptive technologies constantly improve the prospects for production of new vaccines and, given time and investment, these will be delivered.

POSTER COMMUNICATIONS



POSTER SESSION 1

Poster group 1: Helminth Infections of Small Ruminants

251 Effect of the consumption of a tropical tannin-rich plant (*Tzalam*, *Lysiloma latisiliquum*) on the larval establishment of parasitic nematodes in goats

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Gastrointestinal nematode infections are a main pathological threat for livestock in tropical countries. Because of the constant development of anthelmintic resistance in worm populations, the use of tanniniferous plants represents one alternative solution to chemical anthelmintics. Under temperate conditions, the consumption of tannin-rich forages has been associated with an improved host resilience and resistance against nematodes in small ruminants. Nevertheless, few studies have considered the possible use of such tanniniferous browsing plants in tropical countries as being bio-active. In this study, we aimed at i) measuring the consumption of Tzalam (*Lysiloma latisiliquum*), a tanniniferous plant from Yucatan, by goats; ii) examining its effect on the establishment of nematode third-stage larvae (L3), and iii) defining the role of tannins in these effects. An experiment was performed with 3 groups of 6 naive goats fed with 1/ Tzalam, 2/ Tzalam+PEG, or 3/ *Brosimum alicastrum*, a plant with a low level of tannins. After a 7-day period for diet adaptation, each goat received 3000 infective larvae of *Haemonchus contortus* and of *Trichostrongylus colubriformis*. Animals were killed 5 days after infection for worm counts. During the 12-day experimental period, no difference of plant consumption was observed between groups. For both nematodes, the consumption of Tzalam was associated with a reduced larval establishment ($P < 0.001$), assessed by the Mann Whitney test, but not when PEG was added to Tzalam, suggesting a major role for tannins in the effects. Since no difference in appetency was verified, the potential use of Tzalam as bio-active plant could be suggested in tropical farm conditions

252 Use of condensed tannins against gastro-intestinal parasites in naturally infected grazing lambs

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Condensed tannins may reduce the burden of gastro-intestinal parasites in ruminants by direct or indirect anthelmintic effects. The aim of this study was to investigate whether Quebracho extracts had anthelmintic effects in naturally infected grazing lambs.

36 2 month-old French Texel lambs were investigated six weeks after start of the grazing period (T6). They were divided in 3 groups of 12 lambs and underwent treatment at T6 and T10 with orally administrated moxidectin (0.2 mg/kg), with placebo (50 ml H₂O) or Quebracho extract (1mg/kg). Body weight and FEC were determined at T6, T7, T10, T11, T14. Parasitological analysis was performed using the McMaster technique. Separate counts were established for nematodes, *Moniezia expansa* and *Eimeria*. No group-related differences of bodyweight were found. *Eimeria* infestation significantly decreased over time (T6:33000±9700 eggs/g feces versus T14:700±270 eggs/g feces, $p < 0.001$), treatment-related differences were not detected. *Moniezia* infestation was non detectable at T6 and was maximal at T10 (400±200 eggs/g feces); treatment-related differences were not detected. Nematode infestation remained stable in placebo- and Quebracho-treated groups throughout the investigation period (T6:320±96 eggs/g feces versus T14:420±115 eggs/g feces, NS), whereas a significant decrease of FEC was recorded at T7, T10, T11 and T14 in moxidectin-treated lambs (T6:450±116 eggs/g feces versus T7:27±19; T10:90±63; T11: 9±9; T14: 63±39 eggs/g feces, $p < 0.01$).

This study suggests that a single or double oral administration of Quebracho extracts at the dose of 1 mg/kg does not exert an anthelmintic effect in naturally infected grazing lambs. No adverse effects of tannin administration on bodyweight were noted.

253 **Effects of *Azadirachta indica* on sheep infected naturally with helminthes**

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The objective of this study was to evaluate the effects of neem (*Azadirachta indica*) on natural helminthes infection in lambs. Forty, four-month-old entire Santa Ines lambs, with average live weight of 11 ± 2.7 kg were grazed on an *Andropogon gayanus* pasture, over a 20 week period. They were divided into 4 treatments: without drenching (ND), 3g *A. indica*/animal (A3), 6g *A. indica*/animal (A6) and 9g *A. indica*/animal (A9) over 5 consecutive days, with an interval of 28 days between drenchings. Faeces were collected weekly and lamb weight and blood collection were carried out fortnightly. Four weeks after the last drenching all lambs were slaughtered, and worm burdens calculated. No significant differences were observed for lamb performance between treatments. Only treatment A3 showed faecal egg counts (FEC) lower than treatment ND from the 12th week, although this was also not significant. Blood parameters highlighted the progress of the worm infections, with sheep on treatments A6 and A9 ending the experiment showing anemia. There was an increase in the total number of worms associated with increasing levels of neem, especially due to the increase in number of *H. contortus* in detriment to the number of *T. colubriformis* for highest levels of neem (A6 and A9). The increasing doses of neem did not improve the control of endoparasites in sheep naturally infected, increasing the development of *H. contortus* infection.

254 ***Drimys brasiliensis* against nematode parasites of sheep**

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Parasite infection has been considered a limiting factor in the sheep and goat industry and Trichostrongylidae organisms are responsible for economical losses of up to 40%. Their control is exclusively by applying regular usage of chemotherapy, which causes the selection of a more tolerant/resistant parasite population. Fifteen tons of ivermectin is eliminated in the ground (faecal pad, soil, water) per year in Brazil with a half-life of more than 70 days. Thus, more and more agroecological strategies are needed to reduce environmental contamination. The usage of phytotherapy dates from the ancient medical alchemy. The objective of this work was to determine the efficacy of *Drimys brasiliensis* that contain flavonoids and polygodials against nematodes in sheep. Five groups of six Santa Ines sheep were treated orally with a dry-and-grounded *D. brasiliensis* (T1: 0.1g/k; T2: 0.2g/k; T3 0.4g/k; T5: 0.8g/k) and subcutaneous ivermectin (T6: 0.02mg/k). The phytotherapeutic agent had 52% fiber and was administered consecutively for three days. Faecal exams (EPG), coproculture, haematocrit and weight gain was measured at weekly intervals for 28 days. It was observed no positive effect of any measurement after the usage of the natural product. Ivermectin showed an efficacy of 80%. There was a reduction on the haematocrit in all groups with an increase of EPG. The predominant genuses were *Haemonchus*, *Trichostrongylus* and *Strongyloides*. Just yet, the usage of *D. brasiliensis* is not indicated as an alternative for parasite control. A follow up experiment is under execution to demonstrate its contribution together with other parasite control strategies.

255 **African plant-based treatment for gastrointestinal nematodes in sheep**

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A participatory field trial was performed in remote northeastern Uganda to establish a dose and dosing schedule in sheep for *Albizia anthelmintica*, a plant commonly used as a livestock dewormer by East African pastoralists. The dose used by several traditional healers was averaged and found to be 0.9g. 54 young female sheep were divided into six groups, including negative and positive controls. Groups A, B, C, and D each consisted of nine sheep and were given approximately one half (0.4g), one (0.8g), two (1.8g), and five (4.7g) times this dose, respectively. The number and type of parasite eggs in each sheep's feces were counted prior to treatment, then once weekly for five weeks. Five of the sheep in each group were re-treated on day fourteen. Sheep in Groups B, C, and D showed a significantly greater decrease in the number of strongyle-type eggs in the feces, as compared to the negative control, for three weeks after treatment. The five sheep in Group C that were re-treated on day fourteen showed this effect throughout the five-week field trial. Although efficacy against coccidia and the cestode *Monezia* were suggested in descriptive statistics, these effects were not found to be statistically significant. We conclude that in areas where modern anthelmintics are not available, administration of *A. anthelmintica* at 1.8g at three-week intervals can be used to control nematode infections in sheep; and that further research is warranted to clarify efficacy and appropriate dosing for use in treating coccidia and *Monezia* infections.

256 **In vitro and in vivo anthelmintic activity of *Caesalpinia crista* (L.) seed kernels against gastrointestinal nematodes of sheep**

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Graded doses of *Caesalpinia* (*C.*) *crista* seed kernels as crude aqueous methanolic (CAM) extract, chloroform fraction and crude powder were evaluated for anthelmintic activity employing egg hatch test, adult motility assay and faecal egg count reduction test. CAM extract and the chloroform fraction inhibited *Haemonchus* (*H.*) *contortus* eggs hatching with LC50 values of 0.134 and 0.108 mg/ml, respectively. In vitro exposure of adult *H. contortus* to CAM extract and chloroform fraction caused mortality (≤ 0.05) of the worms compared with control (PBS exposed worms). The chloroform fraction had more pronounced effects both in egg hatch test and adult motility assay. In vivo, maximum (90%) nematode faecal egg count reduction was recorded in sheep, minimum and maximum being 60% and 90% in sheep treated with of *H. contortus*, *Trichostrongylus* spp. and *Teladorsagia* spp. in sheep administered per os with crude powder, CAM extract and the chloroform fraction of *C. crista* seed kernels. Maximum faecal egg count reduction was recorded with both crude powder and CAM extract @ 3 g/kg of body weight at 13 days post-treatment. CAM extract exhibited more persistent result compared to crude powder. The results provided a rationale for the use of *C. crista* seed kernels as an anthelmintic in the local ethnoveterinary practices.

257 **Anthelmintic effects of *Newbouldia laevis* on parasitic nematodes of the gastrointestinal tract of sheep**

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Previous in vitro studies indicated that extracts of *Newbouldia laevis* (Bignoniaceae) might have anthelmintic properties against sheep nematodes. Several experiments have thus been performed in Djallonké lambs naturally infected with *Haemonchus contortus* and *Trichostrongylus colubriformis* to confirm in vivo the antiparasitic properties of *Newbouldia* leaves. In the 3 successive trials, the experimental groups presented similar initial egg excretion. Experiment 1 measured the effects of fresh leaves distributed ad libitum 3 times weekly to infected lambs in farm conditions. The results showed a maximal significant reduction of 89 % in faecal egg counts (FEC). Experiment 2 aimed at evaluating the use of ground dried leaves and whether the dose modulate the effects on egg excretion. FECs were measured for 4 weeks in three groups of naturally infected sheep. Two groups received from D14 to D16 either 3.2 or 4.8 g of ground dried leaves/kg live bodyweight (LBW), the third group remained untreated as infected controls. Maximal significant reductions of 84 and 88 % in FECs were found respectively in sheep receiving 3.2 and 4.8 g/kg LBW. In experiment 3, FECs, packed cell volume, worm counts and female fertilities were compared in sheep receiving 1.6g dry *Newbouldia* / kg LBW (D14 to D16) and in an untreated infected group. Significant differences in FEC and PCV were observed in the treated animals from D18 to D31. At necropsy, significant decrease in *H. contortus* number and female fertilities were assessed but not for *T. colubriformis*. Overall, these results support the hypothesis of anthelmintic effects of *N. laevis* leaves.

258 **Evaluation of anthelmintic activity of *Lippia sidoides* essential oil on sheep gastrointestinal nematodes**

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Medicinal plants have been investigated in their anthelmintic properties and demonstrated efficacy against eggs and larvae of gastrointestinal nematodes. The aim of this study was to evaluate the anthelmintic efficacy of *Lippia sidoides* essential oil (LsEO) a plant from Northeast Brazil, on fecal egg counting and worm burden reduction of gastrointestinal nematodes from sheep. LsEO was administered to 44 sheep naturally infected divided in four groups, G1: no treatment; G2: 200 mg kg⁻¹ ivermectin; G3: 230 mg kg⁻¹ LsEO and G4: 283 mg kg⁻¹ LsEO. Fecal samples were collected from each animal to determine epg at 7, 14 and 21 days after treatment. In another experiment, 21 sheep were allocated in a pound and distributed in three groups: G1: no treatment; G2: 200 µg kg⁻¹ ivermectin and G3: 283 mg kg⁻¹ LsEO. Seven days after treatment, animals were euthanized and necropsied to recuperate, count and identify nematodes from abomasum, small and large intestines. In the first test, efficacy of 230 mg kg⁻¹ and 283 mg kg⁻¹ LsEO and ivermectin were 38%, 45.9% and 40.2%, respectively, 7 days after treatment, and 30%, 54% and 39.6%, respectively, 14 days after treatment. In the second experiment, the efficacy of 283 mg kg⁻¹ LsEO and ivermectin were 56.9% and 34.4%, respectively, against *Haemonchus* spp, and 39.3% and 63.6% against *Trichostrongylus* spp, respectively. These results demonstrated that another dose or delivery formulation must be investigated to evaluate completely the anthelmintic activity of *L. sidoides* essential oil.

259 **The effect of sericea lespedeza, fed as pellets, on established infection of *Haemonchus contortus* in sheep**

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Control of *Haemonchus contortus* has traditionally relied on the use of anthelmintics. The development of anthelmintic resistance dictates that alternative approaches are needed. Grazing *sericea lespedeza* (SL, a forage containing condensed tannin), and feeding it as hay and ground hay has reduced fecal egg count (FEC) and worm burden in sheep and goats. This study evaluated the effect of SL, fed as pellets, on naturally acquired *H. contortus* infection in lambs. Twenty lambs were removed from pasture and randomly allocated to two treatment groups (n=10 each). SL pellets were fed over a 35 d period to one group while the other group was fed bermudagrass hay (control). After the 35 d feeding period, both groups were fed bermudagrass hay for an additional 14 d. The control group FEC continually increased while the SL fed group FEC decreased 50% by d 7 and remained significantly ($P<0.05$) reduced over the SL feeding period. During the course of the study, four control animals had to be dewormed (one each on d 14, 21, 35 and 42) due to clinical signs of haemonchosis. After the SL feeding period, FEC did not increase in the SL fed group. This indicated a lack of effect on female worm fecundity which is contrary to previous studies. Due to deworming, there was no difference in FEC between groups at the end of the study; therefore, worms were not recovered. Results indicated that SL, fed as pellets, reduced the worm burden (based on FEC) of naturally acquired infections which could help reduce pasture contamination.

260 **Larvicidal activity of butanol extract from fiber water of green Coconut (*Cocos nucifera* Linn.) on *Haemonchus contortus***

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The control of gastrointestinal parasites of sheep and goats is usually accomplished with anthelmintics. However, the rapid development of anthelmintic resistance, associated with the high cost of the available anthelmintic drugs, has limited the success of gastrointestinal nematodiasis control in small ruminants and thus awakened interest in studying medicinal plants as an alternative source of anthelmintics. Several works have demonstrated tannins act on endoparasites. The fiber water coconut is rich in condensed tannins. Therefore the objective of the present work was to evaluate the larvicidal effect of butanol extract of the water fiber green coconut on *H. contortus*. The butanol extract was obtained through launderings, with butanol solvent, of water fiber using a decantation funnel. The butanol extracts were evaluated at five concentrations: 5.0, 10.0, 20.0, 40.0 and 80.0 mg mL⁻¹. At 40 and 80 mg mL⁻¹, butanol extract inhibited 62.33% and 99.80% of larval development, respectively. Phytochemical tests showed the presence of condensed tannins in the butanol extract. These results suggest the use of butanol extract of water fiber green coconuts may be useful in the control of gastrointestinal nematodes of small ruminants, however, in vivo studies and toxicology tests are necessary.

261 **Relationship between the origin of sainfoin samples, the tannin content and the anthelmintic activity against *Haemonchus contortus***

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Evidence is accumulating to suggest that tannin-rich legume forages have anthelmintic properties and might represent an alternative or a complement to chemical drugs in the control of gastrointestinal nematodes in small ruminants. These effects have usually been related to the plant secondary metabolites, several results supporting the hypothesis that tannin content (TC) plays a key role in the anthelmintic properties of some legume forages. However, it is known that, within the same legume species, factors related to either plant varieties or to environmental conditions, might affect the TC. The general aim of this study was to analyse the relationship between such variations in TC in legumes and the anthelmintic properties against parasitic nematodes, using sainfoin and *Haemonchus contortus* for models. Sainfoin samples of 3 varieties were collected from 2 locations, at 3 different stages and used to produce extracts. The TC of extracts was measured using the radial diffusion technique, the anthelmintic activity against *H. contortus* by the Larval Migration Inhibition assay. Mann and Whitney's tests indicated significant differences in TC and anthelmintic activity depending on the location ($P < 0.01$), but not on the stage and the varieties. Significant Spearman's correlation coefficients were found between the TC and the inhibition of larval migration ($r = 0.413$, $df = 13$, $P < 0.01$). These preliminary results suggest that the variability in tannin content of legume forages might partly explain the variations in anthelmintic activities previously observed. They underline the need for analysis of TC in legume forages before being used as nutraceuticals in farm conditions.

262 **In vitro anthelmintic activity of plant extracts from tropical tanniniferous trees against *Haemonchus contortus***

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The consumption of tropical leguminous tree leaves by small ruminants may have a role in the control of gastrointestinal nematode infections. Several browsing legumes contain plant secondary compounds such as condensed tannins (CT). The anthelmintic effect of CT from temperate legumes has been demonstrated. The anthelmintic effect of four tanniniferous plants (*Acacia pennatula*, *Lysiloma latisiliquum*, *Piscidia piscipula* and *Leucaena leucocephala*) was tested in vitro against *Haemonchus contortus*. Plant extracts (acetone:water, 70:30) were obtained from the fodder of each plant. Firstly, different concentrations of plant lyophilized extracts (150, 300, 600 and 1200 µg/ml PBS) were tested using *H. contortus* L3 larvae based on a Larval Motility Inhibition (LMI) test. Levamisole and PBS were used respectively as positive and negative controls. PVPP was used to verify whether CT from plant extracts were responsible of the effects. Secondly, the effect of extracts on larval exsheathment was tested. Larvae were in contact with extracts at concentration of 1200 µg/ml for 3 h; then were exposed to an artificial exsheathment procedure, the process of exsheathment being observed at 10-minute intervals. A GLM test was used to determine the dose effect in the LMI test. A Kruskal-Wallis test was used to determine the effect of PVPP on LMI test. The LMI test showed a dose dependent effect for *A. pennatula*, *L. latisiliquum* and *L. leucocephala* ($P < 0.05$). No anthelmintic effect was found for *P. piscipula*. PVPP confirmed the role of CT in the observed effects. All four plant extracts caused the inhibition of exsheathment of L3.

263 **In vitro studies using bioassays to examine the effect of aqueous plant extracts on *Teladorsagia circumcincta***

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An increase in anthelmintic resistance to the three broad spectrum drugs has lead to a need to find different methods of sustainable worm control. These alternative methods include vaccines, genetic selection, grazing management, biological control and the use of phytotherapy.

There are a number of different in vitro assays used for determining the potential anthelmintic effects of aqueous plant extracts. These include the egg hatch assay: EHA, larval migration inhibition assay: LMIA, larval feeding inhibition assay: LFIA, larval exsheathment assay: LEA and the parasitic stages motility assay: PSMA. The most sensitive in vitro technique is the LFIA where feeding activity can be disrupted through direct effects on the pharyngeal neuromusculature and/or other target sites for extract products. The LFIA has been used as the primary screen for 513 plant extracts, 119 have demonstrable activity in vitro with LFIA 50 values less than 1.25mg/ml. Nine extracts not only inhibited feeding activity but also seriously affected the cuticular structure of *Teladorsagia* larvae. Using either protein binding inhibitors or protease inhibitors has enabled the identification of some of the principal active components in the extracts, such as the condensed tannins or cysteine proteases.

Although a high percentage of the plant products examined have activity in vitro, the situation in vivo maybe very different due to the complex physiological/ biochemical environments that exist in the ruminant gut. The bioavailability of the active components at the different parasite predilection sites is crucial to the efficacy of the plant extract.

264 **Potential use of Cassava (*Manihot esculenta*) leaves for worm control for small ruminants in the tropics**

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Helminthiasis is one of the causes of mortality and morbidity in sheep and goats in Malaysia. However, due to anthelmintic resistance which is increasing in Malaysia and other countries, other alternatives for worm control is needed. Cassava is a tropical plant which originated from South America. Previous studies conducted in Vietnam (Thi mui Nguyen et. al., 2005) and Cambodia (Seng et al., 2006) have shown the anthelmintic effect of cassava when fed to goats. Therefore, this study was conducted to evaluate the anthelmintic effect of our Malaysian cassava variety) leaves on nematode parasites of sheep. Twenty-four cross bred sheep were randomly selected and equally divided into control (n = 8), fresh cassava group (n =8) and cassava silage group (n =8). Faecal egg counts (FEC) using the modified McMaster technique were carried out 3 times per week and the FAMACHA score for assessing clinical anaemia was conducted weekly for 9 weeks. The results of FEC showed that there was reduction in both fresh cassava and cassava silage group. This result indicates that feeding cassava has an effect in reducing the faecal egg counts. Thus, there is a potential for the use of cassava leaves for worm control in the tropics.

265 **Aprisco-seridó project: a new perspective for goat and sheep farmers on a Brazilian dry area**

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One of the greatest problems of the goat and sheep industry in Brazil are the parasitic infections. In the Northeast Region of Brazil, despite the short rainy season, the lack of investments in the activity, the low degree of instruction of the producers, the indiscriminate anti-parasitic drug usage and the non-selectivity of the treatment only aggravates the problem. With the objective to increase animal production, the protein source in the diet and to implement the agroecological concept, a pioneering project was initiated in the Central and Cristalino-Seridó regions of the Rio Grande do Norte State. The Famacha method was used to diagnose *Haemonchus contortus* and used as part of an Integrated System of Parasite Control (SICOPA) similar to the Target Selected Treatment (TST) program. The project acted in 33 cities with 12 technicians, attending 240 resource-poor farmers totalizing 16.000 sheep and 18.000 goats using a mobile unit during September/2005 and August/2006. The average treatment per animal per interval was of 44.66 and 52.33% for sheep and goats, respectively, totalizing more than 100.000 evaluations. It was verified the rationalization in the use of anti-parasitic drugs, reflected by the significant reduction in the treatment frequency. The adoption of the Famacha method by the Aprisco Project provided an opportunity to qualify and create a new category of technical labor improving the sheep and goat cost/effective production in the region. Technology transfer was well received by the farmers consolidating the diffusion of the SICOPA. A second area with more animals is under investigation.

266 **Development and evaluation of a decision support model for the identification of animals that would benefit from anthelmintic treatment**

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Concern regarding the development of anthelmintic resistance in gastro-intestinal nematodes has sparked investigations into drenching regimes that are based on the targeted selective treatment (TST) of individual animals, rather than prophylactic treatment of the whole mob. The use of TST's may reduce the proportion of anthelmintic resistant eggs deposited onto pasture during a grazing season while minimizing the parasite-induced loss in productivity. While such approaches have proved to be successful at indicating the appropriateness of treatment during *Haemonchus contortus* infections, there is no such system available for species of parasites that are commonly found in temperate environments. Here we report on the development and suitability of a production-based support system that has been designed to provide a practical and effective method of identifying individual animals that would benefit from an anthelmintic treatment. The proposed model takes into account predicted herbage intake and predicted energy deposition to provide an estimate of the efficiency of energy utilization. Preliminary on-farm studies have indicated anthelmintic treatment increases the calculated efficiency values by 16% associated with a 2.5 fold increase in liveweight gain. Compared with a neo-suppressive treatment regime that involved anthelmintic treatment every four weeks, the use of the model as a decision support tool reduced the number of anthelmintic treatments given to a TST mob by 50% while incurring a 15% loss in liveweight gain. Further refinement of the model during the 2007 grazing season and its suitability for use as an indicator of TST on an individual animal basis will be evaluated.

267 **Colour of mucous membrane as an indicator of endoparasite infections in sheep**

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In relation to the pathology of endoparasites different indirect indicators can be used to quantify the total worm burden. In case of parasites, which lead to blood losses (e.g. *Haemonchus contortus*) packed cell volume (PCV) seems to be a useful indicator. Therefore methods were developed earlier to score the colour of mucous membranes (e.g. FAMACHA) as a sign of anaemia. The aim of the present study was to examine the correlation between PCV, FAMACHA score, colour saturation (Chroma) and colour angle (Hue-angle, HA, Minolta Chroma Meter CR-200b). The parameters were taken from German Black Head Mutton (GB, n=118) and Leine sheep (LE, n=114) at an age of five months. Furthermore individual faecal samples were collected at that time to measure faecal egg counts (FEC).

There were no significant differences between the two breeds in PCV ($p=0.768$) and log FEC ($p=0.934$). FAMACHA scores were significantly higher in GB than in LE lambs ($p=0.008$). In consequence chroma and log HA were significantly higher in LE lambs when compared with GB animals ($p<0.05$).

PCV and colour traits (FAMACHA, Chroma and HA) were significantly ($p<0.05$) correlated with higher values in the GB lambs. It is suggested that colour measurements of mucous membranes have to be adapted to different sheep breeds. Irrespective of the breed the FAMACHA system is more adequate to detect animals with a lower PCV ($p<0.01$), whereas Chroma and HA are more adequate when PCV is higher ($p<0.001$).

268 **Nematode targeted selective treatments in goats under temperate climate using body condition scoring and milk yield records**

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Leaving susceptible worms in refugia by only treating those animals that need it may be a way to manage anthelmintic resistance in small ruminants. While the FAMACHA method is efficient in areas where *Haemonchus contortus* dominates, we are still lacking of reliable methods to indicate animals needing treatment where *Teladorsagia* and *Trichostrongylus* spp dominate. In this study, two indicators were tested : body condition scoring and individual milk yield recording. The survey was conducted, from June to October 2006, in three goat farms using pasture and having a milk production registration system. Each 6 weeks, 50 goats (20 high-producing goats, 20 low-producing goats, 10 primpiparous goats) were individually sampled for faeces and scored for body condition. The individual milk yields were monthly recorded. Whatever the flock, body condition was significantly influenced by the milk production level and by the lactation stage. While the mean egg excretion remained below 1000 epg in 2 farms for the whole survey, it increased up to 1800 epg in the third farm. In this last flock, a significant and negative correlation was shown between body condition scoring and egg excretion at the first sampling date (4th-5th month of lactation and 3rd grazing month). At this date, 60% of the goats had a lumbar score lower than 2 : 54% of these goats showed epg values above the cut-off (specificity) ; on the remaining 40%, only 11% had epg values above the cut-off (sensitivity of 89%). Body condition scoring seems to be a good indicator if infection is at a high level. On the contrary, individual milk yield recording failed to show variations due to parasitism, possibly due to a too long period of time between 2 records.

269 **The basis for anthelmintic-targeted selective treatment in high value Sardi sheep in Morocco: Questionnaire and actual infection**

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The targeted selective treatment (TST) approach is centred around the fact that gastrointestinal nematode populations are highly overdispersed. Under these circumstances, the ability to select individual sheep that need treatment should minimise the rate of development of anthelmintic resistance by maintaining an untreated parasite population (in refugia). A questionnaire investigating the structure and management of the farm was conducted on 48 farms of the Chaouia plain in 2006 to determine the acceptability of the TST approach. In addition, details were obtained of methods used to control nematodoses., efficacy of anthelmintics and acceptability of TST. Results suggested that farmers have no concept of resistance and the reduction in anthelmintic effectiveness is mainly attributed to a decrease in the quality of drugs. However farmers are willing to adopt TST as long as it does not interfere with productivity and if they are given training. Parasitological investigations showed that the main parasites in the area are *Haemonchus contortus*, *Teladorsagia circumcincta* and *Nematodirus* sp with the former two species having some benzimidazole (BZ) resistance. The most used anthelmintics are albendazole, levamisole and ivermectin on two to six occasions per season. Sheep used in the initial TST study (2006) had a low faecal egg counts (< 300 epg) which did not require routine treatments. However, in the future, the treatments might be determined by using TST markers (Body condition, Disco and Famacha). The preliminary data suggest that under Moroccan conditions of low infection, TST indicators are loosely related to faecal egg count.

270 **How can targeted selective treatment be applied in commercial sheep flocks in southwest England?**

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Due to the development of anthelmintic resistance new strategies of nematode control are required in order to sustain the British sheep industry. Targeted Selective Treatment (TST), where only certain individuals are treated is a strategy that it is hoped will help maintain anthelmintic efficacy. Lamb weights using an automatic weighing crate and electronic ear tags (Shearwells) and DAG scores were collected on 6 flocks on two farms in the west of England from 1805 lambs. Faecal egg counts were made on representative sub samples of lambs where possible. On one flock lambs were treated with albendazole (90% efficacy) and ewe lambs were kept on the same pasture whilst ram lambs were moved to rested pasture. At the next treatment the efficacy of albendazole in the ewe lambs was (86%) but that of the ram lambs (22%), suggesting that dose and move to cleaner pasture selects rapidly for resistance. Comparison of faecal egg counts with DAG scores showed that there was no overall correlation between these two measures. Comparisons of weight gains with DAG scores also showed no apparent correlation. Since routine faecal egg counts from individual animals are not practical in a farm setting, except for determining anthelmintic efficacy, weight gain appears to be the only practical way of identifying those animals that can be left untreated in the practice of TST. This work formed part of the EU funded PARASOL project.

271 **Parasite control practices on lowland sheep farms in Ireland**

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Introduction : Parasites are an important cause of production loss in ruminants. The growing evidence for anthelmintic resistance seriously threatens the use of anthelmintics and an integrated approach to parasite management is needed. Because of a lack of no published information on parasite control strategies used by Irish sheep producers, this study was undertaken to collate information on parasite control practices that would form the basis for future dialogue on sustainable parasite control practices.

Methods: A questionnaire relating to farm details, grazing and parasite control practices was mailed to a representative sample of lowland sheep producers nationwide (n= 166).

Results : The response rate was 70% (n=116). The majority of respondents (60%) had both a sheep and cattle enterprise, of which 75% indicated mix grazing these animals as oppose to separately. Anthelmintics were administered routinely on a whole group basis in most farms, the frequency of which was greater in lambs. Over 45% indicated usually moving lambs to 'clean' grazing after dosing. Over 40% indicated an element of guesswork in determining the weight of lambs for the subsequent calculation of anthelmintic volume to be administered. While 33% (n=48) indicated a belief that some products were not as effective as in the past, only a small number independently sought confirmation of this.

Conclusion: The evidence indicates that there is a need for a greater appreciation of the principles that inform the sustainable use of anthelmintics. It is imperative that practices that preserve anthelmintic efficacy are incorporated in helminth treatment programmes on each farm.

272 **Alternatives to anthelmintics in parasite control in sheep**

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Efforts to curb production losses caused by anthelmintic-resistant parasitic nematodes in sheep have led to the development of a number of alternative control methods, designed to complement or replace anthelmintics. The alternatives can be classed based on their nematode control mechanisms, and include grazing management, diet enrichment, biological control, vaccination, and genetic resistance. Grazing management and mixed stock rotation represents a traditional method for nematode control. While a moderately successful technique, their integration into current intensive single enterprise farming is becoming increasingly difficult. Diet enrichment is an effective, albeit costly, method of controlling parasitism. Enrichment with protein and plant extracts influences the degree of expression of immunity leading to reduced nematode burden in addition to higher weight gain. Biological control mechanisms disrupts the life cycle of the nematode using predacious fungi. Used as a zoo-technical additive in feed, the preying action of the fungus has a nematode reducing capacity in sheep. Vaccination remains the desired alternative to anthelmintics, however, despite considerable research, a viable vaccine has not been developed. Natural resistance to infection is well described, with considerable evidence for within- and between-breed differences in resistance. Adopting resistant breeds or breeding for resistance, represents a reliable and inexpensive complementary control measure to anthelmintics. Genetic loci associated with resistance have been identified, facilitating both selective breeding for resistance and deciphering of the immunological mechanisms of natural resistance. The choice of control measures will depend on the breed in production, presiding climate and the extent of nematode resistance to anthelmintics.

273 **Survey of nematophagous fungi on sheep farms in Ireland**

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Introduction : Nematophagous fungi (predacious and endoparasitic) have shown potential for biological control of sheep parasites. *Duddingtonia flagrans* in particular has shown great potential but has not been documented in Ireland. The main objectives of this study were to describe the most common nematophagous fungi found in a range of habitats on sheep farms in Ireland.

Methods: Leaf litter, soil, fresh and old faecal samples were collected from permanent sheep pasture on 10 farms. All samples were processed using the Baermann and/or baiting method to detect fungi. Fungi that demonstrated nematophagous ability were isolated and identified using available morphological keys.

Results: A total of 29 nematophagous isolates were recovered of which 26 were identified to species. Twelve were predacious and 17 were endoparasitic. Greatest species richness and abundance were observed in soil compared to faecal samples. The most commonly observed predacious species were *Cystopage lateralis* (n=6) and *Stylopaga hadra* (n=4). The most common endoparasitic species were *Drechmeria coniospora* (n= 9) and *Haptoglossa heterospora* (n=6). Four fungal species were detected from fresh faecal samples. *D. flagrans* was recovered from farms.

Conclusion: Permanent sheep pastures harbour a diverse range of nematophagous fungi which have the potential to limit the survival of parasitic nematodes. The survey provides the first evidence of *D. flagrans* in Ireland.

274 **Biological control of gastrointestinal nematodes of goat with a nematophagous fungus- *Dactylaria* sp.**

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Anthelmintic resistance has been realized as a worldwide problem particularly in small ruminants. Nematophagous fungi are emerging as a probable alternatives for the sustainable control of parasitic nematodes.

A study was conducted to assess the effect of a nematophagous fungi- *Dactylaria* sp. in the feces of goat naturally infected with gastrointestinal (GI) nematodes. *Dactylaria* sp. producing constricting rings was isolated from animal dung and conidial spores were produced on corn meal agar (CMA). Number of spores were adjusted to per gram of feces., EPG and number of eggs in the feces, then the samples were mixed properly, cultured and larvae were counted.

When conidial spores were dosed at the rate of 3000, and 6000 spores per gram of feces, variable results were obtained with a significant reduction (p<0.05) of hatched larvae. While adjusting a constant number of spores (3000) to an increasing level of EPG, a directly proportional relationship between the EPG and hatched larvae occurred.

When spores were adjusted to the number of egg, egg to spore ratio of 1:10 drastically lowered the larval population (89.8%) indicating a highly significant reduction of larvae (p<0.01). In conclusion, spores of *Dactylaria* sp. at egg to spore ratio of 1:10 or higher can effectively control the free-living larvae of GI nematodes of goat which include pathogenic genera too.

275 **Efficacy of the nematode-trapping fungi *Duddingtonia flagrans* and *Arthrobotrys robusta* against infections by gastrointestinal nematodes in sheep at pasture**

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The use of nematophagous fungi has the potential to become an important integrated element in sustainable strategies to control parasitic nematodes in livestock. The efficacy of the nematode-trapping fungi *Duddingtonia flagrans* and *Arthrobotrys robusta*, isolated in Brazil, was assessed against infections by trichostrongyle nematodes in sheep throughout six months. Thirty Bergamacia ewes (11-months-old) were divided into three groups, which were kept in separate pastures. Group 1 was the control, not treated, while animals of groups 2 and 3 were fed, respectively, with *D. flagrans* and *A. robusta* twice a week (Tuesdays and Fridays). Pellets were prepared with the fungus mycelia in liquid culture medium and contained approximately 20% fungus. They were mixed with the animals' diet at the concentration of 2 g pellet per 10 kg live weight. Faecal egg counts (FEC), packed cell volume, and the animals' body weight were determined fortnightly from June to November, 2006. Significant differences occurred only between FEC means: control group presented higher mean FEC than group 2 (*D. flagrans*) on July 25, and higher than group 3 (*A. robusta*) on August 8 and 22 ($P < 0.05$). The mean FEC of group 2 was higher than the mean FEC of group 3 on October 17 ($P < 0.05$). The main parasites found in tracer lambs were *Haemonchus contortus* and *Trichostrongylus colubriformis*. Feeding sheep with pellets containing *D. flagrans* or *A. robusta* had only a small benefit to the prophylaxis of nematode infections under the experimental conditions used in the present study.

276 **Development of a PCR diagnostic test for detection of *Duddingtonia flagrans***

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Introduction: The nematophagous fungus, *Duddingtonia flagrans*, is a potential biocontrol agent against nematode parasites of ruminants. Conventional methods of identification have relied chiefly on morphological identification which is laborious and time consuming. In this study, we describe the development of a species specific PCR diagnostic test for the rapid detection of *D. flagrans*.

Methods: Fungal genomic DNA was isolated from sixteen endoparasitic nematophagous fungal species by mechanical disruption and enzymatic digestion. *D. flagrans* species specific primers were designed from the ITS regions. Sensitivity of the PCR test was determined in soil samples spiked with known quantities of *D. flagrans* chlamydospores. The test was subsequently used to confirm the morphological identification of *D. flagrans* found in faecal samples.

Results: Primers designed were specific to *D. flagrans* and sensitive to a concentration of 100 chlamydospores per gram of soil.

Conclusion: The PCR diagnostic test developed in this study provides a rapid, accurate and sensitive test for *D. flagrans* which will enhance the evaluation of this fungus as a nematode biocontrol agent in the field.

277 **Using a conventional nematode egg counting technique for the estimation of *Duddingtonia flagrans* chlamydospores in sheep faeces**

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A technique to quantify the number of chlamydospores in the faeces of sheep dosed orally with *Duddingtonia flagrans* is described. Nine hair sheep (18.5 ± 3.5 kg BW) were dosed orally with *D. flagrans* chlamydospores. Individual daily doses ranged from 19.5×10^6 (lowest) to 177.5×10^6 (highest). Doses were offered on seven consecutive days. Faeces were collected from the rectum of lambs during chlamydospore administration (days 0 to 6) and during six days post-inoculation (days 7 to 11). Mean number of chlamydospores per gram of faeces was determined in 10 samples per animal per day using a McMaster technique. The total amount of faeces was collected for each animal to determine dry matter. Mean number of faecal chlamydospores and the total faecal production were used to calculate the total number of chlamydospores in the faeces per day. The sum of each day resulted in the cumulative number of chlamydospores in the faeces which was highly associated with the dose of chlamydospores offered ($r = 0.97$; $P < 0.0001$). A second trial investigated the effect of different specific gravities (1.27, 1.28, 1.29, 1.30 and 1.40 g/mL) of sugar flotation fluids on the number of chlamydospores counted in sheep faeces. Chlamydospores were dosed on days 0 and 1. Faeces were obtained on days 0, 1 and 2. Ten samples were processed for each flotation fluid every day. Chlamydospore count was the same for flotation fluids from 1.27 to 1.30 g/mL ($P < 0.05$). Density of 1.40 g/mL reduced the number of chlamydospores counted ($P < 0.001$).

278 **FLOTAC, a novel apparatus for a multivalent faecal egg count technique**

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Faecal egg count (FEC) techniques assess the number of parasitic elements (eggs, larvae, oocysts, cysts) present in the faecal samples, expressed per gram of faeces, and are based on the microscopic examination of an aliquot of faecal suspension from a known volume of a faecal sample. In the present paper a novel multivalent FEC technique, the FLOTAC technique, is described. It is based on flotation in centrifuge and translation of the apical portion of the floating suspension, and gives eggs/larvae/oocyst/cyst counts in quantities of faeces up to 1 gram. A novel apparatus, the FLOTAC, has been developed and patented in order to carry out this technique. It is a cylindrical-shaped device, made of unbreakable plastic, and holds two sample flotation chambers, which are 5 ml each for a total volume of 10 ml, with two ruled grids (18 x 18 mm). Each grid contains 12 equidistant ruled lines that are transparent, and hence permit the counting of parasitic elements under them. The FLOTAC apparatus is also very useful in order to recover parasitic elements after flotation.

279 **Phenotyping selection of resistance Pelibuey lambs to the main tropical nematode *Haemonchus contortus*: Preliminary study**

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A study was conducted to assess the resistance of native Pelibuey straightbred sheep to *Haemonchus contortus* in a tropical region in Mexico. Seventy five Pelibuey lambs from 3 to 4 months-old were infected with 350 and 175 infective larvae of *H. contortus* per kg of body weight by oral route, at day zero and 52, respectively. Infected lambs were grazing for 18 weeks into an experimental paddock in a tropical area. Faecal, blood and sera samples were analysed from each animal every week to determine the number of eggs per gram of faeces (EPG), percentage of the packed cell volume and eosinophils. Twenty nine Pelibuey lambs from a 75 sheep flock were identified as resistance to *H. contortus* after the first infection. The number of EPG was around 1424.5 ± 1176.2 with 32% PVC. In contrast, 46 infected lambs were identified as susceptible with EPG media between 2699 ± 1936 and 4322.9 ± 3818.7 with 18 units of PVC less (25%) than the resistance. In addition, more than 7% of eosinophils were observed after the first five weeks of infection in all the experimental groups compared to the control with 3%. The phenotype of native Pelibuey lambs resistant to *Haemonchus* in this study might help to the control of parasitic nematodes in a tropical climate, where anthelmintic problems have been observed. Furthermore, this study might contribute to the identification of the main genes involved in genetic resistance using Pelibuey breed resistant to Haemonchosis, which are highly adapted to tropical regions.

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280 **Copper oxide wire particles as an adjunct in the control of *Haemonchus contortus* in sheep and goats of pastoralist and smallholder farmers in Kenya**

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The utility of copper oxide wire particle (COWP) boluses in reducing *Haemonchus contortus* infections in lambs was assessed on-farm in semi-arid lowland (SAL) and cool humid highland (CHL) areas of Kenya. COWP boluses were administered at 2g (n=25) and 4g (n=25) dosages to Red Maasai and Black Head Somali crosses maintained in the SAL and at a 2g dosage (n =15) to Corriedale crosses in CHL area. COWP boluses were administered in October 2005 just before the start of the 'short rains' season and again in February 2006 before the 'long rains' season. Tissue copper levels in livers of sheep were measured 3 months after the second treatment. COWP 2g administered towards the end of the 'short rains' season resulted in a 60% and 78% faecal egg counts (FEC) reduction in the SAL and CHL respectively, and 82% FEC reduction for the 4g treatment in the SAL. The 4-g treatment maintained a suppressive effect on FEC up to 6 weeks after treatment. COWP 2g treatments given prior to the 'long rains' season resulted in a 68% and 88% FEC reduction in the SAL and CHL areas respectively. Tissue copper levels were significantly lower in the semi-arid lowland compared to the cool highlands but the levels in both areas were within the normal range of copper in livers of healthy sheep. COWP boluses have the potential to be used as a dewormer by small-holder and pastoralists farmers, as an adjunct in the control of *H. contortus* in sheep.

281 **Copper oxide wire particles as an adjunct in the control of *Haemonchus contortus* in goats of resource-poor farmers in South Africa**

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To assess the anthelmintic efficacy of copper oxide wire particle (COWP) boluses against *Haemonchus contortus* in goats, 19 male indigenous faecal-egg-count-negative Zulu goats, constituting an established infection group, were each given ~1200 infective larvae of *H. contortus* 3 times per week during weeks 1-2 of the experiment. At the beginning of week 7, 6 goats received a 2g-COWP bolus; 7 goats received a 4g-COWP bolus and 6 animals were not treated. A further 20 goats made up a developing infection group. At the start of week 1, 7 of these goats were given a 2g-COWP bolus; 7 goats received a 4g-COWP bolus and 6 animals were left untreated. Each of these goats were given ~400 *H. contortus* larvae 3 times per week during weeks 1-6. In week 11, all 39 goats were slaughtered for worm recovery from the abomasa and small intestines. The 2g- and 4g-COWP boluses were both 94% effective in reducing the worm burdens of established *H. contortus* infections when compared with controls (having mean burdens of 441 worms). In the developing infection group, the 2g-COWP treatment was ineffective but the 4g-COWP treatment was 62% effective in reducing worm burdens relative to controls (having mean burdens of 1050 worms). COWP boluses, which lend themselves to local manufacture by resource-poor farmers, have the potential to be used to control anthelmintic-resistant *H. contortus* in goats.

282 **In vitro predatory activity of “cannibal” nematodes against *Haemonchus contortus* and *Rhabditis* sp.**

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Nematodiasis reduce the potential productions of sheep, affecting the worldwide production. Anthelmintics reduce such parasitosis; however, anthelmintic resistance dramatically diminishes their efficacy. New alternatives of control have been explored; such as the natural antagonists of nematodes, ie nematode-predatory nematodes “Cannibal nematodes”(CN). Predatory nematodes (PN) feed on other nematodes and they could have an implication in the control of animal parasitic nematodes (APN). This research was aimed to evaluate the in vitro predatory activity of a miscellaneous population of PN (*Mononchs*) against *Haemonchus contortus* infective larvae (L3) and nematodes of the free-living nematode *Rhabditis* sp. The assay was carried out using ninety six well tissue culture plates, where *H. contortus* (L3) and *Rhabditis* sp. nematodes were separately exposed to a mixed PN population. Proper controls were considered. Plates were kept for seven days in the lab at 25-30°C. Nematodes were counted after confrontation in the plates and the means between groups were compared using the ANOVA test. Evidence of the predatory activity of CN against *H. contortus* (L3) was observed and then video recorded. The PN showed a low predatory activity against *H. contortus* (4–15%). In contrast, predation against *Rhabditis* sp. was higher (56–77%). The predation of CN population against both nematodes is discussed. The apparent selective predation should be deeply studied considering the different predatory genera. The evaluation of individual specie of CN against either L3 or other stages of *H. contortus* and other APN is proposed.

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283 **Move-than-treat: a new pasture rotation management strategy as an aid to prevent parasite resistance in calves**

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Gastrointestinal parasites cause a serious economical impact to the cattle industry with different clinical manifestations depending on the infection degree and the age of the animal. The objective of this work was to correlate parasitological data and pasture management in 110 naturally infected five-months old calves from March to June 2004 under subtropical conditions. Climate data, eggs per gram of faeces (EPG), fecal culture and the number of larvae on pasture were recorded every 15 days. The animals were kept on a single pasture that had different topography and were moved to a prepared worm-free paddock 4 weeks before the end of the experiment. The EPG showed an increased during the study and coproculture revealed *Trichostrongylus* spp. (60%) and *Cooperia* spp. (40%). Rain and temperature were favorable to larvae survival and development on pasture. The sub-area with the highest larvae count (2280/k/DM) at pasture 1 had a higher concentration of “capim arroz” (*Echinochloa* spp.). The animals were moved to paddock 2 without receiving anthelmintic treatment with an EPG of 1545. Two weeks later, the EPG was 2200 and larvae counts were 3960/k/DM indicating a rapid infestation of the new area. The data suggests that in optimal climatic conditions there is an almost immediate pasture contamination after non-treated animals are transferred to a worm-free pasture depicting the temporary benefit of maintaining a rested pasture. The adoption of this strategy would directly influence the rate of parasite resistance allowing a refugia population to become stable on the new area prior to selection.

284 **Analysis of systemic and mucosal responses to *Teladorsagia circumcincta* in immune and naïve sheep**

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Teladorsagia circumcincta is a highly pathogenic nematode which resides in the abomasum and is the most parasitic nematode in sheep in the UK. It primarily affects young lambs during their first grazing season and is the primary agent involved in parasitic gastroenteritis. The routine treatment for such an infection is the administration of anthelmintic; however, drug resistance is widespread and increasing. Although adult sheep can cycle the infection, especially during they periparturient period, they are not affected clinically and acquired immunity develops following natural and experimental infection. Sheep mount immune responses to various developmental stages of the parasite which result in exclusion of parasites from the abomasal mucosa, parasite stunting and reduced fecundity. Local immune responses are particularly important in establishing immunity however there is a limited amount of information available on the precise responses that mediate larval exclusion. To further understand which local innate, humoral and cellular responses underlie protective immunity in this system, we have developed a model to study an array of responses in sheep undergoing larval exclusion. Responses in these animals have been compared to those of young lambs undergoing a primary challenge infection with *T. circumcincta*. The results of the downstream immunological assays are reported in this presentation.

285 **Identification of antigens from *Teladorsagia circumcincta***

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IgE production is a major component of the Th2 response typically observed during helminth infection however its role in *T. circumcincta* infection is poorly understood. Previous studies have demonstrated an increase in total IgE levels in serum and lymph following challenge of previously infected animals of which a proportion was found to be specific to L3 antigens. This study was designed to identify targets of the IgE immune response from L3 using a combination of 2D PAGE/immunoblotting, tandem mass spectrometry and MASCOT searches. From western blots a panel of 21 IgE reactive spots were selected for further analysis. Identities were obtained for 17 of the spots which resolved to 9 proteins; paramyosin, HSP70, intermediate filament protein B, propionyl coenzyme A carboxylase, putative calponin like protein, tropomyosin, disorganized muscle family member, galectin and a prohibitin-like molecule. Of these 9 proteins only galectin has been previously identified in *T. circumcincta*. Both paramyosin and tropomyosin have been implicated as major immunogenic proteins in other helminths while galectin appears to be a major IgE-reactive antigen in *Onchocerca volvulus*. Our work is now focussed on investigating the interactions between these antigens and the immune response, in particular the MHC, in more detail.

286 **The acute phase protein response to *Teladorsagia circumcincta***

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Acute phase proteins are produced, predominantly by the liver, in response to the pro-inflammatory cytokines IL-1, IL-6 and TNF- α . The acute phase proteins are thought to have a role in innate immunity to a variety of infections. There are conflicting reports as to the presence or absence of a systemic acute phase response in gastrointestinal parasite infection. A number of acute phase proteins have also been shown in other species to be produced extra-hepatically by specific cell types, including intestinal epithelial cells. The principal ruminant acute phase proteins are haptoglobin, serum amyloid A and α -1 acid glycoprotein.

The systemic acute phase response of sheep infected experimentally with *Teladorsagia circumcincta* was investigated by means of biochemical and immunological assays, analysing both blood and gastric lymph from animals undergoing experimental infection. mRNA extracted from the abomasal mucosa of infected animals was analysed by rtPCR for evidence of extra-hepatic production of these proteins within the local tissues. The systemic acute phase response to experimental infection with *T. circumcincta* was minimal, although there was some evidence of local production.

287 **The pathogenicity of nematodiasis in sheep and goats in Pakistan: 1, Parasitological, haematological and clinical findings**

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The response of Bulkhi sheep and local hairy goats of Pakistan to primary infection with prevalent of *Haemonchus contorts*, *Trichostrongylus spp.* and *Oesophagostomum spp.* was compared by examining their worm burden, haematological parameters and pathogenic effects of nematode parasites on these hosts following experimental infection. The results showed that the goats had lower worm burdens and had suffered less severe haematological and clinical disturbance than the Bulkhi sheep. The overall worm counts in sheep were higher than in goats but the count of mucosal phase of larvae, RBC, PCV and level of haemoglobin (Hb) was higher in goats. These differences are possibly immunologic in origin through a genetic resistance and mode of nutrition like browsing (goats) and grazing (sheep) may operate, primarily at level of worm establishment. It can be concluded that pathophysiological changes are correlated with parasitological and clinical observations in the two host species.

288 **Analysis of MHC class II genes in Scottish blackface sheep**

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The class II molecules of the MHC play a central role in presentation of parasite molecules to cells of the immune system. In sheep the DRB1 locus in the class II region of the MHC has been repeatedly associated with resistance to nematode infection but other class II genes could be responsible for the disease association. As a result it is imperative that the polymorphisms of these genes are studied in detail. Variation in the DRB1, DQA1, DQA2 and DQA2-like genes of the Scottish Blackface breed was investigated in 111 rams from 11 different farms involved in the Blackface Elite Sire reference scheme. Direct sequencing of exon 2 (containing the most polymorphic region of the genes) was possible for DQA1 and DRB1 allowing relatively easy allele assignment. This proved more difficult for DQA2 due to the presence of duplicated genes on some haplotypes and a missing codon in two of the known alleles. Cloning and sequencing of DQA2 PCR products resolved this problem and in addition led to the identification of 3 new potential alleles. DRB1 and DQA1 had 21 and 8 alleles respectively. The most common allele in DQA1 was a null allele. The null allele was in linkage disequilibrium with both the resistant (G2) and susceptible (I) alleles of DRB1 suggesting that DRB1 is more likely to be the causative locus than DQA1.

289 **Eosinophil - *Haemonchus contortus* infective larvae interaction: demonstration of larval killing after intra-abomasal infection**

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Introduction. Different authors reported that eosinophils are capable of immobilising infective larvae of nematodes in vitro. However, classifying larvae as mobile or immobile is so subjective that it does not always mean all apparently immobile larvae are dead or those that are mobile are capable of surviving further immune responses if administered to their natural hosts. **Material and methods.** The establishment potential of *Haemonchus contortus* infective larvae was compared between larvae, which had been incubated with eosinophil-enriched cell suspensions and control larvae. As it was not possible to isolate pure eosinophils from sheep blood, we were compelled to evaluate the effects of other blood cells contaminating our eosinophil-enriched suspensions. **Results.** Although, eosinophils and neutrophils were the only cells found adherent to *H. contortus* infective larvae in vitro, eosinophils in the presence of immune serum were primarily responsible for the drastic reduction in larval motility compared to the minor effects of neutrophils and mononuclear cells. Corresponding reductions in faecal egg count and worm numbers were observed when the incubated larvae were transferred into abomasum of sheep. Interestingly, the proportion of larvae that failed to establish was much higher following incubation with induced eosinophils compared with other cells or with immune serum alone. **Conclusion.** Although this study did not address the in vivo role of eosinophils in sheep, the results strongly indicate that sheep blood eosinophils have a larval killing potential in vitro, and a larval mobility test alone may not fully explain the level of damage inflicted on the larvae

290 **Relationship between abomasal histology and parasitological parameters following *Haemonchus contortus* infection in two sheep breeds native to the Canary Islands**

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Introduction: Comparative studies to *Haemonchus contortus* infection in two breeds of sheep native to the Canary islands, the Canaria Hair Breed sheep and the Canaria sheep, have shown the Canaria Hair Breed has an innate resistance to *H. contortus* infection. The aim of this study was to evaluate whether correlations existed between immune cell abomasal populations and haematological and parasitological parameters in these sheep breeds. **Material and methods:** Thirteen Canaria Hair Breed sheep and fourteen Canaria sheep were experimentally infected with 20,000 *H. contortus* parasites. Animals of each breed were killed on day 7 and 28 post-infection. Samples from abomasums for histological analyses were collected and stained with haematoxylin-eosin and Giemsa for tissue cell determination. Faeces and blood were collected weekly before, during and at the time of slaughter. **Results:** Worm burden were positively correlated with faecal egg counts ($r = 0.796$) and *H. contortus* worm length ($r = 0.662$) but negatively correlated with packed cell volume ($r = -0.793$) for both breeds of sheep. No differences were detected in abomasal mast cell counts at 7 and 28 d.p.i. between breeds. Interestingly, the relationship between quantified abomasal tissue eosinophils and faecal egg counts differed between both breeds of sheep; being significantly negatively correlated ($r = -0.857$) only in the Canaria Hair Breed sheep at 28 days post infection. **Conclusions:** Tissue eosinophils at 28 d.p.i. may modulate egg production in Canaria Hair Breed sheep but whether tissue eosinophils are associated with resistance to parasite infection in this breed of sheep remains to be determined.

291 **Comparison of different antigens for the diagnosis of *Haemonchus contortus* in experimentally infected sheep by western blotting**

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In the present study peptide bands of 5 different antigens consisting intestine, uterus, cuticle, whole male and whole female crud antigens of *Haemonchus contortus* were prepared and examined using SDS-PAGE and western blotting. Experimental infection was carry out in 5 lambs with administered 10,000 third stage larvae of *H. contortus* and 2 lambs parasite free were used as control. After the confirmation of infection by faecal examination positive and negative sera were collected and for immunodiagnostic antigens were tested by western blotting. In electrophoretic patterns of different antigens was detected presence of 7, 2, 6, 8 and 5 major peptid bands at molecular weights of at 15 to 110 kDa in uterus, intestine, cuticle, whole male and whole female crud antigens of *H. contortus*, respectively. The protein bands obtained by immunoblotting from positive and negative sera were compared and the molecular weight of specific protein bands for haemonchosis in sheep were determined. Two major peptide bands of 35 and 40 kDa molecular weight in intestine and uterus antigens respectively, were specified for diagnose of infection with *Haemonchus contortus*.

Based on our experience we suggested the used from these antigens with different molecular weight antigen for immunodiagnosis of haemonchosis in sheep as a primary screening test.

292 **Quantification of *Bos indicus* cytokines mRNA levels and its relationship with the cellular and humoral responses in infections by *Haemonchus placei***

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This study was carried out to evaluate the immunological response and cytokines gene expression analysis to *Haemonchus placei* infections in Nelore cattle. One hundred weaned, male cattle, initially 11-12 months old were evaluated. Faecal and blood samples were collected for parasitological and immunological exams. The 7 most resistant and the 8 most susceptible animals were selected based on their individual averages of eggs per gram (EPG) of faeces. Abomasum tissue samples were collected for histological quantification of inflammatory cells and cytokines gene expression analysis (IL-2, IL-4, IL-8, IL12p35, IL-13, TNF-alpha, IFN-gamma, MCP-1, MCP-2, MUC-1) by real-time RT-PCR methodology. The gene expression analysis showed that TH2 cytokines (IL-4 and IL-13) in resistant group and TH1 cytokines in susceptible one (TNF-alpha) were both upregulated. The relationships among *H. placei* worm burden were significative for mast cells, eosinophils, IgG1 against anti-*H. placei* (adult), mucosal IgA anti-*H. placei* (larvae), IL-13 and TNF-alpha. IL-13 was significantly correlated with IL4 ($r = 0,62$) and with TNF-alpha ($r = -0,59$), and this last one with MCP-1 ($r = -0,59$). In resistant group the number of mast cells, eosinophils and IL-13 were negatively correlated ($r = -0.85$, $r = -0.89$ and $r = -0.77$) with *H. placei* worm burden and in the susceptible to IgA anti-*H. placei*-larvae ($r = -0.81$). These results taken together provide evidence to an immune response against *H. placei* mediated by TH2 cytokines in resistant group and TH1 in susceptible one.

293 **Detection of circulating and purified *Cysticercus tenuicollis* antigens in experimental and natural infections of sheep with cysticercosis**

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Detection of circulating and affinity purified *Cysticercus tenuicollis* antigens were performed in sera of sheep with experimental and natural of *C. tenuicollis* infections using ELISA. A higher sensitivity was obtained with circulating antigen than that obtained by the purified antigen. P. M. examination to a total number of 120 sheep slaughtered at Cairo abattoir for collection of its serum and also for the occurrence of *C. tenuicollis*. Serological examination of 120 sheep serum samples were done using ELISA. The results of serological examination showed that 51 (42.5%) and 40 (33.3%) samples were seropositive for *C. tenuicollis* by using circulating and purified antigens, respectively. 33 sheep from 120 PM examined ones naturally infected with *C. tenuicollis* in peritoneum and liver during PM examination. The highly degree of infection occurred in 30,20 samples while 15 and 12 samples showed moderately infection and 6,8 samples showed highly infection by using circulating and purified antigens respectively. The positive O.D. values of the investigated samples were classified into three categories high, moderate and low. Ammonium sulphate precipitate was done to the three groups of samples after using in western blot technique. In three serum samples from *C. tenuicollis* showed specific reactivity with band at 34 kDa by western blot. While, the low and moderate both level of antibodies showed a faint band at 26.5 kDa. At the same time the higher optical density values against *C. tenuicollis* showed three reactive bands at 34, 26.5 and 18.5 kDa. in western blot.

294 **Validation of an indirect ELISA for the diagnosis of the infection by *Teladorsagia circumcincta* in sheep**

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The objective of this study has been to find a suitable ELISA technique to monitor IgA activity during infection by *Teladorsagia circumcincta* in sheep. At present there is no a standard ELISA method to quantify the antibody response against nematodes. In previous studies we have confirmed the importance of the IgA in the resistance of the infection by *T. circumcincta* in sheep. Previously we designed a protocol to measure the optical density in infected animals and in this assay the ELISA has been validated to demonstrate its immunodiagnostic ability.

IgA level was tested with an indirect ELISA against a fragment of a recombinant Tc-PDI (protein disulphide isomerase from *T. circumcincta*). Positive and negative serum samples were obtained from experimentally infected sheep and from uninfected sheep (control).

The validation results showed coefficients of variation for 5 different serum samples which were from 2.3% to 14.4% for intraassay variability and 15.8, 10.1, 11.5, 12.2, 8.1% for interassay variability. The detection limit was established as the mean of 50 negative sample + 2 standard deviations. Accuracy was studied by the evaluation of linearity and parallelism. The coefficients of variation for the linearity were acceptable since were within the range of $\pm 10\%$ (90.1 - 107.5%); the parallelism was also demonstrated comparing the slopes of the curves. ELISA was tested with serums from experimentally *Fasciola hepatica* infected sheep and there was not found any cross reaction.

In conclusion, this indirect ELISA is a useful technique to quantify IgA's sheep in experimental as well as in natural infection.

295 **Species-specific PCR for differential detection of the gastro-intestinal nematode *Ashworthius sidemi***

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The blood-sucking gastro-intestinal (GI) nematode *Ashworthius sidemi* is present in deer populations and herds of *Bison bonasus* in Poland. The parasite is considered to be as pathogenic as *Haemonchus sp.*, consequently making it a potentially important pathogen in wildlife and domesticated animals. Coproscopical examination of eggs and infective larvae is not sufficiently accurate to differentiate *A. sidemi* from other common GI nematodes in ruminants. Furthermore, presence of *A. sidemi* has been reported from a number of neighboring countries. Taking into account the migratory behavior of deer and lack of reliable diagnostic tests it is plausible to expect that *A. sidemi* is an underreported parasite in wild and domesticated animals in Europe.

In order to accommodate proper differentiation, we developed a species-specific PCR method for the detection of *A. sidemi* DNA. Initially, a fragment spanning from the 3'-end of *ssrRNA* to the 5'-end of *lsrRNA* genes was amplified and sequenced (Genbank accession: EF467325).

Based upon the derived sequence information, species-specific primers were designed. These are directed to unique sequences in the internal transcribed spacer (ITS)1 and ITS2 generating a 406 bp fragment. The specificity of the designed primer-pair was confirmed using template DNA from closely related nematodes in the family Haemonchidae and DNA isolated from hosts. The highest achieved sensitivity in heterologous DNA samples resulting in positive PCR runs was 10 pg genomic DNA. In conclusion, we have developed a molecular tool that provides species-specific identification of *A. sidemi*. The achieved levels of specificity and sensitivity are adequate for routine diagnosis and epidemiological surveys of the parasite.

296 **Use of Bayesian MCMC to improve analysis of faecal egg count data**

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Aims: To analyse the distribution of faecal egg counts in a population using Bayesian MCMC, so that resistant animals, effective nematode control strategies and anthelmintic resistance can be more readily identified. **Methods:** A zero-inflated negative binomial model has been developed using Bayesian MCMC, that assumes each count to come from a population-wide distribution. The analysis produces a distribution of the possible values for the mean egg count, overdispersion within the group, and prevalence of infection. With modification, it can also be applied to faecal egg count reduction test data to give a distribution of the possible individual animal and group reductions, and to multiple samples to identify genetic factors for nematode resistance. The size and shape of the distribution is dependant on the evidence available in the data, and is not limited to a symmetric pattern. **Conclusions:** By using a population modelling approach, more information can be extracted from the data than by using group means alone. A Bayesian modelling technique such as this also provides a useful indication of the degree of certainty in the results. The application of these modelling techniques in order to better understand the distribution of egg counts within a population would be of benefit to parasitologists in identifying a genetic component of nematode resistance, influencing nematode control strategies and improving the detection of anthelmintic resistance. **Acknowledgements:** This project was carried out as part of the DEFRA funded VTRI project 0101.

297 **Comparison of plasma pharmacokinetic profile of ivermectin following administration of subcutaneous injection (baymec®) and oral tablet (efektin®) in goats**

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Introduction: Ivermectin (IVM), a member of avermectins, has been used intensively to control parasites in animals and humans worldwide. IVM is available as oral, subcutaneous and topical formulations in the market. The pharmacokinetic behaviour of IVM is significantly affected by route of administration and formulation of the drug. This study evaluates the comparative plasma disposition of IVM following subcutaneous and oral tablet formulation in goats.

Materials and methods: Ten goats were allocated into 2 groups (subcutaneous group and oral tablet group) of five animals each. The animals in Group I received subcutaneously injectable solutions (Baymec®, Bayer) and the animals in the other group received oral the tablet formulation (Efektin®, Sanovel) at the dose of 200 µg/kg bodyweight. Blood samples were collected between 1 hour and 40 days after treatment and the plasma samples were analysed by high performance liquid chromatography (HPLC).

Results: The results indicated that IVM subcutaneous administration produced a significantly higher maximum plasma concentration (C_{max} : 18.16±3.69 ng/ml) with slower absorption (t_{max} : 0.88±0.16 day) and larger area under the concentration vs. time curve (AUC: 44.27±12.55 ng.d/ml) as compared with oral tablet administration (C_{max} : 10.33±4.10 ng/ml, t_{max} : 0.57±0.19 day, AUC: 12.18±7.64 ng.d/ml). Furthermore the terminal half-lives ($t_{1/2z}$) and mean residence times (MRT) also significantly longer in subcutaneous group compared to oral tablet group.

Conclusion: Considering the pharmacokinetic parameters, after oral tablet administration of IVM produced lower bioavailability and shorter persistence than after subcutaneous administration in goats. Higher dosage regime or more often treatment of IVM could be required for oral tablet administration compared with the subcutaneous administration.

298 **Effect of pregnancy on the pharmacokinetic of ivermectin in sheep**

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In spite of the potential use of ivermectin during the last three months of gestation in ewes, there is no information available on its pharmacokinetic behavior in pregnant sheep. Such studies are necessary because the drug's disposition might be altered by the gestation.

The plasma kinetic profile of ivermectin in ewes at the last third of pregnancy was studied after a single subcutaneous administration of 0.2 mg/kg body weight (bw). Two groups of equally balanced in body weight sheep were used for the study. Group I (Control) was maintained unmated; while Group II (pregnant) was estrous synchronized and mated with fertile rams. Both groups were maintained stabled under similar conditions of management and feeding. At 120 days of pregnancy of group II ewes, both groups were treated by a subcutaneous injection of 0.2 mg/kg of ivermectin bw. Blood samples were taken by jugular puncture at different set times between 1h and 40 days post-treatment. After plasma extraction and derivatization, samples were analyzed by high performance liquid chromatography (HPLC) with fluorescence detection. A computerised kinetic analysis was performed and the data were compared using the non-paired Student's *t* test.

Ivermectin was detected in plasma between 1h and either day 25 (control) or day 40 (pregnant) post-treatment. The mean values of pharmacokinetic parameters C_{max} , T_{max} and area under de concentration/time curve (AUC) were similar for both groups of sheep. However, the average of half life of elimination was significantly higher ($P < 0.05$) in the pregnant group (4.4 ± 1.7 days) than in control nongravid (2.1 ± 0.7 days) sheep. Similarly, the mean residence time (MRT) values of pregnant group (8.5 ± 1.1 days) was higher ($P < 0.05$) than those observed in control group (5.3 ± 1.0 days). It is concluded that pregnancy through the changes induced on the maternal physiology delay the permanence of ivermectin in the body of pregnant sheep when it is administrated by subcutaneous route.

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299 **Efficacy and persistent prophylactic efficacy of long-acting moxidectin (inj. sol. 2%) for control of Trichostrongylid infections of sheep**

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Introduction. Objective was to evaluate long-acting moxidectin (CYDECTIN® 2% LA for sheep) against Trichostrongylids in sheep. We performed a blocked, parallel, controlled clinical trial.

Materials and methods. We included 45 ewe-lambs, allocated into Treated (n=30, 1.0 mg moxidectin per kg bw, subcutaneously at base of ear on D0) or Controls (n=15). Animals had been naturally infected (pre-treatment geometric mean epg counts: 233 and 249 respectively) and grazed at same pasture. Fortnightly, we performed established parasitological techniques (epg counts, coprocultures). We used analysis of covariance for post-treatment results, using pre-treatment counts as covariate, treatment as fixed effect, block as random effect.

Results. Geometric mean epg counts in Treated animals were 0 up to D112, 1 on D126, 4 on D140 and 13 on D161. Respective figures for Controls were 369-820 up to D140 and 168 on D161 (p<0.05). In coprocultures, *Teladorsagia*, *Haemonchus*, *Trichostrongylus*, *Cooperia*, *Chabertia* and *Bunostomum / Oesophagostomum spp.* larvae were identified. Overall efficacy of test product was >99% up to D140 and >92% on D161. Minimum persistent prophylactic efficacy (>90%) lasted 140 d for *Teladorsagia*, 119 d for *Haemonchus*, 115 d for *Trichostrongylus*, 72 d for *Cooperia* and 52 d for *Bunostomum / Oesophagostomum spp.*

Conclusions. This is the first reported clinical trial of efficacy of long-acting moxidectin in sheep in Europe. CYDECTIN 2% LA is clearly effective against trichostrongylids of sheep. The particularly long persistent efficacy will provide new possibilities in management of anthelmintic programs for sheep.

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300 **Reproductive benefits in ewe-lambs after administration of long-acting moxidectin (inj. sol. 2%) before mating**

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Introduction. We studied the reproductive consequences of a long-acting antiparasitic (moxidectin; CYDECTIN 2% LA for sheep) given to ewe-lambs before puberty.

Materials and methods. We allocated 45 ewe-lambs (mean age: 5.2 months), into treated (n= 30, treatment on D0, 21 June) or untreated (n=15). Animals had been naturally parasitized, with mean pre-treatment epg counts: 278 and 300 respectively. Three rams of confirmed fertility, were introduced into ewes from 15 August to 15 November. Throughout the study (performed at latitude N 36° 26', in a flock free from brucellosis, *Chlamydia* infection and toxoplasmosis), we monitored parasitological findings (epg counts) and we assessed reproductive performance.

Results. Initially (up to D112) mean epg counts in treated animals were 0; then (up to D224), they were 23-195. Respective figures for controls were initially (up to D112) 480-833 and then (up to D224), 170-470. Reproductive performance parameters for treated and control animals respectively, as follows; days to first mating after ram introduction: 36 d and 46 d (p=0.148); cycling rate: 20% and 7% (p=0.189); mating rate: 77% and 53% (p=0.214); return-to-oestrus rate: 23% and 27% (p=0.815); lambing rate: 73% and 53% (p=0.214); total lambs per ewe: 1.5 and 1.0 (p=0.005); lamb bodyweight per ewe: 5.1 kg and 3.9 kg (p=0.02).

Conclusions. Administration of a long-acting antiparasitic protected efficiently ewe-lambs. Consequently, these showed earlier reproductive activity than controls. Treated ewes also showed a significantly better reproductive performance by producing more and heavier lambs. The particularly long persistent efficacy provides new possibilities in management of sheep flocks.

301 **Effect of gastrointestinal parasitism on the patterns of moxidectin tissue residue depletion, in sheep**

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The effect of gastrointestinal parasitism on the patterns of the edible tissue depletion of moxidectin (MXD) was studied in two groups of lambs. Twenty-four parasitised Suffolk down lambs (20.1 ± 1.0 kg, body weight: bw) were selected. Twelve pairs of lambs were allocated into two groups equally balanced in bw. Group I (non-parasitised) was pre-treated with three repeated administrations of 5 mg/kg of fenbedazole (FBZ) to maintain an healthy condition. While in Group II (Parasitised), the lambs did not received anthelmintic treatment. After a 70 days period, both groups were treated with a subcutaneous (SC) dose of 0.2 mg/kg b.w. of MXD. The animals were slaughtered in groups of 3 at: 7, 14, 21 and 28 days post MXD administration. Samples of the target tissues (liver, kidney, muscle and fat) were obtained from each animal and frozen at -18°C until analysis. Tissue samples were analyzed by HPLC with fluorescence detection after solid phase extraction. The concentrations obtained for each tissue were processed statistically using a computer program based on the EMEA guidelines for estimation of withdrawal periods (WP). Results were compared by a un-paired Student's t-test.

In Group I, FBZ administration increased significantly the bw of lambs in comparison to the parasitised group. After MXD administration, the drug was detected in all of the tissues analyzed up 28 days post-treatment. In both groups, higher concentrations of MXD were obtained in adipose tissue. Higher and more persistent concentrations of MXD were observed in the tissues of non-parasitised group, differences that were significant ($P < 0.05$) in comparison to those observed in parasitised lambs. Taking in consideration the Maximum Residue Limits (MRL) established for MXD of 500 ug/kg in fat (EMA), the calculated WP for the group of healthy lambs (29 days) was higher than that obtained in the parasitised group (25 days). In conclusion, gastrointestinal parasitism through the reduction in body weight gain of lambs significantly reduces the tissue distribution and persistence of MXD. Consequently, the calculated WP of MXD is shorter in parasitised than healthy lambs.

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302 **Effect of multiple anthelmintic drugs on live weight gain in Awassi sheep in subtropical region of Pakistan**

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Parasitism is a major cause of hindering the livestock development in Pakistan. The agro-ecological and geo-climatic condition of Pakistan favors the high prevalence of parasitic infestation. Sheep suffer with heavier worm burdens than goat because of the difference in their grazing habits. Sixty Awassi ewes were randomly allocated, based on faecal egg count, to four groups having 15 animals each. Group 1 was served as control (untreated). Group 2 was treated with 0.75 mg/lb of body weight albendazole (oral drench), Group 3 was treated with combination of 1ml/50 lb levamisole and 0.5ml/55lb of body weight ivermectin (injectable). Group 4 was treated with 1ml / 110 lb of body weight (injectable). Body weight was taken on the basis of pre treatment and post treatment of experimental period. The mean body weight gain after treatment with albendazole, levamisole + Ivermectin, Ivermectin were 7.6%, 11% and 15% respectively. It was observed that parasitic infection might be responsible to arrest the growth. A number of body weight was increased may be due to removal of parasitic load might have had facilitate the weight regain through proper digestion, absorption and metabolism of feed nutrient in the parasite free gastro-intestinal tract.

303 **Efficacy of albendazole against *Coenurus cerebralis* in goats**

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Introduction: Coenurosis is a parasitic disease which affect sheep and goats worldwide. In order to evaluate alternative treatment options the efficacy of albendazole against *Coenurus cerebralis* was tested in goats.

Methodology: Thirteen experimental goats were infected with approximately 3000 eggs of *Taenia multiceps* and distributed into two groups (8 treated and 5 control). Albendazole was given orally two months after infection at the doses rate of 10 mg/kg/day during 3 days. Efficacy was evaluated fifteen days after drug administration by postmortem examination of all animals by protoscolex viability analysis¹. To compare the viability between groups a statistical χ^2 -square test were applied considering $p < 0.05$ as significant.

Results: Total recovery cysts were 58, from that 2 (3.4%) were found in brain and the rest in muscular and subcutaneous tissue. Fifty four cyst (31 from treated and 23 from group) were tested for viability. Protoscolex from treated animals were not viable in 90.3% (28/31) while from control animals were not viable in 13% (3/23) from the control group. There was a significant difference in viability from the treated and control groups.

Conclusion: The results presented herein indicate that albendazole is effective against two months cyst of *C. cerebralis* located in muscular tissue.

1. Casado, N., Rodrigues Caabeiro, F., Hernández, S. 1986. In vitro survival of Echinococcus granulosus protoscoleces. International Journal for Parasitology. 19, 945-947

304 **Influence of pasture type and sheep breed on gastrointestinal parasitism in the Brazilian Savannah**

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This study aimed to evaluate the infection by gastrointestinal parasites in sheep in two sheep breeds (Santa Inês and Bergamácia) on two cultivated pastures (*Andropogon gayanus* and *Panicum maximum* cv Vencedor), in the savannah region of Brazil. Sixty (30 of each breed) adult non lactating ewes were used with a mean live weight of $46,2 \pm 2,6$ kg, distributed equally in two paddocks of 1 hectare of each pasture. The animals were dewormed at the start of the experiment. The experimental period was 4 months in the rainy season. Faeces was collected every fortnight for worm eggs and *Eimeria* sp. count. The *Strongyloides* sp. count was affected by forage, breed and interaction between them, with the lowest infection in Santa Inês on *Andropogon gayanus*. The others species found (*Strongyloidea*, *Moniezia expansa* and *Eimeria* sp.) were not affected by any of the factors studied, suggesting that infection by *Strongyloides* sp. in sheep may be better controlled in Santa Inês on *Andropogon* pasture.

305 **Population dynamics of gastrointestinal nematodes in organic sheep farms in Canada – first year**

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Introduction: Little is known about the epidemiology of gastrointestinal nematodes in sheep in Canada and therefore how they can best be controlled. This research focuses on a three-year epidemiological study to monitor the levels of GIN in organic flocks as well as studying farm management practices that can be used to minimise parasite burdens.

Method: The first year of this observational study started in spring 2006 on 32 farms; on each farm groups of 10 ewes and 10 lambs were sampled and evaluated every month (blood samples only at the beginning and August); pasture samples were also collected monthly; questionnaires were administered on each farm to collect information on every aspect of flock management practices.

Materials: Parameters used were faecal egg counts, pasture larval counts, faecal cultures, worm burden of sentinel animals, PCV and TP, and animal scores (body, dag, faecal consistency, and FAMACHA)

Results: Large variability in faecal egg counts and pasture infectivity were observed amongst the farms. Larval overwintering on pasture was detected in few cases. A third of the farms had to treat the flocks in mid or late summer due to clinical parasitosis. However, blood values were found to be within normal parameters.

Conclusion: Research over the next two years will help to understand the factors associated with flock management in organic farms that contribute to lessen or worsen the incidence of GIN. Data generated will be used to develop models that can be used to predict the efficacy of different control programs on parasite control.

306 **Cross-sectional surveys on helminth infections of goats in Bali, Indonesia**

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Background: Goats mainly kept for meat skewer production are traditionally pastured by day and housed at night in Bali. Knowledge on their helminth fauna is scanty.

Material & Methods: Two cross-sectional surveys were performed to obtain first substantial data on helminth infections of goats in Bali. In the first survey (dry season, August 2005), 50 goats of two age groups from different villages were necropsied. The gastrointestinal tract, liver, pancreas and lungs were examined for luminal helminth stages. In the second survey (end of rainy season, March-April 2006), the abomasum of 31 adult goats were examined for *Haemonchus contortus*.

Results: In the first survey (dry season) the following helminths were identified: *Fasciola gigantica* (young goats 16%, adults 16%; 4–29 worms), *paramphistomid species* (12%, 20%; 7–1450 worms), *Moniezia expansa* (12%, 0%; 3–8 worms), *Haemonchus contortus* (72%, 44%; 10–280 worms), *Trichostrongylus axei* (32%, 25%; 10–1670 worms), *T. colubriformis* (72%, 64%; 10–2770 worms), *Gaigeria pachyscelis* (8%, 16%; 10–40 worms), *Oesophagostomum columbianum* (20%, 20%; 10–50 worms), *Oes. venulosum* (12%, 20%; 10–160 worms), *Strongyloides papillosus* (52%, 56%; 10–1110 worms), *Trichuris spp.* (40%, 4%; 10–1300 worms), and *Capillaria bovis* (64%, 56%; 10–170 worms). Infections with other helminth species including lungworms were not detected in any animal. In the second survey (rainy season), infections with *H. contortus* were detected in 48% of the adult goats (10–330 worms).

Conclusions: Goats in Bali harbour rather low helminth infections.

307 **The first record of the nematode *Ashworthius sidemi* in Sweden**

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Ashworthius sidemi is a blood sucking nematode in the abomasum of a wide range of ruminants. Originally it was known only from sika deer *Cervus nippon* in East Asia, which serves as primary host. From Asia it was spread to various places in the former USSR, Slovakia and the Czech Republic. More recently, *A. sidemi* has also been found in red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*) in France (1986), as well as from the European bison (*Bison bonasus*) in Poland (1997). Furthermore, it has been demonstrated that this parasite can infect moose (*Alces alces*), and in addition sheep are susceptible and maybe also cattle. In March 2007 a fallow deer hind (*Dama dama*) was found dead in a quarantine area in Sweden, situated inside a 40 ha enclosure populated by semi-domesticated fallow deer. At autopsy it was found that this hind was heavily infected by nematodes in its abomasum. These were identified as *A. sidemi* based on the morphology of the posterior end of male worms. Interestingly, the hind was born in Sweden from parents caught in the wild and then imported to Sweden from Hungary. Thus it can be assumed that *A. sidemi* was imported along with the transit consignment of live deer. A small-scale survey has been initiated to determine if any spread has occurred to deer outside the enclosure. So far only three animals have been investigated, and they were all uninfected.

308 **Gastro-intestinal nematodes of small ruminants in Shahrekord district of Chaharmahal and Bakhtiyari province of Iran**

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Introduction: Gastro-intestinal nematodes have an important role in morbidity and /or mortality of small ruminants and loss of meat and dairy production in this part of the country. The appropriate environmental and geographical conditions such as large and luxuriant pastures the province considered to be one of the most important foci of sheep and goat raising.

Objectives: To identify the gastrointestinal nematodes and determine the prevalence rate of each parasite in the animals and investigating the correlation between the infections and such variables as species, area, season, sex ,age .

Materials and Methods: The study was carried out on 500 stool samples collected from the animals, based on a randomly cluster sampling method in two seasons, autumn and spring. Direct wet mounts, concentration methods and Stoll method were applied for the examination of stool samples.

Results: The overall prevalence rate of the infections was about 64%, and the rates were 66.5% and 59.5%, in sheep and goats, respectively. Also, the rate of the parasitism was grater in the female animals than the males (66.8% vs.56.4%). While *Marshallagia* was the most prevalent nematodes of both animals (38.3%), *Strongyloides* constituted the lowest rate (20.7%). Although the infections occurred in all types of grazing areas of the province ,but there was a significant correlation between the plain areas and the infection compared with the other areas, mountainous, mountainous – plain areas ($p < 0.05$).

Conclusion: Infection with gastro-intestinal nematodes is an important problem in small ruminants in plain areas of the region which should be noticed efficiently.

309 **Study of genetic variability of *Haemonchus contortus* in alpine Italian ruminant populations**

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Haemonchus contortus, a generalist abomasal nematode, has been shown to affect the livestock productivity in domestic ruminants and the health status of wild hosts. Molecular techniques are useful to investigate genetic differentiation in parasite populations, host specificity and evolutionary history of parasite species. In total 77 adult males of *H. contortus* collected from isolates of 3 different Italian alpine areas were used for partial mitochondrial NADH dehydrogenase subunit 4 gene (ND4) analysis. Sequences were compared with 49 GenBank sequences, obtained from sheep in United States. On the basis of the results on ND4, a subset of isolates was examined also for Internal Transcribed Spacers 1 and 2 (ITS1 and ITS2) nuclear genes. Analyses on ND4 gene showed 2 clusters, in which isolates appear from each considered host species (chamois, roe deer, goat, sheep) and geographical origin. American sequences are aligned in only one group. A high intra-specific variability was pointed out even in each cluster. However molecular data on ITS1 and ITS2 have not confirmed the mitochondrial results. For this reason, to assess if it could be referred to an ancestral polymorphism or due to host-adaptation mechanisms, a further work using microsatellites is in progress. Moreover, the low host-specificity and the high gene flow among the *H. contortus* populations, led us to further check the a priori assumption that domestic sheep is the reservoir of this parasite. It arises the importance of managing parasite infections of domestic flocks to prevent spill-over to wildlife.

310 **Seasonal trend of *Haemonchus contortus* in small ruminants breed grazed under semi-extensive farming system, Pakistan**

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The present study was carried out to work out seasonal trend of *Haemonchus contortus* infection in naturally infected ewes and goats breeds reared under semi-extensive grazing system of Pakistan. In this study (December 2004-January 2006) was designed by using coprological and hematological examinations of randomly selected 968 ewes and 961 bucks at four different studied sites. Overall status of *H. contortus* infection showed that sheep readily acquired significantly higher level of *H. contortus* infection ($P < 0.05$) as compared to goats. Both host species illustrated a clear trend of seasonal *H. contortus* infestation pattern getting a highest peak of infection in the rainy season (July-October), while lowest level of infection was noted in December (cold month) and June (dry hot summer). In ewes, site wise highest log faecal egg counts (LFECs) with marked reduction in packed cell volume (PCV) and hemoglobin (Hb) levels were recorded at Islamabad site followed by Attock, Jehlum and Chakwal sites. However, in goats the LFECs trend was found to be highest in Islamabad followed by Jehlum, Attock and Chakwal districts respectively. Results revealed significant variation in the infection incidence exhibited between sheep and goat breeds from site-site while no significant variations were observed between breeds at the same site. However, Hairy (Jattal) bucks and Salt-Range (Latti) ewes displayed significant reduced LFECs and individual larval culture along with higher packed cell volume (PCV) and haemoglobin (Hb) levels as compared to other breeds. Moreover, FAMACHA® chart eye scoring in relation with haematocrit ranges is found to be more applicable for identifying the anemic ewes than goats. This knowledge provides the fundamental knowledge for the design of integrated control strategies for haemonchosis in small ruminants grazing in semi-extensive system.

311 Development of free-living stages of *Teladorsagia* (= *Ostertagia*) *circumcincta* at varying temperatures

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Introduction: To determine the rates of development of *T. circumcincta* at varying temperatures, both constant and a daily range representing summer, autumn and winter in New Zealand.

Methods: Faecal samples were obtained from 3 experimentally infected sheep. 3 reps of 10g faeces/sheep were each placed in a 55mm diameter petri dish within a larger 85mm diameter petri dish containing 5ml water. These were then placed inside humidified containers at the following constant temperatures: 4, 7, 13, 17, 25 and 37oC. Faecal samples were harvested at intervals using a Baermanns apparatus at 25oC. Larvae emerging by 24 hours were identified and counted.

Faecal temperatures over a calendar year were measured every 15 minutes in an artificially created 220g faecal pat on pasture located either in full sun or constant shade. The rate of larval development was measured in a variable temperature incubator at a constant high humidity using the mean temperatures in full sun for January, April and July.

Results: The developmental success rates (range) to L3 were: 4oC (1-4%), 7oC (10-16%), 13oC (25-70%), 17oC (74-94%), 25oC (40-77%) and 37oC (0%). For January, April and July the mean daily ranges of temperatures were 14-32oC, 11-22oC and 6-13 oC respectively. Development was maximal at 50-70% by Day 8-12 for January, 70-90% by Day 16 for April and 53-65% by Day 48 for July.

Conclusions: The optimal development temperature was 17oC reducing to minimal development at 4oC and 37oC. Development was higher in April (autumn) than January (summer) and July (winter).

312 A survey of seasonal infestation with *Przhevalskiana* larvae in slaughtered goats and sheep in South-western Iran

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In this research slaughtered sheep and goats were investigated during August 2003-July 2004 at a slaughterhouse in Masjed-Soleyman, South-Western Iran. The number of infested animals with *Przhevalskiana* larvae and their age and sex was recorded. Collected larvae from infested animals were stored in Alcohol-Glycerin solution and were studied to determine their morphological properties and species. The data was analyzed by the chi-square test. Rate of infestation was 0.2 and 5.3% in sheep and goats, respectively. Infestation rate in male and female goats and sheep was significantly different ($p < 0.05$). Prevalence of hypodermosis in sheep was significantly different in spring and winter, while infestation prevalence in goats was significantly different in four seasons of the year. Myiasis was seen more frequent in 2-3 and 1-2-year-old age groups of sheep and goats, respectively. The causative agents of goat and sheep hypodermosis were *Przhevalskiana aegagri* and *Przhevalskiana crossii*. The onset of fly activity was from late June to mid July.

313 **Use of *Caenorhabditis elegans* to study the metabolism of ivermectin by nematodes**

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Ivermectin is a commonly used anthelmintic for the treatment of parasitic infections in both humans and animals. It belongs to the macrocyclic lactone class of drugs and was licensed in the early 1980's. Due to its widespread use over the last 25 years, resistance has become increasingly common, with the small ruminant industry most severely affected.

Metabolism of anthelmintics as a possible mode of resistance is an area which has thus far not been investigated in any detail. However, the cytochrome P450s, a large group of haem containing monooxygenases, are known to play an important role in resistance to DDT and other insecticides in *Drosophila melanogaster*.

Due to the difficulty in studying parasitic nematodes directly, we have decided to use *Caenorhabditis elegans*, a free-living nematode, as a model organism due to its close phylogenetic relationship with *Haemonchus contortus*.

By using a *C. elegans* whole genome microarray, followed by real time quantitative PCR, we have investigated the genes whose expression is increased following exposure to ivermectin. The results of these experiments will be presented.

314 **Comparative genomics of nematodes: *Caenorhabditis elegans* as a tool to study the *Haemonchus contortus* genome**

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We have been using genome information from *Caenorhabditis elegans* to begin to identify and characterise genes from the parasitic nematode *Haemonchus contortus*. The *H. contortus* genome is being sequenced at the Sanger Institute and currently 4.5x coverage is complete. The aim of the project is use comparative genomics along with functional analysis in *C. elegans* to help annotate the *H. contortus* genome and study gene function in this parasite. We have focussed initially on the genes of the β -tubulin family, some of which are important targets of benzimidazole anthelmintics. Mutations in the isotype-1 and isotype-2 β -tubulin genes of *H. contortus* have been shown to be associated with benzimidazole resistance. However the potential role for other β -tubulin genes has not been investigated. We have been able to find two additional β -tubulin loci within the *H. contortus* genome which now gives a total of 4 genes for this family. Our findings on the organisation of the β -tubulin gene family in *H. contortus* will be presented. We are also interested in identifying genes involved in the RNAi silencing pathway in *H. contortus* and comparing these with *C. elegans*. RNAi can be used to knockdown mRNA levels of some genes in parasitic nematodes; however it does not seem to be as effective as in *C. elegans*. It is possible that the RNAi pathway may not be conserved in all nematode species. Our latest results of mining available nematode databases for *C. elegans* RNAi pathway genes shall be presented.

315 **Investigation of the cytochrome P450 family in the parasitic nematode *Haemonchus contortus***

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Cytochrome P450s are encoded by a multi-gene family that includes important drug-metabolising enzymes in many organisms. The involvement of such enzymes in the metabolism of anthelmintics by parasitic nematodes is unknown and their potential role in anthelmintic resistance has not yet been investigated. We are characterising the *H. contortus* cytochrome P450 gene family in order to investigate their potential role in these processes. Bioinformatic analysis of the *H. contortus* genome sequence (currently at 4.5-fold coverage) reveals over thirty family members and we have undertaken phylogenetic analysis to identify those we considered most likely to be involved in anthelmintic metabolism. Four genes are currently being characterized in detail. Full length cDNAs are being isolated and BAC clones containing the loci identified. Functional analysis and comparison of the genes in anthelmintic susceptible and resistant parasites will follow.

316 **In vitro development of the fourth stage of *Haemonchus contortus* as a biological study model**

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Haemonchus contortus is the main tropical parasitic nematode, which cause severe damage to domestic ruminants. The fourth stage of *H. contortus* is the first blood feeder larvae, localized among the mucosa tissue, followed by the further development of immature and mature adult stages. The in vitro development of *H. contortus* fourth stage could be helpful in providing larvae as a biological model to carry on the evaluation of different studies of control against endoparasitic gastro-intestinal nematodes. Thus, infective larvae of *H. contortus* (L3) were incubated at 39°C in a 5% of CO₂ atmosphere using specific culture media for two months. Morphometric changes were recorded each week, followed by the analysis of crude-extract and a secreted-excreted product by the gel activity assays such as SDS-PAGE and gelatine Zymograms. Twenty one days later, morphological changes were observed, like the formation of oral cavity and intestinal tube. Also, different statistical measures ($p < 0.05$) were recorded between L3 and the subsequent development stages of *H. contortus*. The SDS-PAGE showed similar protein masses between the fourth and the adult stage around 200, 100, 90, 35, 30 y 20 kDa and three proteolysis of approximately 36, 58 and 95 kDa were visualized as a clear area where gelatin has been digested using secreted and crude extract antigen. In conclusion, the development of the fourth stage of *H. contortus* was obtained and maintained by two months; however, important genes identification might be done to compare to the adult stage to use it as a model in the control of Haemonchosis. Thanks to Conacyt, Mexico for their financial support: Project No. C01-67.

- 317 **Transcription studies on depsiphilins in different stages of *Ancylostoma caninum*, *Haemonchus contortus* and *Ostertagia ostertagi* using quantitative real-time PCR**
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In *Haemonchus contortus*, the heptahelical transmembrane cyclooctadepsipeptide receptor Hc 110-R was identified as a target protein for emodepside. It shows a high degree of sequence identity to the latrophilin like protein 1 of *Caenorhabditis elegans*, for which a binding of emodepside in the N-terminal region was detected. Furthermore, orthologues to these receptors were identified in *Ancylostoma caninum* and *Ostertagia ostertagi*, named depsiphilins. Recently a dose-dependent effect of emodepside on worm development in *C. elegans* was shown. Also a reduced emodepside sensitivity, measured via a locomotion assay, in early larval stages (L1 – L3) was detected. The time required for hatching of eggs exposed to emodepside did not significantly decrease. Own studies also showed via egg hatch test, that hatching is not affected in several gastrointestinal nematodes. This leads to the question, if the emodepside-binding receptor is differentially expressed in the different stages of nematodes. A transcription study via quantitative real-time PCR was performed to achieve information about this. In this study, eggs, first and second stage larvae respectively, infectious third stage larvae, male and female adult worms of *A. caninum*, *H. contortus* and *O. ostertagi* were investigated in a quantitative real-time PCR using TaqMan probes. For quantification external standards in combination with housekeeping genes as normalisers were used, as well as an internal calibrator. The resulting data showed no significant differences in transcription levels of depsiphilins within different stages of the three nematode species analysed. Thus the differing sensitivity of eggs, larvae and adult worms to emodepside is not correlated with the abundance of this receptor. Drug accessibility or additional target sites are to be investigated in this respect.

Poster group 2: Helminth Infections of Cattle

- 318 **Decision trees for worm control in cattle, sheep and horses as teaching tools**
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A number of years ago a decision tree for the control of gastrointestinal nematodes and lungworm in first grazing season cattle was developed in the Netherlands, which is accessible now for farmers and veterinarians on the website of the Animal Health Service in Deventer. The advantage of this decision tree is that it results in a farm-specific recommendation for control, based on grazing management, rather than in more general recommendations for parasite control that may not fit at all in the planning of the farmer.

Besides being a useful tool for recommendations at farm level it also appears to be a good tool for teaching as it helps the students to understand the ins and outs of nematode control in cattle.

Recently more complete decision trees have been developed for worm control in sheep and in horses, which also have already been used for teaching final-year students.

The decision trees for horses and sheep will be demonstrated on the computer. Those interested in receiving them can address one of us. m.eysker@vet.uu.nl; h.ploeger@vet.uu.nl; d.c.k.vandoorn@vet.uu.nl. However, in the near future (end of 2007-2008) more streamlined versions of the decision trees will become accessible on the University web pages.

319 **Delivery of anthelmintic drug combinations – A rumen delivery technology to meet current needs**

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Introduction: Current opinion suggests that underdosing and single drug anthelmintic treatments are two factors that promote parasite resistance in ruminants. To address the current nematode resistance problem in ruminants an appropriate treatment involving the combination of at least two different classes of anthelmintic drugs, each at sufficiently high dose levels, is needed. This is particularly problematic for long term rumen bolus where a high drug load is limited by the maximum drug load capacity of the product.

Recently, a new bolus suitable for the long-term release of drugs in ruminants has been developed which has been shown can contain very high drug loads.

Methods: The drug release rate of model drugs with different physicochemical properties (water soluble, insoluble, mineral and organic) from the new rumen bolus was investigated in fistulated cattle. Boluses containing 50% drug load were withdrawn after predetermined times and analyzed for residual drug content to obtain % drug release.

Results: Linear drug release was observed for both the highly water soluble (sodium salicylate) as well as for the insoluble model drug (kaolin). Magnesium sulphate release rate was slightly curved.

Inter-individual animal variability was observed to be very low indicating a highly consistent drug release.

Conclusion: The new rumen bolus was shown to deliver high drug loads to animals very consistently, irrespective of the physicochemical nature of the drug. These characteristics make it suitable for the development of a high dose, multi-drug treatment product for the delivery of anthelmintics.

320 **Pharmacokinetics of moxidectin long-acting formulation in cattle: disposition in plasma and hair**

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The plasma kinetics disposition of moxidectin following a subcutaneous administration with a Long Acting formulation (Cydectin® 10%, Fort Dodge Animal Health, France) at the recommended dose of 1 mg.kg⁻¹ body weight was evaluated in Charolais cattle breed (5 females weighing 425–450 kg) for 120 days. Furthermore, its concentration was measured in hair for the same period. After plasma extraction and derivatization, samples were analysed by HPLC with fluorescence detection. The peak plasma concentration (C_{max}) was 55.71 ± 15.59 ng.ml⁻¹ and 444.44 ± 190.45 ng.g⁻¹ for plasma and hair respectively. The mean calculated time of peak occurrence (T_{max}) was 3.40 ± 3.36 days and 2 days for plasma and hair respectively. The mean residence time (MRT) was 28.93 ± 2.87 days and 13.32 ± 2.48 days for plasma and hair cattle. The area under concentration-time curve (AUC) was 1278.95 ± 228.92 ng.day.ml⁻¹ and 2663.82 ± 1096.62 ng.day.g⁻¹ for plasma and hair respectively. At the last sampling time (120 days), the concentration was 1.91 ± 0.26 ng.ml⁻¹ and 0.69 ± 0.52 ng.g⁻¹ for plasma and hair respectively. For the first time the moxidectin pharmacokinetics parameters in hair after a subcutaneous administration was described. The moxidectin profile concentrations in hair reflected that registered in plasma. The previous studies of efficacy have to be correlated to the extended period of absorption and distribution by the LA formulation due to the 5-fold higher dose rate in comparison with the 1% injectable formulation (0.2 mg.kg⁻¹ body weight).

321 **Toxicity evaluation of a 2.25% ivermectin + 1.25% abamectin solution in calves younger than four months of age**

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Avermectins are molecules derived from a natural macrocyclic lactone compound, obtained from fermentation of *Streptomyces avermitilis* fungi, and are widely used for endo and ecto parasite control. Abamectin has been recommended worldwide only for use in bovines above four months of age. Pharmacokinetic studies show plasmatic differences between conventional and long acting avermectin molecules. The present study assessed the toxicity of a long action formulation of 2.25% Ivermectin + 1.25% Abamectin in different dosages, in bovines younger than four months of age. Sixteen male, 30 day old, HPB calves were distributed into four groups: GI - 450 mcg/kg Ivermectin + 250 mcg/kg Abamectin; GII - 900 mcg/kg Ivermectin + 500 mcg/kg Abamectin; GIII - 1350 mcg/kg Ivermectin + 750 mcg/kg Abamectin; GIV - physiologic pH solution (control). All bovines were submitted to clinical examination twice daily for 14 days. Blood and cerebrospinal fluid samples were taken on days 0, 1, 7 and 14 post-treatment. Biochemical parameters measured in blood were total protein and AST/GOT, GGT and AP enzymes. The cerebrospinal fluid was quantified for pH, density, leucocytes, erythrocytes, protein, glucose and CK/CPK, ALT/GPT and LDH enzymes. No clinical alterations were detected in the medicated bovines. The results of the laboratorial exams (blood and cerebrospinal fluid) of the treated animals were not significantly different ($P>0.05$) from those found in the control group. Although other studies, specifically designed, are needed, the results of the present study allow the conclusion that the long action 2.25% Ivermectin + 1.25% Abamectin formula is well tolerated by bovines younger than four months of age.

* Solution 3,5% L.A. – Intervet do Brasil Veterinária Ltda.

322 **Preliminary evaluation of the efficacy of albendazole and ivermectin against gastrointestinal nematodes of cattle and goats in south of Mozambique**

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Introduction: Albendazole and ivermectin are used to control gastrointestinal nematodes in ruminants in Mozambique. The resistance to benzimidazole drugs has been observed in goats¹. The aim of this study was to evaluate the efficacy of albendazole and ivermectin against gastrointestinal nematodes in cattle and goats in South Mozambique.

Methodology: Sixty cattle and sixty goats naturally infected with gastrointestinal nematodes from two farms were distributed into 3 groups of 10 animals each according to the treatment: oral albendazole (5 and 7.5 mg/kg for goats and cattle) respectively ivermectin (0.2mg/kg S.C.) and control. Faecal samples were collected at 0, 10 and 14 days after treatment. The faecal egg count reduction test and egg hatch assay (EHA) were used to evaluate the efficacy².

Results: The percentage of reduction (R%) for albendazole was 100% and 95% and 100% and 56% for cattle and goats in farm 1 and 2, respectively. For ivermectin were higher than 96% in both animal species and farms. Efficacy dose (ED50) values in EHA confirm the existence of benzimidazole resistance for goats in one farm. *Haemonchus* and *Oesophagostomum* were the genus found on faecal samples after treatment.

Conclusion: The efficacy of ivermectin in both farms was observed. However, resistance to albendazole was observed for goats in one farm.

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323 **Evaluation of cymiazole+abamectin pour-on formulation against bovine gastrointestinal nematodes and *Rhipicephalus (Boophilus) microplus* ticks under experimental and field conditions in Brazil**

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A combination of Cymiazole and Abamectin as a pour-on formulation was evaluated for its efficacy and persistent activity against bovine gastrointestinal nematodes and cattle tick *Rhipicephalus (Boophilus) microplus* under experimental and field conditions in South eastern region of Brazil.

In the first study, the product was evaluated in cattle naturally infected with nine nematode species (*Haemonchus similis*, *Haemonchus placei*, *Cooperia spatulata*, *Cooperia punctata*, *Cooperia pectinata*, *Trichostongylus axei*, *Trichostongylus colubriformis*, *Oesophagostomum radiatum* and *Capillaria bovis*). The post mortem examination of treated and control animals showed that the product was 100% effective against *Haemonchus similis*, *Cooperia spatulata*, *Trichostongylus axei*, *Trichostongylus colubriformis*, *Oesophagostomum radiatum* and *Capillaria bovis* where as the efficacy against *Haemonchus placei*, *Cooperia punctata* and *Cooperia pectinata* was found to be above 90%. In second study, the product was evaluated in cattle artificially infested with *Rhipicephalus (Boophilus) microplus* larvae (stall test). The product was >95% efficacious during 26 days post treatment observation period and > 90 % efficacious during 30 days post treatment observation period. However the efficacy dropped to 76.34% during 39 days post treatment observation period. The product was evaluated in the third study against natural infestation of *Rhipicephalus (Boophilus) microplus* and the efficacy achieved was >95% during day 3 to day 21 post treatment when it achieved 100% efficacy. In the fourth study on cattle naturally infested with *Rhipicephalus (Boophilus) microplus*, 97.5% tick control was achieved during 21 days observation period post treatment, and it remained above 90% during 28 days post treatment observation period. The results obtained show that the assessed product can be used for the control of bovine nematodes and tick infestations in Brazil.

324 **Evaluation of the 0 + 8 doramectin pour-on programme against lungworm in cattle**

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Repeated treatment of cattle with doramectin was evaluated. Three groups of 10 calves were turned out on equivalent set-stocked paddocks between May and September 2006. Animals in group-A remained untreated, group-B received a topical application at turn-out, whereas group-C was treated at turn-out and again after 8 weeks on pasture. Individuals were weighed and faecal samples and blood were analysed every 4 weeks. Weight gains were identical in B and C after the first 8 weeks and animals had gained approximately 5 kg more compared to A. The following 8 weeks there was a drop in performance, especially in B that after 16 weeks were approximately 5 and 30 kg lighter compared to animals in A and C, respectively. However, two animals with severe dictyocaulosis in A were treated in mid July. The mean weights were similar in A and B both at housing and when the animals had been stabled for 4 weeks. They were then approximately 20 kg lighter compared C. Although lungworm larvae and elevated antibodies were observed in all groups after 8 weeks, L3s and antibody levels were much higher in A. After 12 weeks no L3 were observed in A and C due to immunity and treatment, respectively. In contrast, a slight increase was seen in B as was also confirmed by increased antibody levels. Antibody levels gradually decreased in A and B during the last 12 weeks, whereas an increase was noticed in C. However, this was not associated with shedding of larvae.

325 **Parasitic infections in dairy cattle around Hanoi, Vietnam**

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In Northern Vietnam, dairy cattle are mainly kept confined at all time and are fed by cut and carry methods, or are occasionally allowed to graze the dikes. In the present study the occurrence of parasitic infections in the provinces surrounding Hanoi, Northern Vietnam, was examined. In total 201 farms were visited and 316 stool, 239 blood and 260 milk samples were collected from both young and adult animals. Coproscopical examination indicated a high prevalence of nematode eggs (*Cooperia* spp., *Haemonchus* and *Oesophagostomum*): 66% in animals (n=154) between 3 and 24 months and 54% in animals (n=90) older than 24 months. The prevalence of *Fasciola* was 28% and 39% in animals between 3 and 24 months and older animals respectively, while the prevalence of *Paraphistomum* was around 80% in both groups. Of the calves younger than 3 months (n=72), 48% was positive for *Eimeria* spp. and 48% for *Giardia*, mainly identified as *G. bovis* on the beta-giardin gene. The blood samples were examined with ELISA for the presence of *Anaplasma*, *Babesia* and *Theileria* antibodies, and a prevalence of 28%, 54% and 58% was found, respectively. In the milk samples *Neospora* antibodies were detected in 30% of the animals.

Conclusion: parasitic infections occur frequently in dairy cattle around Hanoi and should be considered as possible pathogenic agents in the dairy industry around Hanoi. To minimize the potential detrimental effects, screening and treatment programs, along with treatment evaluation programs should be implied.

327 **Morphological differentiation among three *Thelazia* species (Nematoda: Thelaziidae) by scanning electron microscopy**

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Thelaziosis is an ocular infection of several mammals caused by nematodes of the genus *Thelazia* (Spirurida, Thelaziidae). The adults live in the lachrymal ducts and/or conjunctival sacs of infected animals and transmission occurs by means of different species of muscids. *Thelazia rhodesi*, *Thelazia skrjabini* and *Thelazia lacrymalis* affect livestock (i.e. mainly cattle and horses) inducing conjunctivitis, keratitis and ocular discharge. Scanning electron microscopy (SEM) was used to study the surface ultrastructure of adult worms of these nematodes. The worms were removed and washed in 2% sodium cacodylate buffer. The worms were next placed in 4% glutaraldehyde. The nematodes were put into fresh 4% glutaraldehyde for 2 h, post fixed in 2% osmium tetroxide in cacodylate buffer at 4°C for 2 h, and dehydrated through a series of increasing ethanol concentrations. They were then dried with liquid CO₂ in a critical point drier. Each worm was placed on a numbered aluminium stab, which was coated with gold in an ion coating and viewed with a JEOL JSM-840 Scanning Electron Microscope. This article describes the morphological features of adult *T. rhodesi*, *T. skrjabini* and *T. lacrymalis* by scanning electron microscopy and highlights the most relevant differences for their identification.

328 Occurrence and pathology of *Gongylonema* infection in cattle in north of Iran, Mazandaran Province

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Little is known about *Gongylonema* prevalence, seasonal distribution and its pathology in cattle of north of Iran.

Esophagus of 136 cows-96 Native and 42 Holstein- were tested in a one year period (2006-2007) at post partum examination.

Gongylonema infection in 36 cows out of 138 animals (26%) from North of Iran coast of Caspian sea was identified. The occurrence was more in female animals (31.2%) than males (14.2%). The locations of worms were classified to three categories (anterior, middle, posterior) and there was a predilection by the worms for sites towards the distal portion of the esophagus (43%).

All of the *Gongylonema* worms were identified as *G. pulchrum* species.

The pathology of affected parts of esophagus was tested after sampling, sectioning and staining. The affected areas showed fibrinous inflammation but a little gross pathology was seen. The infection rate was more in native cows than Holstein cows. A seasonal distribution was seen and infection rate in spring (50%) was more than other seasons significantly.

Our identified infection rate was more than the other work in Iran on cows of north west Iran in Urmia abattoir (11.3%) that maybe because of climate of our district that is more suitable for Scarabid beetles.

329 Immunological responses and cytokines gene expression analysis to *Cooperia punctata* infections in resistant and susceptible Nelore cattle

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This study was carried out to evaluate the immunological response and cytokines gene expression analysis to *Cooperia punctata* infections in Nelore cattle with different degrees of resistance to natural infections. One hundred weaned, male cattle, initially 11-12 months old and kept on the same pasture was evaluated. The 7 most resistant and the 8 most susceptible animals were selected based on their individual averages of eggs per gram of faeces (EPG) and worm burden. Small intestine tissue samples were collected for histological quantification of inflammatory cells and cytokines gene expression analysis (IL-2, IL-4, IL-8, IL-12p35, IL-13, TNF- α , IFN- γ , MCP-1, MCP-2, MUC-1) by real-time RT-PCR methodology. The resistant group presented higher IgG1 levels against infective larvae (L3) and adult *C. punctata* antigens. The resistant group showed higher mucosal IgA anti-*C. punctata* (L3 and adult) and globule leucocytes values compared to susceptible group ($P < 0.05$). *C. punctata* worm burden were negatively correlated with eosinophils, globular leucocytes, IgG1 anti-*C. punctata* (adult), mucosal IgA anti-*C. punctata* (adult), IL-2 and IFN- γ ; ($P < 0.05$). The gene expression analysis showed that TH2 cytokines (IL-4 and IL-13) in the Resistant group and TH1 cytokines in the Susceptible one (IL-2, IL-12p35,

IFN- γ and MCP-1) were both upregulated. These results taken together with the immunological data provide evidence to an immune response against *C. punctata* mediated by TH2 cytokines in resistant group and TH1 in susceptible one.

330 **Gender specific transcription of asp antigens in *Ostertagia ostertagi***

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Activation-associated secreted proteins (ASP) are members of a nematode specific protein family with homology to a group of evolutionary related secreted proteins called the SCP/Tpx-1/Ag5/PR-1/Sc7 family. ASPs have been studied as potential vaccine candidates in different parasites, including *Ostertagia ostertagi*. The function of these proteins is still unclear, but a role in transition to parasitism and a role as allergen is often suggested. However, the presence of ASP-like proteins in the free-living nematode *Caenorhabditis elegans* indicates that these proteins might have a more general biological function. Moreover, ASP-like sequences have been found in lists of male enriched genes of different nematodes and they show homology to mammalian testis specific proteins (Tpx), which suggests a role in reproduction. The aim of this study was to investigate the possible reproductive function of ASP molecules in *O. ostertagi* by analyzing the expression pattern of different ASPs in male and female adult worms. The ASP molecules investigated were a two domain ASP (Oo40) and three different single domain ASPs (Oo-ASP1, Oo-ASP2, Oo24). Quantitative RT-PCR data indicated that the transcript levels for the three single domain ASPs are highly enriched in males compared to females. The Oo40 on the other hand was only transcribed in the L4 stage. Immunohistochemistry showed that Oo-ASP1 and Oo-ASP2 are localized in the reproductive tract of both male and female worms. The localization of Oo24 is currently under investigation. These data suggest that at least some of the ASPs in *O. ostertagi* might have a role in reproduction.

331 **Identification and characterisation of a novel ASP-like protein (OoAL1) from *Ostertagia ostertagi***

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It has been shown that the bovine abomasal parasite *Ostertagia ostertagi* drastically modulates its environment: epithelial cell damage, accumulation of inflammatory cells and changes in stomach pH. Although the mechanisms used by the parasite to change the abomasal environment are largely unknown, an important role has been attributed to parasite excretory-secretory (ES) material. In this study we have identified a novel ES protein of approximately 17 kDa which contains the SCP/Tpx-1/Ag5/PR-1/Sc7 protein motif. Although this protein motif is also present in the activation associated secreted protein family of nematodes (ASPs), sequence analysis indicated that the 17 kDa protein is distinct from all the previously identified ASPs. Therefore, the protein was named OoAL1 (*O. ostertagi* ASP-like protein). OoAL1 contains a signal peptide and has one predicted N-glycosylation site. The highest levels of transcription were detected in the adult male worms, while the levels in females and L4 larvae were respectively three- and tenfold lower. No transcription could be detected in the L3 stage. Western blots of somatic extracts and ES products of different developmental stages of *O. ostertagi*, developed with anti-OoAL1 antibodies, indicated the presence of OoAL1 in the ES products of adult worms. At least two genes are present in the *O. ostertagi* genome encoding OoAL proteins. Analysis of the nematode genome and EST databases indicated that this type of ASPs is unique for *O. ostertagi* and the closely related *Teladorsagia circumcincta*. The function of these novel nematode proteins is unknown.

332 Identification of galectins involved in the infection process of *Ostertagia ostertagi* in cattle

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Galectins are evolutionary conserved beta-galactoside binding proteins. Host galectins are upregulated during infection and modulate both innate and adaptive immune responses. In contrast, no function could yet be assigned to parasite galectins. Hypothetically, parasite galectins could mimic host galectins, thereby modulating the immune response. Parasite galectins can bind to the extracellular matrix and glycosidic complexes on host cells and therefore might also be important for adhesion and invasion.

To identify host and parasite galectins involved in the infection process of *Ostertagia ostertagi* in cattle, abomasal mucosa was collected from worm-naïve calves and experimentally infected calves at different times post-infection, together with *O. ostertagi* L3 and L4 larvae and adult worms. Four bovine galectins were shown to be present in abomasal mucosa of infected calves (GAL-1, -3, -4 and -9) and five new *O. ostertagi* galectins were identified (Oo-gal-1 to -5). Parasite galectin transcription levels in the *O. ostertagi* parasitic L3, L4 and adult stages were compared to the transcription levels in the pre-parasitic L3 stage by real-time qPCR. Oo-gal-2 and Oo-gal-5 were upregulated during infection, in L4 and exsheathed L3 and L4, respectively. A qPCR analysis of the bovine galectin transcription levels in the abomasal mucosa of infected calves compared to that in worm-naïve calves is currently under investigation. Galectins from both cattle and *O. ostertagi* that are upregulated during infection will be expressed as recombinant proteins. Polyclonal antibodies against these recombinant galectins will be used for immunolocalisation and in vitro studies will be conducted to assess the galectin immunomodulatory effects.

333 No antigenic variation in the major sperm protein (MSP) of the nematode *Dictyocaulus viviparus*

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It has become increasingly clear that populations of the bovine lungworm are genetically structured. The aim of this study was to investigate if this genetic variability also is reflected in immunogenic proteins. We decided to focus on MSP, because this is an abundant protein used in diagnostic immunoassays. MSP is also well characterised and reasonably short, which makes it possible to study the nucleotide diversity by direct sequencing. Thus, MSP is an ideal model to test the hypothesis of whether and to what extent the overall genetic variation observed in mtDNA and by AFLP is reflected in a functional protein involved in immune recognition. Accordingly, MSP was sequenced with standard methods using worm DNA from eight adult worms from each of nine populations that previously had been defined as genetically heterogeneous. As a comparison we also included MSP sequences of the closely related *D. capreolus* and *D. eckerti* as well as from other nematodes deposited in GenBank. In contrast to previous results, this study shows that the MSP sequence of *D. viviparus* is similar to other nematodes. Furthermore, almost no sequence variation was detected in MSP between worms from different sub-populations or in the other *Dictyocaulus* species investigated. Finally, a functional test of a recombinant variant of MSP showed that the expressed protein was recognised by antibodies in sera from infected cattle. Overall, almost no antigenic variability was observed. This has practical implications in the development of recombinant vaccines and immunodiagnosics.

334 **In vivo transfer of young adult *Dictyocaulus viviparus***

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With the broncho-alveolar lung lavage technique it is possible to study local immune responses against pathogens infecting bovine lungs, including the lungworm *Dictyocaulus viviparus*. Here, we demonstrate for the first time that it is possible to use the same technique for transferring living young adult lungworms from donor to recipient calves. In several experiments we collected living young adults and transferred these within hours to recipient calves. It was ensured that the ratio of male and female worms was close to 1:1. Results from faecal examination for larval shedding and serology using a *Dictyocaulus*-specific ELISA demonstrated that most transfers resulted in actively reproducing adult worms. The possibility of in vivo transfer of the lung parasitic stages allows studies on the development of immunity resulting from adult worm burdens only, without the inference of the pre-lung larval penetration and migration phase of the infection. It also opens up possibilities to study effects of single-sex infections or worm reproduction experiments with varying sex-ratio's.

Poster group 3: Fasciolicides

335 **A recombinant-based ELISA for the evaluation of two fasciolicides in autochthonous *Rubia gallega* bovine**

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The autochthonous cattle breeds are commonly maintained under field conditions because of its well-adaptation to the climatic conditions of the origin area. The herds are maintained outside and are only brought into the paddocks during the night, which enhances the possibility of infection and challenge by several parasites as the liver-trematode *Fasciola hepatica*, especially in areas with frequent rain and mild temperatures. In a previous investigation, the usefulness of a 2.9 kDa F. hepatica-recombinant protein for the early detection of active fasciolosis in sheep under field conditions has been proved. In the present work we analyze its usefulness for evaluating the efficacy of two fasciolicides, netobimin and albendazole, on *Rubia Gallega* autochthonous cattle. Blood samples were collected from three groups of 10 cattle during 14 weeks after the administration of the anthelmintics. The immunoenzymatic ELISA with a 2.9 kDa recombinant F. hepatica protein, and with crude excretory/secretory antigens (FhES) was used for determining the IgG humoral response. The efficacy of the fasciolicides was estimated by calculating the Efficacy Percentages 1 (EP1, % reduction IgG with the rFhAPS) and 2 (EP2, % reduction IgG with the FhES).

The ELISA showed that the IgG kinetics decreased following the administration of the two fasciolicides, but control-uninfected values were not reached. The lowest values of IgG were reached against the rFhAPS, and a constant value around 0.6 was obtained after the administration of the two fasciolicides. The highest values of EP1 were recorded in the cattle receiving netobimin (2nd to the 6th w.a.t.), but percentages above 40% were not observed.

This work was partly supported by a Scientific Collaboration with Schering-Plough, Madrid, Spain.

336 **Metabolic interference enhances the plasma profiles of triclabendazole metabolites in sheep**

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Understanding triclabendazole (TCBZ) kinetic and metabolism patterns is critical to optimise its flukicidal activity. TCBZ is metabolised by both flavin-monooxygenase (FMO) and cytochrome P450 (CYP) enzymatic systems in the liver. Interference with these metabolic pathways may be useful to increase the systemic availabilities of TCBZ and its metabolites, which may improve the efficacy against *Fasciola hepatica*. The plasma disposition of TCBZ metabolites was evaluated following TCBZ co-administration with FMO [methimazole (MTZ)] and CYP [piperonyl butoxyde (PB) and ketoconazole (KTZ)] inhibitors in sheep. Twenty (20) healthy female Corriedale sheep were allocated into 4 treated groups: a) TCBZ alone (5 mg/kg, IV); b) TCBZ (5 mg/kg, IV) + MTZ (3 mg/kg, IV); c) TCBZ (5 mg/kg, IV) + PB (30 mg/kg, IV) and d) TCBZ (5 mg/kg, IV) + KTZ (10 mg/kg, oral). Blood samples were taken over 240 h post-treatment and analysed by HPLC. TCBZ sulphoxide and sulphone were the metabolites recovered in plasma after all treatments. MTZ did not affect the pharmacokinetics of TCBZ. TCBZ sulphoxide peak concentrations significantly ($P < 0.05$) increased after TCBZ+PB (62 %) and TCBZ+KTZ (40 %) treatments compared to those measured in the TCBZ alone treatment. TCBZ sulphoxide plasma availability (measured as AUC) was also higher ($P < 0.05$) in the presence of both PB (99%) and KTZ (41%) metabolic inhibitors. CYP-mediated inhibition of TCBZ oxidation in the liver accounted for the increased systemic availability of its active metabolite TCBZ sulphoxide. These results should be considered among other strategies to improve the use of this trematocidal drug in ruminants.

337 **A comparison of the efficacy of doramectin, closantel and levamisole in the treatment of the “oriental eye fluke”, *Philopthalmus gralli* in commercially reared ostriches (*Struthio camelus*)**

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Introduction: Commercially reared ostriches at Msengi farm situated in Chinhoyi area in Mashonaland West province of Zimbabwe were reported to be infected with the “oriental eye fluke”, *Philopthalmus gralli*. This was the first record of the fluke in Zimbabwe. Trials were embarked to identify a suitable drug for the treatment of this fluke.

Materials: A total of 12 ostriches confirmed to be infected with the fluke through clinical examination of the eyes were randomly divided into three equal groups with each group receiving a different treatment protocol. The three drugs used were doramectin, levamisole and closantel. Each of the drugs was used in combination with chloramphenicol as an eye ointment.

Methods: Levamisole was administered topically into the eye, and doramectin and closantel were administered intramuscularly.

Results and Conclusions: The results indicated a positive response to levamisole-treated-birds and there was no noticeable response to doramectin and closantel treatments. It was concluded that levamisole was effective for the treatment of *P. gralli*. However more research needs to be done to elucidate the mechanism of action of the levamisole to the fluke

338 **In vitro activity of some plant extracts against newly excysted *Fasciola hepatica* metacercariae**

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The aim of the present study was to evaluate under in vitro conditions the activity of 20 plant extracts against newly excysted *Fasciola hepatica* metacercariae. Plants were selected on the base of anthelmintic activity, traditional use, availability and solubility as well as permanence in the liver. For the in vitro evaluation, 24 well NUNC immunoplate containing RPMI-1640, bovine sera plus antibiotics were used. Extract concentrations were placed in triplicate at 500 y 50 mg/l. Finally approximately 10 newly excysted flukes per well were placed, the plates being incubated in a CO₂ incubator at 5% atmosphere during 3 days. For each plate, four control wells were included containing non-treated flukes. All procedures were undertaken under an air flow cabinet. Efficacy was measured on days 1 and 3 considering the motility of the live or dead trematodes evaluated with the aid of an inverted microscope. Results indicated that from all extracts evaluated, *Tilia sp.* showed an average efficacy of 100 and 100%; *Eryobotria japonica* 100 and 95%; *Coffea Arabica* 100 and 100%; *Crataegus mex* 95 and 90%; *Byrsonima crassifolia* 100 and 100%, for concentrations of 500 and 50 mg/L, respectively. From this preliminary evaluation it is concluded that further and more detailed evaluation at lower concentrations should be undertaken to know the real efficacy of these plant extracts.

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339 **Fasciocidal activity of artemisinin derivatives**

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Fascioliasis caused by liver flukes of the species *Fasciola hepatica* and *F. gigantica* is a zoonotic disease of great veterinary importance. Triclabendazole is the current drug of choice, but resistance is already widespread. Hence there is a pressing need to identify new fasciocidal drugs. We evaluated the fasciocidal properties of the artemisinins (artesunate, artemether) and a synthetic 1,2,4 trioxolane (OZ78) *in vitro* and we examined dose-response relationships of these drugs employing the *F. hepatica*-rat model. Worm burden reductions were assessed against untreated control animals. In addition, adult *F. hepatica* were observed by scanning electron microscopy (SEM) following drug incubation *in vitro* and *in vivo* treatment. Exposure to 10-100 µg/ml of artesunate, artemether or OZ78 *in vitro* resulted in the death of all trematodes by 48-72 h. Death of parasites occurred faster and damage to the tegument was more pronounced when haemin was added to the incubation medium. Complete worm burden reductions were obtained *in vivo* with single 100-400 mg/kg doses, respectively. In conclusion, artesunate, artemether and OZ78 show promising fasciocidal activities, a finding which raises hope for novel approaches towards trematocidal drugs. Studies evaluating the efficacy of artemether in *F. hepatica*-infected sheep are ongoing in the Campania region of Italy and preliminary findings will be discussed.

340 **Pharmacokinetic evaluation of the ivermectin-triclabendazole combination in sheep**

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Drug combinations are now available worldwide to increase the spectrum/efficacy of different anthelmintic preparations. However, information on potential pharmacokinetic interactions occurring after co-administration of different antiparasitic drugs is scarce. The current work was designed to evaluate the pharmacokinetic disposition of ivermectin (IVM) and triclabendazole (TCBZ) given separately or co-administered intravenously to sheep. Fifteen (15) adult female Corriedale sheep (25-30 kg) were randomly allocated into three experimental groups. Animals received either IVM (50 µg/kg) (Group A), triclabendazole (5 mg/kg) (Group B) or IVM (50 µg/kg) + TCBZ (5 mg/kg) (Group C) by intravenous administration. Blood samples were taken between 10 min and 7 days post-treatment. IVM, TBCZ, TCBZ sulphoxide (TCBZSO) and TCBZ sulphone (TCBZSO2) plasma concentrations were measured by HPLC. Data was subjected to kinetic and statistical analyses. The IVM plasma disposition was affected by its co-administration with TCBZ. The IVM residence time and elimination half-life were significantly enhanced (between 68 and 83 %) compared to the IVM alone treatment. Additionally, IVM modified the disposition of the TCBZSO metabolite. TCBZSO peak plasma concentration was significantly higher after the co-administration IVM+TCBZ ($23.2 \pm 3.54 \mu\text{g/ml}$) in comparison to the TCBZ alone treatment ($12.6 \pm 2.13 \mu\text{g/ml}$). The competition between IVM and TCBZ-TCBZSO by the cellular protein transporters (ABC) involved in hepatic and/or intestinal excretion processes may explain the pharmacokinetic interaction observed after their intravenous co-administration. Further research is needed to characterise the involvement of hepatic and intestinal ABC transporters on the disposition and clinical efficacy of these anthelmintic drugs used in combination.

341 **The relative potential of verapamil and ivermectin in combination with triclabendazole, to overcome triclabendazole resistance**

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Triclabendazole (TCBZ) is the drug of choice to treat *Fasciola hepatica*. However, in recent years reports of TCBZ resistance have been increasing. This potential problem comes into focus when the rapidly increasing levels of *F. hepatica* infection and the emergence of *F. hepatica* as a major zoonosis are considered. Over expression of P-glycoprotein (Pgp) (an ABC-transporter) has been linked to resistance to other benzimidazoles, closantel and ivermectin in nematodes. This may also be a potential method of triclabendazole resistance. This study uses two P-glycoprotein inhibitors in combination with triclabendazole sulphoxide (TCBZSO) to treat a known TCBZ-resistant isolate of *F. hepatica* (Oberon) in an attempt to assess whether resistance can be reverse. Resistant flukes were incubated in vitro for 2h with R(+)-verapamil at a concentration of 10^{-4} and then transferred to a combination treatment of verapamil and TCBZSO either at a concentration of $15 \mu\text{g/ml}$ or $50 \mu\text{g/ml}$ for a further 22h. A second experiment using a 2h pre-incubation in ivermectin ($1 \mu\text{g/ml}$) followed by a 22h incubation in an ivermectin and TCBZSO ($15 \mu\text{g/ml}$) combination was also set up. Scanning and transmission electron microscopy was used to assess the potential of both combinations in overcoming TCBZ resistance and these results will be compared to a previous study which has shown verapamil to increase the efficacy of TCBZSO in vitro against the TCBZ resistant Sligo isolate.

342 **Susceptibility of a newly isolated strain of *Fasciola hepatica* to closantel in sheep**

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Introduction: Ovine fascioliasis causes extensive damage to the liver when young flukes migrate through the parenchyma and rupture blood vessels, resulting in hepatic fibrosis and hyperplastic cholangitis. Closantel is currently marketed as oral suspension or injectable solution for the treatment and control of *Fasciola hepatica* in sheep and cattle, respectively.

Materials and methods: Sixteen, 6-months old Dorset sheep were artificially infected twice with 100 metacercariae of *F. hepatica*. The metacercariae were harvested from snails, infected with miracidia from embryonated eggs, isolated from infected sheep. The strain of *F. hepatica* was reported to be resistant to closantel. After confirmation of infection through faecal egg counts, eight sheep were left untreated, eight were treated with 10 mg closantel per kg body weight using the Flukiver® 5% oral drench formulation, 84 days post infection. Two weeks later, the fluke burden was determined in each sheep.

Results: Faecal egg counts started to become positive 70 days post infection, reaching 62.5% positive samples 84 days post infection in both groups. The infection was shown to be adequate with all control animals harbouring fluke. The geometrical mean worm burden in the control group was 33.1, ranging from 19 to 69. In none of the closantel treated sheep, fluke were recovered, demonstrating 100% efficacy of the treatment.

Conclusion: A 100% efficacy was demonstrated of a single, oral 10 mg closantel per kg body weight dose. It can therefore be concluded that this strain of *F. hepatica*, which was isolated as a potentially resistant strain, was susceptible to closantel.

343 **A field efficacy study to determine the efficacy of ivermectin/closantel injection (Norbrook Laboratories Limited, Produce code P-CIV-170) against naturally acquired infections of *Fasciola hepatica* and gastrointestinal nematodes in cattle**

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Two studies were conducted to determine the efficacy of a single dose of a novel formulation of Ivermectin/Closantel Injection for the treatment of mixed infections of *Fasciola hepatica* and gastrointestinal nematodes in cattle under field conditions. Efficacy was determined by faecal egg counts in 20 parasite free study 'tracer' calves that had grazed for four weeks on contaminated pastures. In each study, 10 'tracer' calves were treated 10 weeks after housing, the other group were untreated. Study calves were sacrificed two weeks after treatment. Efficacy of the treatment against *Fasciola hepatica* was 98.4% and 99.4% respectively in the two studies, with efficacy against gastrointestinal nematodes *Ostertagia ostertagi* 100% in both studies, and against *Cooperia oncophora* 99.1% and 99.4% respectively, confirming the effectiveness of the treatment in naturally acquired infections.

344 **The effect of a parenteral ivermectin/closantel injection on the growth and reproductive development of early immature *Fasciola hepatica* in cattle**

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A study was conducted whereby sixteen calves were infected with *Fasciola hepatica* and divided into two groups. On day 35 all 8 calves in Group A were injected subcutaneously with an ivermectin/closantel combination. Group B animals acted as control group. Both groups were sacrificed between days 70 and 72 to enable counting and examination of the flukes. Treatment reduced the immature fluke population by 42.6% and size by 43.9%. The mean reproductive score for untreated flukes was 8.76 and for treated flukes 5.64 ($p < 0.001$). Egg shedding from treated flukes was significantly less than that from controls ($p < 0.05$), but there were no differences in hatchability, suggesting that closantel treatment did not induce functional defects in the gonads or accessory reproductive organs.

Histological examination confirmed a reduction in development of testes, ovaries and vitellaria in treated flukes, with a consequent reduction in egg production, which may have epidemiological importance in addition to the reduction in fluke numbers.

345 **Evaluation of efficacy of moxidectin/triclabendazole oral drench against mixed infections of gastro-intestinal nematodes and liver fluke in sheep under field conditions in Spain**

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Gastro-Intestinal (GI) Nematodes and Liver Fluke are two of the most frequent parasitic infections in sheep in the north of Spain causing important losses in sheep production. Previous studies carried out by our research group, show that the prevalence of GI nematode infections is about 100% and that for liver fluke is between 50-60%. Many strategies have been proposed to preserve the efficacy of existing drugs and slow down the spread of resistance, one of the most used is the drugs combinations. The objective of this study has been to evaluate the efficacy of Moxidectin 0.1% and Triclabendazole (5%) Oral Drench combination (Fort Dodge) against this both parasites under field conditions. The study involved 60 ewes with positive nematode and fluke egg counts, ranked in pairs of ascending nematode egg counts. Group 1 (n=30) was treated with Moxidectin/Triclabendazole on day 0; group 2 (n=30) acted as control. Both groups were kept in the same farm throughout the trial. Faecal nematode and fluke egg counts were carried out on days 21, 28, 35, 42, 49 and 56 post-treatment. Pooled group faecal samples were cultured for larval identification on all sampling dates. Antibodies against *Fasciola hepatica* were measured by means of an indirect ELISA on days 0, 28 and 56 post-treatment. The efficacy for nematode infection of the combination was $\leq 99\%$; that for liver fluke was of 100% except on day 28 pt, being 99,72%. The more frequently nematode species present were *Teladorsagia circumcincta* and *Trichostrongylus* spp in pre-treatment and post-treatment.

Acknowledgement: We acknowledge Fort-Dodge Animal Health for the funding of this trial.

- 346 **The role of beta-tubulins in inferring resistance to triclabendazole in *Fasciola hepatica***
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Trichinellosis, a disease caused by the nematode *Trichinella*, occurs in more than 150 animal species in areas with different geographical and ecological characteristics. In China, *Trichinella* infection in animal is widespread, although little is known about the existing *Trichinella* species genetic variation. By amplification of different target regions (expansion segment V, 5S ribosomal DNA intergenic spacer and mitochondrial small and large subunit ribosomal RNA) by means of PCR, two *Trichinella* species were identified in China. In Henan, Tianjing, Haerbing (North of China), Xian (West of China) and Yunnan (South of China) *Trichinella spiralis* was identified in five isolates originating from domestic pig, while *Trichinella nativa* was identified in Jilin province (North-East of China), in a dog isolate. The present results confirmed the previous identification of only two *Trichinella* species in mainland China. Furthermore, the sequence analysis of the 5S ribosomal DNA intergenic spacer for the *Trichinella spiralis* isolates indicates the existence of a genetic variation among them. Work funded by the National Natural Science Foundation of China (NSFC 30328020) and AFRCST (PRA BT 03-02).

- 347 **An electron microscopical study to assess the effects of the 1,2,4- trioxolane, OZ78, at a surface and ultrastructural level on the parasitic trematode *Fasciola hepatica***
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Fascioliasis (Fasciolosis) is a zoonotic disease of great veterinary importance and one which poses a significant threat to public health. An estimated 91 million people are at risk and up to 17 million people may be infected. In livestock production animals, there are considerable economic losses due to weight loss, reduced milk yield and animal fertility. Triclabendazole (TCBZ) is the drug of choice for treating fasciolosis because of its high efficacy against both juvenile and adult fluke. Resistance to triclabendazole in *F. hepatica* was first described in Australian livestock in the mid 1990s and resistance is now spreading across Europe. Most recently, triclabendazole resistance has been reported in Spain. Although resistance in human infections has not yet been reported, it may only be a matter of time. Heavy use and the resulting resistance, means TCBZ and other treatment options for fascioliasis are still unsatisfactory and hence there is an urgent need for novel flukicidal drugs. This study examines the drug induced effects following in vitro incubation with 100 µg/ml for 48 h of the 1, 2, 4- trioxolane OZ78 on the tegumental surface of *Fasciola hepatica*, using scanning electron microscopy, and at the ultrastructural level, using transmission electron microscopy. Results showed a limited amount of disruption to the tegumental surface, whilst in contrast a significant level of internal disruption was observed. The gut and, in particular, the reproductive organs were heavily disrupted with considerable autophagic breakdown of the tissues, and other internal morphological changes were observed.

348 **The evaluation of the activity of compound alpha against two isolates of *F. hepatica* in sheep**

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Triclabendazole (TCBZ) is currently the drug of choice for the treatment of liver fluke infections in cattle and sheep. It is the only flukicide that claims efficacy against all stages of liver fluke, from early immature to adults. However, TCBZ resistance has been reported in many countries throughout Europe. Compound alpha is an experimental fasciolicide that has been shown to be very efficacious against both adult and juvenile fluke. In vitro studies using a TCBZ-resistant (TCBZ-R) isolate found that alpha sulphoxide, unlike TCBZ sulphoxide, is capable of disrupting the tegument and associated tegumental cells of 12-wk- and 4-wk-old fluke. The in vitro data prompted an in vivo trial, pitting alpha against TCBZ-R and TCBZ-S fluke infections in sheep. The trial involved 104 indoor-fed sheep which were allocated to 12 groups and dosed with either 250 TCBZ-S metacercariae or 250 TCBZ-R metacercariae. Alpha (15 mg/kg body weight) or TCBZ (10 mg/kg body weight) was administered at 3 days, 4 weeks or 12 weeks post-infection. All lambs were euthanased and examined at 16 weeks post-infection. Although alpha failed to show any efficacy against any stage of TCBZ-R *F. hepatica* infections, it did have a higher degree of efficacy against TCBZ-S infections. Fluke were removed from sheep 24 h, 48 h and 72 h post-treatment and prepared for electron microscopy studies. Microscopical changes to the fluke tegument and selected ultra-structure during the time-course experiment will be presented.

349 **Fasciolicide activity in sheep of three experimental compounds selected by computer aided design**

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The aim of the present study was to evaluate the fasciolicide activity of three experimental drugs selected by an In Silico system called TOMOCOMD-CARDD, in sheep. Drugs were identified by the computer system and after statistical selection twenty four, Pelibuey sheep were infected on days 0 and 30 each with 200 metacercariae of *F. hepatica*. When the infection reached 8 and 4 weeks of age respectively, four groups of 6 animals each were formed. Group 1 (G1) received thiacetazone 150 mg/animal/p.o. G2 was treated with 3,5,5, trimethyloxazolidine 2,4-dione at 450 mg/animal/p.o. G3 received guanabenz acetate at a dose of 1.5 mg/animal/p.o. G4 served as an untreated control. Monitoring of the study was followed by individual coprological examinations and slaughter of the animals 15 days after treatment to collect and count flukes from the liver. Efficacy was measured on the percentage of fluke reduction on treated animals relative to the control group. Results indicated an efficacy of 80.0, 43.8 y 100% for 8 week-old flukes and 62.1, 57.9 y 100% for 4 week-old flukes, respectively. It is important to note that guanabenz acetate besides the high exerted activity, showed to be highly toxic killing two animals at approximately 24 hrs. post treatment. It is concluded that further investigations should be conducted to perform computer aided prediction of drugs aimed to detect fasciolicide activity.

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350 **Comparative ovicidal effect of albendazole and triclabendazole on *Fasciola hepatica* eggs**

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The benzimidazole compounds albendazole (ABZ) and triclabendazole (TCBZ) are both effective against *Fasciola hepatica*, although ABZ is only effective against adult flukes. Additionally, ABZ is a broad-spectrum nematocidal compound with well-known ovicidal activity. However, as far as we know no data on the ovicidal effect of TCBZ against *F. hepatica* eggs are available. The work reported here evaluated the comparative ovicidal effect of ABZ and TCBZ on *F. hepatica* eggs (500 eggs/ml, n= 4) recovered from bile of sheep artificially infected with either a TCBZ-susceptible (Cullompton) or a -resistant (Sligo) *F. hepatica* isolate. Eggs were incubated with TCBZ, TCBZ-sulphoxide (TCBZSO), ABZ, ABZ-sulphoxide (ABZSO) (5 nmol/mL) or without drug (untreated control). The drug molecules were removed by several wash cycles after 12 h. Miracidial development (egg hatch) was evaluated after 15 days of incubation (25°C) by direct microscope observation. A similar egg hatch rate was observed in the untreated control and in eggs incubated with TCBZ or TCBZSO collected from sheep infected with both the TCBZ-susceptible and -resistant isolates. However, a significant ($P<0.05$) inhibition of the egg hatch was observed in ABZ- and ABZSO-incubated eggs. In conclusion, ABZ and ABZSO had a clear inhibitory effect on *F. hepatica* egg development. However, the most extensively used flukicidal compound, TCBZ, and its main sulphoxide metabolite, did not affect the egg hatch even in susceptible flukes. These findings complement previous work on the understanding of the comparative flukicidal activity of TCBZ and ABZ, and may be important to optimise fluke control in the field.

Poster group 4: Miscellaneous

351 **International collaboration in support of standardization, validation, and harmonization of quality assured diagnostic parasitology testing for food safety and trade**

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The Centre for Food-borne and Animal Parasitology (CFAP) is an International Standards Organization (ISO)-accredited laboratory performing parasitology assays for animal health, food safety, and trade purposes, including those for cryptosporidiosis, cyclosporiasis, cysticercosis, piroplasmiasis, toxoplasmosis, trichinellosis, and tritrichomonosis. Laboratories accredited according to ISO guidelines to conduct such quality assured testing are required to participate in proficiency sample exchanges to demonstrate analyst competence, and to use validated and internationally accepted methods, such as those prescribed by the World Organization for Animal Health (OIE), WHO and EU. International participation in ring trials and in the provision of reference materials and reagents contributes to a more comprehensive validation of a method and better defines its fitness for harmonized international use. An immunohistochemical (IHC) assay for bovine cysticercosis, recently validated at our laboratory using degenerated cysticerci from experimental and natural infections, identified 100%, 95%, and 73.3% of specimens obtained from heart, skeletal muscle and liver, respectively. Overall, this assay identified 91.7 % of the known positive specimens, more than twice those identified using a standard histological method. These data and subsequent requirements for international proficiency sample exchanges and ring trials for the IHC method, and for other assays, will be presented.

352 **Veterinary parasite collections at Harvard University's Museum of Comparative Zoology**

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The Museum of Comparative Zoology (MCZ) at Harvard University has maintained collections of veterinary importance since the mid 1800s, within the Departments of Invertebrate Zoology (parasites), and Entomology (vectors). The most extensive veterinary collections are of avian and mammalian ticks (Acarina) and nematodes, followed by cestodes, trematodes, several minor groups of helminths, crustacean parasites of fish, and protozoan parasites of various hosts. The MCZ participated in several major expeditions in the early 1900s, including the Kelley-Roosevelt expedition to Indo-China, Coolidge's Asiatic Primate Expedition, Natterer's filariasis expedition to Brazil, the British Vernay Malaysia Expedition, and Putnam's Congo expedition. Hundreds of veterinary and zoonotic parasites are maintained from these and other expeditions throughout the world. Hosts represented include companion animals, major livestock species, laboratory species, gallinaceous and anserine fowl, reptiles and amphibians, exotics/zoo animals, commercially important fishes, and wildlife. Specimens are curated either whole in vials, or on microscope slides as whole mounts or histopathological sections. The primary current emphasis of MCZ's work with veterinary parasites is on voucher specimens from epidemiological, experimental, and clinical research. Veterinary researchers from around the world are welcomed to deposit specimens, and those who are interested in examining specimens from the MCZ collections are welcomed to request a visit to the MCZ or to request loan of specimens, subject to MCZ's policies posted on our website at <http://www.mcz.harvard.edu>. We are in the process of listing all specimens on our online searchable database, which is accessible to any interested veterinary researchers or clinicians.

353 **DNA barcoding for filarial nematodes: a web-based tool for the molecular identification of parasitic infections**

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Filarial nematodes are major plague causing some of the most serious parasitic diseases, called filariasis, affecting human beings and other animals. More than 150 million people have already been affected and many domestic animals are infected. The standard method for diagnosing of active filarial infections is the identification of microfilariae in a blood smear by microscopic examination or serologic methods. Both these methods are potentially misleading for the species identification. DNA amplifications using species-specific primers, when correctly performed (i.e. absence of product contaminations), is considered a valid method for diagnosis in medical and veterinary contests. This approach, recently proposed for pluricellular organisms as DNA barcoding, has gained more and more favour, in particular among parasites and pathogens. DNA barcoding do not require skilled taxonomists, and allow to correlate any life stages to a single molecular entity. Here we propose a Web tool for identification of filarial species implementing concepts of DNA barcoding. Based on a dual gene approach and a validated set of sequences, our identification engine allow to perform tassonomic assignments at species level. The identification engine is available at the site <http://www.zoonemalab.unimib.it>.

354 **Parasite contamination of soil samples taken from the household areas of a Mexican rural community endemic for neurocysticercosis**

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In a previous study, high neurocysticercosis (NC) prevalence was determined by a CT scan study in the community of Tepetzintla. The aim of the present work was to evaluate the magnitude of fecal and parasite contamination by *Taenia spp.* in the soil of households of this community during the 4 seasons of the year. The latrine, courtyard, kitchen, washbasin, laundry and corrals of 14 to 27 households were sampled in each season. High parasite egg intensity of *Taenia spp.* was found in 24.2% of sampled areas. The highest percentage of *Taenia spp.* eggs was found in spring and the lowest in summer. Significantly higher levels of *Taenia spp.* eggs were found in kitchen soil samples. A significant correlation was found between the presences of *Taenia spp.* eggs in household soil during the summer with CT scan NC diagnoses in the inhabitants of these homes. Coproparasitological exams and anti-cysticercal antibodies were determined in a cohort of the inhabitants of the sampled houses. Antibody levels and coproparasitological results were not found associated with NC. Overall, these results illustrate the alarmingly high degree of fecal contamination of potential risk to human health in rural communities. This assessment could be of use in control programs of this zoonosis.

355 **Prevalence of soil-transmitted helminths in women in developing country**

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Objective: In developing country like Nepal, prevalence of parasite increasing. to find the occurrence pattern and prevalence of the soil transmitted helminths in women of child bearing age group.

Methods and Materials: The study was conducted in western part of Nepal and India border region, an area at the altitude of 2100 meter from sea level. The faecal sample of 759 women of childbearing age (15 to 45 years) were taken randomly and examined for the ova of soil transmitted helminths. Result: The occurrence pattern was 53.0%, 20.0% and 2.7% for Hookworms, *Ascaris lumbricoides* and *Trichuris Trichuria* respectively. Both *Ascaris* and Hookworm prevalence rates noticeably increased with increasing age, with the highest infection rate between the age of 36 - 45 years while *Trichuris* infection reached the highest in women of 15-25 years of age. Conclusion: There is a high prevalence of hookworm and ascaris in women of childbearing age. One of the main factors illiteracy, poverty, poor hygiene, lack of information. However necessary intervention is needed according to WHO guidelines which is very urgent for developing country like Nepal.

356 **The effect of *Nigella sativa* oil against *Aspicularis tetraptera* and *Hymenolepis nana* in naturally infected mice**

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This study was performed to investigate the effects of *Nigella sativa* oil (NSO) on *Aspicularis tetraptera* and *Hymenolepis nana* in male Swiss albino mice. The infection with these parasites was natural. The natural infections were determined by the cellophane tape method (on the anal region) and by the centrifugal flotation method of stool samples. The infected mice were divided into four groups; two treatment groups (Group 1 had *A. tetraptera*; Group 2 had *H. nana*) and two control groups (Group 3 had *A. tetraptera* but not treated; Group 4 had *H. nana* but not treated). Each treatment groups had 9 mice and each control groups had 7 mice. NSO was given at the dose of 250 µl/kg body weight orally for two consecutive days. The stool samples of the mice in all groups were examined a day before treatment, on the day of the treatment and daily for 7 days after the treatment. All the mice used in the experiment were sacrificed on the 7th day after the last treatment. It was observed that NSO caused reduction in both eggs of the parasites in feces and the number of the parasites in the intestine after the necropsy. The difference was significant between Group 1 and Group 3 ($p < 0.05$), but it was not significant between Group 2 and Group 4 ($p > 0.05$) from the point of view of the number of the parasites detected after the necropsy.

357 ***Nippostrongylus brasiliensis* infection in the lactating rat: A model to assess nutritional sensitivity of periparturient breakdown of immunity**

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During late pregnancy and early lactation, many mammals exhibit a relaxation of immune responses to gastrointestinal parasites culminating in increased worm burdens and egg excretion. In ruminants this periparturient relaxation of immunity (PPRI) increases pasture contamination and thus infection pressure to the immunologically naïve offspring. PPRI may be due to preferential partitioning of scarce nutrients, particularly protein, to reproductive functions such as lactation rather than immune functions. At times of protein scarcity, an increase in protein supply and/or reduction in protein demand would be expected to ameliorate PPRI. This hypothesis has been proven by studying secondary infection to the nematode *Nippostrongylus brasiliensis* in lactating rats which show a comparable PPRI to small ruminants. Lactating rats fed a low protein (LP) diet show increased parasite burdens in comparison with non-lactating LP rats following secondary infection with *N. brasiliensis*. Secondary infected lactating rats fed high protein (HP) foods exhibit lower parasite burdens than those fed LP foods, whilst rats nursing three pups exhibit decreased parasite numbers compared with rats nursing six or nine pups. This model is now being utilised to investigate the immune reactions underlying the PPRI. Initial studies have demonstrated that lactating HP rats develop greater immune responses than lactating LP rats, as indicated by increased antibody levels in mucosal secretions, local eosinophilia and goblet cell hyperplasia. Current work with this model concentrates on profiling local cytokine levels to assess nutritional sensitivity of the Th1/Th2 balance.

358 **Morphological description and molecular analysis of *Filaria martis* Gmelin 1790 (Spirurida, Filariidae) affecting beech marten (*Martes foina*)**

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Introduction: *Filaria martis* causes a poorly known subcutaneous filariasis in mustelids. So far, few information is available about lesions that *F. martis* causes in beech martens, on its morphology, biology, the occurrence of the infection and on arthropod/s acting as intermediate host. The aims of this work were to improve the current knowledge on this nematode by reporting the occurrence of the infection by *F. martis* in beech- martens from southern Italy, to describe the nematode morphology by supplementing the existing data with those obtained from the SEM microscopy and to provide data for the molecular identification of *F. martis* based on the mitochondrial cytochrome c oxidase subunit 1 (cox1) sequence. Materials and methods: From 1997 to 2006, 29 beech-martens from two sites of southern Italy (sites A and B) have been necropsied. Ectoparasites and nematodes were collected and morphologically identified. A variable region of the cox1 of *F. martis* has been characterized to compare females presenting caudal tips smooth without spines (i.e. Morphotype 1 –Mrph. 1) and with spines (i.e. Mrph. 2). Results: All ticks collected were identified as *Haemaphysalis erinacei*. Eleven animals from Site A were found infected by *F. martis* nematodes in subcutaneous tissue in both membranous capsules or free under the inner skin surface. The most important morphological characters of *F. martis* have been reported and discussed. The molecular analysis showed 100% homology among cox1 sequences from Morph. 1 and 2 thus indicating that the shape of female posterior edge may vary among specimens of *F. martis*. Conclusion: The results here presented provide new insights into the biology, ecology and morphological characteristics of this nematode.

359 **Residual determination of 5-chloro-2-methyltio-6-(1-naphtyloxy)-1h- benzimidazole in bovine tissue by HPLC**

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With the aim to determine the kinetics of 5-chloro-2-methyltio-6-(1-naphtyloxy)-1h-benzimidazole called compound alpha in cattle tissue through High Performance Liquid Chromatography (HPLC), eight crossbred adult cattle were divided in four groups of 2 animals each. Groups 1 to 3 received a single oral dose of 12 mg/Kg of compound alpha. Afterwards each group was sacrificed at 3, 6, 14 y 14 hours post-treatment, respectively to collect samples of muscle, liver, kidney, and renal fat. Samples were transported to our laboratory in 250 ml. plastic flasks for further evaluation. They were individually analyzed to determine the compound residues by HPLC. Results indicated the presence of compound alpha in a elimination constant of muscle in 0.07, in duodenum, 0.11 and in fat 0.10 and half life of 9.9 , duodenum 6.28 and fat 6.53 hours. For the sulphoxide and sulphone metabolites the following constants were determined: Elimination in liver 0.14 and in fat 0.179 and the half life in liver was 4.74 and in fat 3.87 hours. With regard to the concentrations they were in muscle 0.01 ng/g and 0.16 in fat, being 100 and 8 times lower to the maximum permissible (1 ppb), being the renal fat where the highest concentrations were detected. It is concluded that compound alpha is lipid-soluble under in vivo conditions being the detected residuals non risky for humans.

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360 **In vitro activity of the cyclooctadepsipeptides emodepside and PF1022A in comparison to ivermectin and levamisole in gastrointestinal nematodes**

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This study included three different in vitro assays for testing the effects of cyclooctadepsipeptides (COD) on egg hatch, larval development and larval motility. The egg hatch assay (EHA) and the larval development assay (LDA) were carried out using stages of *Haemonchus contortus* and cyathostomins of the horse. The larval motility inhibition test (LMIT) was performed with larval stages from *Cooperia oncophora*, *Ostertagia ostertagi*, *H. contortus*, *Ancylostoma caninum* and cyathostomins to comparatively assess the effects of levamisole (LEV), ivermectin (IVM) and emodepside. Ovicidal effects of COD in the EHA have not been observed even at the highest concentrations used. The LDA showed the inhibiting activity of emodepside on larval development which differed only slightly between the species tested. The LMIT demonstrated the inhibitory effects of emodepside on larval migration with similar EC₅₀ values within the species. The most susceptible species was *A. caninum*, the least susceptible *H. contortus*. For all drugs tested a clear order of potency was observed: IVM was the most potent anthelmintic, LEV the least potent one. With EC₅₀ values of 2.25-8.28 μ M emodepside ranged between the two. In the present study the LDA and LMIT have been successfully established to document the activity of emodepside. To date, the in vitro activity of this compound has only been tested with laboratory isolates. In the future it will be necessary to study field populations in order to monitor the efficacy of emodepside.

361 **Interactive effects between growth potential and protein nutrition on resilience and resistance to parasites in mice**

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Breed improvement in farm animals through genetic selection is often attained through a narrow breeding goal, e.g. selecting for higher growth only. An increasing body of evidence suggest that the latter may reduce resilience and resistance to gastrointestinal parasites. Here, we assess whether protein supplementation may overcome such effects. A 2x2x2 factorial design (n=8) was used, with two levels of growth potential, infection and protein nutrition. Weaned male mice were derived from the Roslin lines, divergently selected for more than 20 generations into high (ROH) or low (ROL) growth potential. At day 40 of age, mice were dosed with 250 infective *Heligmosomoides polygyrus* larvae or sham-infected with water (day 0). The individually housed mice were fed foods with either 50 (LP) or 250 (HP) g protein per kg. Food intake and body weight were assessed twice weekly until day 28, when colon egg counts and worm burdens were taken. Infection reduced intake and body weight gain for the ROH mice but not for the ROL mice (P<0.001). However, these effects were more pronounced in LP mice (P<0.001) than in HP mice (P<0.05). Protein supply did not affect worm burdens and number of nematode eggs in the colon contents, but the latter were higher for ROL mice than for ROH mice (P<0.01). Although observed effects on host resilience were not consistent with those on host resistance, the results support the view that selecting for growth performance reduces host resilience, and that protein supplementation can ameliorate this penalty to some extent.

362 **Disseminated cestodiasis of mammals involving anomalous epithelial development in metacestodes**

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For almost a century, sporadic veterinary case reports have described disseminated cestodiasis in a variety of hosts, resulting from proliferation of aberrant plerocercoid metacestodes. The hosts involved most often have been dogs in various parts of North America, Europe, and Australia. Other hosts include various primates from Africa, rodents from Europe and North America, and a single case from a horse in North America. Similar cases have been reported from humans in North and South America and Asia, and from a snake in North America. In most cases, these have been diagnosed as belonging to the genera *Spirometra* (order Pseudophyllidea) or *Mesocestoides* (order Cyclophyllidea), but aberrant morphological features often preclude identification even to the order level. In the present study, specimens were examined from many of these cases around the world, as well as from other hosts and locations, including spiny echidnas from Australia, and shrews from Europe. Analysis included standard histological preparations as well as analysis of stained whole mounts by differential interference contrast microscopy. Results demonstrated various developmental anomalies, with aberrant development of the tegumentary and excretory epithelia in both proliferative and apparently non-proliferative metacestodes, but the latter usually having abnormally large hindbodies. These epithelial aberrancies occurred in metacestodes with normal scoleces and those lacking scoleces, but usually involve internalization of host inflammatory exudate by the greatly exaggerated tegumental folds. These results suggest a role for these epithelial aberrancies in fostering parasite dissemination and/or abnormal somatic growth within the host, leading to increased pathology.

363 **Trichinellosis in Switzerland**

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Human trichinellosis is a food-borne zoonosis exhibiting significant health and economical problems predominantly in countries with high pork consumption. During the past ten years the number of human outbreaks around the world has increased in certain areas. In Europe, more than 20,000 human cases have been detected between the year 1991 and 2000. Conversely, trichinellosis has not been reported for many decades among Swiss domestic pigs. The last autochthonous cases of human trichinellosis in Switzerland date to a time period prior to the first half of the last century. Nevertheless, *Trichinella* occurs in a sylvatic cycle in Switzerland. Molecular and genotyping investigations on the taxonomy of present Swiss *Trichinella* isolates always yielded *Trichinella britovi* as a species. In an earlier study, foxes had been tested using the artificial digestion method with a prevalence finding of 1.3%. Similar investigations in Swiss lynxes yielded a parasitological prevalence of 27%. Based upon this epidemiological background, a risk-based surveillance project is presently running under the sponsorship of the Swiss Federal Veterinary Office, including a detailed parasitological and serological (E/S-ELISA and Westernblot) investigation of representative populations of domestic pigs, wild boars, foxes, lynxes and other wild carnivores. In order to obtain reference laboratory specimens to standardize parasitological and serological tests, experimental infections in pigs were carried out with *Trichinella spiralis*, *T. britovi* and *Trichinella pseudospiralis*. Seroconversion occurred on day 21 p.i. for *T. spiralis* and on day 28 p.i. for *T. britovi* and *T. pseudospiralis*. As expected, all sera of the three species cross-reacted with the *T. spiralis* E/S-antigen, thus indicating that this kind of antigen will be suitable to catch hold of all three species by serological means.

364 **Heterogeneity of human trichinellosis and animal *Trichinella* infection in Romania: a geographical paradox due to ancestral food habits and political changes**

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With an incidence of 55,1 cases/106 man-year of trichinellosis over a period of 8 years et a prevalence of 8 cases/104 tested animals for pigs, 9 cases/103 for wild boars, 13,1 cases/102 for bears, Romania represents the country with the most extensive *Trichinella* infection in the world. The uneven repartition of populations of German origin, who colonized specific areas of Romania (Transylvania) beginning with the end of the 12th century, and their particular food habits (raw pork meat et sausage consumption) was taken into account though, when analysing trichinellosis epidemiological data in Romania. Analysis of human incidence and of animal prevalence data result in an apparent geographical paradox: whereas the trichinellosis incidence rate is twice higher in Transylvania than in the other counties, the *Trichinella* infection prevalence in pig is much lower. Hypotheses and facts contributing to the heterogeneity of human trichinellosis and *Trichinella* infection cases in Romania are discussed. Work founded in part by the EU project “TRICHIPORSE” (contract QLK1-CT-2001-01156) and in part by the EU project MedVetNet (Workpackages TRICHINET and TRICHIMED).

365 **Molecular identification of *Trichinella* species circulating among wild and domestic animals in Romania**

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Trichinellosis, a food-borne parasitic zoonosis, which affects more than 11 million people worldwide (Dupouy-Camet, 2000), is one of the most serious helminthic zoonosis of Romania. In spite of the presence of both a domestic and a sylvatic cycle, all *Trichinella* spp. isolates identified so far were assumed to be *Trichinella spiralis*. Therefore the present study was conducted to identify *Trichinella* spp. circulating among wild and domestic animals, by PCR-based methods. The potential influence of climate on the distribution of *Trichinella* species was also evaluated. *Trichinella* sp. larvae were detected in 54 (31.8%) of the 147 wild and 23 domestic mammals examined. No *Trichinella* sp. larva was detected in the breast muscle samples of the 182 birds examined. *Trichinella spiralis* and *Trichinella britovi* were the only two species identified in the 40 isolates which showed a positive PCR amplification. *Trichinella britovi* is the most prevalent species (n=26, 65%), followed by *T. spiralis* (n=14; 35%) which was predominant among domestic animals (n=9; 75%), while *T. britovi* was prevalent among wildlife isolates (n=24; 85.7%). No mixed infection was found. The distribution of *Trichinella* species in Romania does not show a species-specific cluster, like described in Bulgaria, both species being present over the entire examined counties. The highest prevalence of *Trichinella* infection was detected in wolves (31.4%; 11/35), followed by the European wild cats (14.3%; 4/28) and red foxes (7.0%; 5/71).

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366 **Isolation, biochemical analysis and localization of a 24 kDa Glutathione S-Transferase from *Trichinella***

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Survival of *Trichinella* sp., a pathogenic nematode parasite, is likely dependent upon the detoxification activities of the glutathione S-transferases (GSTs). A DNA sequence similar to the GST gene family was first identified from a subtracted cDNA library of *T. spiralis* new born larvae (NBL) by suppression subtractive hybridization, and was used as a probe for the screening of a *T. spiralis* adult worm with hatched larvae (Ad5) cDNA library. The identified clones shared 47% to 52% of identity with the 24 kDa pi class intracellular GSTs of other nematodes. Moreover, the comparison of *T. spiralis* GST sequence (tsgst24) with those amplified from *T. britovi* (tbgst24) or *T. nativa* (tngst24) revealed a high interspecies nucleotide conservation (96% of identity). The GST of *T. pseudospiralis* (tpsgst24) presented 88% identity with tsgst24. The open reading frame coding for *T. spiralis* GST24 was expressed in a prokaryotic system and the soluble recombinant protein was purified by glutathione-affinity columns. TsGST24 enzymatic activity was proved to be different from the activity of mammalian pi class GSTs, and other pi class related nematode GSTs. The distribution of TsGST24 was examined by immunohistochemistry, using the anti-GST antibody, in *T. spiralis* muscle larvae (ML) and infected tissues. TsGST24 was located in the larval stichocytes and in the genital primordium. The nurse cell was completely unstained, suggesting that Ts GST is not vehiculed outside the parasite.

Work founded by the EU project TRICHINET/TRICHIMED workpackage (MedVetNet).

367 **Species identification of *Trichinella* isolates from China**

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Trichinellosis, a disease caused by the nematode *Trichinella*, occurs in more than 150 animal species in areas with different geographical and ecological characteristics. In China, *Trichinella* infection in animal is widespread, although little is known about the existing *Trichinella* species genetic variation. By amplification of different target regions (expansion segment V, 5S ribosomal DNA intergenic spacer and mitochondrial small and large subunit ribosomal RNA) by means of PCR, two *Trichinella* species were identified in China. In Henan, Tianjing, Haerbing (North of China), Xian (West of China) and Yunnan (South of China) *Trichinella spiralis* was identified in five isolates originating from domestic pig, while *Trichinella nativa* was identified in Jilin province (North-East of China), in a dog isolate. The present results confirmed the previous identification of only two *Trichinella* species in mainland China. Furthermore, the sequence analysis of the 5S ribosomal DNA intergenic spacer for the *Trichinella spiralis* isolates indicates the existence of a genetic variation among them. Work founded by the National Natural Science Foundation of China (NSFC 30328020) and AFCRST (PRA BT 03-02).

368 **Sylvatic trichinella infection in Finland - red fox (*Vulpes vulpes*) and raccoon dog (*Nyctereutes procyonoides*) as indicator species**

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The prevalence of sylvatic *Trichinella* infection in Finland varies by region. The dominating *Trichinella* species in Finnish wildlife is *T. nativa*. Other species found are *T. spiralis*, *T. britovi*, and *T. pseudospiralis*. According to hypothesis, the raccoon dog acts as an important reservoir in Finland. Due to the winters, raccoon dogs do not thrive in northern Finland while foxes reside in the entire country. The role of the raccoon dog in the epidemiology of *Trichinella* infection was tested by studying the prevalence of infection in red foxes in three climate zones.

The material consisted of 662 raccoon dogs and 1110 red foxes collected by hunters between 1999 and 2005. Muscle samples from each animal were artificially digested to determine the density of *Trichinella* infection. Identification, based on multiplex-PCR, was performed on 55% of positive samples (n=206).

Twenty-eight % of raccoon dogs were *Trichinella* positive with median lpg (larvae per gram of muscle) of 40 (range: 0.05-760). The prevalence in red foxes was 19 % with median lpg of 9.5 (range: 0.02-342). The results of the present study indicate that the foxes from raccoon dog dense area (southern Finland) had 79-fold risk (odds ratio, OR=78.8, CI95% 36.1-171.7) to be *Trichinella* positive compared to foxes from raccoon dog free area (northern Finland).

T. nativa was present in 82 % of PCR positive foxes and 74 % of raccoon dogs. All four *Trichinella* species previously reported from Finland were detected in PCR.

The raccoon dog seems to play a pivotal role in epidemiology of *Trichinella* infection in Finland.

Poster group 5: Arthropods of Companion Animals

369 **Longitudinal systematic survey of fleas on dogs**

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A longitudinal systematic survey aimed to study the presence and distribution of fleas on dogs was carried out in the Campania region of southern Italy. Between February 2005 and February 2006, dogs were examined for fleas at four private veterinary clinics, with a twice weekly frequency. All the dogs brought to the clinics for routine health care procedures were examined (total number = 1,376), and registered as to sex, age, weight, hair length, utilization, live together with other dogs, indoor/outdoor night status, and geographical origin. From each positive dog, fleas were collected in tubes containing alcohol at 70% and preserved at room temperature until identification. Fleas were detected in 246 (17.9%) out of the 1,376 tested dogs. A total of 960 fleas were sampled and two species were identified, namely *C. felis felis* (258 males and 624 females) and *C. canis* (6 males and 72 females). *C. felis* was the most frequent species (16.3% of dogs); *C. canis* was found on 1.5% dogs. Mixed infection wasn't detected. The results of the logistic regression model showed a significant association ($P<0.05$) between the flea positivity and the following independent variables: life together with other dogs and with cats and utilization, i.e. increasing prevalence from pets to guard, hunting and stray dogs. The monthly flea prevalence values were related to the monthly minimum, mean and maximum temperatures in the study area. Although the prevalence was higher during the period between June and October, flea infestation was detected throughout the year.

370 **Evolution of flea infestation in dogs on an eleven years period in west of France**

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Very little information is available on the evolution of frequency of fleas in dogs although the frequent question about change of efficacy of antiparasitic drugs and influence of climatic changes. Data were collected from computerized information from the clinics of Unit DPM. These data are systematically collected and prospectively validated in a GCP system and can be therefore retrospectively analysed. Flea infestation (fleas and/or flea faeces) was systematically assessed on every dog by thumbing and combing methods. The period of study covered the years 1996 to 2006. The percentage of infestation was calculated for monthly (except for July and August) or yearly variations.

Results : From 7537 dogs 660 were infested (8.8%). Average monthly infestation varied from a 14.5% in October to a minimum of 4.6% in June. There is a progressive decrease from October to March. A maximum of infestation was seen in October 1997 (51.2%) and flea were totally absent from only 8 of the 110 months. The annual percentage of infestation regularly decreased from 1997 (17.3%) until 2006 (4.0%)

As observed from a previous study *, the evolution of flea population is clearly seasonal in dogs. Interestingly there is a decrease in prevalence. These do not support a detectable global problem of reduction in efficacy of antiparasitic drugs nor negative effects due to climatic changes until now in the area.

*BOURDEAU P, BLUMSTEIN P. and FROMEAUX C. : Non seasonal prevalence and epidemiological characteristics of flea infestation in dogs and cats. *Veterinary Dermatology* (2000), 11, (suppl). 37.

371 **Are corticosteroids involved in generalized demodicosis in dogs?: Informations from 55 cases**

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Myth or reality ? It is frequently mentioned that corticosteroids as used in practice may cause generalized demodicosis in dogs or secondary bacterial infection. The aim of this study was to evaluate this hypothesis. Computerized data from clinics of Unit DPM, organized on GCP approach with systematic prospective validations, were retrospectively analysed. Two groups of dogs were selected. A = adults dogs with generalized demodicosis : Diagnosis was based on clinical signs, isolation of *Demodex* in multiple scrapings from different areas; B = reference : Dogs presented at the same period but without demodicosis. The presence of an associated pyoderma and treatment with systemic corticosteroids (before or after the development of skin lesions) were recorded. Results were compared using Chi 2 test ($p < 0.05$).

A total of 108 dogs were included (55 A, 53 B). In group A 21 (38.2%) dogs had received corticosteroids (A+), but only 3 before the demodicosis, compared to respectively 14 (26.4%) (B+) and 1 in reference group. Pyoderma was associated in 15 from A+ (71.4%) and 21 (61.8%) from A-. These results were respectively 8/14 (57.1%) in B+ and 13/39 (33.3%) in B-. No statistical difference was observed between the two groups considering the frequency of use of steroids. Infection was significantly ($p=0.01$) higher in A group compared to B but not between A+ and A- nor B+ and B- dogs. As a conclusion the use of corticosteroids cannot explain the onset of lesions nor secondary bacterial infection in demodectic dogs.

372 **Distribution of cat flea eggs and larvae in the carpeted environment**

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The wide distribution of fleas and their role as vectors for pathogens, and their significance for public health, make a successful control definitively necessary. The cat flea maintains its life cycle indoors feeding on pets. However, it must be emphasized that only about 1-5% of the flea population is on the hosts, the remaining 95-99% (eggs, larvae, and pupae) are spread around the habitat. In the first study, three cats were kept in a carpeted room, and had been infested four times with 50 cat fleas weekly. After five weeks the spatial distribution of developmental stages in the carpet, and on resting sites were checked by vacuum cleaning. The hatched eggs were focused around feeding area and resting sites. The largest number of hatched eggs from any single sample was 565 from the male cats' resting box. The distribution of larvae was nearly the same as that of hatched eggs. The presence or absence of flea larvae in an area was related to sunlight exposition, temperature or activity areas of the cats. Concentrations of flea eggs and larvae on the carpeting were highest in sleeping and resting areas. The second study was performed in order to evaluate the efficacy of vacuuming for removal of the developmental stages of cat fleas. Our data indicate that vacuuming can remove 40-80% of eggs that are located in the carpet pile, but vacuuming may remove only < 5% of flea larvae in the carpet. The power of vacuum cleaners (1200,1500,1800 W), and time of vacuuming after having infested carpet ground with a definitive number of eggs and larvae (1 and 6 hours) did not correlate with the amount of removed eggs and larvae. However, the length of carpet fibers is closely associated with the removal of the developmental stages. Vacuuming in the household carpeting may remove only a small proportion of eggs and larvae.

373 **Flea infestation in cats: evolution on an eleven years period through prospective/retrospective evaluation**

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The aim of this study was to evaluate the incidence of flea infestation in cats on a long period of time in west of France. Data were collected from computerized information from the clinics of Unit DPM. These data are systematically collected and prospectively validated in a GCP system and can be therefore retrospectively analysed. Flea infestation (fleas and/or flea faeces) was systematically assessed on every cat by thumbing and combing methods. The period of study covered the years 1996 to 2006. The percentage of infestation was calculated for monthly (except for July and august) or yearly variations. From 1973 cats, 473 were infested (24.0%). Average monthly infestation varied from a maximum of 31,3% in September to a minimum of 18.8% in February followed by an increase until June 23,3%. Fleas were totally absent in only 7 from the 110 months. The annual percentage of infestation was maximum in 1998 (33,1% of cats) with a progressive and persistent decrease until 2005 (15.5%). However in 2006 infestation rate was 19%.

As observed from a previous study on a 4 year period* the evolution of flea population in cats differs from dogs and remains around a monthly value of 25%. The significance of an increase of prevalence in 2006 after a persistent period of decrease on 9 years needs further information.

* BOURDEAU P, BLUMSTEIN P. and FROMEAUX C. :

Non seasonal prevalence and epidemiological characteristics of flea infestation in dogs and cats. *Veterinary Dermatology* (2000), 11, 37.

374 **Investigation of the microscopic anatomy of *Ixodes ricinus* and *Ctenocephalides felis* with view to identification of sites for microbial pathogen localisation**

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We aim to redress the lack of information on vector-pathogen interactions by constructing a toolbox of techniques for identification, visualisation and quantification of microbial pathogens within vector tissues during blood-feeding. Preliminary work has concentrated on investigating the microscopic anatomy of model vectors (*Ixodes ricinus* and *Ctenocephalides felis*). Whole *I. ricinus* and *C. felis* were fixed in 4% formaldehyde before being processed for sectioning and embedding in wax. Using a microtome, 4µm sections were taken and transferred to slides, dewaxed, and then stained either with haematoxylin and eosin (HE), haematoxylin van Geison (HVG) or toluidine blue stains. Dissections of whole vectors were carried out in saline solution, using a standard dissection microscope. Visualisation of the internal anatomy in whole-organism stained sections was clear and distinct, allowing the precise identification of specific organs and tissues. HE and HVG staining produced the best results, with clear contrasting colouration between organs and tissues. Toluidine blue staining was less clear but allowed the staining of delicate and fragile sections. All major organs were identified, including the gut track, salivary glands, malpighian tubules and nervous system. Dissection of vectors was time-consuming and skill-intensive but the anatomical knowledge gained by basic dissection and microscopy lays the groundwork for the detection and tracking of pathogens as they invade the arthropod vector. Whole-organism sections will allow microbial pathogen visualisation using pathogen-directed immunofluorescence and in situ hybridisation, whilst dissections will allow individual organs and tissues to be isolated for real-time polymerase chain reaction assays for tissue-specific quantification of microbial load.

375 **Clinical and immunity aspects in experimental infection and reinfection with *Microsporum canis* in cats**

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The aim of the present study was to investigate the presence of general and local immunity consequently of experimental infection with *Microsporum canis* in cats. The infection and reinfection with *Microsporum canis* was performed on a group of 8 male and female cats. At the first infection, the cats were 2-3 months old, and the reinfection was performed one year later. The 8 cats were separated in 2 groups: for the 1st group (4 cats) the infection was made at the thoraco-abdominal region, and the reinfection at the head; for the 2nd group (4 cats), both infection and reinfection were performed at the head. The used dose, identically for infection and reinfection, was as high as 10⁵ *M.canis* conidia per region, 3 days consecutively. The monitoring of the local lesions was performed at 21, 28, 48, 69 and 90 days p.i. A special notational system for the gravity of the local lesions was applied in order to obtain a lesion score.

In the first infection, the average infection period was of 84 days for the 1st group and 98 days for the 2nd group (p = 0.46). In the case of reinfection, the period was 44 days for the 1st group and 29 days for the 2nd group (p = 0.035).

The lack of interferences between the lesion scores in case of the first infection demonstrates that the cutaneous region where the experiment was performed plays a major role in the evolution of microsporoses lesions in cats.

The statistically valid differences between the lesion scores, when the region of infection doesn't change (head to head), as well as the lack of phases in the evolution of a lesion at reinfection, proves the existence and importance of local immunity in this group.

The decrease in gravity of the lesions, as well as the significant decrease of the infection period in both groups in case of reinfection, demonstrates indirectly the existence of a general gained immunity.

376 ***Demodex gatoi* associated contagious pruritic dermatitis in cats – A report from six households in Finland**

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Demodex gatoi is unique among demodectic mites. Instead of harbouring hair follicles, it dwells in the keratin layer of the epidermis causing a pruritic and contagious skin disease in cats. Little is known of occurrence and treatment of *D. gatoi* associated dermatosis.

Here we describe *D. gatoi* in 10 cats including six Cornish Rex, two Burmese, one Exotic and one Siamese, living in six multi-cat households in Finland. Intense pruritus was the main clinical sign. In addition, scaling with broken hairs, alopecia and self-inflicted excoriations were observed.

The diagnosis was based on finding demodectic mites with stubby appearance, typical of *D. gatoi*, in skin scrapings/tape strips or in skin biopsies. Other pruritic skin diseases such as allergies and dermatophytoses were ruled out accordingly. In one cattery, despite of finding several mites from one cat, all six cats remained symptomless.

Amitraz used weekly at a concentration of 125-250 ppm for 2-3 months was successfully used in three catteries, 2% lime sulphur weekly dips for six weeks in one cattery and peroral ivermectin (1 mg eod for 10 weeks) in one cattery. Previous trials in four catteries with imidacloprid-moxidectin, selamectin or injected ivermectin given once or twice a month turned out to be ineffective.

These cases show that it *D. gatoi* associated dermatosis may be an emerging contagious feline skin disease in Finland. Although pruritus is common, some cats may harbour the mites without showing clinical signs. In addition, due to translucency of the mites and feline grooming habits, the diagnosis may be challenging.

377 **Assessment of the ear mite, *Otodectes cynotis* infestation and the efficacy of Advocate® in the treatment of otoacariosis in a Hungarian cat shelter**

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Two field studies were carried to assess *Otodectes cynotis* infestation and to evaluate the efficacy of Advocate® spot-on (1.0% moxidectin and 10% imidacloprid, Bayer AG) against ear mites in a cat shelter. Fifteen out of 100 cats selected randomly were found to be infested with live mites. Seven animals showed severe clinical signs of otitis externa. These cats were restless, shaking their heads and scratching their ears. Alopecia, wounds caused by self injuries and dermatitis were seen in and around the ears of two cats having most severe clinical signs. The other infested animals had only some dark brown exudates accumulated at the deeper part of the external ear canals.

Ten and five cats naturally infested with *O. cynotis* were randomly assigned to the treatment and the untreated control groups, respectively. Each cat of the treated group was weighed and treated with the recommended dosage of Advocate® spot-on on day 0. Clinical inspections including direct and otoscopic examination of the ear canals and sampling with cotton-tipped swabs were performed on days 9, 16 and 30. On day 9 three cats were infested with live ear mites. The efficacy of Advocate® against *O. cynotis* was 100% on days 16 and 30. No or some dried up mites occurred in samples of treated cats without any clinical signs of otoacariosis. The external ear canals of untreated cats continued to contain large amounts of dark brown, waxy cerumen and their ear swab samples contained live ear mites until the end of the study.

378 **Clinical evaluation of the efficacy and safety of topically applied advantage Multi™ (imidacloprid/moxidectin) against ear mites (*Otodectes cynotis*) in client owned cats**

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A topically applied formulation containing 10% imidacloprid/1% moxidectin (Advantage Multi™ / Advocate®) has been developed for monthly treatment to cats for the prevention of feline heartworm disease; and for the treatment and control of flea infestations, ear mite infestations, and intestinal nematode infections.

A multi-centered clinical field study was conducted to evaluate the safety and efficacy of Advantage Multi against ear mites (*Otodectes cynotis*) in client owned cats. Veterinarians and staff from 6 veterinary clinics (U.S.) participated in the study. Single and multi-cat households were enrolled in the study. In multi-cat households, all cats were evaluated for safety, but only one cat in the household was randomly chosen for evaluation of efficacy. Ninety-three (93) households with 332 cats were evaluated for safety, and 77 households with 301 cats were evaluated for efficacy. Each household was randomly assigned to a treatment group: Advantage Multi or Revolution® (selamectin, Pfizer).

Treatments were administered twice, approximately 30 days apart, by the cat's owner. Otoscopic examinations were performed on the cats by the investigators for the presence of ear mites prior to treatment, and approximately 30 days after each treatment.

Advantage Multi provided 92.5 and 98.1% ear mite efficacy following the first and second treatments, while Revolution provided 91.7 and 95.8 % efficacy following the two respective treatments. No serious adverse events related to treatment with either product were observed during the study.

379 **Efficacy of selected spot-on insecticides in the treatment of cats from animal shelters infested with ticks (Acarina) and fleas (Siphonaptera)**

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Most insecticides do an excellent job of eliminating existing ticks and fleas from the host during the initial application. The problem is that reinfestation is a common occurrence. Tick and Flea control was achieved through repeated spot-on insecticides application. In the tests of the study 24 cats were experimentally allocated to 3 groups. All cats were naturally infested with ticks (*Ixodes ricinus*) and fleas (*Ctenocephalides canis* and *Ctenocephalides felis*). Cats were experimentally allocated to 3 groups and treated with Fipronil (Front line spot-on), Fenthion (Tiguvon spot-on) and Permethrin (Exspot sol. spot-on). The efficacy after flea treatment reached 89.2 % to Fipronil during 35 to 46 days. Tiguvon treated cats reached average of efficacy 91.9 % to fleas and in cats treated with Permethrin spot-on the mean efficacy was 86.8 %. According to their living habitat and hunting habits, cats may also acquire infestations with ticks, *Ixodes* species. Used tick control products were available for repelling and killing ticks. The efficacy of acaricides mentioned above ranged from 68.3 to 83.4 %, but their duration of action were only from 2 to 4 weeks.

The study was supported by the basic research of National reference laboratory for pesticides at the University of Veterinary Medicine in Kofice and grant agency CR - FRVS 2007/413.

380 **0.4% dimeticone spray, a novel control material to kill fleas by physical means**

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Introduction. The cat flea, *Ctenocephalides felis*, is a common pest of domestic animals and can be a cause of irritation and health problems in humans. Most products to control these pests rely upon neurotoxic insecticides, and many lack persistence to affect juvenile stages of the flea life cycle. Recent legislation, through the Biocidal Products Directive, has led to the removal of some consumer products from the market and emphasized the need for a non insecticidal solution to the problem.

Materials and methods. High molecular weight dimeticone, 0.4% in a volatile silicone base, was used as an aerosol sprayed on carpet. Cat flea adults and all development stages from a laboratory culture colony were exposed in vitro and in semi field conditions.

Results. All mobile stages of the flea life cycle placed on a pre-treated surface tried vigorously to escape contact with the dimeticone. Insects became physically immobilized within minutes without recovery. Exposed eggs failed to hatch and adult fleas were unable to escape from treated cocoons.

Conclusion. 0.4% dimeticone spray has been shown to have a physical control activity against all stages of the cat flea. Tests showed this new product to have a knock down capability comparable to that of permethrin spray, whilst persisting in test arena area for months after application. The product has the capability to eliminate a flea infestation with one application.

381 **Pharmacokinetics of metaflumizone in the plasma and hair of cats following application in a topical spot-on formulation**

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Metaflumizone is a novel insecticide with potent activity against fleas. A novel, topical spot-on formulation containing 20% w/v metaflumizone (ProMeris® for Cats) is effective for the treatment of existing infestations of fleas and provides control against reinfestation for up to 8 weeks. Two studies were conducted to determine the distribution of metaflumizone in the plasma (Study 1) and hair (Study 2) of cats. Six purpose-bred cats were used in each study. Product was applied as a single spot on the back of the neck at the base of the skull on Day 0 to provide approximately 40 mg/kg body weight of metaflumizone. Plasma was harvested in Study 1 and hair samples were collected in Study 2 just prior to dosing and periodically through 56 days PT. Metaflumizone concentrations in plasma were generally below LOQ (<50 ng.mL⁻¹) and were frequently not detectable (<1.1 ng.mL⁻¹). Plasma collected three days post-treatment from one cat had a metaflumizone concentration of 57.8 ng.mL⁻¹. Analysis of hair samples indicated that metaflumizone was widely distributed in the hair with maximum concentrations within two days PT, after which they gradually decreased. Low but quantifiable levels were still present at the end of the 56 day study. Data from the present studies indicate that the ectoparasitic activity of ProMeris for Cats is due to exposure of the parasites to metaflumizone on the surface of the host (skin and hair), not to exposure via the circulatory system (taking of a blood meal) of the host.

382 **Pharmacokinetics of metaflumizone and amitraz in the plasma and hair of dogs following application in a topical spot-on formulation**

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Metaflumizone is a novel insecticide with potent activity against fleas via topical application to dogs. Metaflumizone has been combined with amitraz in a novel spot-on formulation containing 15% w/v of each compound (ProMeris®/ProMeris Duo® for Dogs) for flea and tick control on dogs. Two studies were conducted to determine the distribution of both compounds in the plasma (Study 1) and hair (Study 2) of dogs. Six adult Beagle were used in each study. Product was applied as a single spot between the scapula on Day 0 to provide approximately 20 mg/kg body weight of each active ingredient. Plasma was collected in Study 1 prior to dosing and periodically through 56 days PT. Hair samples were collected from five sites in Study 2 periodically 1 to 56 days PT. Amitraz was generally not detectable in plasma (<3.2 ng.mL⁻¹). Metaflumizone was generally detectable (>1.0 ng.mL⁻¹) in plasma, but not quantifiable (<50 ng.mL⁻¹). Analysis of hair samples indicated that both metaflumizone and amitraz were widely distributed within one day PT at basically similar levels. Maximum concentrations generally occurred between two and seven days post-treatment. Low but quantifiable levels were still present at the end of the 56 day study. Data from the present studies indicate that the ectoparasitic activity of ProMeris/ProMeris Duo for Dogs is due to exposure of the parasites to metaflumizone and amitraz on the surface of the host (skin and hair), not to exposure via the circulatory system (taking of a blood meal) of the host.

383 **The efficacy of pyriprole topical solution against the cat flea, *Ctenocephalides felis*, on dogs**

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Three laboratory studies were conducted to demonstrate the efficacy of pyriprole topical solution (Prac-tic®; Novartis AG, Basel Switzerland) against fleas. All studies were well controlled, randomized and blinded.

In study NAH-03-0026 dogs were housed in simulated home environments and treated with pyriprole topical solution or placebo on Days 0 and 30. On Days 1, 7, and 14 each dog was infested with 50 fleas. On Days 7, 14, 21, 30 and 45 fleas were combed off, counted, and up to 200 returned to the dog. On Day 60, fleas were combed off, counted, and discarded. No live fleas were found on any of the treated animals. Thus pyriprole topical solution prevented the build up of flea infestations on dogs.

In study NAH-05-0008 two groups of dogs were treated with pyriprole topical solution (18 dogs) or placebo (6 dogs) on Day 0. On Days 11, 18, 26, 39, 46, 53 each dog was infested with 1000 fleas. Flea eggs were collected 3 and 4 days post each infestation. Pyriprole killed 100% of adult fleas before they were able to lay eggs for 42 days post treatment. Greater than 97% of fleas were killed through Day 50.

In study 444IRL-0103, dogs were treated with pyriprole topical solution (32 dogs) or left untreated (32 dogs). Dogs were infested with ~100 fleas on Study Day -1. Fleas were counted at +12 hours, +24 hours, +36 hours and +48 hours after treatment. Pyriprole killed greater than 90% of fleas at 12 hours and greater than 99% of fleas at 24, 36 and 48 hours after administration.

384 **The efficacy of pyriprole topical solution against tick (*Rhipicephalus sanguineus*, *Ixodes ricinus*, *Dermacentor reticulatus*) infestations on dogs**

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Three randomized controlled and blinded laboratory efficacy studies were conducted to demonstrate the efficacy of Pyriprole topical solution (Prac-tic®; Novartis AG, Basel Switzerland) against major European tick species. In each study 8 dogs were treated with pyriprole topical solution on Study Day 0. Eight dogs were assigned to the control group and were either treated with a placebo formulation or left untreated. All dogs were infested weekly for 4 weeks post treatment with 50 adult ticks and tick counts were performed 48 hours post each infestation. Efficacy against *Rhipicephalus sanguineus* was 100% throughout the study. Protection against *Ixodes ricinus*, as well as *Dermacentor reticulatus* infestations was greater than 98% for 4 weeks post treatment. An infestation with *Dermacentor reticulatus* one Day before treatment was eliminated with greater than 95% efficacy on Study Day 2.

Pyriprole topical solution is highly effective in dogs for the prevention of infestations by three clinically important tick species for 4 weeks post treatment.

385 **The safety profile of pyriprole and prac-tic®**

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Novartis Animal Health, Basel, Switzerland

Pyriprole is an ectoparasiticide of the phenylpyrazole class and is the active ingredient in PRAC-TIC, a spot-on solution for treatment and prevention of flea infestations and elimination of ticks on dogs.

Pyriprole is rapidly converted via hepatic metabolism into its sulfone and sulfoxide derivatives. The metabolite profile of pyriprole is basically identical in rats and dogs and independent from the route of administration. Pyriprole and its major metabolites bind with high affinity to GABA-gated chloride channels.

Pyriprole is moderately toxic after a single oral administration in rats. After single dermal administration, toxicity is low in rats and moderate in rabbits. Pyriprole and PRAC-TIC are not irritant to skin and eyes and do not cause skin sensitization. Pyriprole is not genotoxic, not teratogenic and does not induce relevant reproduction toxicity or developmental neurotoxicity.

Adverse effects of pyriprole are associated with transient effects on the nervous system, such as incoordination, muscle tension, sensory induced convulsions and seizures. Secondary effects on the liver and thyroid were only observed in rodents, which are known for their species-specific sensitivity towards disturbances of the liver-pituitary-thyroid axis.

In a repeat dose target animal safety study it was concluded that monthly administration of PRAC-TIC to puppies was very well tolerated at 3-fold of the maximum therapeutic dose. At the 5-fold dose adverse nervous signs were only observed on a single occasion in one animal only and completely resolved within one day.

PRAC-TIC was also shown to be safe for humans (including toddlers) and for the environment.

386 **The effect of water and shampooing on the efficacy of pyriprole topical solution**

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In several studies it was demonstrated that water immersion and shampooing of dogs does not reduce the duration of protection of pyriprole topical solution (Prac-tic®; Novartis AG, Basel Switzerland) against flea- (*Ctenocephalides felis*) and tick (*Rhipicephalus sanguineus*) infestations on dogs. All studies were well controlled, randomized and blinded. Control groups were either left untreated or were placebo treated. All animals were infested weekly with fleas or ticks for 4 weeks post treatment and parasites were counted 24 or 48 hours post each infestation.

The effect of weekly water immersion on the efficacy against fleas and ticks was studied. Flea efficacy was 100% throughout the study, except of Study Day 23, where 99.9% efficacy was reached. In the tick study 100% efficacy was reached for 4 weeks post treatment.

The effect of shampooing post treatment was evaluated in two studies, against fleas and ticks respectively. In the flea study dogs were shampooed 24 hours post treatment and the efficacy was greater than 98% for 4 weeks. In the tick study dogs were shampooed 2 days post treatment and the efficacy was greater than 99% throughout the study.

The effect of water immersion 8 hours post treatment was examined in a further study against ticks. The efficacy was greater than 98% for 4 weeks post treatment.

Weekly immersion in water, bathing 8 hours post treatment and shampooing 24 hours post treatment did not reduce the protection period of pyriprole topical solution against ticks and fleas on dogs.

387 **Efficacy of imidacloprid 10% and imidacloprid 10% plus moxidectin 2.5% against natural lice (*Trichodectes canis*) infestations in dogs**

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Two negative controlled GCP studies of identical design were conducted in adult mixed breed dogs to evaluate the efficacy of imidacloprid containing formulations when used at the minimum therapeutic dose rate to treat an existing natural infestation of dog lice (*Trichodectes canis*) and to control lice infestation for five weeks, following a single topical administration.

In each study, twenty adult mixed breed dogs were divided into two equal treatment groups. Animals assigned to one group were treated with 0.1 mL/kg bodyweight of either imidacloprid 10% (study 1) or imidacloprid 10% + moxidectin 2.5% (study 2) on Study Day 0, the other group served as untreated control.

The efficacy of treatment was assessed at the two days following treatment and subsequently in weekly intervals up to 5 weeks by comb counts on predefined sites.

The results of these studies show that a single topical administration of imidacloprid 10% when applied in a mono or in a fixed combination formulation with moxidectin 2.5% at a dose rate of 10 mg imidacloprid/kg body weight is effective against dog lice (*T. canis*). In both studies efficacy was >95% two days following administration; and 100% (imidacloprid) or >99% (imidacloprid+moxidectin), respectively, from one week until five weeks following administration.

388 **Efficacy of a novel formulation of metaflumizone plus amitraz (ProMeris® / ProMeris Duo®) for the treatment of demodectic and sarcoptic mange in dogs**

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Amitraz is commonly used as a dip for the treatment of mange in dogs. We report on its first use as a spot-on formulation, combined with metaflumizone, against demodectic and sarcoptic mange. Sixteen dogs infested with *Demodex sp.* and sixteen infested with *Sarcoptes scabiei* were allocated to groups of eight each. Upon inclusion in the study mite numbers were estimated from skin scrapings and the clinical signs of mange evaluated and described for each dog. All dogs were treated topically at 20 mg kg⁻¹ of both metaflumizone and amitraz; groups differed only in the interval of treatment, namely 28 or 14 days. *Sarcoptes*-infested dogs were treated on either two or four occasions, whereas *Demodex*-infested dogs were treated on four or six occasions. Following treatment, mites were counted and clinical signs evaluated at regular intervals. Efficacy was evaluated in terms of the reduction in mite numbers and clinical signs of mange. Treatment of *Demodex*-infested dogs at 28-day or 14-day intervals resulted in a reduction in mite numbers of 94% and 99%, respectively and marked improvement in clinical signs. In the *Sarcoptes*-infested dogs treatment resulted in a rapid reduction in mite numbers and improved clinical signs. Cure rates were 75% and 83% for the 28-day and 14-day treatment regimens, respectively. The results of these studies indicate that ProMeris® / ProMeris Duo® for dogs is an easy to use, safe and efficacious acaricide that can potentially be administered in conjunction with other supportive therapies to treat demodectic and sarcoptic mange.

389 **In vitro and in vivo bioassay systems of insecticides applied to dogs against the European house dust mite *Dermatophagoides pteronyssinus***

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House dust mites are well-known to cause asthma and other respiratory allergies in humans as well as in pet animals. The most common species of house dust mites are *Dermatophagoides pteronyssinus* and *D. farinae* which have been reported to be the predominant dust mites in Korea as well as other countries world wide. Although avermectin derivatives such as ivermectin, moxidectin or selamectin are widely used as both internal and external parasiticides for both pet and livestock animals, few studies are available on the efficacy of these drugs against house dust mites. In this study, both in vitro and in vivo bioassay systems were developed to evaluate the efficacy of avermectin derivatives and other ectoparasitic drugs administered to dogs against *D. pteronyssinus*, the European house dust mite. Mites were co-cultured with either hair from dogs applied with pour-on or spray type antiparasitic drugs or with drug-applied filter papers for 24 hours at 25 C with 75% humidity, and the death rate of mites was compared with untreated controls. Pour-on or spray type products containing moxidectin, selamectin, fipronil, benzyl benzoate, imidacloprid, or permethrin showed moderate to excellent anti-house dust mite activities in vitro. However, hair samples collected from drug-treated dogs showed variable results when co-cultured with house dust mites. Promising anti-house dust mite activity was observed with hair from dogs treated with pour-on type drugs such as the combination of imidacloprid and moxidectin.

Poster group 6: Neospora

390 **Pathological and immunological findings on placenta after infection with *Neospora caninum* in a pregnant mouse model**

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Neospora caninum has been described as a major cause of bovine abortion and the main route of transmission in cattle is transplacental. In order to study in depth the role of placenta during infection, we decided to investigate pathological and immunological changes undergoing in pregnant BALB/c mice infected on days 0, 7 and 14 of gestation (groups A, B and C, respectively). Foetuses and placentas were collected for evaluation of parasite DNA detection and load, lesion severity and cytokine expression (IFN γ and IL4). In group A, parasite DNA was found in foetal and maternal placenta but not in foetuses. The main lesion observed was necrosis accompanied by an slight increase in the IFN γ expression. In group B, parasite DNA was detected in either foetuses or foetal and maternal placentas and parasite burden was higher than in groups A and C. Necrosis was found in the maternal and foetal placenta, together with an increase the IFN γ expression and a reduction in IL4 mRNA levels. In group C, a low parasite burden was detected in foetal and maternal placenta and parasite DNA was only detected in one foetus. A high inflammatory infiltration was observed in maternal placenta accompanied by a decrease in IL4 expression. In conclusion, when *N. caninum* infection was carried out at early gestation an increase in IFN γ expression was observed, whereas after infection at late gestation, a down regulation in IL4 mRNA levels was found. After infection at mid-gestation, an increase in IFN γ expression together with a decrease in IL4 levels was observed. These findings could be related with the capacity of placenta to control parasite multiplication and with the vertical transmission previously observed. Acknowledgement: We would like to thank Vanesa Navarro Lozano and Raquel Expósito Molina for their excellent technical assistance and Ricardo García Mata for statistical analyses. This work has been supported by a grant from the Spanish Government (AGL2001-1362) and was part of COST-Action 854. Inmaculada C López Pérez has been financed by Spanish Ministry of Science and Technology. This experiment complied with the current local laws of animal protection in Spain.

391 ***Neospora caninum* infection in birds: experimental infections in chicken and embryonated eggs**

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Neospora caninum causes economical impact in cattle raising farms since it is implicated as the major cause of bovine abortions. Although infection by the parasite has been widely described in mammals, the role of birds in its life cycle is still obscure. Therefore, this work aimed to evaluate the infection by *N. caninum* in different chicken models. Experimental infections were conducted in seven day old chickens, laying hens and embryonated eggs, where samples were analyzed for parasite burden, IgG antibodies and lesions promoted. Chickens demonstrated an asymptomatic infection, although with seroconversion and systemic replication of the parasite. In laying hens, no signs of vertical transmission were observed. However, embryonated eggs inoculated by the allantoic cavity route demonstrated susceptibility to infection, with mortality rates around 50% independently of the inoculum dose. Additionally, dogs became infected after ingestion of different amounts of inoculated eggs, producing oocysts and specific IgG antibodies. The results herein presented demonstrate that chickens may be intermediate hosts of *N. caninum* and that embryonated eggs could be a useful model to study the parasite's biology.

392 Seroprevalence of *Neospora caninum* in cattle from western Australia

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Introduction: *Neospora caninum* is an important cause of abortion and reproductive failure in cattle. Although numerous studies have examined the seroprevalence of *N. caninum* in cattle throughout the world, and in parts of Australia, no studies have yet to examine the seroprevalence of *N. caninum* in cattle from Western Australia. **Methods:** Blood samples were collected from 503 dairy cattle and 141 beef cattle in 22 herds located in the south west portion of Western Australia. Herd sizes ranged from 70 to 1300 cattle (median 431) and 15 to 51 blood samples (median 28) were collected from each herd. Serum was analysed for the presence of antibodies against *N. caninum* using the IFAT and a commercially available ELISA. **Results and Conclusions:** Results from both the IFAT and ELISA demonstrated an overall *N. caninum* seroprevalence of 2.17% in cattle from Western Australia. The *N. caninum* seroprevalence in individual herds ranged from 0 - 12% with 36% of herds having at least 1 seropositive animal. The results of this study indicate there is a low prevalence of *N. caninum* infection in cattle from Western Australia. Thus, *N. caninum* does not appear to be an economically important parasite for the majority of cattle herds in this region.

393 Cross-sectional survey on the seroprevalence of *Neospora caninum* infection in Bali cattle (*Bos javanicus*) in Bali, Indonesia

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Background: Knowledge on the occurrence and seroprevalence of *Neospora caninum* infection in bovines in Indonesia including Bali is missing till now.

Material & Methods: Serum samples of 438 Bali cattle (*Bos javanicus*) collected from three slaughterhouses in Bali between January and June 2005 were examined for *N. caninum*-specific antibodies using the p38-ELISA.

Results: *N. caninum*-specific antibodies were detected in 6.2% of the cattle sera. The seroprevalence was not significantly related to the sex or age of the animals.

Conclusions: These results indicate, for the first time, natural infections with *N. caninum* in Bali cattle (*B. javanicus*) and suggest the occurrence of this protozoal infection in Indonesia.

394 **Ocurrence of *Neospora caninum* infection and other intestinal parasites in at-risk dog populations in Spain**

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In order to research the significance of *Neospora caninum* infection in at-risk dog populations, we studied the presence of specific antibodies in sera and faecal oocysts shedding in stray and dairy farm dogs from two different regions in Spain. Specific antibodies (IFAT-Nc50) were detected in 120 out of 275 dog sera (43.6%) with titres ranging from 1:50 to 1:800. Differences in seroprevalence between dairy farm dogs (47.5%, 67/141) and stray dogs (39.5%, 53/134) were not significant ($P>0.05$, χ^2 test). Farm dogs showed significant higher titres than stray dogs ($P<0.01$; t-student test). In farm dogs, we observed a positive association between seropositivity of dogs and dairy farms (Odds ratio=2.1). On the other hand, a total of 226 faecal samples were tested using standard sucrose flotation technique. We observed *Hammondia heydorni*/*N. caninum*/*Toxoplasma gondii*-like (HNT) oocysts in one farm dog but the aetiology could not be confirmed either by species-specific PCR tests or by bioassay in *Meriones unguiculatus*. In addition, prevalence of other intestinal parasites was also studied by coprological or different PCR techniques. The overall prevalence was significantly higher in stray dogs ($P<0.01$; χ^2 test). Specifically, stray dogs had significantly higher prevalence of *Ancylostomatidae*, *Toxocara*, *Toxascaris* and *Taenidae* ($P<0.05$; χ^2 test). Prevalence differences of *Thichuris* and intestinal protozoan parasites (*Cystoisospora*, *Giardia*, *Cryptosporidium* and HNT) were not significant ($P>0.05$, χ^2 test). The high *N. caninum* seroprevalence observed in stray and farm dogs was not associated with *N. caninum* oocyst shedding. This finding could just indicate that exposure to *N. caninum* is frequent in at-risk dog populations. On the other hand, these dog populations are an important source of environmental contamination with other intestinal parasite forms, which could be of significance to animal or human health. Acknowledgements: We would like to thank Belén Antonio and Marta Alonso for her excellent technical assistance. This work was supported by the projects PR1/06-14467-B (Complutense University of Madrid) and RTA04-047-C2 (INIA). We gratefully acknowledge the Servicios municipales de Saneamiento of La Rioja, the Laboratorio Regional de Sanidad y Producción Animal in Lugo and farm veterinarians for samples and data collection.

395 **Antibodies to *Neospora caninum* and *Toxoplasma gondii* in domestic cats from Brazil**
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The occurrence of antibodies to *Neospora caninum* and *Toxoplasma gondii* was determined in 400 domestic cats (Siamese, Persian and undetermined breeds) from the Municipality of Araçatuba-SP, Brazil, through the indirect fluorescence antibody test (IFAT). Of the 400 cats, 100 were seropositive to *T. gondii* (25%, titer / 64) and 98 to *N. caninum* (24.5%, titer / 16). The rate of seropositive cats for *T. gondii* was correlated with age ($X^2 = 35.7$; $P<0.001$), with a higher number of infected animals at older ages. Of the 219 cats younger than one-year-old, 13.2% were seropositive for *T. gondii*, while 39.2% were positive in the 181 older animals. The presence of *N. caninum* was also correlated with age ($X^2 = 8.8$; $P<0.01$), with 18.7% (41/219) and 31.5% (57/181) of positive animals at ages below and above 12-month, respectively. An association between the occurrences of both protozoa in the felines was also observed ($\chi^2 = 19.6$; $p<0.001$).

396 **Detection of antibodies to *Neospora caninum* in the blood of bison (*Bison bonasus bonasus* L.) eliminated from the breeding program in 2004-2007 in Poland**

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Antibodies against the protozoan parasite *Neospora caninum* were detected in the blood of European bison (*Bison bonasus bonasus* L.) living in Poland. Samples were taken from 121 animals of different ages and both sexes eliminated from the breeding program and shot in 2004-2007.

Sera were tested using a commercial ELISA kit (IDEXX Laboratories, Inc., Westbrook, Maine, USA) routinely used for the detection of anti-*Neospora* antibodies in cows.

Positive results (when S/P ration was greater than 0.5, according to kit description) and uncertain results (between 0.5 – 0.3) were verified by Western blot analysis. Antibodies against *N. caninum* antigens were detected in all analysed samples.

According to the results obtained in ELISA and Western blot, among the 121 samples collected in 2004-2007, 16 were positive (prevalence 13.2%).

Results indicate that *Neospora caninum* exists in a sylvatic cycle in Poland. Additionally, the effect of this parasite on the health status and conservation of the European bison should be considered.

397 **Acute protozoal myopathy in a dog due to *Neospora caninum***

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A 7 month-old male Irish Setter was presented to the University Teaching Hospital with a 4 day history of sudden, progressive weakness in all 4 limbs. The dog presented severe non-deambulatory tetraparesis and generalized muscle atrophy in all 4 limbs. Postural reactions were normal, but quite difficult to elicit because of the serious muscular weakness. Spinal reflexes were very weak, and the patellar reflex was absent. Due to serious masticatory muscle atrophy the dog had difficulty in eating and swallowing. Other cranial nerve functions were normal. The dog also had difficulty breathing, especially during the inspiratory phase. Two days after admission, the dog developed megaesophagus and inspiration pneumonia. Blood exams showed marked increase in CK (>12000 U/L) and GOT (512 U/L) and severe leukocytosis. Serology for *N. caninum* was positive at a titre of 1:200 (IFAT) and serology for *Toxoplasma gondii* was negative. The dog was immediately put on clindamycin (10 mg/kg/BID), ceftriaxone (20 mg/kg/BID) and fluid therapy. The dog progressively worsened and died of respiratory arrest 8 days after the onset of clinical signs. At necropsy megaesophagus was confirmed and all tissues were sampled for routine histology. An acute inflammatory response was observed in numerous organs and tissues including skeletal muscles, smooth esophageal muscle and CNS. Within the same tissues, cysts and scattered tachyzoites were observed that reacted with both available polyclonal antibodies (goat and rabbit origin) raised against *N. caninum*. *N. caninum* serology of the mother and the other littermates was negative, suggesting postnatal infection.

398 **Effect of *Neospora caninum* infection on *Bos taurus* and *Bos indicus* retropharyngeal lymph node mRNA expression**

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Neospora caninum is an important cause of abortion in cattle worldwide. High seroprevalence rates are observed most of the infected are asymptomatic, with tissue cysts in Central Nervous System (CNS). Vertical transmission occurs during several gestations and frequently associated to neuromuscular disease in young calves.

The objective of this experiment is to evaluate immune related genes at CNS draining lymph node (LN) in *N.caninum* negative calves during acute infection. Four Holstein-Friesian and three Nelore calves received 10(7) culture-derived live tachyzoites of *N. caninum* (NC-1 strain) by intramuscular injection, three extra calves of each breed kept as uninfected controls received Vero cells injection. Calves were killed six days after infection (DAI) and tissues collected for gene expression analysis by Real-Time RT-PCR. Two of the Holstein and four of the infected Nelore calves showed specific IgM at the 6 DAI by the indirect immunofluorescent assay. No IgG was detected. Interferon-gamma (IFN-g), tumor necrosis factor-alpha (TNF-a), inducible nitric oxide synthase (iNOS), interleukin-4 (IL-4), IL-10, transforming growth factor-beta 1 (TGF-b1) results were normalized to GAPDH as endogenous control. IFN-g expression was upregulated at both infected groups, more intense at Holstein's (Student's t, alpha=0.1). No statistical difference was detected at TNF-a, IL-10, TGF-b1 and iNOS gene expression. IL-4 expression was increased (alpha=0.1) at Holstein but not at Nelore calves. *N. caninum* DNA was not detected by PCR on those samples. Discrete local response variation is observed among Holstein and Nelore tissues. Further studies with CNS tissues are in process (Financial support: FAPESP 05/01659-0 and 06/00197-5).

399 ***Neospora caninum* infects the canine macrophage cell line dh8**

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Neospora caninum is an apicomplexan protozoan that is responsible for abortion in cattle and severe neuromuscular disease in the definitive canine host. Immunity to *N. caninum* has been widely studied in cattle, both in vivo and in vitro. There are however few studies concerning the immune response to infection in the dog. The canine macrophage cell line DH82 has been reported to be useful as an in vitro model for the study of intracellular pathogens. Indeed, this cell line has been shown to be capable of phagocytosis, production of various cytokines following lipopolysaccharide stimulation, including TNF alpha and interleukins 1, 6 and 8, and modulation of MHCII expression following infection with *E. canis*. Here, the authors report the in-vitro infection of DH82 cells with *N. caninum*. Briefly, 100,000 cells/ml were incubated until confluence and then infected with tachyzoites of the NC-1 isolate of *N. caninum* at a parasite:cell ratio of 3:1. Twenty-four hours after infection, cells were collected, incubated for 10 minutes with an anti-*N. caninum*-FITC antibody and analyzed in flow cytometry. Results show that approximately thirty percent of the cells were infected with *N. caninum*. This study demonstrates that the DH82 cell line is receptive to *N. caninum* infection and this could be useful as an in-vitro model for the evaluation of the immune response to infection in the canine host.

400 ***Neospora caninum* infection of bovine pbmc-derived macrophages**

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Neospora caninum is an apicomplexan protozoan that is responsible for abortion in cattle and severe neuromuscular disease in the definitive canine host. Host-parasite interaction has been widely studied in-vitro in several different cell types, mainly using murine models. Infection of murine macrophages in particular has shown that major histocompatibility complex (MHC) class II-up-regulated macrophages play a crucial role in the protective host immune responses to *N. caninum* infection. Here the authors report the generation of peripheral blood mononuclear cell (PBMC)-derived macrophages from healthy Holstein cattle and subsequent infection with *N. caninum*. Briefly, 2.5×10^6 PBMC, obtained by Ficoll density gradient, were seeded on polylysine-coated cover slips and incubated for 2 hours. Non-adherent cells were removed and adherent cells were then incubated for a further seven days in culture medium for differentiation to macrophages. Infection was carried out with the NC-Liv isolate of *N. caninum* (1.75×10^6 tachyzoites/well). Non-infected macrophages served as negative controls. Twenty-four hours after infection, cover slips were recovered and stained with either May-Grunwald Giemsa, anti-*N. caninum* polyclonal antiserum or anti-MHCII monoclonal (VMRD, WA) antibody. Microscopic examination and immunocytochemistry showed that *N. caninum* efficiently infected PBMC-derived macrophages and replicated within the cytoplasm. Interestingly, several macrophages also contained *N. caninum*-positive debris, suggesting parasite destruction on the part of the infected cell. MHCII staining appeared similar between non-infected and infected cells. Further studies on *N. caninum*-bovine macrophage interaction could shed light on the immune response to infection in cattle.

401 **Persistence of colostral antibodies to *Neospora caninum* in infection free calves**

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Neospora caninum is considered the main cause of bovine abortion worldwide. The correct laboratorial diagnose is very important to identify the infected animals and to apply control measure. The objective of this study was to show the persistence period of colostral antibodies in calves. Eight newborn Holstein Friesian calves, males, were selected from *N. caninum* seronegative dams. Pre-colostral blood samples were collected of these calves and all of them were seronegative to *N. caninum*. They were fed with two liters of pooled colostrum from seropositive cows within two hours after birth. Blood samples were collected and tested weekly until the animals turned negative. Serum samples were tested for antibodies to *N. caninum* using indirect fluorescence antibody test at 1:50; 1:100 and 1:200 dilutions. Antibodies were not detected from three out of eight calves and they were excluded from the study. The remaining 5 calves seroconverted in all dilutions at the fifth day after colostrum ingestion. At 1:50 dilution, one calf remained positive for 21 weeks, two for 20 weeks and one for 13 weeks. At 1:100, one calf was positive for 15 weeks and the remaining 4 calves for 13 weeks. At 1:200, each calf was positive for 1, 7, 12, 12 and 13 weeks, respectively. These results demonstrate that the colostral antibody to *N. caninum* may persist until 21 weeks after birth in calves and it's very important to exclude the calves at the first four months of age in the seroprevalence studies to avoid the false-positive results.

402 **Detection of antibodies to *Neospora caninum* in cattle by enzyme-linked immunosorbent assay with truncated NcSRS2 expressed in *Pichia pastoris***

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In veterinary medicine, *Neospora caninum* is an important pathogen causing abortion in cattle and neuromuscular paralysis in dogs. In cattle, the main route of infection is transplacental. The surface antigen 1-related sequence 2 of *N. caninum* (NcSRS2) is considered as an immunodominant antigen. In this study, the truncated NcSRS2 (NcSRS2t) lacking a C-terminal hydrophobic tail was expressed in *Pichia pastoris* as a secreted recombinant protein and purified. Its diagnostic potential in an enzyme-linked immunosorbent assay (ELISA) was evaluated using field serum samples collected from cattle in Belgium. The recombinant protein based ELISA was compared to an in house ELISA using crude tachyzoite lysate. Samples of precolostral sera or adult sera were analysed. 446 samples were analysed (170 precolostral sera, 276 adult sera); the sensitivity and specificity was reached to 87% for a cut-off of 3 for precolostral sera and to 83% for a cut-off of 7 for adult sera. False positives were observed with cows' sera when using the recombinant protein. These results indicate that NcSRS2t recombinant protein based ELISA can be useful for the diagnosis of *N. caninum* infection in transplacentally infected calves.

403 **Phenotypic characterization of spleen dendritic cells in BALB/c mice infected with *Neospora caninum* tachyzoites**

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Neospora caninum is a cyst-forming coccidian parasite first described as the causative agent of neuromuscular disorders in dogs. Clinical infections caused by this parasite were subsequently described in other animal hosts including bovine species. There is now ample evidence that a Th1-type immune response mediates protection to acute *N. caninum* infection whereas a Th2-type immune response correlates with increased susceptibility.

Dendritic cells (DCs) are responsible for the priming of naive T cells. Therefore, we assessed the expression of co-stimulatory and MHC-class II molecules in the surface of these cells in the spleen of BALB/c mice infected with *Neospora caninum*. Mice were euthanized 5 days after intraperitoneal inoculation of 1_10E7 *Neospora caninum* tachyzoites and quantification of DCs was made by FACS analysis. Samples were enriched in DCs using CD11c microbeads. An increase in the number of DCs in the spleen of infected mice as compared with respective controls was observed. These cells showed an up-regulation of the co-stimulatory molecules CD80 and CD86 and a decrease in the surface expression levels of MHC class II molecules.

Altogether, these results show that DCs are specifically stimulated by *N. caninum* tachyzoites and suggest that this parasite can modulate MHC class II expression.

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404 **Efficacy of the vaccination with killed whole *Neospora caninum* tachyzoite or tachyzoite-bradyzoite antigens in a pregnant BALB/c mouse model**

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The parasite *Neospora caninum* is an intracellular protozoan that causes abortion in cattle world-wide. Parasite live vaccines can have problems with safety, short shelf-life and large-scale production, thus the development of killed vaccines could be very advantageous, but generally they show less efficacy. Vaccine preparations containing different stages of the parasite life cycle could improve the efficacy. In this study, we used either killed whole tachyzoites (TZ) or a tachyzoite-bradyzoite mixture (TZ-BZ) from Nc-Spain 7 isolate of *N. caninum* incorporated into either a water-in-oil emulsion (adjuvant A) or aluminum hydroxide-immunostimulatory Ginseng dry extract (C). In a previous work using a non-pregnant BALB/c mouse model, adjuvants A and C combined with TZ antigen have shown some protection during chronic or acute *N. caninum* infection, respectively. BALB/c mice were immunised subcutaneously before pregnancy twice at 3-wk intervals and challenged at mid gestation by inoculation of live NC-1 tachyzoites. To evaluate efficacy, neonatal mortality and vertical transmission were evaluated during a 30 days post-partum period. Specific anti-*N. caninum* IgG1 and IgG2a responses and IL4, IL10 and IFN- γ expression were also studied in dams. Data obtained showed that groups vaccinated with A/TZ reduced significantly neonatal mortality and vertical transmission compared with A/TZ-BZ and controls groups. On the contrary, adjuvant C displayed low neonatal survival and high vertical transmission rates either with TZ or TZ-BZ antigens. All groups vaccinated with TZ-BZ combined with A, C or PBS showed an exacerbation of the infection and pups survived for a shorter time. This fact was associated to a Th2-type immune response in dams.

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405 **Reduced parasitaemia after infection with *Neospora caninum* in mice immunised with NcSRS iscoms**

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We have previously reported that an experimental vaccine containing recombinant NcSRS2, one of the immunodominant surface antigens of *Neospora caninum*, bound to immune stimulating complexes (iscoms) induces immunity sufficient to reduce clinical symptoms and brain parasite load of infected mice. In the present study, we have further investigated the effect this vaccine by analysing the level of parasitaemia during the acute stage of infection. A real-time PCR assay based on the detection of the Nc-5 repeated sequence of *N. caninum* and the DNA-binding dye SYBR Green was used to detect and quantify parasite DNA. Mice were immunised twice with NcSRS2-iscoms, and after challenge infection blood was collected daily for 9 days. During this period the amounts of parasite DNA detected in immunised mice were significantly lower ($P < 0.001$), and the duration of parasitaemia appeared to be shorter, than in non-immunised controls. Furthermore, the degree of parasitaemia seemed to correlate well with the amount of *N. caninum* DNA in the brain 21 days post inoculation and with disease severity measured as changes in body weight. Thus, the protective immunity induced by the NcSRS2-iscoms was sufficient to reduce the level of parasitaemia, which probably reduced the number of parasites reaching the brain, and could be the reason for the reduction in brain parasite load and clinical symptoms. Furthermore, real-time PCR was found to be a sensitive means for rapid assessment of *N. caninum* in blood.

* Pinitkiasakul et al (2005) Veterinary Parasitology 129, 25-34; Pinitkiasakul et al. (2007) Vaccine 25, 3658-3668

406 **Protective immunity induced by recombinant antigens rNcSAG4 and rNcGRA7 against chronic and congenital neosporosis in an experimental murine model**

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Neospora caninum is currently considered one of the main causes of reproductive failure in breeding cattle. Thus efforts have been invested in developing recombinant vaccines against neosporosis focused on a tachyzoite stage specific protein -NcSAG1- or shared proteins -NcSRS2, NcMIC1, NcMIC3 and NcGRA7- by both tachyzoite and bradyzoite stages. Bradyzoite specific antigens have been also suggested to be employed in the design of vaccines. However to date only two *N. caninum* bradyzoite stage specific antigens -NcSAG4 and NcBSR4- have been described. In the present study the protective immunity developed by rNcSAG4 was evaluated for the first time. rNcGRA7 was also included in the vaccine preparations and both recombinant proteins, expressed in *Escherichia coli*, were formulated alone or in combination with or without Titermax® adjuvant (a water-in-oil emulsion). A pregnant mouse model was employed to evaluate transplacental transmission and a mouse model for cerebral neosporosis was also used to study the chronic phase of infection. Serological analysis showed that groups immunized with rNcGRA7 developed antibodies directed against native parasite antigens whereas mice immunized with rNcSAG4 hardly detected the native protein and generated antibodies recognizing mainly the recombinant antigen. In addition groups inoculated with rNcGRA7 developed a strong IgG1 response and the presence of the adjuvant was required to generate IgG2a antibodies before challenge. Also higher values of the mean rate of survival corresponded to the offspring from dams immunized compared with control group. Efficacy of recombinant antigens against chronic neosporosis in adult mice and vertical transmission in their offspring will be presented and discussed in depth.

407 **Characterisation of the effects of pentamidine-derivatives in *Neospora caninum*-infected cell cultures**

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Pentamidines have a long-standing history as anti-trypanosomal, anti-leishmanial and anti-malarial drugs. In this study, we report on the effects of a series of pentamidine-derivatives against the apicomplexan parasite *Neospora caninum*. For this, HFF monolayers were infected with *N. caninum* tachyzoites, at 1 h p.i. drugs were added once at concentrations of 0.05 – 1 µg/ml, and proliferation of tachyzoites was measured by real-time PCR at day 3 p.i.. Of 20 pentamidines tested, the highest efficacies were noted for DB768 (IC₅₀ = 0.213 µM) and DB750 (IC₅₀ = 0.237 µM), with maximal proliferation inhibition at 1.7 µM. These two drugs were chosen for further studies. Tachyzoite proliferation was also stopped when treatments were initiated at days 2 or 3 p.i.. At 1.7 µM, a treatment duration of 24 h was sufficient to exert a true parasitocidal effect. Electron microscopy of treated infected cultures revealed distinct alterations in parasite ultrastructure including vacuole formation, electron-dense cytoplasmic inclusions and membrane stacks, distortion of the PV tubular network, and lipid droplet formation. Pentamidines acted exclusively upon intracellular parasites, since pretreatment of *N. caninum* tachyzoites for 1 hour had no effect on infectivity. Treatments of uninfected HFF with DB768 and DB750 did affect neither host cell proliferation nor viability. However, 24 hour-pretreatment of uninfected HFF monolayers with both drugs, followed by infection with *N. caninum* tachyzoites and subsequent culture, resulted in complete inhibition of parasite proliferation. This suggests that either these compounds or respective active metabolites were still present after removal of the drugs, or that drug treatments impaired some functional activities in HFF, which were essential for parasite survival.

In trypanosomatids, the pentamidine anti-protozoal activities have been shown to be based on cleavage of the kinetoplast DNA, and parasite membrane-associated high-affinity transporters accumulate the drugs within the parasite, reaching much higher concentrations than in the host cells. Since this mechanism of action cannot account for the activity against *N. caninum* tachyzoites and the effects seen upon pretreatment of host cells suggest an involvement of the host cell, other targets must be affected. DB750 has been coupled to epoxy-agarose, and affinity chromatography using *N. caninum* extracts and/or HFF lysates is being performed in order to identify pentamidine-binding proteins, and thus potentially novel pentamidine drug targets.

408 **Identification of polymorphic regions in several strain of *Neospora caninum***

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Several strains of *Neospora caninum* have been isolated from cattle and dogs, a definitive host. Despite this, however, there is currently limited information concerning genetic variability among strains. A genetic characterisation of *N. caninum* would allow identification and evaluation of several different biological aspects of the parasite, including virulence, immunity and transmission dynamics. Starting from sequences available from the project on cDNA sequencing of *N. caninum* (USDA-WashU *Neospora* EST Project), primers for the amplification of three immunomodulatory surface antigens were designed in order to obtain amplified fragments from field isolates for studies on polymorphism in nucleotide sequences. This genetic variability from field isolates of *N. caninum* could be responsible for varying virulence and differing immune responses. However, sequences of these surface antigens resulted identical in three different isolates of *N. caninum*. Microsatellite sequence analysis constitutes another suitable tool for the identification of genetic polymorphism. We identified microsatellite targets discriminating the tested isolates of *N. caninum*. A bioinformatics analysis on the *N. caninum* sequences available in data bases is in progress to obtain other hypothetical microsatellite regions for the screening of new field isolates supplied to our laboratory.

409 **A BALB/c nu/nu mouse model to evaluate the viability of *Neospora caninum* tachyzoites in bovine extended semen**

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Experimental infections with refrigerated and frozen bovine extended semen straws artificially contaminated with different numbers of *Neospora caninum* tachyzoites of the NC-1 strain were conducted in BALB/c nu/nu mice to assess the capability of the parasite to induce an infection in these animal models in order to assess the sensitivity of this bioassay methodology. Five groups of 6-weeks-old female athymic mice were used and they were intraperitoneally infected with 10, 102, 103, 104, and 105 tachyzoites resuspended in either refrigerated and frozen extended semen from a *N. caninum* seronegative bull. Positive (103 tachyzoites diluted in PBS) and negative (only extended semen) control groups were also included. All animals were sacrificed at 10 weeks postinoculation. *Neospora* DNA was found in the brains of mice inoculated with refrigerated and frozen semen straws contaminated with at least 103 and 104 tachyzoites, respectively. Clinical signs associated to neosporosis were detected in some mice inoculated with refrigerated extended semen contaminated with 104 and 105 tachyzoites. The processing of the extended semen straws by addition of the extender and by freezing of the insemination straws could have an adverse effect in the infectivity of the protozoa. Therefore, the nude mice model used is only suitable to evaluate the viability of *N. caninum* in bovine extended semen when high numbers of parasites are present.

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410 **Isolation and genetic characterization of *Neospora caninum* from asymptomatic calves in Spain**

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Neospora caninum is a cyst-forming parasite that has been recognised worldwide as a cause abortion in cattle. In spite of the ubiquitous distribution of this parasite and its wide host range, the number *N. caninum* isolates obtained to date is limited. In vitro isolation of the parasite has been arduous and, in many occasions, unsuccessful. In addition, most isolates were obtained from clinically affected cattle and therefore this population could be biased towards more virulent isolates. In order to study in depth the biological diversity within *N. caninum* and obtain naturally attenuated isolates a new isolation approach from healthy animals was undertaken.

Firstly, transplacentally infected calves from seropositive herds were selected by IFAT of pre-colostral sera. Seropositive calves were sacrificed and the presence of the parasite in brain confirmed using a specific nested-PCR of the ITS-1. Afterwards, Balb/c athymic mice were inoculated with brain homogenates and daily examined for the development of neosporosis. Mice with severe clinical signs were sacrificed, peritoneal cavity was flushed and the fluid was inoculated onto MARC-145 cell monolayers which were daily observed for the presence of tachyzoites. This technique was successful since a total of ten new isolates were obtained from calves from different regions of Spain. These isolates were numbered from NC-Spain1H to NC-Spain10 according to the order of in vitro isolation. A previously described microsatellite technique was applied to both brain homogenates and cell culture tachyzoites to confirm the identity of the isolates. Most isolates showed unique genetic profiles, except Nc-Spain1H-Nc-Spain10 and Nc-Spain3H-Nc-Spain4H pairs which exhibited identical genotypes and were isolated from distinct calves of the same herd. Future pathogenic characterisation of these isolates will allow us to investigate the relationship between virulence and the outcome of infection.

411 **Anti-neosporal effect of *S. flavescens* and *T. japonica* extracts against *N. caninum* on mice, goats and dogs**

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We tested this effective fractions to mice, sheep and Korean native goats to know anti-neosporal efficacy. In this study, we infected Korean *Neospora* (KBA-2) to mice, pregnant goats and suckling dogs and evaluated anti-neosporal efficacy of HPLC fractions of herb extracts (*S. flavescens*, *T. japonica*). We surveyed clinical signs, survival rate, abortion rate and histo-pathological lesions. The *Neospora* infected BALB/c mouse showed rough hair, anorexia and motor ataxia from 14 days and all BALB/c mice were dead after 17-25 days. Necrotic or non-suppurative inflammations were found in brain, pancreas, lung, liver and spleen. Especially necrotic encephalitis index was 8.15 that means severe lesion. There was no difference between control group and herb extract treated group in average survival days, but survival rate in treated mice improved more than control group. Especially the mouse which was treated with high dose of *S. flavescens* (7.125ng/ml) and *T. japonica* (7.125ng/ml) showed improved survival rate to 25% and 41.7%, respectively and showed decreased brain lesion score to 1.81 and 2.23, respectively. We infected *Neospora* to pregnant goats and tested anti-neosporal efficacy. Two goats which were *Neospora* infected but not treated showed abortion after 27 day, and multi-focal necrotic encephalitis was found in fetus brain. Six goats out of 8 pregnant goats that were treated high dose of *S. flavescens* (7.125 ng/ml) and *T. japonica* (7.125 ng/ml) showed abortion. But there is difference in average abortion days. Average abortion day of high dose of *S. flavescens* (7.125 ng/ml), low dose of *S. flavescens* (2.850 ng/ml) and high dose of *T. japonica* (7.125 ng/ml), low dose of *T. japonica* (2.850 ng/ml) was 51 days, 45 days, 78.5 days, 50.5 days, respectively.

We infected Korean *Neospora* (KBA-2) to dogs, but could not evaluate anti-protozoa efficacy in suckling dogs because pathogenicity of *Neospora* was very low. HPLC fractions of *S. flavescens* and *T. japonica* showed good efficacy in protection of *Neospora*, and improved survival rates in mice. We could found out that *S. flavescens* and *T. japonica* could delay abortion days in pregnant goats but it could not prohibit *Neospora*. infection or treat neosporosis.

Through this experiment, HPLC fractions of *S. flavescens* and *T. japonica* showed high anti-neosporal efficacy. Further research on the herbal materials should be carried out.

412 **Comparative *Bos taurus* and *Bos indicus* tissue mRNA response against *Neospora caninum* infection**

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Differences between *Bos taurus* and *Bos indicus* resistance to tick and helminth parasites is well studied but little information is available referring to *Neospora caninum* infection. Due to limitations of serological and pathological analysis to discriminate early tissue responses during acute tachyzoite infection, gene expression to interferon-gamma (IFN-g), tumor necrosis factor-alpha (TNF-a), inducible nitric oxide synthase (iNOS), interleukin-4 (IL-4), IL-10, transforming growth factor-beta 1 (TGF-b1) and GAPDH were measured at local lymph nodes (LN) and compared to non-infected controls. Four Holstein-Friesian and four Nelore calves were inoculated with 10(7) culture-derived tachyzoites of NC-1 strain through intramuscular injection, three extra calves of each breed kept as uninfected controls received Vero cells injection. Six days after inoculation they were killed and both right and left popliteal LN collected for Real-time RT-PCR. Ipsilateral popliteal LN enlargement was observed in *N. caninum* infected calves, but not in control group. Specific IgM was detected by indirect immunofluorescent assay in sera of two Holstein and four Nelore infected calves, no IgG was detected. Interestingly *N. caninum* DNA was only detected by using PCR at IgM negative and infected Holstein LN samples. Real-time RT-PCR results were normalized by using GAPDH as endogenous control and revealed increased IFN-g gene expression at Nelore infected calves ($\alpha=0.05$), but not in Holstein. No statistically significant variation was observed at TNF-a, iNOS, IL-4, IL-10 and TGF-b1 expression. Preliminary results suggest an earlier immune activation at Nelore calves inducing IFN-g and IgM production and inhibition of the parasite replication (Financial support: FAPESP 05/01659-0).

Poster group 7: Vector-Borne Protozoa

413 **Occurrence of *Borrelia* and *Babesia* spp. in hard ticks collected from dogs in Hungary**

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Hard ticks (Acari: Ixodidae) are vectors of different pathogens of veterinary and public health importance in many European countries. Emerging canine tick-borne diseases like babesiosis and Lyme-borreliosis have also drawn both public and scientific attention to these arthropods. In Hungary, there has been scant information about species and prevalence of tick-borne pathogens of ticks infesting dogs.

Ticks were collected from dogs appearing for clinical consultation in veterinary practices and clinics in different geographical regions of the country. In total, 2145 ticks were removed from 766 dogs. *Ixodes ricinus* (N=1106) and *Dermacentor reticulatus* (N=811) were the most common species of which females were selected for molecular analyses. One to 12 specimens were used in each sample for DNA extraction. Polymerase chain reactions were performed with BSL-F/BSL-R primers for detecting *Borrelia* spp. in *I. ricinus* and with PIRO-A1/PIRO-B primers to amplify *Babesia* spp. in *D. reticulatus*. Randomly selected PCR products were sequenced to identify the pathogens.

DNA of *Borrelia* spp. could be detected in 6 from 108 *I. ricinus* samples and 43 from 144 *D. reticulatus* samples were PCR-positive for *Babesia* spp. Sequencing revealed the highest similarity with *Borrelia afzelii*, *Borrelia garinii* and *Babesia canis canis*, respectively.

414 **Isolation and in vitro culture of zoonotic *Babesia* sp. EU1 from roe deer (*Capreolus capreolus*) and evidence of parasite transmission in *Ixodes ricinus***

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Babesia sp. EU1 was first characterized at the molecular level in 2003, from human cases of babesiosis in Italia and Austria. Since, EU1 has been recovered from roe deer in Slovenia as well as from *I. ricinus* in Slovenia and Switzerland. Indeed, this *Babesia* species merits increased attention as a potential agent of emerging tick-borne disease, in particular because his suspected vector, *I. ricinus*, is the most prevalent and widely distributed tick in Europe and frequently bites humans. In order to evaluate the public health importance of EU1, it is essential to determine with certainty its vector, animal reservoir hosts, as well as its geographic distribution. With this aim in view, we analyzed the presence of EU1 both in roe deer and in *I. ricinus* in Western France. *Babesia* sp. EU1 was, for the first time, successfully isolated in vitro and cultured in autologous erythrocytes from two roe deer. It was also adapted for in vitro culture and cloned in sheep erythrocytes. In addition, this study provided some evidence of parasite vertical and transstadial transmission in *I. ricinus*, as parasite DNA was recovered in eggs and larvae obtained from females collected on infected and also on uninfected roe deer. Such results suggest that this tick is both a vector and a reservoir of EU1. Sequence analysis of the 18S rRNA from parasites obtained either from the cultures or from tick samples showed 100% identity to the human EU1 strain.

415 **Chemotherapeutic evaluation of babesicides products (IC 040, Berenil and Vivater LA) in calves experimentally infected with *Babesia bigemina* and *Babesia bovis* in Brazil**

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The present work aimed to comparatively evaluate the effectiveness of the experimental formulation IC 040 (Akzo Nobel Ltda. – Intervet Division) with the babesicides Berenil®(Akzo Nobel Ltda. - Divisão Intervet) and Vivatet®(Clarion Biociências Ltda.), in 24 calves cross breed (*Bos taurus* x *Bos indicus*) with degrees of blood from 3/4 to 7/8, with age between one week and two months and weighing between 40 and 80 kg. The animals were inoculated by the intravenous route, with pathogenic samples of *B. bigemina* and *B. bovis*, previously maintained under cooling by 2 hours before the inoculation, coming from a bovine one with acute infection. The rectal temperature and blood smears were used as parasitemia indicators. After the presentation of the classical symptom of the babesiosis, the treatments were made, in the dose 1ml/10Kg for the three formulations. All clinical signs of babesiosis, disappeared 24 hours after the treatments. With a single administration of IC 040, it can be obtained the expected results, translated by absence of circulating parasites. In the group treaty with the formulation IC 040 the fall of the observed rectal temperature, happened like to the others, staying for up to 30 days inside of the values considered normal. The hematological exams related to the stained blood smears with Giemsa revealed the complete disappearance of parasites of the species *B. bovis* and *B. bigemina* in the samples of capillary blood, picked of all the medicated animals, persisting the negative for more than 40 days.

416 **Piroplasmosis as a possible cause of mortality in giraffes**

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Introduction: Piroplasms have previously been reported from giraffes in Kenya and Namibia. In one case theileriosis was suspected of causing mortality. Diagnosis was based on the presence of small intraerythrocytic piroplasms on blood smears. Recently, six cases involving adult giraffes, from different geographic areas in South Africa, showed sudden onset of disease. The course of the disease was peracute and appeared to affect many organ systems. Some of the clinical signs noted were: severe depression, recumbency and severe weakness, hypothermia, dyspnoea, rumen stasis and oedema of the sclera and conjunctiva. Some of the post-mortem findings included frothy nasal discharge, haemoglobinuria and haemopericardium. Microscopic examination of thin blood smears revealed the presence of small piroplasms.

Materials and Methods: DNA was extracted from blood and spleen samples. The V4 variable region of the 18S rRNA gene was amplified and analyzed using the Reverse Line Blot (RLB) assay. RLB results indicated that the PCR products did not hybridize with any of the *Babesia* or *Theileria* species-specific probes present on the blot, but only hybridised with the *Babesia/Theileria* genus-specific probe. Full-length 18S rRNA genes were amplified, cloned and the recombinants were further analysed by sequencing analysis. Sequencing data were analysed using the Staden package and aligned with published sequences of related genera using ClustalX. Phylogenetic trees were constructed using neighbor-joining in combination with the bootstrap method.

Results and Discussion: Sequence similarity analysis indicated a *Babesia* species infection was present in three of the giraffe samples whereas *Theileria* species infections occurred in the other.

417 ***Trypanosoma evansi* in cattle farms in Malaysia**

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Outbreaks of clinical surra occurs occasionally in animals in Malaysia with mortalities reported in cattle, buffaloes, horses, pigs, deer and the Sumatran rhinoceros. A study was conducted to determine the prevalence of *Trypanosoma evansi* in two large and five small cattle farms with a previous history of clinical trypanosomiasis within the same year. Blood samples were taken from 5-35% of animals in each farm and examined for tryps by wet mount, HCT, QBC, thin stained film and serum tested with CATT/*T. evansi* and Ag-ELISA. The prevalence based on parasite detection was low (0-13%) with low parasitaemias, however based on seroprevalence was much higher (12-55%). The mean prevalence rate of *T. evansi* was significantly higher in dairy cows than in their calves and yearlings, with the highest in lactating animals. The Brangus breed imported from Argentina was 11 times more susceptible to *T. evansi* compared to the indigenous cattle, both breeds being managed on the same farm. Among the sampled cattle which appear clinically healthy there was no correlation between *T. evansi* infection and body scores nor PCV values. Generally, animals which succumbed to the disease were those that were physiologically stressed such as just after parturition, during early lactation and also during the rainy season.

418 **Haematologic response of donkeys to *Trypanosoma evansi* infection**

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Camel trypanosomosis due to *Trypanosoma evansi* infection is singled out as the only disease that hindered longitudinal or cross-sectional development and exploitation of camel in the arid and semi-arid zone.

The epidemiology of “Surra” in Sudan is far from being fully understood. Due to the drought that had stricken the country in the past years, camel husbandry and grazing areas has been completely changed. Camels encroach deeper south in search for water and pasture and hence contact with increased prevalence of biting flies. Animals such as cattle, sheep, goats and donkeys are frequently seen mixed with camels. The contribution of these animals to the epidemiology of camel trypanosomosis in Sudan has not yet been evaluated. Moreover, donkeys constitute an important agricultural resource in rural areas and an important income generating in urban areas.

The present study examines the susceptibility of donkeys to *T. evansi* infection and hence determines their contribution to the epidemiology of camel trypanosomosis.

Two groups of donkeys were inoculated with two stocks of *T. evansi*. One stock, isolated from eastern Sudan and the other from western Sudan. Clinical signs, levels of parasitaemia, anaemia and other haematological values were recorded daily for two weeks after detection of parasites in the peripheral blood. Thereafter they were recorded three times a week until the end of the study period. Parasitaemia levels were recorded daily until the end of the study period. Both stocks of *T. evansi* produced high levels of parasitaemia, around $\times 10^6$ parasites /ml in four peaks. With the exception of 3-4 periods of remissions, parasitaemia persisted in the peripheral blood until death. PCV, RBC and Hb concentration were similarly dropped to more than 50% of the preinfection values. All animals died of infection in less than two months. P.M. picture showed gross changes in most vital organs such as heart, liver and lungs. Histopathological studies are not yet completed. It is concluded that (at least in the present study) donkeys are highly susceptible to *T. evansi* infection. The possibility that they may actively contribute to the epidemiology of camel trypanosomiasis in Sudan is discussed. It is suggested that field surveys to determine the natural prevalence of *T. evansi* in donkeys in areas known to harbor higher prevalence of the disease in camels should be conducted.

419 **Trypanosomiasis among free ranging Indian tigers: a global challenge for veterinarians** Singh D.P.

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Trypanosoma evansi infection (Trypanosomiasis, surriosis) among free- ranging Tigers (*Panthera tigris tigris*) as a new host have been reported in the form of morbidity and mortality in the history of wild life parasitology in Ranthambore tiger reserve (RTR) India. An attempt was made to keep a close watch on tigers by video camera and tranquillized the ailing tigers showing clinical signs like corneal opacity, even blindness, anoroxia & muscular spasm of limbs by Dart system. Biomaterial samples (blood, lymph & vital organs) collected from 3 ailing & 6 carcass of tigers which reveal the presence of this pathogenic trypanosome in 11%, 33% by wet blood & lymph smear examination (WBLE) and 22% by micro haematocrit centrifugation technique (MHCT). On post mortem marked progressive-anaemia, myocarditis, enlargement of lymph nodes, liver and kidney. Presence of this protozoan observed up to 18 hrs in the carcass blood. This study alarms that the two third of the world population of Indian wild tigers are at the verge of extinction due to this pathogenic protozoan parasitic infection and for control this genetic erosion & ecological hazard suggest a high techno-diagnostic research strategy at cellular, molecular, genetic, therapeutic, prophylactic, vector control and eco-developmental labels in the co-operation with global developed nations & organizations.

420 **A survey on the cardiac arrhythmias in the cows with theileriosis**

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Theileriosis is a dangerous protozoal diseases especially in ruminants including cows, sheep and goats ; In this diseases cardiac arrhythmia are probably due to anemia and electrolytes imbalances. In this research 70 cows with theileriosis were studied. Firstly infected cows were diagnosed based on clinical signs and observed parasite in smear blood samples which are prepared from auricular vein. The two samples of blood were obtained from the related cows. The first sample with anticoagulant for doing hematological test and determining PCV, total RBC, total WBC and differential count of white blood cell, and the second sample was whole blood, in which after separation blood serum by centrifuging, the biochemical tests such as total protein, calcium, phosphorus, Alkaline Phosphates, Aspartate aminotransferase, Sodium and potassium levels were measured. In cow with theileriosis, after 30 minute rest and before treatment, ECG by using electrocardiography, 3 channel and Base apex lead about 30 second we obtained and type of arrhythmia was determined. In this study 13 cases of atrial premature beat, 9 cases of sinus tachycardia, 6 cases of sinus arrhythmia, 2 cases of first degree atrio-ventricular block and 1 cases of ventricular premature beat, were observed.

According to the kinds of arrhythmia and electrolyte imbalance concluded that more arrhythmia in cows with theileriosis are physiologic and treatment of infected cows as well as balancing of electrolytes will remove arrhythmias.

421 **The potential of East Coast Fever (ECF) vaccine on improved and indigenous cattle in Tanzania**

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A study was conducted to determine the protection ability of East Coast fever (ECF) vaccine in several regions of Tanzania. A total of 1216 immunised cattle were sampled from four regions of Tanzania for analysis of *Theileria parva* antibody levels. Animals that were examined came from Arusha (225 cattle), Coast (208), Mara (311), and Morogoro (472). In addition, there were 29 and 38 controls from Coast and Morogoro regions, respectively. Blood sample was taken from jugular vein into plain vacutainer tubes. The sera were analysed using an indirect ELISA test. The cut off point for ECF antibody titre was titrated to be ≥ 20 percent positivity (PP). Results show that the overall efficacy of the vaccine was 70% whereas 46% of non-immunised controls were also protected. Cattle with ≥ 20 PP were Arusha 72 (32%), Coast 151 (73%), Morogoro 375 (79%), and Mara region 255 (82%). The protection percentages were comparable for Morogoro and Mara, which were significantly higher when compared to Coast and Arusha ($p < 0.01$). The mean PP differed among the regions when compared to each other ($p < 0.001$). The animal age and time span after immunization did not significantly influence the mean PP ($p > 0.05$). The sero-prevalences of other tick-borne diseases were conducted for samples from Arusha only and were as follows: *Babesia bigemina* (24%), *Anaplasma marginale* (19%), *Cowdria ruminantium* (11%) and *Babesia bovis* (0.4%). These results demonstrate that ECF immunized cattle would confer different level of immunity probably owing to geographical location, tick challenge, frequency of acaricide application and/or management system. It is concluded that ITM is a potential control method of ECF and that the prevalence of other TBD could be higher than previously thought especially so for the *C. ruminantium*.

422 **Infectivity, immune response and possible protective capacity of *Leishmania infantum* strains isolated from HIV-*Leishmania* co infected patients in the canine model**

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Introduction: Zoonotic visceral leishmaniasis (ZVL) is a parasitic disease caused by *Leishmania infantum*. Isoenzymatic characterization of *L. infantum* isolates from HIV patients has showed that some strains are new variant that have not been described to cause VL in the dog, the main reservoir of the parasite. At the present work we have evaluated on the dog, the infectivity, pathogenicity, immune response and the possible protective capacity of two new variants isolated from immunocompromised patients. Materials and Methods: Strains LLM-759 (zymodeme MON-190) and LLM-480 (zymodeme MON-228) and the highly virulent strain LLM-724 (zymodeme MON-1) were used. Dogs were experimentally infected with 108 *L. infantum* promastigotes and the clinical, immunological, and parasitological status were monitored monthly during 1 year. After that year dogs infected with the strains LLM-759 and LLM-480 were challenged intravenously with the virulent strain and monitored during 180 days. Results: Dogs infected with the strains of zymodemes LLM-759 and, LLM-480 did not showed clinical, immunological and parasitological status of disease. However the dogs inoculated with the strain LLM-724 showed alterations in all the parameters studied and a parasitological positive status one year after infection. Presence of the parasite was confirmed by culture and PCR in those dogs infected with LLM-480 at day 180 after challenge with the virulent strain, while dogs infected with LLM-759 were parasitologically negative Conclusion: These results confirm that the strains *L. infantum* LLM-759 and LLM-480 are not able to induce the outcome of the disease in the canine host and the possible protective capability of the strain LLM-759.

423 **Evaluation of enzyme-linked immunosorbent and flow cytometry assays for the serological diagnosis of visceral leishmaniasis using crude and recombinant *Leishmania* proteins**

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Introduction: Dogs are the main reservoir of zoonotic visceral leishmaniasis (ZVL), caused by *Leishmania infantum*. An accurate diagnosis of canine infections is essential for the veterinary community and for ZVL control programs, with a special regard to the *Leishmania*/HIV co-infection in humans. Method: The presence of specific antibodies was tested by an enzyme-linked immunosorbent assay (ELISA) using *L. infantum* recombinant proteins (CPX and MTPX), and by ELISA and flow cytometry using total extracts of promastigote or amastigote forms.

Materials: Naturally exposed dogs were classified in symptomatic or asymptomatic *Leishmania* infected, and animals infected with other pathogens. Dogs from an area where ZVL is not endemic were used as negative controls. Results: CPX-ELISA provided a very high sensitivity for the detection of symptomatic dogs, similarly to that obtained with total extracts by either ELISA or flow cytometry. In contrast, MTPX was barely recognised. Flow cytometry was the most sensitive technique for the detection of asymptomatic dogs. No differences were found when promastigote or amastigote extracts were used. However, the specificity of this technique was low. In fact, cross reactions were found with sera from dogs with other infections and even with samples from negative controls. In the detection of asymptomatic dogs, CPX showed higher sensitivity compared to total extracts in ELISA, although it remained lower to flow cytometry analysis. Conclusion: Flow cytometry proved to be the most sensitive technique, yet lacking the required specificity. We suggest that CPX, due to its high specificity and sensitivity, might be a good candidate for a field marker of ZVL.

Acknowledgments: The recombinant proteins were purified from clones provided by A. Tomas group (IBMC).

424 **Serological survey of *Leishmania* infection in dogs from 3 municipalities of central Portugal**

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Introduction: In view of the previous detection of some canine leishmaniasis (CanL) cases in the district of Santarém and given the absence of any quantitative data on canine *Leishmania* infection from this region of central Portugal, the objective of this study has been the evaluation of seroprevalence in dog populations from the municipalities of Alcanena, Constância e Santarém. **Method:** Anti-*Leishmania* antibodies were detected by FAST (fast agglutination screening test) and FAST-positive samples were further tested by DAT (direct agglutination test).

Materials: From April to August 2006, serum samples were obtained from 204 dogs (114 females and 90 males) of various breeds and ages: 153 from the municipality of Alcanena, 30 from the municipality of Constância and 21 from the municipality of Santarém. Animals were surveyed during the anti-rabies vaccination campaign or at the Veterinary Medical Clinic in Alcanena. **Results:** Eighteen (8.8%) out of the 204 animals analysed had positive titres of anti-*Leishmania* antibodies (positive FAST and DAT > 400); and 9 (50%) out of the former presented clinical signs of the disease, representing 4.4% of the whole population. Seroprevalences were 9.2% in the municipality of Alcanena, 0% in the municipality of Constância and 19.0% in the municipality of Santarém. The difference between the percentage of seropositive dogs from Alcanena and Santarém was not significant ($p < 0.05$, Fisher's exact test); the mean seroprevalence in these two contiguous municipalities was 10.3% (18/174).

Conclusion: Data referring to the municipalities of Alcanena and Santarém suggest the existence of an additional zone of endemic CanL in Portugal.

425 **Evaluation of a DOT-immunoblot assay for detecting Leishmanial antigen in *Phlebotomus papatasi* in Iran**

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Introduction: Leishmaniosis is a polymorphic disease of the skin and viscera caused by an intracellular Protozoan. Zoonotic Cutaneous Leishmaniosis (ZCL) is a major health problem in rural areas of Iran.

Methods : A simple and highly reproducible dot-immunoblot assay was developed to detect leishmanial antigen in *Phlebotomus papatasi* that were naturally infected with *Leishmania* major. The test was sensitive to as little as 10 ng of antigenic protein and also appeared to be specific, in that it gave a positive result with some *P. papatasi* (the primary vector of *L. major* in Iran) and *L. major* but not with *P. sergenti* or other pathogens. When used to investigate a large number of sandflies collected from areas of the Iran where cutaneous leishmaniasis is endemic

Discussion : The assay appeared sufficiently sensitive and specific to detect the naturally infected insects. The simplicity, reproducibility, high sensitivity and high specificity of the assay should make it useful for field studies.

Poster group 8: Protozoa, miscellaneous

426 Mapping of quantitative trait loci affecting resistance/susceptibility to *Sarcocystis miescheriana* in swine

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Marked differences in resistance/susceptibility to *Sarcocystis miescheriana* have been shown between Chinese Meishan and European Pietrain pigs, and these differences are associated with high heritabilities. Identification of genes and polymorphisms causal for these differences would help to improve our knowledge of host-parasite interactions and host defense mechanisms. A first step on this way may be the mapping of responsible chromosomal regions as quantitative trait loci (QTL). This survey represents the first QTL study on parasite resistance in pigs. QTL mapping was performed in 139 F2 pigs of a Meishan/Pietrain family, before and after challenge with 50,000 sporocysts of *S. miescheriana*. Phenotypes of the F2 pigs were defined by clinical and clinical-chemical traits, and specific antibody levels, before (day 0), and after challenge, where the pigs passed through three stages representing acute disease (day 14), reconvalescence (day 28) and chronic disease (day 42). Numbers of bradyzoites per gram of musculus longissimus dorsi were determined at day 70 p.i. Fourteen genome-wide significant QTL were mapped to several chromosomal areas. Among others, major QTL were identified for bradyzoite numbers in skeletal muscles ($F=17.4$; $P<0.001$) and for *S. miescheriana*-specific plasma IgG2 levels determined 42 days p.i. ($F=20.9$; $P < 0.001$). These QTL were mapped to different regions of chromosome 7, i.e. to the region of the major histocompatibility complex (bradyzoites) and to an immunoglobulin heavy chain cluster, respectively. These results provide evidence for a direct and causal role of gene variants within these gene clusters in differences in resistance to *S. miescheriana*.

427 Molecular studies on *Sarcocystis* species in reindeer from Norway and Iceland

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Introduction: The first aim of this study was to sequence the complete small subunit (ssu) rRNA gene of the six known *Sarcocystis* species in reindeer, i.e., *S. grueneri*, *S. rangi*, *S. tarandivulpes*, *S. tarandi*, *S. rangiferi* and *S. hardangeri*, in order to validate and expand previous species descriptions based on sarcocyst morphology.

The second aim was to screen reindeer in Iceland for *Sarcocystis* and compare any species present with the *Sarcocystis* population in Norway, which is the country of origin of the Icelandic reindeer population.

Material and methods: Cardiac and skeletal muscles were sampled from carcasses of 18 adult reindeer from northern Norway and from 11 calves and adults in Iceland. *Sarcocysts* were excised from fresh tissue and classified into species based on morphology. Total DNA was extracted from two randomly selected cysts of each species, from both countries. The complete ssu rRNA gene was amplified by PCR and sequenced.

Results and conclusion: All six *Sarcocystis* species were found in the Norwegian reindeer samples. *Sarcocysts* of *S. hardangeri*, *S. rangi* and *S. tarandivulpes* were found in the Icelandic samples. Intraspecies ssu rRNA gene sequence variation was small, but the species differed from each other by insertions, deletions and single nucleotide polymorphisms and thereby the classification of at least six *Sarcocystis* species in reindeer was supported. Cyst morphology and gene sequences of the *Sarcocystis* species from Iceland were identical to isolates of the same species in Norway in spite of more than 200 years of separation of the two reindeer populations.

428 Serodiagnosis of sarcocystosis in water buffalo by elisa test and its comparison with digestion method

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To compare of the Elisa test with digestion method for diagnosis the sarcocystosis in water buffalo and to estimate its sensitivity and specificity, 300 slaughtered water buffaloes were examined. So blood samples were taken for elisa test. Muscles from oesophagous were taken for microscopic method (digestion method). Sensitivity, specificity of elisa and 95% confidence intervals between to tests were determined. Specific anti-*Sarcocystis* antibodies were detected in 54.3% (163 of 300) of cases whereas microscopic infection were 57% (171 of 300) respectively. No significant difference was observed between the positive results in two examined methods ($p < 0.001$). Sensitivity and specificity of elisa were 97% and 90.5% respectively. So we concluded that the elisa test appears to provide an effective and reliable means for detecting sarcocystosis in naturally infected water buffaloes and its simplicity and ease of performance make it particularly suited for use in large-scale epidemiological surveys on livestock.

429 Tissue cyst forming protozoa of camels in Egypt

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Sarcocystis and *Toxoplasma* are investigated in camels. Three hundred blood samples are collected from camels slaughtered at abattoirs, related to three districts at Behera province, Egypt. Incidence of *Sarcocystis* in investigated camels is determined by microscopic examination of pathological sections prepared from esophagus, heart, tongue and diaphragm. Higher incidence is found in esophagus (66%) followed by diaphragm (39.2%), heart (29.2%) and tongue (15.7%) respectively. Histopathologic morphology of detected cysts of *Sarcocystis camali* is discussed. Serological diagnosis of *Sarcocystis camali* is done using ELISA, where antigen characterization is achieved by using 12% SDS polyacrylamide gel electrophoresis. 74% of tested sera are found seropositive for *Sarcocystis camali*. Age susceptibility to *Sarcocystis* is also discussed in investigated camels. Toxoplasmosis is diagnosed serologically by IHT as a screening test, where 26.3% are found seropositive.

430 ***Bartonella* and Bartonellosis - Forgotten and Coming Back**

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Nowadays doctors often have to face diseases common for animals as well as for humans. Aiming at elimination of hemoparasitosis we have studied specimens of peripheral blood of 142 patients with recurrent inflammatory diseases of various genesis, subfebrile condition, agnogenic fever and lymphoproliferative diseases.

Smears were stained according to standard procedures: Romanovsky-Giemsa stain, May-Grunwald -Giemsa and Leikodif sets, Czechia.

9,2% of patients had *Haemobartonella felis* adherent to erythrocytes. The number of epicellular parasites varied from solitary in mild cases, to affection of practically all erythrocytes in grave condition.

It is known that hemobartonellas cause percutaneous zoonosis – “Cat scratch disease” - in apparently healthy people, and lead to bacteremia and other systemic manifestations: angiomatosis, endocarditis, peliosis hepatis, etc in the immunosuppressed.

Hemobartonella, earlier referred to rickettsia, now reclassified and identified as hemotropic mycoplasma – *Mycoplasma haemotrophic* – of *Mycoplasma pneumoniae* group, *Mycoplasma* genus, Mollicutes class, Mycoplasmataceae family. The specified new group of parasitic mycoplasma possesses pathogenic potential, earlier unknown in representatives of Mollicutes. *Mycoplasma* persistence in organism can be perpetual; even in case when the symptoms of the infection disappear, *mycoplasma* are found in various organism tissues. It is supposed that the main depot of persistent mycoplasma is bone marrow.

In view of the significant role; *pneumoniae* plays in human infectious pathology, and growing immunosuppression of population, it can be assumed that the importance of hemoplasma in human pathology will be increasing.

431 **Development of immunofluorescence and in situ hybridisation techniques for the detection of *Bartonella* and *Anaplasma* organisms within vector tissues**

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Techniques for the visualisation of pathogens within vector tissues are lacking. Pathogen-specific immunofluorescence and in situ hybridisation were used as methods to view the route of pathogen invasion and the subsequent distribution of the pathogen within vector organs after the ingestion of an infected blood meal. The vector-pathogen models used were *Anaplasma phagocytophilum* infection of *Ixodes ricinus*, and *Bartonella henselae* infection of *Ctenocephalides felis*. Both assays were initially evaluated on a simplified vector-pathogen model consisting of tick and flea cell cultures infected with the associated pathogen, before being evaluated on whole vector sections. The immunofluorescence detection of pathogens in infected cell cultures used polyclonal anti-*A. phagocytophilum* and anti-*B. henselae* antibodies sourced from naturally infected bovines and felines respectively. Primary antibodies were paired with fluorescently-labelled anti-feline or anti-bovine antibodies, for an indirect immunoassay, and concentrations were optimised using the standard chequer-board approach. For in situ hybridisation detection of pathogens, suitable genomic 16s ribosomal targets were identified from sequences held in Genbank, and corresponding probes were fluorescently labelled. Sample preparation and hybridisation of the probe were optimised for each target pathogen. Visualisation of both techniques was carried out using an epifluorescence microscope and both techniques allowed the successful visualisation of the pathogens within the vector tissues. It is planned to combine these visualisation techniques with the quantification ability of pathogen- and vector-specific real-time PCRs. When used in conjunction, these techniques will produce an invaluable toolbox to illuminate the dynamics of vector infection following blood feeding.

432 **Molecular detection of *Ehrlichia canis* and *Anaplasma platys* in dogs living in rural areas of the State of Minas Gerais, Brazil**

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Ehrlichia canis and *Anaplasma platys*, the two best known etiological agents of canine ehrlichiosis, have a worldwide distribution and are thought to be transmitted by *Rhipicephalus sanguineus* ticks, the main biological vector in urban areas. Despite the importance of canine ehrlichiosis, very little is known about its epidemiological aspects in rural areas in Brazil. The present study describes results of an epidemiological survey carried out in three distinct regions of the State of Minas Gerais, Brazil, where blood samples were collected from a total of 248 dogs living in farms. DNA was extracted from blood samples and Real Time PCRs were performed for detection of *E. canis* and *A. platys*. The variables age, breed, sex, presence of ticks, species of tick and packed cell volume (PCV) were also analyzed. The percentages of positive dogs for *E. canis* and *A. platys* were, respectively: 26.5 and 19.6% (n=102) in Nanuque region, 24.5 and 4.1% (n= 49) in Belo Horizonte region, and 1.0 and 7.2% (n= 97) in Lavras region. Dogs infested with *R. sanguineus* (OR=3.0; P=0.00; IC 95%= 1.4-6.5) and with *Amblyomma cajennense* (OR=2.6; P=0.01; IC 95%= 1.3-5.5) showed higher rates of infection with *E. canis*, while animals infested with *R. sanguineus* (OR=4.4; P=0.00; IC 95%= 1.9-10.0) had higher rates of infection with *A. platys*. The results point to the importance of canine ehrlichiosis in rural areas and to the need of further studies related to its transmission and maintenance in nature.

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433 **Presence of erlichiosis and anaplasmosis in dogs in Sinaloa, Mexico**

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Canine Ehrlichiosis is a disease caused by the rickettsia *Ehrlichia canis*, which is transmitted by the *Rhipicephalus sanguineus* tick(1). Despite it being considered one of the most common diseases in dogs, there is little information about its prevalence in this zone. Anaplasmosis, a disease caused by organisms belonging to the Anaplasmataceae family, which has a high prevalence in bovine cattle and sheep in Sinaloa state: 25.5 % (2), is also transmitted by ticks. However, prevalence of this disease in dogs in Sinaloa is unknown, or if it is even present. Because the clinical signs are similar in both pathologies in dogs, it is believed that canine anaplasmosis has not been diagnosed due to the lack of reliable test techniques. The objective of this work was to perform sampling in dogs to assess the presence of canine anaplasmosis and corroborate Ehrlichiosis prevalence. In order to achieve this, the ELISA test was utilized using Idex snap 4dx® kit. A prevalence of 71% samples were positive for Ehrlichiosis, and 21.43% of the samples were positive for anaplasmosis. In some cases, positive results for both micro-organisms were observed. It is concluded that the presence of canine anaplasmosis in this zone is a reality and must be considered as one of the differential diagnosis by small animal practitioners. It is recommended to perform surveillance studies for the positive cases to canine anaplasmosis and mixed infections of ehrlichiosis and anaplasmosis, as well as perform a prevalence study in canine anaplasmosis in a larger number of dogs.

434 **Effect of a monthly imidacloprid-permethrin treatment against *Ixodes ricinus* on the seroprevalence of *Anaplasma phagocytophilum* in dogs**

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Anaplasma (A.) phagocytophilum (Rickettsiales), the causative agent of canine granulocytic anaplasmosis (CGA), is an ixodid tick-transmitted, gram-negative, intracellular organism. The objective was to evaluate whether an appropriate imidacloprid-permethrin treatment (Advantix®) during the tick season prevents from *A. phagocytophilum* infection in dogs living in areas with high *Ixodes ricinus* populations. A total of 27 dogs (serologically/PCR-negative for *A. phagocytophilum* at the beginning of the trial) were treated with Advantix® - Spot-On every 4 weeks from May 2006 March 2007. A tick infestation was only noticed in 7 of these dogs several days before the subsequent treatment. Twenty six treated dogs remained sero- and PCR-negative, whereas one dog became seropositive without any sign of disease. The positive control group consisted of 147 dogs which were not or only irregularly treated with different ectoparasiticides. Seventy eight (53.1%) of these dogs were seronegative and 69 (46.1%) were seropositive. Four (5.1%) of the seronegative and 11 (15.9%) of the seropositive, inappropriately treated dogs were PCR-positive. Nine control dogs were diagnosed with CGA based on PCR and clinical and laboratory abnormalities suspicious for CGA. It was concluded that imidacloprid-permethrin was useful to protect dogs from tick infestation and consequently from *A. phagocytophilum* infections.

435 **Seroprevalence of *Encephalitozoon cuniculi* infection in pet rabbits in France**

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Microsporidia comprise unicellular eukaryotes all obligate intracellular parasites which can infect almost all animal groups and which are now recognized as major opportunistic emerging pathogens in humans. Rabbits are supposed to be frequently infected by the species *Encephalitozoon cuniculi* and may be considered as a reservoir of pathogens for humans. The purpose of the present study was to assess the seroprevalence of *E. cuniculi* in pet rabbits in France. A total number of 124 blood samples were collected as part of a routine health screen or before general anaesthesia at the veterinary College of Alfort and at 3 private veterinary practices in Paris (or close suburbs). Blood samples were taken from 88 asymptomatic rabbits and 36 rabbits showing neurological (head tilt, seizures or ataxia), renal or ocular signs suggestive of encephalitozoonosis. Specific anti-*E. cuniculi* antibodies were detected by indirect immunofluorescence. A specific protein dot-blot test was also performed and the results of the two serological tests were compared. In the asymptomatic group, 58 of 88 (66%) animals were seropositive. In the group with clinical signs, 29 of 36 (81%) animals were seropositive. There was no significant difference between the two groups. The serological status of the rabbits was not correlated with the age nor the sex of the animals.

- 436 **Nf-cHsp70 is essential for proliferation and cytotoxicity of pathogenic *Naegleria fowleri***
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Naegleria fowleri, a free-living amoeba of soil, ponds and fresh water, feeds on bacteria present in these environments. It also exists as a virulent pathogen causing primary amoebic meningoencephalitis in humans, mice, and other mammals. To evaluate the role of heat shock 70 protein (Hsp70) in the pathogenicity of *N. fowleri*, we cloned, characterized a constitutive and inducible heat shock 70 gene from pathogenic *N. fowleri*, and named as Nf-cHSP70. The Nf-cHSP70 was localized in the cytoplasm, pseudopodia and phagocytic food-cups. To investigate role of Nf-cHSP70, we inhibited the Nf-cHsp70 protein with benzylidene lactam compound, KNK437, which has effect on Hsp70 synthesis and knock-downed Nf-cHsp70 gene with antisense oligomers which has designed with a start region specific antisense oligonucleotides (24 mers) and modified with phosphothioate. KNK437 inhibited the induction of *N. fowleri* HSP70 in a dose-dependant manner. After treatment with 300uM of KNK437, the proliferation of *N. fowleri* was reduced to 79.4% compared with untreated control (100%). Hsp70-Knock downed *N. fowleri* with antisense oligomer showed reducing proliferation to 70% compared with untreated control (100%). Hsp70 inhibited *N. fowleri* showed reducing cytotoxicity against CHO target cells as to 50%. It suggests that the cloned cHsp70 plays an important role in the proliferation and cytotoxicity of pathogenic *N. fowleri*.
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- 437 **Immune responses and mortality of mice immunized with the rNfa1 protein post-infection of pathogenic *Naegleria fowleri***

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Pathogenic *N. fowleri* causes a fatal disease, called as primary amoebic meningoencephalitis, in humans and experimental animals. To observe the protective immunity for *N. fowleri* trophozoites infection in a mouse model, BALB/c mice were intraperitoneally injected with 50 µg rNfa1 mixed with Freund's complete adjuvant in a total volume of 200 µl. The mice were boosted with 25 µg rNfa1 mixed with Freund's incomplete adjuvant 2 weeks after the primary immunization. A final booster of rNfa1 mixed with Freund's incomplete adjuvant was given 2 weeks after the second immunization. And then 10000 trophozoites of *N. fowleri* were intranasally inoculated into mice. Mice were divided into four groups; Group I, Normal; Group II, Adjuvant+PBS; Group III, rNfa1+Adjuvant; Group IV, rNfa1 only. Up to 25 days after infection, mortality rates in group III and group IV were 23.1 % and 25 %, in contrast to the 100 % in group I and group II, respectively. Survival times of mice in group I, II, III and IV were 14.8, 17.2, 22.3 and 19.7 days, respectively. Sera obtained from immune protected mice had a significantly increased level of immunoglobulin G (IgG). Analysis of IgG-subclass profiles revealed that IgG1 showed the greatest increase followed by IgG2b, IgG2a, and IgG3. Splenic cells from Group III (rNfa1+Adjuvant) immunized mice secreted significantly high levels of both Th1 cytokine gamma-interferon and Th2 cytokine interleukin-10 after stimulation with rNfa1 in vitro. These findings may contribute to the understanding of rNfa1-induced Th1/Th2-mediated effector mechanisms required for the protection of *N. fowleri* infection.

438 **Regurgitated pellets of *Merops apiaster* as reservoir of infective *Nosema ceranae* spores**

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The European bee-eater, *Merops apiaster*, is a migrant insectivorous bird abundant mainly in arid and semi-arid areas. Bee-eaters predominantly eat insects, especially bees, wasps and hornets. *Nosema ceranae* is a *Microsporidia* of *Apis mellifera* honeybee. It has been described as highly pathogenic for this new host but few data are available. In this study, spore detection in regurgitated pellets of *Merops apiaster* is described, and spore infectivity demonstrated. Five regurgitated boluses and two faeces samples of *Merops apiaster* were collected from the covers of naturally infected by *Nosema ceranae* hives in spring 2006 and 2007. The presence of microsporidia spores was first confirmed in pellets or faeces for phase-contrast microscopy (PCM) while PCR shown *Nosema ceranae* specie. Spore infectivity was demonstrated after infection of healthy honey bees. Newly emerged *Nosema* free honeybees were infected collectively with 100,000 spores and infection determined in one bee per group after 3, 7 and 21 days p.i. Infected bees were positive to PCM spore detection both after 3 days p.i. and 7 days p.i. Quantification of spores showed a 10 fold increase in the number of spores from day 3 to day 7. After 20 days p.i. all the infected bees became visibly less active, with sluggish movements, or immobile. The next day all of them died. No similar signs were observed in control group, with all bees alive except one by day 21 p.i. Epidemiological consequences of the presence of *Nosema* spores in regurgitated pellets of *Merops apiaster* are discussed. Supported by JCCM 05-280/PA-47 and API/FEGA-MAPYA FOUNDS-06-009.

439 **Detection of infective *Nosema ceranae* (Microsporidia) spores in corbicular pollen of forager honeybees**

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Nosema ceranae is a *Microsporidia* recently described as a parasite in *Apis mellifera* honeybees in Europe and scarce epidemiological data are available. In this study, spore detection in both pollen baskets and pollen collected from commercial traps is described (PCM, TEM and PCR methods) and spore infectivity demonstrated. Forager bee samples were obtained from weak colonies from a naturally infected *Nosema* colony. Corbicular pollen was removed from the honeybees' corbicula by using sterile tweezers. Commercial pollen was also analysed. The presence of microsporidia spores was first confirmed in bee and pollen samples, for phase-contrast microscopy (PCM), lately by Transmission Electron Microscopy (TEM) analyses while PCR shown *Nosema ceranae* specie. Newly emerged *Nosema* free honeybees were infected and infection determined in one bee per group after 3, 7 and 21 days p.i. Forager bees, corbicular pollen and commercial pollen were positive to *Nosema* sp. spores. Infected bees were positive to PCM spore detection both after 3 days p.i. and 7 days p.i. Quantification of spores showed a 10 fold increase in the number of spores from day 3 to day 7. After 20 days p.i. all the infected bees became visibly less active, with sluggish movements, or immobile. The next day all of them died. No similar signs were observed in control groups, with all bees alive except one by day 21 p.i. Epidemiological consequences of the presence of *Nosema* spores in pollen as a source of nosemosis for the colony are discussed. Supported by JCCM 05-280/PA-47 and API/FEGA-MAPYA FOUNDS-06-009.

440 **Life cycle of *Nosema ceranae* in *Apis mellifera***

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Nosema ceranae has been recently described as a parasite of the ventriculi of *Apis mellifera*. Samples from experimental and natural infections were processed for light and electron microscopy and life cycle described.

The first extracellular stage of *Nosema ceranae* includes mature spores that are small, ovocylindrical from 3.3 to 5.5 μm and from 2.3 to 3 μm in size. A typical diplokaryon appears surrounded by 20-23 coils of the polar filament. Under appropriate conditions, the spore is activated in bee gut and triggered to evert its polar filament, pierces a bee intestinal cell, injects the sporoplasm into it and phase II starts.

Phase II is the first phase of intracellular development. *Nosema ceranae* maintains direct contact between plasmalemma and host cell cytoplasm lacking any type of interfacial envelope. Once injected, the sporoplasm forms an elongated multinucleate cell that divides by multiple fission.

Phase III or the sporogonic phase involves sporonts, sporoblast and spores. Material secreted by the parasite forms an electro-dense addition to the plasmalemma. Then cell undergoes one nuclear division process by binary fission producing two sporoblast cells that mature to produce a spore.

Sometimes cytokinesis is not linked to karyokinesis, four diplokaryon are also produced. In less than three days after infection, either mature spores as emptied spores can be observed inside infected cells. Intracellular germination of spores is taking place in *A. mellifera* infected with *N. ceranae* with an expeditious horizontal transmission between epithelial cells.

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441 **Prevalence of *Tritrichomonas foetus* infection in cats in France**

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Feline trichomonosis is caused by a flagellated protist, *Tritrichomonas foetus*, the primary causative agent of bovine urogenital trichomonosis. In cats, *T. foetus* colonizes the colon, resulting in chronic, large bowel diarrhoea mainly in young animals. *T. foetus* also can be cultured from the faeces of asymptomatic cats. Environmental stress, immunologic immaturity and crowding may be significant risk factors for disease sensibility.

In order to investigate the presence of *T. foetus* in French catteries and to identify risk factors for feline trichomonosis, an epidemiological survey was conducted on 151 cats from 19 catteries in the region Ile-de-France (around Paris). Cultivation of feline faeces was obtained using a commercially available system "In PouchTM TF test" (BioMed Diagnostics, Oregon USA). The prevalence of *Tritrichomonas foetus* was found to be 10 %. The presence of *T. foetus* was more frequently observed in cats with digestive clinical signs and in young cats.

442 **The use of PCR to confirm non-*T. foetus* trichomonads in cultured preputial samples of a bull, and its modification for routine diagnostic use**

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Trichomonosis is a venereally transmitted reproductive disease of cattle caused by the protozoan flagellate *Tritrichomonas foetus*. The currently accepted diagnostic test for *T. foetus* is microscopic examination following the culture of samples collected from the prepuces of bulls or the vaginas of cows. Reliable diagnosis of *T. foetus* infection requires proper collection and handling of samples, use of appropriate growth media and conditions, and correct identification of any trichomonads recovered. Fecal contamination at sample collection, and morphological similarity amongst flagellated protozoa are causes of false positive diagnosis of bovine trichomonosis. Recently, a preputial diagnostic sample collected from a bull and cultured in modified Diamond's medium was found to contain many *T. foetus*-like trichomonads. Microscopic examination of a wet mount sample revealed that the organisms were motile and similar in size, shape, and flagellae as *T. foetus* control specimens. A modified Giemsa stain of the organisms indicated that at least some of them contained more than three flagellae. In order to rule out the possibility of *T. foetus* in the sample, a PCR assay was performed. A modification of a previously published method was used to amplify an ITS1 region of the cultured flagellates and compare it in size and sequence with those of *T. foetus* amplicons. The results confirmed that the trichomonads in the diagnostic sample were not *T. foetus*. The PCR assay has since been further modified to improve its sensitivity and reliability for routine diagnostic use. A ring trial is being planned to evaluate its performance on field samples.

443 ***Trichomonas* spp. as a possible cause of a new recognized Canary Infraorbital Synusitis syndrome in Italy**

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The breeding of ornamental canary (*Serinus canarius*, Linnaeus, 1758) is well represented in Italy. The medium animal stock is constituted by 35 couples of birds, producing 7-8 offspring per year. In the past 10 years, breeders have repeatedly reported a syndrome, here called Canary Infraorbital Synusitis (CIS), affecting the eyes of the canary. The CIS is characterised by a subtle emergence, with only 1 or 2 birds infected per breeding flock. Usually the first symptom is a slight mono- or bilateral conjunctivitis, followed by the formation of a yellow, hard and painless tumescence under the eyes. Autopsy reveals a mono- or bilateral sinusitis located mainly in the infraorbital sinus. Bacteriological examination performed from the sinus material showed more frequently *Escherichia coli*, *Klebsiella oxytoca*, *Enterobacter cloacae* and *Staphylococcus aureus*, but focused antibiotics treatments never resolved the symptomatology of CIS. Virological examination and *Mycoplasma* culture were always negatives. In a fresh smear of purulent material of one of these cases, a protozoan Trichomonas-like was seen. The protozoa was successfully cultured in a Trichomonas medium (Trichomonas medium No20, Oxoid). The protozoan was then searched and found on all the subsequent 17 breeding flock examined. The hypothesis of a protozoan caused syndrome was reinforced by the successful treatment of the flocks with dimetridazole and furazolidone drugs in association, which resulted in a complete remission of symptoms.

444 ***Balantidium coli* in dairy cattle: Clinical signs and successful treatment**

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Balantidium coli is a parasite belongs to the flagellated protozoa's which cause disease in human and animals by contaminated water and food. Non-human primates and swine are the reservoirs of this parasite and the infected cyst can result to infection in other animals and causing bloody diarrhea in some of them by contaminated sewages.

Some cases of transmitting the human to human infection by drinking contaminated water are reported. This parasite is announced as one of the most important zoonoses agents between human and animal in New Guinea in 2005 and between the wild boars of Luristan Province the range of contaminating is reported as 25% (Solaymani et al). This parasite cause gastrointestinal disease in cats, dogs and other animals also is one of the parasitic agents causing enteritis in these animals. Immunodeficiency is known as one of the reasons of spreading this parasite to the other organs, specially the lungs.

According to our knowledge and because of the presence of some clinical cases with the signs of large amount of watery feces, big abdominal space (Pearapple shape abdomen) and inappetance, that was seen in the cattle of Shahrekord area (Taghanak), we decided to have an epidemiological study on this disease.

During 3 months, fecal samples were taken from 95 dairy cattle and the samples were sent to the libratory. The results of direct examination of feces, shows that 8 (7.6%) cattle were contaminated by the parasite and the presence of parasite was proved by examination of dog feces from this region. In continue we tried to treat the infected cattle by combination of oral sulfadimidine (Behrood Atrak co.) for five days, vitamin E and selenium compound (I.M.) and Oxytetracycline 10% for five days (I.V.). After twenty days, sampling of feces was done again and the samples were exammed directly with microscope which shows successful treatment.

445 **Development and application of nested PCR assay for detection of dairy cattle-derived *Cyclospora* sp.**

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Cyclospora organisms are intestinal pathogens of humans that are increasingly recognized in many parts of the world. *Cyclospora*-like oocysts, found firstly in feces of dairy cattle in China, were identified as a new species belonged to *Cyclospora*. Based on partial 18S rDNA of the cattle-derived *Cyclospora* sp., two pairs of primers were designed. These primers selectively amplified a 168-bp DNA fragment of the 18S rRNA gene of cattle-derived *Cyclospora* sp. With these primers, a nested PCR assay for detection of cattle-derived *Cyclospora* sp. was developed. The results showed that the nested PCR assay was specific and there is no cross-reaction with other parasites, such as *Eimeria* spp., *Cryptosporidium* spp., *Giardia* sp., *Toxoplasma* sp., *Trichuris* sp. and cattle ciliate. The assay was able to detect as low as 2.85×10^{-2} fg of control positive DNA. The results of detection of clinical samples revealed that the assay was coinciding with microscopic examination and it will be an effective tool for detection of *Cyclospora* sp. in cattle feces. This work was supported by grants from National Natural Science Foundation of China (grant no. 30371082, 30671577) and Natural Science Foundation of Guangdong Province (grant no. 32286).

446 **Standardization of a real time PCR for differential diagnosis of *Hepatozoon canis* and *H. americanum* in dogs**

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Canine hepatozoonosis is a tick-borne disease caused by *Hepatozoon canis* and *H. americanum*. The geographic distribution, the vectors and pathogenesis of the two protozoan infections are distinct and the classification of *H. americanum* as a new species was based on clinical signs, tissue tropism, and pathological and morphological findings. A differential diagnosis can be made on the basis of genetic sequences; however, it is laborious and costly. This paper presents the development and standardization of a Real Time PCR to differentiate between *H. canis* and *H. americanum*. Successive alignments of 18S rDNA consensus sequences of *H. canis*, *H. americanum*, *Babesia vogeli*, *B. canis*, *B. rossi*, *B. gibsoni*, *B. microti*, *Boophilus microplus*, *Amblyomma variegatum*, *Rhipicephalus sanguineus*, *Ixodes ricinus*, *Mus musculus* and *Homo sapiens* were performed. Three distinct primers and probes specific for *H. canis* and *H. americanum* were selected. Different annealing temperatures and concentration of magnesium chloride, DMSO, BSA, dNTP and probes were tested. Specificity was determined using positive controls for *H. canis*, *H. americanum*, *B. vogeli*, *B. canis*, *B. rossi*, *B. gibsoni*, *Theileria annae*, *Ehrlichia canis*, *Anaplasma phagocytophilum*, *Leishmania infantum*, *Borrelia burgdorferi*, *Dermacentor reticulatus*, as well as negative samples (uninfected dogs). The reactions were highly sensitive and specific for *H. canis* or *H. americanum* and no other species of parasites were amplified. The PCR has a potential use as a differential diagnostic test for detection of *H. canis* and *H. americanum* infections. Further evaluations will be carried out to validate its use in epidemiological surveys.

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447 **Prevalence of bovine theileriosis and babesiosis in irrigated area of Morocco**

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Epidemiological investigations were carried out in 4 irrigated area of Morocco: Gharb, Doukkala, Haouz and Tadla in order to show the relationship between ticks born diseases and agricultural management, and its effects on changing patterns of these diseases. Blood samples were collected from a total of 3421 cattle. Serum sample were tested for antibodies to *Babesia bovis* by cELISA and for *Babesia bigemina* and *Theileria annulata* by IFAT. In the Gharb we recorded a predominance of *Babesia bovis* and *Babesia bigemina* with respective seroprevalence of 25% and 22.94% followed by theileriosis (18.82%). In Doukkala Haouz and Tadla theileriosis was dominant with respective seroprevalence of 36.5%, 31.67% and 23.1%. A retrospective study have shown that babesiosis was almost absent in Tadla, Doukkala, Haouz. The increase in seroprevalences of babesiosis in these regions (respectively, 37.59%, 28.18%, 14.51%) might be explained in part by irrigation and agricultural management, and non controlled movement of animals. Indeed *B. bovis* and *B. bigemina* were largely introduced in the arid and semi arid bioclimatic stratum.

448 **Canine Babesiosis (*Babesia canis*) in France : A national survey. Clinical presentation of the disease**

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A national survey on Canine Babesiosis was conducted in 2005 on 1) distribution and epidemiology (cf. Bourdeau P. et al: WAAVP 2005), 2) clinical characteristics, 3) diagnostic methods 4) therapeutic/prophylactic options. The questionnaires contained a list of 17 clinical signs that had to be classified as: extremely frequent (> 90% of cases); frequent: (50-90%); occasional (10-50%); rare to absent (<10%) and the clinical pattern was evaluated as typical (hyperthermia + haemoglobinuria + anemia) or atypical forms. From the 892 veterinary clinics 91 % diagnose at least 1 annual case (1% > 200 cases, 40 % 21 to 200). A score system (0-300) was calculated and compared between clinics: (A) in an enzootic area, (B) with > 50 cases/year and (C) global. The results are grossly the same for A,B;C indicating the little influence of life in enzootic area for the clinical manifestation. The 7 major symptoms are: prostration then anorexia, hyperthermia, dark urines, pale mucosae and splenomegaly (A and B) and locomotor problems (A and B) and the 3 less common are: skin edema, respiratory troubles and skin necrosis. For 53% of clinics more than 75% of cases are typical forms. However the atypical presentations seem to be increasingly frequent for 40% of clinics (54% in B, 46% in A). Co-infections have been diagnosed in 28% of the clinics (mainly ehrlichiosis and borreliosis). Ticks are present on less than 25% of affected dogs for 60% of veterinarians and 35% have never seen ticks on the day of diagnosis.

449 **Canine Leishmaniosis (*Leishmania infantum*) in France: A national survey. Informations obtained on treatments used by veterinarians and criterias of efficacy**

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The treatment of Canine General Leishmaniosis is based on various molecules and protocols. A national survey, conducted in 2004 (cf. Bourdeau P et al: Canine Leishmaniosis in France : European Multicollloquium of Parasitology 2004) evaluated these aspects through detailed questionnaires sent to veterinary clinics. The results from 992 clinics. Were analyzed. Glucantime is used by 96.9% (systematically in 67,1%) and allopurinol 92,5% (systematically 74%). Other molecules mentioned are : Metronidazole (19,9%), Fluoroquinolones (17.6%, only for occasional use), Lomidine (16.9 %) and finally Amphotericin B (5.5%). Glucantime is associated to Allopurinol used in 80.8%. The dosages, combinations, frequency of use or even route of administration of these two molecules are very variable. A symptomatic treatment is frequently associated (41,2 to 44,6%), mainly corticosteroids (mentioned in 64.7%) used in cases of kidney or ocular lesions. An Improvement of efficacy of treatment and management of CL is indicated by 62,3% of clinics. Tolerance was considered good / frequent side effects occasional side effects and was respectively : glucantime: 52 – 39 - 9%; allopurinol : 91.5 - 7.9 - 0.6 ; amphotericin B: 20 – 40 - 40%. Criteria used to stop the treatment are clinical cure 76.2% serology 54.5%, electrophoresis 25.5% and biology (blood, urine) 25.9%. Reason to restart the treatment are : clinical relapse in 38.8%, or even without relapse 37.5% : mainly based on worsening of serology (80%), worsening of electrophoresis (27%). The use of these therapies allow to estimate the survival of treated dogs < 3 months 8.6%, 3-6 months 4.7%; 6-12 months 7,2%; 1-2 years 13.5% ; 2-5 years 35,7% and > 5years 30.3%.

450 **Bovine Theileriosis: Evaluation of 372 cases during spring and summer in south of Iran**

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Bovine theileriosis caused by *Theileria annulata* is a dangerous and important disease in tropical and subtropical areas. There is large economical losses in these areas every year including in Iran. The vector of the disease, the tick of *Hyalomma* is found in most parts of Iran. The prevalence of the disease correlates with the abundance of the vector in spring and summer.

In this study prevalence of the disease in terms of month of the year, age, sex and breed of the animal were studied. Results showed that in spite of all the means of prevention and vaccination, the disease has been known as the most important disease in the region. In relation to the month, the maximum and minimum prevalence of disease was in may and June, respectively. In relation to age, 76.2 percent of infected cattle were one month old or younger. In relation to the breed, infection of Holstein, crossbred and native cattle were 1.6, 94.4 and 4.0 percent respectively. In relation to sex, infection rate of males were 55.4 percent and female was 44.6. From the aspect of clinical signs, all cattle showed anorexia and enlargement of lymph nodes and the other signs were in different percentages. In 58 percent of blood smears the infection rate of red blood cells with the intraerythrocytic form of *Theileria* was less than 10%, in 38% more than 10 percent and in 4% the results were uncertain due to technical faults in staining. All taken smears of lymph nodes were positive for Koch's blue bodies.

Poster group 9: Vector-Borne Protozoa

451 **Infection of the fishes with *Ligula intestinalis* and *Bothriocephalus acheilognathi* in Sattarkhan Dam, northwestern Iran**

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After an extensive fish mortality in Sattarkhan Dam, in East- Azarbaijan province during the year 2003, we collected 160 specimens of the fish *Alburnus fillipi* and 5 specimens of the fish *Alburnoides biponctatus* to examine the cause of mortality. The samples measured biometrically and examined them for internal and external infections. We found that of the 160 specimens of *Alburnus fillipi*, 131 (81.8%) were infected with *Ligula intestinalis* and 26 (16.25%) specimens were infected with *Bothriocephalus acheilognathi*. Of 5 specimens of *Alburnoides biponctatus*, 4 (80%) were infected with *Ligula intestinalis* and no *Bothriocephalus acheilognathi* observed. This is the first report of the *Ligula* and *Bothriocephalus* parasites infecting *Alburnus fillipi* in Iran.

452 On cestode and nematode parasites of cyprinoid fish from Zayandehrood dam, centre of Iran

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Introduction: Tapeworms (Cestoda) are a group belonging to the phylum Platyhelminthes. These are all internal parasites and mature cestodes usually live in the digestive system of its final host. Tapeworms are both found in freshwater and seawater fish. Also, Nematodes are long cylindrical worms of unsegmented body. Mature parasites most often live in the digestive system of the host and lay eggs that go out with the faeces. The first host is often an arthropod, which is then eaten by the fish. Fish can both be final or intermediate hosts depending on the species of tapeworm or Nematode.

Material and methods: During this study in summer 2005, 150 Cyprinid fishes belonging to the five species were examined for helminth parasites. The fishes were hunted from the arrival, median area and boarder of the zayandehrood dam. In order to expose the gastrointestinal tract, the abdomen of the fish was opened laterally and the intestine was lifted up carefully. Then, the intestine was aseptically removed. In order to discharge the excrement, the gut was gently rinsed and the contents pushed through a 100 µm nylon mesh. The content of each gut was filtered for any present worm. Parasites were fixed and stained using routine method (Carmine acid method), and identified.

Results and conclusion: We found adult cestodes (*Caryophyllaeus fimbriceps*) and nematodes (*Rhabdochona railliet*) (Nematoda: Rhabdochonidae) and both parasites were reported for the first time in Centre of Iran. Also, the prevalence of contamination to cestodes and nematodes were (14%) and (12%), respectively.

453 Parasites of wild and cultured fish from Sepidrood River, north of Iran

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Introduction: Parasites commonly found in freshwater fishes primarily belong to Protozoa, Platyhelminthes, Acanthocephala, Nematoda, Hirudinea and Crustacean. Most of these parasites are external parasites of the skin, fins or gills, while few are internal parasites living in the epidermal intralamellar gills, intestine or intramuscular tissues.

Material and methods: A total of 370 cultured and wild fishes belonging to the sixteen different species were examined for parasites. The fishes were collected for the purpose of detecting protozoan and metazoan parasites from the Sepidrood River. Then, the parasites were fixed and stained using routine method and identified.

Results and conclusion: The protozoans, *Ichthyophthirius multifiliis* were found in gills of wild fish (*Carassius carassius*) and cultured fish (*Cyprinus caprio*, *Ctenopharyngodon idella*, *Hyphophthalmichthys molitrix*), and *Trichodinidae* sp were detected in gills of wild fish (*Capoeta capoeta capoeta*) and in gills of cultured fish (*Cyprinus caprio*, *Ctenopharyngodon idella*, *Hyphophthalmichthys molitrix*, *Carassius auratus*, *Aristichtys nobilis*).

The metazoan parasites included, 1 species of Crustacean (copepods of *Lernaea cyprinaecea*) in the gills of cultured fish (*Cyprinus caprio*, *Ctenopharyngodon idella*), 2 species of Cestoda (*Caryophyllaeus laticeps* and *C. fimbriceps*) in the intestines of Cyprinidae fish; and 18 species of Monogenea belonged to five family (Gyrodactylidae, Dactylogyridae, Ancyrocephalidae, Traenchoidea and Masocaraeidae) were also found on the gills and fins of the fishes. Also, the distribution of monogenea parasites in various fish species was identified.

454 **Identification of anisakid nematodes with zoonotic potential by isotopic and non-isotopic SSCP of ITS-2 rDNA**

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Using genetic markers defined previously in the second internal transcribed spacers (ITS-2) of nuclear ribosomal DNA (rDNA), isotopic and non-isotopic PCR-coupled single strand conformation polymorphism (SSCP) were evaluated for the specific detection of each of three anisakid species [*Anisakis simplex* (s.l.), *Contracaecum osculatum* (s.l.) and *Hysterothylacium aduncum*] from different host species and geographical locations in Poland and Sweden. While subtle microheterogeneity was observed within each of *Anisakis simplex* (s.l.) and *H. aduncum*, distinct SSCP profiles were displayed for each of the three species, allowing identification and differentiation of the three taxa. Subsequent sequencing of the ITS-1, 5.8S and ITS-2 rDNA revealed that *Anisakis simplex* (s.l.) represented *A. pegreffii*, and *Contracaecum osculatum* (s.l.) represented *C. osculatum* C. Application of the non-isotopic SSCP assay to larval anisakid specimens from different hosts and geographical locations in China revealed three distinct SSCP profiles, one of which was consistent with that of *A. pegreffii*, and the other two had different SSCP profiles from that of *C. osculatum* C and *H. aduncum*. Sequencing results supported the SSCP findings and revealed three species, i.e., *Anisakis typica*, *Anisakis pegreffii* and *Hysterothylacium* sp.. Together with other methods, each of these molecular tools will be useful for investigating the ecology of these anisakid nematodes. Project support was provided by grants from the China National Science Funds for Distinguished Young Scientist (grant no. 30225033).

455 **Survey on epicomensal parasites of *Penaeus indicus* in Bushehr province**

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Penaeid shrimp reared in semi-intensive, intensive, or super-intensive culture systems, or in systems of poor water quality, often develops some form of gill or surface fouling disease. All, or nearly all, of the organisms involved in gill and surface fouling disease syndromes are free living organisms, and are not true pathogens. These organisms are called epicomensals or epibionts, because they use shrimp as a substrate for attachment. For the most part, these organisms do no direct damage to the shrimp, but instead cause problems indirectly by attaching to the gill or cuticular surfaces. They may kill shrimp by either preventing proper water flow over the gills, by interfering with gas exchange across the gill surfaces, or by interfering with molting, feeding, or locomotion. In this survey 120 juvenile and adult *Penaeus indicus* from Bushehr rearing ponds were reviewed. Gill and appendages of shrimps were examined by wet smear and in some cases by pathological sections. According to results of this study incidence of epicomensal protozoa in shrimps were 60.8% .In appendages intensity and incidence of these protozoa were higher than gills. The most incidence and intensity were belonged to Zoothmnum (Incidence 55.83% in appendages, 19.2% in gills), Epistylis and Vorticella respectively. A few numbers of these protozoa are common in hatcheries and rearing ponds, but if the numbers of them rise, it can be indicated bacterial and viral infection or high inorganic components and environmental stress in shrimps.

456 **External fouling disease in shrimp culture *Fenneropenaeus indicus* of I.R.IRAN, Bushehr Province**

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Protozoans frequently foul the exoskeleton and gill cavity of shrimp. Severe fouling is usually associated with poor water quality, but may be due to reduced preening which occurs when animals are diseased. In a research project during April until July 2005 different stages and ages of shrimps in hatcheries and farms in Bushehr province in south area of Iran evaluated for shrimp fouling disease. Samples collected from hatcheries (11 hatcheries in Bushehr province) and grow out ponds of Bushehr sites (6 sites of shrimp farms) in south area of Iran. Samples from hatcheries was include spawners, eggs, larvae and post larvae and in farms in 30, 60, 90 and 110 days after stocking, preopods, pleopods and gills of samples screened for fouling disease, number of samples in spawner was 30 and in others was 100. Results showed, in hatcheries 70% of spawner with 2-4 severity showed fouling disease include *Epistylus*, *Zoothamnium*, and *Vorticella*, in eggs we didn't see any protozoan and larvae stages 30% and post larvae had 28.2% of infection. In grow out ponds shrimps that was in 60 days age had further infection (78% with severity 1-3) compare with other groups (30 days= 52%, 90 days=64%, 110 days=43%), but didn't have any mortality in each groups. Fouling disease itself is of little consequence to the health of the shrimp unless it is so severe that it causes improper gill functioning, and it can be managed by water exchange.

457 **Parasitic fauna of *Liza* spp (mugilidae) in Southern Caspian sea and freshwaters of Iran**

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The members of the family Mugilidae which are extremely euryhaline are almost marine or brackish water but less frequently freshwater fishes. Three species of this genus namely *Liza salianes*, *Liza auratus* and *Liza cephalus*, were introduced from Black sea to the Caspian Sea from 1930 – 1934. In southern part of Caspian sea they became well acclimatized, and they are at present of great economic importance. Another endemic *Mugilid species*, called *Lisa abu* is semi anadromous fish which inhabits in freshwater and still waters in Tigris region of Iran. Mugilid fishes in Iran are parasitized by various parasites belonging to different genera including *Ligophorus* sp1 and sp2 and *Microcotyle* spp in the gills, *Saccocoelium obesum*, *Caryophyllaeus fimbericeps* in the intestines and *Contracaecum spiculigerum* in body cavity, and several protozoa also can infect captured fishes in pond fisheries. In the present paper parasitic fauna of Mugilid fishes in Iran are presented and the specificity of known species are discussed. Some spp also are in the procedure of species determination.

458 **Parasites of cultured trouts in Iran**

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Trout is one of the most popular and important fishes in Iran aquaculture industry. The parasitic fauna of 200 specimens of Rainbow trouts (*Oncorhynchus mykiss*) with various sizes was investigated. Specimens were collected from trout farms located in different areas including Kelardasht, Chalus and Urumia (Caspian basin), Yasuj (Tigris basin) and Chahnimeh lake (Sistan basin). Totally nine parasite species were found and identified to genus or species level. Rainbow trout specimens were infected by two *Trichodina* spp on fins, skin and gills, *Chilodonella* sp on skin, *Dermocystidium salmonis* in gills, *Lernae* cyprinicea on the skin and fins and *Diplostomum spathaceum* in lens of eyes. Other parasites that were existed in this species of trout included *Ichthyophthirius multifiliis*, *Ichtyobodo necator* and *Gyrodactylus derjavini*. The parasites fauna and their prevalence in trouts studied with regard to the fish size, season, water current and finally the size of habitats. However among them, *Gyrodactylus derjavini* and *Ichthyophthirius multifiliis* were found to be the most dangerous parasites and they cause much problems in trout production industry of Iran. The goal of present study is to elucidate faunatic data about parasites of trouts in Iran.

459 ***Giardia duodenalis* in clams (*Ruditapes philippinarum*) and mussels (*Mytilus galloprovincialis*) from Northern Adriatic Sea**

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Several viruses, bacteria and protozoa have been reported in the Mediterranean bivalve mussels, which are known to filter large amount of water and particles.

M&M – In winter 2005/06 and summer 2006, 17 pools of 30 molluscs each (270 *Ruditapes philippinarum* clams and 240 *Mytilus galloprovincialis* mussels) were collected in Northern Adriatic sea (Po river delta and Venice Lagoon). Five and twelve pools were sampled before and after purification process, respectively. *Giardia* cysts were searched in hemolymph by IF (Merifluor kit, Meridian Diagnostic, Cincinnati, OH, USA) and in digestive glands by a nested-PCR technique for the *Giardia* 16S-rRNA gene according to Hopkins et al. (1997) and Read et al. (2002). For both mollusc species expected infection rate was calculated.

Results – The IF revealed 3 positive pools (2 clams and 1 mussel) all before purification process from the Po river delta. The number of cysts detected was very low (range=0.5-1 cyst/ml hemolymph). The PCR confirmed the positivity of these 3 pools and 2 additional ones were found. One *Giardia* isolate was identified as *G. duodenalis* by sequencing. The expected infection rate was 1.15% for total molluscs, 1.34% for clams and 0.95% for mussels.

Conclusion – Only molluscs before purification process were found positive, while clams and mussels properly purified were not. This could be due either to the purification activity or to the different origin of the molluscs. Considering that *Giardia* genotypes A and B can be zoonotic, cooking bivalve molluscs before eating is strongly recommended.

460 **Gastrointestinal parasites in phocid and otarid populations from South Shetland Islands and Antarctic Peninsula**

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Marine mammals are regarded as good bioindicators of environmental changes. However, little is known about the health status of the Antarctic marine mammals. The investigation of the presence of gastrointestinal parasites in Antarctic pinniped populations is therefore of high interest. On the one hand, some of these parasites are zoonotic and include man amongst their hosts (e.g. *Anisakis spp.*, *Cryptosporidium spp.*, *Giardia spp.*). On the other hand, the determination of their presence and frequency would enable the assessment of the health status of these animal populations as well as to evaluate the impact of the increasing human activity in the Antarctic continent.

In this study, we investigated the presence of gastrointestinal parasites in faecal samples of phocids (*Leptonychotes weddelli*, *Lobodon carcinophagus*, *Hydrurga leptonyx*, and *Mirounga leonina*), and otarids (*Arctocephalus gazella*) from Deception Island, and other areas in the South Shetland Islands and Antarctic Peninsula. Preliminary results show that these animals present high infection rates by gastrointestinal parasites. Comparatively, the prevalence is higher in the Weddell seal (90%) and the southern elephant seal (78%) than in the Antarctic fur seal (36%). No differences were found between the Antarctic fur seal populations from Deception Island and those from other zones. With respect to the groups of parasites, nematodes were detected in a high percentage (89%-94%) of all marine mammal species analysed, whereas amongst protozoan parasites *Isospora* and *Cryptosporidium* oocysts were only found in a Weddell seal and a southern elephant seal, respectively. Cestode eggs were detected in 52% of Weddell seals and in two samples of leopard seal analysed; in 15% of southern elephant seals and 5% of Antarctic fur seals. Efforts are currently being made to identify isolates to a species level and the results will be presented in the congress.

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461 **Host specificity and host range in *Dactylogyridae* (Monogenea) parasites of freshwater fishes of Iran**

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Among 225 known parasites species in freshwater fishes of Iran, more than 110 species belongs to *Monogenea*, among them, however member of *Dactylogyridae* family comprised more than 80 known species. In spite of *Dactylogyrus spp* from European and East African fishes which show very strict host specificity and most of them infect only one fish species, *Dactylogyrus* species found on Iranian cyprinids exhibit less pronounced host specificity and infect some closely related fish species. For instance *D. pulcher* and *D. lenkorani* which are typical Ponto-Caspian species can be found in several fish hosts. *D. pulcher* observed in *Capoeta capoeta*, *C. trutta*, *C. damascina*, *Barbus lacerta*, *Aspius vorax* and *Albornoides bipunctatus* (Totally 6 fishes belong to 4 genera and 6 species) and *D. lenkorani* is found in *C. capoeta*, *C. damascina*, *C. trutta* and *C. aculeata*. Although old regions such as Tigris contains numerous endemic fish species (at least 54 fish species and subspecies) and some of those in turn harbor specific endemic parasites, which *Dactylogyrus holciky* specific to *Chondrostoma regium* is a good example for such situation. The present paper is aimed to provide a comprehensive review of the host specificity and host ranges of *Dactylogyridae* in both native and exotic fish species of freshwaters of Iran and presents a phylogenetic relationship among host and parasites in Iran.

462 **Parasites of fishes in Choghakhor Lake, Iran**

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Twenty ecto-and endo- parasites were found in different organs of 8 native and exotic fish species in Choghakhor lagoon situated in the west of Iran. Overall 130 fish specimens belonging to *Cyprinidae*, *Cyprinodontidae* and *Poeciliidae* families were examined during the years 2005 and 2006. Of them, 4 protozoans including *Ichthyophthirius multifiliis*, *Trichodina* sp and two unknown Cysts were recorded and two Myxosporean parasites including *Myxobolus musayevi* and *Myxidium pfeifferi* were found. Of the metazoan parasites 6 Monogenean species, mostly belonging to the *Dactylogyridae*, *Gyrodactylidae* families were found and identified to species and genus level. The number of helminthes reached 15 by adding 3 Digenean metacercaria: *Diplostomum spathaceum*, *Tylodelphys clavata* and *Ornithodiplostomum* sp and 3 Cestoda species including *Bothriocephalus gowkongensis*, *Kawia* sp and a pseudophyllidean cestode. Two Crustacean species (*Argulus foliaceus* and copepodid stage of *Lerne cyprinacea*) were also found in native and non native fishes. Among the parasites above *D. spiralis*, *T. clavata*, *Ornithodiplostomum* sp are reported for the first time in Iran. Seasonal prevalence, Host and organ specificity of parasites are discussed.

463 **Parasitic fauna of *Capoeta damascina* (valenciennes, 1842) in Beheshtabad river, Iran**

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Twelve ecto-and endo- parasites were found in different organs of one of native fish species of Beheshtabad river situated in the west of Iran. Overall 90 fish specimens were examined during the year 2006. Of them, 2 protozoans including *Ichthyophthirius multifiliis*, *Trichodina* sp were recorded and one Myxosporean parasites *Myxobolus musayevi* was found. Of the metazoan parasites 4 Monogenean species, mostly belonging to the *Dactylogyridae*, *Gyrodactylidae* families were found and identified to species and genus level (*Dactylogyrus lenkorani*, *D. pulcher*, *Dactylogyrus* sp and *Gyrodactylus* sp). The number of helminthes reached 8 by adding 2 Digenean parasite: *Allocreadium isoporum* and *Allocreadium pseudaspaii*, and 1 Cestoda and 1 nematode species *Bothriocephalus gowkongensis* and *Rhabdocona* sp. One Crustacean species (*Lamproglana chinensis*) species was also found in fishes.

Seasonal prevalence, Host and organ specificity of parasites are discussed.

464 **Characterization and Sequencing of ITS-1 gene of *Tylodelphys clavata* (von Nordman 1832)**

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Parasites morphologically consistent with *Tylodelphys clavata* collected from the vitreous humor of *Chondrostoma regium* one of native fish species inhabits in Choghakhor lagoon at the west of Iran. Samples examined by PCR of the ITS-1 region using 1 pair F45 and R4 Primers. The ITS region is now the most widely sequenced DNA region in most of the organisms. It has typically been most useful for molecular systematics at the species level, and even within species. The expected amplification band (560-bp) was observed on agarose gel. To assess accuracy of result and also to confirm obtained data, the amplified sample were cloned and then sequenced. The blast result confirmed that all amplified product are corresponding to *Tylodelphys clavata* without any pseudo data could be generated by false priming/amplification or carry over contamination. It should be noted that the homologues sequences were found by blast search were not the same those previously had deposited in GenBank by Anandan and colleagues that might indicates the heterogeneity of studied gene among different variants of this parasite in Iran.

465 ***Cryptosporidium* in oysters sold in markets and commercial stores in Mexico-city**

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Introduction: The oocysts of *Cryptosporidium parvum* a zoonotic water borne can be removed by the bivalbe mollusc from contaminated water and retain in gills and hemolimpha.

Methods: Aleatory study done between July 2005 and January 2007 in 355 specimens of *Crassostrea virginica* from 71 markets and commercial stores from Mexico-City.

Slides were examined microscopically under direct light and the molecular structures were characterized. Ten percent of the samples(n=35) were analyzed by PCR.

Results: Of the total number of samples(n=355), 44.3% were positive for *Cryptosporidium-sp.* oocysts in *C.-virginica*. From the control group (*C.-parvum*) 825 pairs-of-bases were obtained, whereas from the oyster samples, 500pb were obtained, 650pb and 825pb.

The control (*C.-parvum*) gave rise to a band of 825pb. The sample from track 7 exhibited a band of 825pb. Samples from tracks 3, 5 and 6 gave origin to 500pb amplicones and the one from track 10 originated a segment of 650pb.

Conclusion: There was a high incidence of *Cryptosporidium* oocysts. Oysters are able to concentrate resistant parasitic organisms from water contaminated with fecal matter. The specimens studied contained *C.-parvum* since with the use of specific primer one sample amplified a segment of 825pb, similar to the control.

Segments of lower molecular weight obtained from the remaining samples permit to speculate that oysters are contaminated by other organisms of the apicomplexa Phylum which may belong to the class *Cryptosporidium* or to closely related classes from the phylogenetic point of view.

466 **Helminths and copepods parasites of skip jack tuna *katsuwonus pelamis* (Pisces, Scombridae) disembarked at the harbour of Abidjan (Atlantic Coast of Ivory Coast)**

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The following helminths parasites of Skip jack tuna (Linnaeus, 1758) from the harbour of Abidjan in Ivory Coast, are reported in this work: Monogenea: *Allopoecylus macrova* (Unnithan, 1957); Digenea: *Didymocystis coatesi* (Ariola, 1902), Cestoda: *Tentacularea coryphaenae* (Bosc, 1797), Nematoda: *Anisakis* sp. (Dujardin, 1845), Acanthocephala: *Rhadinorhynchus katsuwonis* (Harada, 1928), Copepoda: *Caligus pelamidys* (Kroyer, 1863). Trematodes were the majority of the parasites specimens collected (72,41%). As endoparasites, *Anisakis* sp. and *Tentacularea coryphaenae* were the dominant species with highest prevalence (100%).

Poster group 10: Parasites of Poultry and Swine

467 **Prevalence of ecto and endoparasites in pregnant and weaning sows under four management systems**

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Porcine gastrointestinal parasitism and ectoparasitism are limiting the pigs growing up in countries worldwide. They increase production cost due control programs practice and visceral confiscate at slaughterhouse; those problems are larger in extensive and outdoor management systems. In order to obtain prevalence values of ectoparasites and endoparasites (*Ascaris suum*, coccidias and strongyles) in pregnant and weaning sows under four management systems, was carried out the present study. A number of 64 line Camborough 22 sows had similar age and weight, were randomly set out in four management systems (I: cage; II: farmyard, III: deep bedding, IV: outdoor); Twenty (five of each system) feces samples were taken (through rectum) monthly during six months, they were studied by the Mc Master technique. To ectoparasites diagnose, all animals in each system were observed and parasitic specimens collection were carried to further morphological identification. Endoparasites infections media prevalence values by system respectively were: *A. suum*: system I= 0%; II= 4%; III= 8.3%; IV= 0%; coccidias: I= 20.8%; II= 40%; III= 41.66%; IV=54.16%. Only strongyles infections in the system (I= 4.16%) were observed. Were statistical differences among coccidia infections by system ($P<0.05$); those systems with more humidity, shown higher prevalence. The ectoparasites observed were: *Cochliomyia hominivorax* (Myiasis), *Musca domestica* and *Tabanidae*. Were differences among myiasis prevalence by system only ($P<0.05$), may be due climatic conditions and piglets births time.

468 **Distribution of *Ascaris suum* genotypes within the pig host is affected by pig and location in the small intestine**

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We investigated the effect of parasite genetics on establishment and distribution of *Ascaris suum* in pigs. We used PCR-RFLP to discriminate between mtDNA from 4 *Ascaris* females (Types A, B, C, and D) collected at a Danish abattoir. We inoculated 26 pigs with 500 eggs from each female and 6, 6, and 14 pigs were slaughtered at days 14 (before expulsion), 17 (during expulsion), and 28 pi (after expulsion), respectively. At slaughter the small intestine was divided into 8 sections of similar length and the larvae were isolated. Six (100%), 5 (80%) and 3 (21%) pigs were positive for *Ascaris* at the 3 days of slaughter, respectively. The numbers of larvae genotyped from each pig at each of the three days ranged from 245-412, 109-655 and 18-569, with totals of 1908, 1616 and 675, respectively.

We used logistic regression to analyse the effect of parental mtDNA genotype on the distribution of their offspring (i.e. A, B, C or D) within sections of the small intestine and between pigs. The relative numbers of larval genotypes was strongly affected by pig and section ($P < 0.0001$) at days 14 and 28 pi. and by pig at day 17 pi. ($P < 0.0001$). Furthermore, there was a significant difference in representation of the four larval types at day 14 pi. compared with 28 pi. ($P=0.046$).

In conclusion, we found evidence for selection of worms carrying specific mtDNA genotypes after (day 28 pi.) compared with before expulsion (day 14 pi.). Furthermore, the relative numbers of worms bearing the four mtDNA genotypes was uneven between individual pigs providing evidence that interactions between host and parasite play a role in infection dynamics.

469 **Effect of *Ascaris suum* infection on performance of fattening pigs**

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Production losses due to roundworms are rarely investigated in commercial pig farms. Therefore, the aim of our study was to compare performance in fatteners with and without *Ascaris suum* infections. Two Danish farms producing fatteners and showing moderate *Ascaris* prevalence were selected for the study. In each farm, pens with fatteners were randomly assigned to either treatment with Flubenol®, Janssen Ani-mal Health, or a placebo. Pigs were treated by administering Flubenol® or placebo mixed in the feed for 5 consecutive days (5 mg/kg bodyweight) immediately after introduction to the finishing unit and 5 weeks later. Fecal egg excretion, liver white spots at slaughter, average daily gain, feed conversion and lean meat percentage were recorded per pen. *Ascaris* egg excretion was detected in none of the pens where pigs were treated with Flubenol® and in 18.2% of pens in the placebo group. The prevalence of white spots was higher in the treated pigs compared to placebo pigs. No significant differences were present in average daily gain, feed conversion or lean meat percentage between the Flubenol® and the placebo group. Considerable batch-to-batch variation was observed, and a possible negative influence of other disease (diar-rhea) on performance could not be excluded. Treatment with Flubenol® twice during fattening prevented *Ascaris* egg excretion but did not reduce the occurrence of liver white spots, most likely due to lack of immunity at the end of the fattening period. To improve performance significantly, deworming should be carried out every 5 weeks and maintained for several rounds.

470 **Effect of Solubenol® on *Ascaris suum* infections in fattening pigs**

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The efficacy of water portable flubendazole (Solubenol®, Janssen Animal Health) against *Ascaris suum* infections in fattening pigs was investigated in a commercial pig herd. A Danish fattening pig farm with high *A. suum* prevalence (white spot prevalence >60%) was selected for the study. In two fattening units of approximately 400 fatteners each, pens were randomly assigned to either treatment with flubendazole or a placebo. Pigs were treated by administering anthelmintic or placebo diluted in the drinking water for 5 consecutive days (5 mg/kg bodyweight). All pigs were treated three times during the fattening period with 5-week intervals, starting on the first day after introduction to the finishing unit. The study comprised four consecutive fattening rounds in both units. Fecal egg excretion, liver lesion scores (white spots) at slaughter, average daily gain, feed conversion and lean meat percentage were recorded per pen. The preliminary results show that treatment with Solubenol® reduced the prevalence of *Ascaris* egg excretion to 7.1%, compared to 33.3% in the placebo group. The prevalence of white spots was lower in pigs treated with Solubenol® compared to pigs treated with placebo, but only declined significantly (>50%) in the last fattening round. Performance data on average daily gain, feed conversion and lean meat percentage are not yet complete but will be presented. This demonstrates that strategic deworming needs implementation over several production rounds to significantly reduce infection pressure. Strategic treatment with Solubenol® over four rounds reduced *Ascaris* egg excretion and white spot prevalence in fattening pigs.

471 **The effect of *Ascaris suum* infection on the protective response to vaccination against *Mycoplasma hyopneumoniae* in pigs**

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Since their first introduction more than a century ago, vaccines have become one of the most cost-effective tools available to prevent and manage infectious diseases in human and animal populations. It is vital to understand mechanisms that may impair optimal vaccine efficacy. The aim of the study was to investigate the bystander effect of concurrent *A. suum* infection on the protective immune response of a *Mycoplasma hyopneumoniae* (Mh) vaccine against a subsequent challenge infection. Pigs were trickle infected with 25 *A. suum* eggs/kg/day twice per week throughout the study, vaccinated against Mh, and inoculated with Mh four weeks after vaccination. All pigs without worms (group 2) seroconverted 3 weeks after vaccination (100%), as opposed to only 33% of vaccinated pigs with *A. suum* infection (group 4). Only 78% of the latter pigs seroconverted during the study whereas seroconversion remained 100% in the parasite free, vaccinated pigs. Vaccinated pigs with *A. suum* had a higher mean percentage of lung pathology and variation was significantly higher in these pigs compared to vaccinated pigs without worms. Our present study indicates that *A. suum* is able to significantly compromise the effect of an Mh vaccine. The impact of reduced vaccine efficacy caused by a common gastrointestinal helminth emphasises the importance of parasite control because of their immune modulating potential. More focus should be put into this area of research to outline mechanisms, and thus to be able to predict possible interactions.

472 **Parasite specific IL-4 responses in *Ascaris suum* and *Trichuris suis* infected pigs evaluated by ELISPOT**

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In many experimental settings it is useful to be able to measure changes in specifically induced cytokines at post-mRNA level over time. Specific measurement of IL-4 in particular is important for studies on nematodes due to its key function in driving the TH2 response. The objective of the present study was to develop an ELISPOT method to measure parasite specific IL-4 producing cells during experimental *Ascaris suum* and *Trichuris suis* infections in pigs. Two separate experiments were carried out, with *A. suum* and *T. suis* trickle infections, respectively. After parasite exposure, we were able to measure statistically significant increases in specific IL-4 production as well as blood eosinophils over time. Egg excretion and worm burdens at necropsy were measured. This method enables repeated and parasite specific measurement of IL-4 at protein level, which is a valuable tool for future experimental settings.

473 **Prevalence of taeniasis swine in suburban localities of Ludhiana, India and its association with risk factors**

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Introduction: Taeniasis swine i.e. infection with *Taenia solium* tapeworm is of great public health and economic relevance because of its potential to cause neurocysticercosis (NCC). Prevalence of *Taenia solium* is associated with certain food habits such as eating raw or undercooked pork and poor socio-economic conditions. Ingestion of ova shed by tapeworm carrier can result in human cysticercosis. Present work was contemplated to determine the prevalence of taeniasis swine in the areas under study and its association with various risk factors.

Method: A total of 127 human stool samples from persons residing in sub urban localities of Ludhiana were collected. Stool samples were examined by sedimentation and floatation techniques.

Material (s): Saturated salt solution, Human stool samples

Results: About 11 persons were found positive for presence of *Taenia spp* indicating 8.67 per cent prevalence rate. Prevalence rate of taeniasis swine and other gut parasites was 2.36 and 50.39 per cent, respectively. Infection was more in undercooked or raw pork consumers than consumers of properly cooked pork. More percentage of males were found positive than females. Prevalence of swine cysticercosis was 6.36 per cent (n=236) in pigs of the same localities and infection rate was more in pigs reared under extensive system of management as they had more exposure to human faeces.

Conclusion: Prevalence rate of taeniasis swine was observed to be high and it reflects the risk of neurocysticercosis in the city. Therefore, proper control strategies should be initiated to check the occurrence of taeniasis swine and swine cysticercosis.

474 **The Glutathione-S-transferase of *Oesophagostomum dentatum*: isolation, characterisation and possible biological function**

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Glutathione-S-transferases (GSTs) are multifunctional enzymes of eukaryotic cells with various housekeeping functions including detoxification of xenobiotics, drug metabolism and protection against oxidation of fatty acids. In nematodes predominantly GSTs of the sigma-class have been identified, and they are often regarded as potential vaccine targets due to their ubiquitous nature and their structural and biochemical differences to mammalian GSTs. However, their specific functions for nematodes are still unknown. They are possibly involved in the metabolism of bioactive lipids (eicosanoids) produced by nematodes which are frequently secreted and regulate basic biological functions in these organisms. GST activity was demonstrated in the cytosolic fraction of *Oesophagostomum dentatum*, the nodular worm of pigs. Two proteins (23 and 25 kDa) were isolated via glutathione affinity chromatography and sequenced and showed a high similarity to putative *Caenorhabditis elegans* GSTs. The isoelectric points were pH 4.5 (23 kDa) and 5.8 (25 kDa), respectively. All developmental stages investigated (infectious sheathed third-stage larvae, early and late parasitic larvae, adult males and females) show GST activity that could be inhibited by sulphobromophthaleine (SBP) in concentrations of 150 μ M or higher. Larval development was also inhibited by SBP in vitro. Further studies will examine the effect of GST inhibitors on worm viability and the possible functions mediated by these enzymes.

475 **Effects of parasitism due to *Metastrongylus* sp. in wild boar (*Sus scrofa*)**

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A study was conducted to evaluate the effects of *Metastrongylus* sp. infestation in Wild boars. Apart information on : sex, age (class I,II ,III respectively <1, 1-2 and >2 years old), weights of carcass (W)and lungs (w), evaluation of parasitism (identification and P =weight of parasites)and samples of 1 carpus and 1 tarsus (to evaluate size by measuring T= length of talus) were performed. The effects of parasitism were assessed on 107 wild boars in relation with ratio w/W (normally 1% or slightly above) and T. Little effect on W is noticed in class I even when P > 1g. In Class II, 8 from 11 animals have moderate parasitism (P < 0,7g) ; a defect in W is however noticed in one (P =1.3g). In Class III the infection is limited (P < 0.4g). In Class I an increase of w with moderate P is an indicator of high inflammatory reaction, The ratio w/W varies from 1.5 to 2.5% in class I and is close to 1% in class II. Low values of T (< 34 mm) are noticed only (but not systematically) in animals with P > 0.5g. This relationship is also present in class II. W and w do not vary simultaneously suggesting 3 phases in the infection: A) recent infestation with inflammatory response resulting in increase of w without effect on W ; B) controlled infestation (normalisation of p) with first signs of thinning (W) or effects on growth (T); C) latent infestation, eventually with decrease of w (lesions) but major effects on W.

476 **Epidemiological study of *Dermanyssus gallinae* infestation in egg laying flocks of Mashhad area, Iran**

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An epidemiological study was done from 2002 to 2003, in order to determination of prevalence of *D.gallinae* infestation in egg laying flocks in Mashhad area. Twenty-four flocks were randomly selected and has been sampled one month interval for three times. First, every houses of farm were carefully inspected about presence of absence mite infestation and then dust samples were collected in everywhere of houses, simultaneously; ten blood samples were collected for measurement of packed cell volume (PCV) in hens in each house .In this study, *D.gallinae* infestation was detected in eleven farms (43.45%). The PCV of hens in infested farms were below the normal range ($p<0.05$). This infestation was caused to decrease egg production with a rate of 17% to 25%. In this study , some variable related to hygiene management in infested and non- infested farms were compared and analyzed ,the results of this study were shown that the hygiene management was poor in infested farms ($p<0.05$). In based on the high prevalence of *Dermanyssus* infestation in egg laying farms in Mashhad area, it needs to do a control program for this mite in the infested farms.

477 **Identification and localisation of tropomyosin and paramyosin in *Dermanyssus gallinae* (De Geer)**

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Dermanyssus gallinae, the poultry red mite is a blood-feeding parasite that infests many bird species. From an economic point of view the most important host of the mite is poultry, in particular pullets and to lesser degree broilers. Mite homologues of protein antigens discovered in ticks and other mites could be used to generate an immune response against poultry red mite. Homologues of two powerful allergens paramyosin and tropomyosin characterised in the house dust mite *Dermatophagoides pteronyssinus*, have been investigated in the poultry red mite. Western blots of PAGE gels of the soluble fraction of whole mite protein extract from *D. gallinae* were prepared and probed with antibodies raised in mice against both proteins. Bands were present at 100 kDa for paramyosin and at 38 kDa for tropomyosin. Immunolocalisation of paramyosin and tropomyosin in sections of mites showed their ubiquitous distribution throughout the mite.

478 **In vitro effect of some synthetic acaricides and plant extracts against *Dermanyssus gallinae***

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The poultry red mite is an important haematophagous ectoparasite of domestic birds, with high occurrence in layer farms and free-range systems. The study was performed during June 2005 - September 2006, on *Dermanyssus gallinae* populations, collected from layer farms and free-range systems, from 5 localities in Transylvania area, Romania. We have tested the comparative in vitro acaricidal activity of 3 synthetic acaricides (amitraz - 0,25; 0,5; 1; deltamethrin 0,5; 1; 2 diazinon - 0,5; 1; 2), and 11 plant extracts (savory, basil, coriander, fir, pine, cade, peppermint, eucalyptus, cedarwood, lemon, lavender) 10%; 20%; 30%, using direct contact method. The best efficacy against *Dermanyssus gallinae*, collected from both breeding systems, was obtained with amitraz, and generally speaking the synthetic acaricides had better effect against the ticks collected from extensive systems. The tests performed on 2 layer farms has revealed a chemical resistance to pyrethroids. No resistance was observed on the mites collected from extensive systems. Some plant extracts (peppermint, basil, savory, coriander) were found very effective against the poultry red mite.

479 **Persistence and efficacy of traps impregnated with phoxime for the control of *Dermanyssus gallinae***

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The poultry red mite, *Dermanyssus gallinae*, is an important emerging parasite problem in layer operations in Sweden. The current lack of effective measures to control the mite causes reoccurring mite problems in poultry facilities once afflicted by this blood-feeding parasite which result in production losses due to irritation, anaemia and may even cause the death of its host. As the parasite persists in the poultry house between flock cycles it may also act as reservoir host of pathogens for the replacement pullets.

The study was performed in five different egg-producing operations during flock cycle, ranging between 64 – 82 weeks of production. Cardboard traps (1000x40x3 and 200x70x3 mm) containing 1% phoxime were placed adjacent the nests where mites gathered, out of reach for the hens, in three floor systems and two enriched cage systems for layers.

The results of the study showed that 99% of the mite populations were reduced after 6 weeks in floor systems harbouring already well-established populations of mites by a single application of impregnated traps, measuring 1000x40x3 mm. Control levels ranging between 96-99% were then maintained by replacing all phoxime-impregnated traps every 4th or 7th week throughout the production period. In the enriched cages, 96% of the mites were reduced within 3 weeks and control levels between 93-96% were then maintained when traps were replaced every 4th or 7th week. Moreover, when directing the control measures, i.e. replacing traps or refill traps with phoxime where mite gatherings were detected only, the parasite was controlled for 21 weeks.

480 **Evaluation of methods of control of poultry red mite infestations in two caged layer houses in the United Kingdom**

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Dermanyssus gallinae has been associated with a number of blood borne pathogens of poultry e.g. *Salmonella* spp., *Campylobacter* spp, *Pastuerella* spp., Newcastle disease virus, chicken pox virus and *Erysipelothrix* spp. The full economic cost of these mite infestations including associated diseases is probably highly understated. In the absence of a totally effective product licensed for control of *D. gallinae* infestations, an integrated management program should be adopted. Such a program may include measures of ensuring effective biosecurity, methods of achieving constant surveillance and prompt treatment of infestations when mites are evident.

Glass vials of mites at sentinel locations in two caged layer houses were used to evaluate two methods of treating red mite infestations. Heat and fumigation of the premises at turnaround were assessed. Mite mortality was assessed after each treatment. Heat treatment was a target temperature of 45°C (for more than four hours), achieved using electrical heaters. Fumigation was by spraying with 10% formalin for a minimum of 6 hours. Glass Pasteur pipettes were modified to act as vials for a reference isolate of *D. gallinae*.

Corrected mean percent mite mortality data demonstrated fumigation to be less effective than dry heat but effectiveness was improved when combined with dry heat. The data demonstrated the combined treatment regime to be an effective method of treatment of poultry red mite infestations in the battery houses studied. This regime could improve biosecurity against mite infestations and associated infections such as Salmonellosis.

481 **Investigating the residual toxicity of lavender essential oils as acaricides against the poultry red mite (*Dermanyssus gallinae*)**

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The poultry red mite, *Dermanyssus gallinae*, is a serious pest of poultry throughout the world. Managing this pest by conventional means (i.e. synthetic acaricides) has become increasingly problematic.

As a possible alternative to synthetic acaricides, research in the UK and Korea has identified several plant essential oils that are toxic to *D. gallinae*, with this toxicity expressed in the vapour phase. This could potentially make these essential oils particularly effective as acaricides for *D. gallinae*, which spend large amounts of time concealed in the cracks and crevices of poultry housing, where conventional acaricides may not penetrate. However, essential oils are highly volatile. Therefore any acaricidal effect they exert could be short-lived in practice.

The aim of this experiment was to investigate the residual toxicity of six different lavender essential oils to *D. gallinae*. In sealed Petri-dishes, mites were exposed to filter papers impregnated with essential oil at a concentration of 0.14mg/cm³. Having corrected for control mortality, when filter papers were used immediately after impregnation, 66% to 90% *D. gallinae* mortality was observed after 24 hours, depending upon the essential oil used. If impregnated filter papers were left in a fume cupboard for 24 hours prior to use, mortality rates fell to 11% or less for the same essential oils.

The results suggest that whilst certain essential oils may be effective as acaricides for *D. gallinae*, their residual toxicity is likely to be short-lived. Further work is investigating the LD50 doses and the repellent effects of some of these essential oils.

482 **Temporal synergism controls highly resistant poultry red mite**

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The poultry red mite (*Dermanyssus gallinae*) is one of the most important ectoparasites affecting broiler and egg layers in all types of production system across the UK and Europe. Management regimes continue to rely heavily on the use of chemical control, such as the synthetic pyrethroids. These pesticides have become increasingly less effective at controlling the red mites in recent years due to the development of resistance; in some cases no available pesticides confer effective control (Walker, A., 2001).

Pesticide resistance is often due to the enhancement of metabolic enzyme systems within the pest, particularly non-specific esterases and microsomal oxidases. If synergists are allowed sufficient time to fully inhibit these enzymes (temporal synergism) then the sensitivity of insect pests to pesticides can be increased by several orders of magnitude (Moores et al., 2005; Young et al., 2005; 2006).

A 42-fold increase in the level of susceptibility was found when a 12 hour pre-treatment of 100ppm PBO was applied to a standard laboratory susceptible strain of chicken red mites and a 750-fold enhancement was found with resistant field strains under the same treatment.

This demonstrates the exciting potential temporal synergism could have in the area of ectoparasite control.

483 **Leucocytozoon infection in poultry in Vietnam**

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Among 159 chicken received for necropsy during the period of 2004 - 2006, 67 were found to be infected with *Leucocytozoon*; one kind of blood parasite; in various areas of the carcass. *Leucocytozoon* appeared as multifocal dark dots in muscle and visceral organs of chicken.

Microscopic lesions characterized by numerous megaloschizonts, one developmental stage of parasite. Size of megaloschizonts ranged from 35x40 to 100-500 µm. Megaloschizonts were commonly found in muscle, fat, lung, kidney and liver (> 80%). The general morphology of megaloschizonts in these cases corresponds closely to the description for the species *Leucocytozoon caulleryi* Mathis and Leger 1909. Size and shape of megaloschizonts vary according to the stage of development and to the kind of the parasitized organ. When being parasitized by *Leucocytozoon*, the organs either kept normal condition or had pathological changes depending on the size of megaloschizonts and/or on the type of organ. The most common lesions were found to be hemorrhage, atrophy of tissues, necrosis and inflammatory reaction. Other developmental stages of *Leucocytozoon* have not been found. Severe affection by *Leucocytozoon* resulted in deaths of broilers and dropping egg yield in hens. This is the first paper relating to the morphological and pathological characteristics of *Leucocytozoon* infection of poultry in Vietnam.

484 **Prevalence and pathology of parasitic gastrointestinal infections of poultry in Punjab**

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The present study deals with the pattern of infection and pathology of different gastrointestinal parasites from 27,285 birds in different age groups necropsied at the poultry disease diagnostic laboratory, Guru Angad Dev Veterinary and Animal Sciences University, (GADVASU) Ludhiana, India, received from various private and government owned poultry farms of Punjab state and adjoining areas during the period from January, 2001 to December 2004. A total of 137 outbreaks of parasitic infections were recorded. It was notable to record that all outbreaks were seen in broiler chicks raised mainly in deep litter system. The incidence of coccidiosis was maximum (100/137), out of which maximum outbreaks were recorded in the month of January (26/100) followed by March, February, September and April, respectively. The highest prevalence (46.0 %) was recorded in young birds of 1-6 weeks, followed by 6-12 weeks (33.0 %) and least above 12 weeks (21.0 %). The mortality rate was highest in winter (December-January) and spring seasons (February- March), followed by rainy (August- October) and summer (April - July). Incidence of cestodes and nematodes revealed maximum number of outbreaks in summer followed by rainy and spring season, respectively. No trematodes and acanthocephalan infection was reported during the study. Pathology in different infections has been discussed with reference to different gross and histopathological changes observed in different parts of the intestine.

485 **Nematodes natural infection in *Neotropical cormorant*, *Phalacrocorax brasilianus* Gmelin, 1789 (Aves; Phalacrocoracidae) from Rio de Janeiro, Brazil**

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The Neotropical Cormorant, *Phalacrocorax brasilianus* Gmelin, 1789 is a piscivorous bird found in all South America. In Southern Chile, River Cruces Ramsar, was recorded high prevalence of *Contracaecum rudolphii* Hartwich, 1964 in adults Neotropical cormorants. These adults can transmit parasites of their upper digestive tract by regurgitation to chicks in the nest, so adults and chicks share the same nematode community. In 2006 *C. rudolphii* was recorded for the first time in Lago Guaíba, Municipality of Guaíba, RS, Southern Brazil. The aim of this report is amplify the records of *C. rudolphii* in *P. brasilianus* in Brazil. Two Neotropical cormorants, one from Paracambi and other from Maricá. both from Rio de Janeiro State, were found with caquexia and eventually died. During necropsy, many worms were found in proventriculus and ventriculus formed eosinophylic granulomas. Nematodes were collected and examined under light microscopy after clarification with Aman's lactofenol. These nematodes were identified as *Syncuaria squamata* (Linstow, 1883) and *C. rudolphii*. In Neotropical cormorants from Paracambi both species were found and in the host from Lakes Region (Maricá) only *C. rudolphii* with high level of infection (adults and larval forms = 700 worms). This parasite could influence the bird health alone or when associated with other infection causing severe damage or even facility the bird mortality. The high infection in birds should be considered as a potential risk for aquiculture.

486 **The role of preparation technique, culture media and incubation time for embryonation of *Heterakis gallinarum* eggs**

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In a first experiment intact female *H. gallinarum* worms were kept for development of the unembryonated eggs in four different media and incubated under constant temperature (20 – 22°C) for 2, 4, 6 or 8 weeks. Afterwards the body wall of the worms were ruptured and the number of embryonated and unembryonated eggs were determined. Based on that the percentage of embryonated eggs were calculated. After 8 weeks of incubation 27.6 % of the eggs cultivated in 0.5 % formalin developed into third larval stage. For worms cultivated in 0.1 N sulphuric acid, 26.7 % and in 0.1% potassium dichromate culture 29.4 % developed, respectively ($p > 0.05$). In contrast only 18.6 % cultivated in 2 % formalin were embryonated ($p < 0.05$). In a second experiment *H. gallinarum* eggs were harvested directly from worms uteri and cultivated afterwards in different media (2 % formalin, 0.1 N sulphuric acid, 0.1% potassium dichromate) at 20 to 22° C for 6 weeks. Compared with the results of experiment one after 6 weeks of incubation a significantly ($p < 0.05$) higher percentage of eggs kept in 2.0 % formalin and 0.1% potassium dichromate media developed into the third stage larvae, whereas no significant differences were found for 0.1 N sulphuric acid at that time. The results suggest that preparation technique, media and time of incubation has an essential influence on the development rate of *H. gallinarum* eggs.

487 **Bird caecal worm, *Heterakis gallinarum* (Ascarida: Heterakidae): ultrastructural observations**

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Heterakis gallinarum Schrank, 1788 occurs in the caeca of the fowl, guinea-fowl, pea-fowl, turkey, duck, goose and numerous other birds. The direct effects of *H. gallinarum* are slight and only in heavy infection may there be thickening of the caecal mucosa with a number of petechial haemorrhages on the surface. The principal economic importance of *H. gallinarum* lies in its role as a carrier of *Histomonas meleagridis*, the causal agent of blackhead or entero-hepatitis of turkeys. The life cycle is direct and when the host swallows an infective egg the infection takes place. Earth worms may play transport host role. Scanning electron microscopy (SEM) was used to study the morphological aspects of adult worm of this nematode in detail. The worms were removed and washed in 2% sodium cacodylate buffer. The nematodes were put into fresh 4% glutaraldehyde for 2 h, post fixed in 2% osmium tetroxide in cacodylate buffer at 4°C for 2 h, and dehydrated through a series of increasing ethanol concentrations. They were then dried with liquid CO₂ in a critical point drier. Each worm was placed on a numbered aluminium stab, which was coated with gold in an ion coating and viewed with a JEOL JSM-840 Scanning Electron Microscope. There were large lateral alae extending some distance down the sides of the body. There were three large lips at the anterior end of both sexes. Each ventral lip had two cephalic papillae, which were absent on dorsal lip. There was one amphidal aperture on both ventral lips. At the anterior end, an excretory pore was observed. In the female, the vulva was located directly behind the middle of the body and the tail carried an anal pore and two phasmids. In the male, the tail had large caudal alae. There were a prominent, circular precloacal sucker, twelve pairs of anal papillae, and two unequal spicules.

488 **Studies on endoparasites of some migratory birds**

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The present study was carried out to determine the prevalence of endoparasites infecting some migratory birds , 228 migratory birds (belonging to 12 species) collected from different localities around Burullus lake in Kafr El - Sheikh governorate Egypt. The overall prevalence of internal parasites in the examined birds was 67.98%. The nematodes showed the highest infection rate (49.5%) , followed by trematodes (43%) , cestodes (11.8%) , enteric protozoa (2.2) and Acanthocephala (0.4%) . The recovered trematodes were *Apharyngostrigea ibis* - , *Nephrostomum ramosum*, *Apatemon gracilis*, *Psilochasmus aglyptorchis*, *Notocotylus pacifera*, *Echinoparyphium recurvatum* , *Echinostoma species*, *Heterophyes equalis* , *Echinochasmus brevivetellus* , *Psilochasmus oxyurus* , *Paracephalagonimus minutum* , *Cyclocoelum microstomum*, *Echinostoma revolutum* and *Bodocotyle* species , while the obtained cestodes were *Diorchis bulbodes* , *Choanotaenia infundibulum* , *Hamatolepis teresoides* , *Monotestilepis* species and *Choanotaenia ola*, the recovered nematodes were *Amidostomum raillieti*, *Synhimantus invaginatus*, *Epomidostomum uncinatum*, *Capillaria anatis* , *Heterakis gallinarum*, *Porrocaecum wui*, *Heterocheilus* species and *Cyrnea dollfusi* , the recovered Acanthocephala was *Centrorhynchus conspectus* and the obtained protozoa was *Isospora* species . The intensities , seasonal variation , morphological description and the role played by migratory birds in the pollution of the environment were completely studied and discussed.

POSTER SESSION 2

Poster group 11: Helminth Infections of Horses

489 The prevalence of intestinal parasites of horses in NSW, Australia

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Gastrointestinal parasites of horses are of veterinary significance in Australia. This study aimed to provide baseline data on the prevalence of internal parasite infection horses in NSW. Surveys on the prevalence and abundance of internal parasites were conducted in different areas in NSW, Australia. From the period of January to April 2006, 18 horses were examined for internal parasites at post-mortem. Parasites were collected from gastrointestinal organs, cleared by lactophenol and identified by using the keys of Lichtenfels 1975 and 1998. A total of 15 of 18 horses were infected with internal parasites (prevalence 83.3 %). The most prevalent parasites were *Gastrophilus intestinalis* larvae (14 horses with prevalence 77.7%) followed by cyathostomins in 13 out of 18 horses (72%). Sixteen species of cyathostominae were recovered and the five most prevalent species were *Cylicocyclus nassatus*, *Coronocyclus coronatum*, *Cyathostomum tetracanthum*, *Cyathostomum catinatum* and *Cylicocyclus ashworthi*, respectively. Other parasites species were also found in these horses as following (prevalence): *Triodontophorus* spp. were present in 4 horses (22.2%); *Strongylus vulgaris*, *Parascaris equorum* and *Habronema muscae* were recorded in two horses (11%); *Strongylus equinus* and *Oxyuris equi* were present in one horse (5.6%); *Anoplocephala perfoliata* were found in 5 horses (27.7%) and *Anoplocephala magna* were recovered from one horse (5.6%). Work will continue over the next 12 months especially through analysis of larval cyathostomins in this horse population.

490 Is *Strongylus vulgaris* still a problem in Sardinian horses?

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Forty-six Sardinian horses (19 female, 27 male), aged from 10 months to 24 years, regularly slaughtered, were examined for strongyle infections. Fecal samples and all the aortic system, including the origin of cranial mesenteric artery and the originating branches, and renal arteries were collected at slaughtering. Forty-two horses (91%) were found positive at fecal examinations (fecal egg counts arithmetical mean 449, range 15-2865). Four point three percent (4.3%) of over 51000 cultured larvae from fecal samples was identified as *Strongylus vulgaris*. Overall, 225 arterial lesions due to *S. vulgaris* larvae were found in all examined animals. One to 13 lesions per horse (arithmetical mean 4.9) were found mainly in the wall of ileocolic artery (28.44%), in their colic branch (16.44%), in the common section of colic arteries (13.78%), right colic artery (9.78%), middle colic and cecal lateral arteries (both 6.22%), jejunal and ileal arteries (5.33%), middle cecal artery (4.89%), cranial mesenteric artery (3.56%), aorta (1.78%), etc. One to 39 *S. vulgaris* fourth and fifth larval stages were found in 18 examined horses (39%). Larvae were found in ileocolic artery (65.8%), in their colic branch (16.1%), in common section of colic arteries (8%), cranial mesenteric artery (4.3%), right colic and middle colic artery (both 2.1%) and caecal lateral artery (1.6%). The results showed that *S. vulgaris* is still present in horses in Sardinia. Although this parasite was found with a quite low frequency (4.3%) within the strongyle population, the parasite was able to cause quite severe lesions in the hosts.

491 **Development and migration of Cyathostominae infective larvae in Coast-cross grass (*Cynodon dactylon*) in a subtropical region of Rio de Janeiro, Brazil**

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The aim of the present research was to evaluate the development and migration of Cyathostominae L3 in coast-cross sward, in three different times a day, in subtropical climate of Baixada Fluminense, RJ, Brazil. Feces were collected direct from the rectum of naturally infected horses and placed on coast-cross grass every month from July 2003 to September 2004. Seven days after, feces and grass samples were collected every week at 8 a.m., 1 and 5 p.m., weighed and processed by Baermann technique. The survival of L3 was higher in dry season 14 and seven weeks to feces and sward respectively, and to rainy season, the survival was nine and three respectively. In the rainy season higher number of infective larvae were recovered of feces (30,100 L3 kg⁻¹dh) when compared with dry season (12,250 L3 kg⁻¹dh), to pasture the L3 recovered was smaller (0,600 L3 kg⁻¹dh, and 0,763 L3 kg⁻¹dh) respectively. The multivariable analysis of main components had shown the influence of time and ambient variables on L3 recovery from feces and grass. The strong relationship was observed among the number of infective larvae on feces and time but in a low proportion to L3 number on sward and time. Rainfall, air and soil temperatures influenced the L3 number recovered. The infective larvae recovered three times a day had no significance by Kruskal-Wallis analysis.

492 **Equine parascariasis under the tropical weather conditions of Ethiopia: a cross-sectional coprological and post-mortem study**

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Parascaris equorum infection has a worldwide distribution and it is of concern for veterinarians and horse owners because of economic losses caused by reduced weight gain, and occasional deaths caused by intestinal obstruction or rupture. Although *P. equorum* has been reported in donkeys from various regions of the world, no studies have been made specifically to determine the epidemiology, the pathogenecity and the immunology of parascarid infection in equids in general and donkeys in particular under tropical weather condition of Ethiopia. The aim of the present study was, therefore, to investigate the epidemiology of this parasite in working equids and in Ethiopia. Further investigation was made whether the prevalence and intensity of infections are age-dependent or not in donkeys.

Materials and methods: A cross-sectional coprological survey in different geographical regions and a retrospective postmortem investigation were conducted to study the epidemiology of *Parascaris equorum* in the equine population of Ethiopia. Faecal samples from 803 working donkeys and 402 horses were collected for *P. equorum* egg recovery. Data on post-mortem worm recovery from 112 donkeys died of different health problems and accidents in the years 1995 to 2004 were also analysed.

Results and conclusions: Coprological study showed 51.1% and 16.2% prevalences of parascaris infection in donkeys and horses, respectively, while at post-mortem examination there was 55% prevalence in donkeys. There was no statistically significant difference between the different age groups of donkeys either in infection prevalence or infection intensity ($P>0.05$). More importantly adult donkeys and horses were found shedding high faecal egg count and adult donkeys harbouring increased worm burden. Of the 55% infected donkeys 43% were above 4.5 years old suggesting, unlike in horses, age-dependent immunity may not develop in donkeys or they may have been immuno-compromised. The findings indicate the need for a thorough experimental study to understand the immune status of working equids in general and donkeys in particular, to parascaris infection under tropical weather condition of Ethiopia.

493 **Acquired cervical scoliosis in 6 horses associated with dorsal grey column chronic myelitis caused by aberrant *Parelaphostrongylus tenuis* migration**

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Introduction: We have described a neurologic syndrome related to an aberrant migration of *Parelaphostrongylus tenuis* in 6 horses. This nematode, endemic in the US, matures in the spinal cord of whitetail deer. Aberrant migrations in other hosts result in devastating neurological signs but had not been described in the horse. The syndrome of cervical scoliosis with mild spastic hemiparesis and ataxia is explained by a novel neuroanatomical mechanism, which we have also found in other species.

M&M and Results: Clinical signs developed over 1-3 weeks, resulting in permanent, pronounced scoliosis. Consistent microscopic findings were a continuous, unilateral, chronic myelitis centered around one dorsal gray column of the cervical and cranial-to-caudal thoracic spinal cord. This lesion occurred on the convex side of the deformity and its topography and character were most compatible with parasitic migrations. In one case which was seropositive for *P. tenuis* E/S antigen, the intralesional nematode was identified.

Conclusion: The development of scoliosis was thought to relate to a sensory denervation of cervical paraspinal muscles and ligaments, caused by the loss of their general proprioceptive afferents from neuromuscular spindles that synapse in the dorsal gray column. The loss of the general somatic afferent nociceptive neurons resulted in an ipsilateral hypalgesia while the ipsilateral mild spastic hemiparesis and ataxia reflected a lesion in the adjacent dorsal and lateral funiculi. We speculate that spinal nerves may serve as route of access to the dorsal gray column and that selective parasite migration in the dorsal gray columns may reflect a tropism.

494 **Epidemiology and control of strongyle infection in a native donkey breed in Portugal**

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Introduction: Miranda do Douro donkey breed is important in agriculture and rural tourism in northern Portugal. This field trial aimed the study of its major gastrointestinal parasites.

Material: The faecal samples of 12 donkeys from a farm in the northeast of Portugal were monitored in 2005-2007.

Methods: Faecal samples were collected every month since October 2005 till February 2007. Qualitative coprology methods and EPG counts with McMaster slide were performed. L3 larval stages were isolated after faecal culture. The efficacy of a deworming scheme with Doramectin was assessed through faecal egg count reduction test (FECRT) and egg reappearance period (ERP) $\geq$ 200 EPG.

Results: Only strongyles were found as gastrointestinal parasites. After deworming in October 2005, three major EPG peaks were recorded in 2006: April (4000 EPG), June (6100) and November (5900). FECRT on day 15 after deworming was 100% and the ERP determined was 53 days. A second deworming in November 2006 showed a FECRT of 81%. L3 larval stages of *Cyathostomum s.l.* were the main ones before and after deworming (92% prevalence). *Trichostrongylus axei* (15%), *Triodontophorus spp.* (13%), *Gyalocephalus capitatus* (8%), *Poteriostomum spp.*, *Strongylus equinus*, *S. edentatus* and *S. vulgaris* (3-5%).

Conclusions: 1) The intestinal strongyle population showed a seasonal variation of the egg shedding, with 3 peaks, in April, June and November. 2) *Cyathostomum s.l.* genus was the most prevalent one. Doramectin showed a 100% efficacy initially, however its continuous use may become ineffective or may induce the onset of strongyle resistant populations.

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495 **Identification of high egg shedding donkeys and associated risk factors**

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The Donkey Sanctuary has 2300 donkeys resident on its farms in the UK. We have recognized the ongoing problem of overuse of anthelmintics and have taken positive steps to reduce their use. It has been shown that a small minority of equids are responsible for the majority of pasture contamination with the young and old often being suspected as being the 'guilty parties'. In order to rationalize our approach to parasite control a representative farm was selected and all donkeys (n=200) were faecal egg counted on a 4-weekly basis for a year. Animals were dewormed according to faecal egg counts (FEC) with only those with counts of over 300 eggs per gram (epg) treated. Analysis of the data allowed animals to be identified that were disproportionately contributing to pasture contamination. 'High shedders' were defined as animals requiring anthelmintic treatment on three or more occasions in a year (≤ 300 epg), this accounted for only 30% (n=61) of the population. It was observed that these 'high shedders' contributed 55% of the faecal egg output for the entire population despite targeted treatment. Age of the donkeys had a significant effect ($P < 0.01$) on number of anthelmintic treatments with geriatric animals (>25 years) needing more treatments in a year. Sex had no significant effect on shedder status. By identifying high shedders a directed approach can now be taken to controlling pasture contamination and identification of additional risk factors may further rationalize the approach to managing such a mixed donkey population.

496 **The strongylid nematodes of working donkeys from Ethiopia**

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The donkey (*Equus asinus*) is the main beast of burden in many countries. However, few attempts have been made to study donkeys worldwide, particularly the gastrointestinal parasites, as compared to that of the horses and ponies. An opportunity arose to study the intestinal helminths of working donkeys from specimens kindly available from the Donkey Sanctuary working in Ethiopia. The samples were obtained from three donkeys at post-mortem examination. Two of the donkeys were euthanased after car accidents and the third after colic surgery. All nematodes were collected from the intestines and fixed in 10% formalin. 2606 strongylid specimens were identified using their morphological characteristics.

Twenty-seven species of Strongylidae from 9 genera were identified. Five of these belong to the subfamily Strongylinae and 22 to the subfamily Cyathostominae. The number of species per donkey ranged from 16-21. *Cyathostomum tetracanthum*, *Cyathostomum montgomeryi*, *Cylicocyclus asini*, *Cylicostephanus minutes*, *Cylindropharynx longicauda* were the dominant species. They were identified from all donkeys and consisted of 29.8%, 21.3%, 10.8%, 6.8% and 6.4% of the total number examined, respectively. Fourth stage larvae of *C.asini*, *C.auriculatus*, *C.tetracanthum*, *C.montgomeryi* and *Cylindropharynx intermedia* were also identified. Despite the small sample of donkeys examined, the result obtained has revealed the richness of the parasite fauna of donkeys in Ethiopia. The number of *Strongylidae* species found in this study is similar to those reported from other regions of Africa (Matthee et al., 2000, 2004). Future studies to determine the full picture of the parasitic fauna of donkeys in Ethiopia is recommended.

497 **Ocular infection of donkeys with *Setaria equina***

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Seven adult donkeys of different sexes, ages and from different localities in Kafr El-Sheikh and Algharbia governorates were admitted to the clinic of Surgery Department with the history of unilateral eye affections. Upon close examination of recent cases, a white worm was found rapidly moving or swimming within the anterior chamber of the affected eye, but in old cases the cornea became so severely opaque that the movement of the parasite inside could be observed only occasionally at the narrow edge of limbus of the cornea. The parasites were surgically removed from the eyes of five donkeys, cleared, mounted and identified as *Setaria equina* based on light microscopic features. The ocular infection with *S. equina* reported herein may document the first aberrant cases in the Egyptian animals.

498 **Cyathostomin infection of horses and donkeys in Portugal based on the study of L3 subpopulations of *Cyathostomum sensu latum***

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Introduction: Horse cyathostomins L3 identification is important since some of them (type A with 8 intestinal cells) come from prevalent species (v.g., *Cylicocycclus nassatus*, *Cylicostephanus longibursatus*, *Cyathostomum catinatum*). Some recent findings concerning different *Cyathostomum sensu lato* L3 subpopulations are presented.

Methods: Faecal samples of horses and donkeys were collected (20 samples/group) for faecal cultures. Prevalence/abundance analysis of *Cyathostomum sensu lato* L3 was performed. Differences were assessed with Mann-Whitney test, $p < 0.05$.

Material: Faecal samples from feral (1 group) and domestic (4) horses and donkeys (3) in Portugal.

Results: Eight types of *Cyathostomum sensu lato* L3 were recorded: five with 8 cells (A, B, C, D and G) and other three referred with 6, 7 and 9 cells (E, F and H). The majority belonged to *Cyathostomum* A (65-98% abundance), followed by C and D (6-8%). The larval types with lower expression were B and E to H. Type A L3 was the most prevalent one (93-100%), but C and D were prevalent too. Dewormed horses had a clear dominance of type A. However, B is more relevant in non treated horses (19-85%), which have higher diversity of larval types. The comparative analysis of feral and domestic horses revealed significant differences, namely concerning type A ($p < 0.001$).

Conclusions: a) Different subpopulations of *Cyathostomum s.l.* and L3 type A is dominant. b) There are significant differences between horses regularly dewormed or not; c) This method allows an indirect evaluation of the impact of control measures on cyathostomins biodiversity.

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499 **Cyathostomin infection in a horse production system in Portugal using dry and irrigated pastures**

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Introduction: Horse cyathostominosis is a growing problem in Portugal. The aim of this work was to evaluate the risk of infection with cyathostomins in a farm with stabled and pasture horses.

Methods: A prevalence study was carried out on different age groups in a horse stud farm. EPG, faecal cultures and pasture contamination studies (L3/kg) were performed. Spearman test correlated parasitological and meteorological data.

Material: Faecal samples from stabled and pasture horses and herbage from dry and seeded irrigated pastures.

Results: Mares and yearlings presented highest EPG counts in April (5000) and in September-October (2500-4000) and the foals presented EPG peaks in April, July and December (1000-3000). Stabled horses didn't show an EPG peak (1600 EPG annual average) but it increased gradually till December. *Cyathostomum s.l.* L3 were the most abundant and prevalent: 90-98% abundance and 72-97% prevalence. These L3 were found in higher numbers in dry natural pastures (99,98% of total L3) during Spring (5400 L3/kg) and mainly Autumn (18600 L3/kg), correlating positively with the rainfall levels. Spring, Autumn and mainly Summer (4200 L3/kg) were the risk months for the seeded irrigated pastures (99,3% of total L3), correlating positively with the temperature and the EPG of stabled horses.

Conclusions: In this horse production system, there's a risk of infection all over the year: spring, summer and autumn pastures are highly contaminated and stabled and pasture horses have high cyathostomin infections, contributing to a high pasture contamination, even in the coolest months.

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500 **Preliminary detection of infection by gastrointestinal nematodes in horses from NW Spain**

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Nowadays, the horse industry is increasing because its diversity, and involves business, sport, gaming, entertainment or recreation. Horses are being used in wild pastures and countries for the forest management, utilizing unwanted vegetation for production. For this reason, this industry deserves the attention of the general public, and the governments also. Control of internal parasites is needed for knowing the health status of these animals. Prevalence data from equine populations are difficult to obtain, and chemoprophylaxis is not based on previous parasitological analysis. A coprological survey for determine current prevalence of several species of gastrointestinal nematoda in equids (n= 269) was conducted in NW Spain. Faecal samples were individually collected and analyzed by the coprological flotation and sedimentation techniques. Coprocultures were also done to identify the main strongylid genera affecting the horses. Data were considered by taking into account the age of the animals, sex, aptitude and management. The main genera identified were *Trichonema*, *Cyathostomum* and *Triodontophorus*. The prevalence of strongyloid parasites was 59%, 3% for *Parascaris* and 2% for *Oxyuris*. The 88% of the positive horses to strongyloids had small strongyles, 1% large strongyles and 11% both. The highest prevalence for ascariasis was observed in the youngest horses (<2 yr), for oxyuriasis in the >10 yr animals, and for strongylosis in the 2-10 yr ones. Females were significantly more parasitized than the males. The greatest prevalence for ascariasis was recorded in breeding horses, and that for oxyuriasis and strongylosis in animals maintained under extensive-forest conditions.

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501 **Annual survey on oribatid mites soil density in Spain and possible link with the risk of tapeworm infection in horses**

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The purpose of this survey was to quantify oribatid mites (intermediate hosts of tapeworm) during one year in soil samples collected monthly from 20 different sites in Spain, representing various climatic conditions. Two kg. soil samples were collected at each site. Temperature and relative humidity (RH) were recorded every month at 2 cm. below the ground surface. One hundred gr of the sample were processed using Berlese Funnel for 24 hours, and invertebrates collected. Oribatid mites were selected under a stereomicroscope and mounted in Hoyer's medium. Soil organic matter was analysed once in March from each site. Clustering and correspondence analysis were performed to classify monthly records for the 20 sites from the 4 variables (oribatid density, soil temperature, RH and organic matter) and to look for links between oribatid density and the 3 soil variables. Oribatids were found in all sites, 165 out of 200 soil samples being positive. Seven different genera, all of them involved in tapeworm transmission to horses, were identified. Oribatid density was low during the whole year in 5 sites. In other sites the maximum density was observed either in May-October (4 sites), August-November (4) or September-October (7). Positive correspondence was found between oribatid density and soil temperature (higher density if temperature > 19°C). Optimal soil RH and organic matter for mites density seemed to be 71%-81% and > 2.7% respectively. These findings could be used to predict risk of tapeworm infection in horses from seasonal climatic data.

502 **Coprological and serological diagnosis of *Anoplocephala perfoliata* in horses from Southern Alberta, Canada**

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Anoplocephala perfoliata infection in horses (*Equus caballus*) has potentially serious consequences, and it has become important to determine infection risk factors. Prevalence and distribution of this tapeworm in western Canada is not well described. Spatial and temporal occurrence of *A. perfoliata* in faecal samples varied among seasons with prevalence of 18% in the winter and 8% in the summer. Significantly more pastured horses were infected than non-pasture horses during both sampling seasons. Regional differences were also noted with drier pastures of the eastern region having significantly lower prevalence than central and western regions. Detection of eggs in faeces is relatively insensitive, and was evaluated against coproantigen and western blot techniques. Samples collected at a local abattoir served as the gold standard for evaluating these techniques. Feces and sera were collected and the caecum was inspected for presence of tapeworms. Examination of caecae indicated prevalence of 7%. Western blots analysis using 12/13kDa worm excretory/secretory (E/S) antigen showed that all worm-positive horses were also serologically positive. A high percentage of worm negative horses were also serologically positive, indicating recent previous exposure and highlighting the need for antigen detection. Coproantigen diagnostic results will also be presented.

503 **The prevalence of *Anoplocephala perfoliata* in abattoir and stabled horses**

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An earlier survey suggested that about 52 % of abattoir horses in the south west of England were infected with *Anoplocephala perfoliata* (Morgan et al Vet.Rec. 2005). Since that survey praziquantel has been introduced to the UK market but the effect it may have had on the prevalence of *A. perfoliata* is unknown. Numbers of tapeworms were counted in horses at a local licensed abattoir in winter 2005-6 and faecal samples were collected 24 hours after horses had been treated with praziquantel in the winter of 2006-7. Eggs were counted by the Flotac system that is sensitive to 1 epg. 34% of 125 horses examined at the abattoir were positive (range 1-1892 worms). 57% of faecal samples from 74 yarded horses were positive(>1 epg) for tapeworm eggs with 43% >10 epg. The data from the abattoir suggests that there may have been a fall in numbers of infected horses following the introduction of praziquantel but this is not apparent from samples from local horses. The types of animals at the abattoir may not be representative of local horses as animals at the abattoir are usually old, but faecal samples from all ages were tested from the yards. The results indicate that the Flotac system could be useful when horse owners wish to determine whether their horses have been infected with mature *A. perfoliata* and counts should indicate the degree of infection. Supported with a gift from Virbac.

504 **Some factors related to the infection by *Anoplocephala magna***

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The objective of this study was to ascertain the influence of some factors including age and season in the infection of horses with tapeworms. Two age groups were established: foals (<2 years) and adult horses (>2 years). A total of 157 digestive tracts from horses slaughtered in Central Spain were dissected from October 2005 to February 2007 (69 foals, 53 adult and 35 horses of unknown age). Tapeworms were collected for identification and counting of *Anoplocephala* species. Prevalences were compared according to age and between seasons by chi-square test.

A. perfoliata and *A. magna* were detected respectively in 27.4% and 14.0% of horses whatever the age. Prevalences were higher in foals (resp. 30.4% and 17.4%) than in adults (resp. 20.8% and 3.8%), difference being significant for *A. magna* ($p < 0.05$).

A. magna was only detected on 2 adults among horses whose age was known: a pregnant mare and a mare which foaled six months previously. The foal was necropsied at the same time and was also positive for *A. magna*. No significant differences on prevalences were found between seasons neither for *A. perfoliata* prevalence nor for *A. magna* whatever the age. Geometric means of *A. perfoliata* and *A. magna* burdens were respectively equal to 15.0 (range: 1-476) and 4.2 (range: 1-137) whatever the age (no significant differences between foals and adults). While more studies are needed, the finding of *A. magna* infection in foals and pregnant/postparturient mares could be linked to the immune status of the horses.

505 ***Anoplocephala perfoliata* infections in horses: parasitic burdens and pathology**

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The gross pathological alterations caused by *Anoplocephala perfoliata* in the ileocaecal junction were investigated in 35 horses slaughtered in Central Spain. According to the intensity of the damage, lesions were scored as grade 1 (congestion, thickening and inflammation of mucosae in the area of tapeworm fixation), grade 2 (focal pseudomembranous enteritis, with fibrin membranes around the attachment sites) and grade 3 (necrotizing enteritis in the area of tapeworm attachment). Parasitic burdens were also scored as high (> 100 tapeworms), moderate (30 to 100) and low (< 30) and the relationship between lesion grade and parasitic burden analyzed (chi-square test). Although a statistical association ($p < 0.0005$) between parasitic burden and grade of lesion was observed, as high burdens were always associated with severe lesions, grades 2 and 3 lesions were also observed amongst the horses with low parasitic burdens, which were predominant in the study. Six ileocaecal junctions with lesions of grade 2 or 3 were selected for histological and immuno-histochemical studies. Histologically, lesions of grade 2 showed a non-specific chronic enteritis with infiltration of eosinophils affecting lamina propria and submucosa. Lesions of grade 3 showed a fibrin-necrotic enteritis compromising all layers of the studied area. In both cases, neuronal degeneration of ganglions belonging to submucosal and myoenteric plexa of Meissner y Auerbach were observed, more intensely in grade 3. The fact that these histological and neuronal alterations can occur in horses with low *Anoplocephala* burdens is particularly relevant, taking into account their potential role in the spasmodic colics.

506 ***Trichinella* sp. infection in horses of Romania: serological survey and parasitological examination**

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Herbivorous animals are usually, by virtue of their diet, outside the major transmission cycles of *Trichinella* spp. However, in the last 32 years, the horse appeared as a novel vector of *Trichinella* spp. infection to humans as 15 outbreaks associated with the ingestion of horsemeat were documented in France and Italy. Romania, one of the main country exporting horses into the European Union, experienced a dramatic increase of *Trichinella* sp. infections in both domestic pigs and humans in the 1990's. Some *Trichinella spiralis*-infected horses were exported into the EU during this period. The aim of this study was to evaluate the prevalence of *Trichinella* sp. infections in horses from various Romanian regions with indirect and direct tests. In 2001 of the 3,000 serum samples tested, none resulted positive by ELISA using three different antigens (crude; excretory/secretory, ES; and stg-BSA antigens). In 2002, of the 2,992 serum samples tested, 17 (0.56%) showed in some laboratories, optical density values higher than that of the cut-off by an ELISA using ES antigens and only one was confirmed by western blot (WB). Four out of the 17 ELISA positive horses, including the horse with a confirmed serology by WB, were subjected for intensive meat examination at slaughter, but no *Trichinella* sp. larva was detected. No *Trichinella* sp. larva was detected by trichinelloscopy and artificial digestion in the 25,838 horses slaughtered in Alexandria and Timisoara between 2001-2004.

Although specificity of ES ELISA seems to be high in horses during the first months after infection, serological results must be interpreted critically in term of false negative results after a long-term infection as observed in experimental studies. The false positive results obtained by serology suggest that serological methods cannot be used yet to detect *Trichinella* sp. infection in horses. Furthermore, the lack of detection of *Trichinella* sp. infected horses by artificial digestion, suggest the very low prevalence of infection of these nematodes in horses of Romania.

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507 **Easiness of administration and underdosing risk of endectocide oral pastes in horses**

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Underdosing is a major contributory factor to the development of anthelmintic resistance in horses. This study aimed to compare the ease of administration of different macrocyclic lactone anthelmintics and the correlation between the actual dose ingested and real body weight (bw) of the horse. Four hundred and eighty horses, randomly assigned to 8 groups, were administered the entire syringe content from one of the 8 tested products: ivermectin pastes (products A-B-C-D), moxidectin paste (product E), ivermectin/praziquantel pastes (product F-G), moxidectin/praziquantel paste (product H). Each syringe allowed respectively treatment of 700 kg (products A-F), 600 kg (products B-C-D-G) or 575 kg bw (products E-H). Horses were weighed (mean bw: 624 kg) and easiness of administration, defence reactions, salivation, flehmen and paste spitting were scored after dosing. Actual dose ingested was calculated from bw and estimated amount of paste rejected. Underdosing was defined as ratio between actual and recommended doses below 90%. Groups were compared by the chi-square test with multiple comparisons Bonferonni method. Defence reactions, salivation and flehmen rates were not significantly different between products but product C was more difficult to administer than products A-B-E-F-G ($p < 0.05$). Significantly different spitting rates were found between products, being lower than 10% for products A-B-F-G and ranging between 29% and 68% for other products. Significantly less horses were underdosed with products A and F (less than 10%) than with other products (from 15% to 73% of horses). In conclusion underdosing could be avoided by using adequate syringe capacity and products easily ingested by horses.

508 **A survey of parasite control methods used by horse owners in Portugal**

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Introduction: The use of anthelmintics in horses has increased since the 1980's. This survey aimed a better knowledge concerning horse parasite control measures in Portugal.

Methods: The survey was performed during 2002-2005 in horse events or during veterinary consultation and horse owners answered a written questionnaire.

Material: One hundred and twenty private horse owners in Portugal.

Results: Most replies were from owners with horses for recreational purposes (34%), with 1-5 horses (43%) and access to pasture (71%). The majority of owners dewormed horses twice a year (56%) and used only one type of AH (63%). Macrocyclic Lactones (ML) were the most used (78%), followed by Pyrantel (PPYR) (21%) and Benzimidazoles (BZD) (1%). 37% rotated to another AH, but only 6% to a drug of a different group. Only 17% of owners evaluated the efficacy of treatments through coprological tests and 18% referred pasture hygiene measures.

Conclusions: 1) low deworming frequency (75% deworm 1-2 times/year), but using always the same AH or a chemical related AH; 2) Ivermectin was the most common drug used; 3) None of the owners rotate AH on annual basis and only 6% changed to another class of AH; 4) adequate pasture hygiene was not applied and the majority of owners had no knowledge of such control measures. Portuguese horse owners need to know better how to control parasites and horse practitioners need to assume a greater role in prescribing and monitoring deworming programs to achieve a more effective parasite control and prevent resistance to ML.

509 **Time of re-appearance of faecal tapeworm eggs output in horses treated with an ivermectin-praziquantel combination and left in their contaminated environment**

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This study aimed to measure the time of re-appearance of tapeworm eggs in horses left in their natural environment after treatment. Twenty-six horses with positive faecal tapeworm egg counts were included from 5 stud yards in Germany. They were treated once with an oral gel of ivermectin (200 µg/kg bw) and praziquantel (1.5 mg/kg bw) combination (Equimax, Virbac). Faecal samples were then collected monthly for one year to detect tapeworm egg output by a centrifugation/flotation method. Probability of tapeworm eggs re-appearance was estimated using the Kaplan-Meier method. All horses remained negative during the 2 months after medication, confirming treatment efficacy. Then the percentage of negative horses decreased gradually from 3 to 12 months. Probabilities of tapeworm eggs re-appearance were 0.37 and 0.46, 8 and 12 months after treatment respectively. Kinetics of tapeworm eggs re-appearance was consistent with the re-infection exposure of horses, the prepatent period of tapeworms and the lack of remanence of praziquantel. As tapeworm coprological diagnosis lacks sensitivity, the re-infection probabilities are likely to be underestimated. Thus taenicial treatment may be required less than one year after initial one.

510 **The activity of GenesisTM Equine in naturally infected ponies**

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Nematodes and cestodes (primarily *Anoplocephala perfoliata*) are the two most important groups of helminths parasites of horses. Combination anthelmintics (macrocyclic lactones and praziquantel) are increasingly popular for their control. To evaluate a new combination product, 16 naturally infected wild Dartmoor ponies were used which had been removed from the moor to prevent overgrazing. Since the ponies had never been treated with anthelmintics they had a wide range of parasites. After 7 days acclimatisation in stalls the animals were randomised by faecal egg counts and 8 were weighed with a heart girth tape and administered a paste delivering 0.2 mg/kg ivermectin and 2.5 mg/kg praziquantel. No adverse reactions were observed. The remaining 8 animals acted as untreated controls. Ponies were sacrificed 14 days after treatment and the numbers of parasites enumerated. Genesis Equine was 99.9% effective against bots (*Gasterophilus intestinalis*), 100% against *A. perfoliata*, 100% against *Parascaris equorum*, 100% against luminal stages of large strongyles (*Strongylus vulgaris* and *S. edentatus*) and 99.96% effective against luminal stages of immature and adult cyathostomins. The results indicate that the paste formulation is highly effective against nematodes, cestodes and bots of horses.

511 Plasma disposition and faecal excretion of netobimin following oral administration in horses

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Introduction: Netobimin (NTB) is a pro-drug of albendazole (ABZ) used against liver flukes, tapeworms and gastrointestinal and lung nematodes in ruminant species only. Netobimin is converted into ABZ by gastrointestinal microflora following oral administration to ruminants. Almost all of the ABZ absorbed from the intestine is rapidly metabolized into its anthelmintically active albendazole sulphoxide (ABZSO) and inactive sulphone (ABZSO₂) metabolites by liver. The present study was designed to determine the plasma disposition and faecal excretion of NTB and its respective metabolites in horses following oral administration.

Materials and methods: Netobimin was administered orally to 8 horses at 10 mg/kg bodyweight. Blood and faecal samples were collected from 1 h to 120 h post-treatment and analysed by high performance liquid chromatography (HPLC). Using a chiral phase-based HPLC, plasma disposition of ABZSO enantiomers produced was also determined.

Results: The metabolic pattern of NTB in horses was similar to ruminants. Neither NTB nor ABZ were present and only ABZSO and ABZSO₂ metabolites were detected in the plasma samples. Maximum plasma concentration of ABZSO ($0.53 \pm 0.14 \mu\text{g/ml}$) and ABZSO₂ ($0.36 \pm 0.09 \mu\text{g/ml}$) were detected at (t_{max}) 10.50 and 19.50 h respectively following administration of NTB. The area under the curve (AUC) of the two metabolites was similar to each other. Netobimin was not detected, and ABZ was predominant in all faecal samples. Unlike the plasma samples, the enantiomers of ABZSO were close to racemate in faeces.

Conclusion: Netobimin is completely converted to ABZ probably by the gastrointestinal flora and albendazole absorbed is completely metabolised to its sulphoxide and sulphone metabolites by first-pass metabolism. The specific behaviour of the two enantiomers probably reflects different enantioselectivity of the enzymatic systems of the liver which are responsible for sulphoxidation and sulphonation of ABZ. Considering the pharmacokinetic parameters only NTB could be used as an anthelmintic in horses.

Poster group 12: Anthelmintic Resistance

512 Analysis of the L256F mutation in a new ivermectin resistant *Cooperia oncophora* isolate

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Cooperia oncophora is one of the most common intestinal parasitic nematodes of cattle in temperate climates. These parasites are mainly controlled with macrocyclic lactones, such as ivermectin. However, this control strategy is threatened by the widespread appearance of ivermectin resistant isolates. Therefore, in recent years research has focussed on the search for a molecular marker to detect resistance at an early stage. A previously proposed marker is the L256F mutation in the GluCl α 3 gene of *C. oncophora*, which was shown to be associated with resistance in a UK lab selected isolate. Because it is unclear if the same polymorphism is present in other resistant isolates, the aim of the present study was to analyse the presence of L256F polymorphism in a newly isolated *C. oncophora* ivermectin resistant lab isolate. In a preliminary experiment the GluCl region of 17 individual resistant and 17 susceptible L3 larvae spanning the L256F polymorphism were PCR amplified and sequenced. None of the worms, either susceptible or resistant, carried the actual L256F mutation. However, there were silent polymorphisms present around that region. The current aim is to develop a pyrosequencing method to analyze a large number of larvae simultaneously for polymorphisms in the L256F region. This method will be used to analyze both the susceptible and fully resistant isolate together with the semi-resistant *C. oncophora* isolates collected during the selection trial (0,0125/0,025/0,050/0,075/0,1 mg/kg). This will indicate whether the L256F is present or whether there is any selection pressure on the GluCl α 3 gene.

513 **Ivermectin resistance in cyathostomins in UK horses**

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The failure of macrocyclic lactones (MLs) to control cyathostomins has been reported in Thoroughbreds in Brazil (Molento et. Vet.Rec. in press) and the beginning of resistance has been suggested from studies in Germany (von Samson-Himmelstjerna et al 2007). The objective of the work was to look for evidence of the beginning of resistance in the UK. Horse yards were contacted and asked to send faecal samples 28 days after treatment with ivermectin. Egg counts were performed using the Flotac cell, a method capable of detecting 1 epg. Sample sizes ranged from 2 to 10 per yard and came from private owners, riding stables and Thoroughbred studs. Fifteen yards were checked. On 3 yards no eggs were found. Including a further 10 yards the mean egg counts for all 87 horses were 1 epg. However, two yards had mean egg counts of 14.2 and 22.2 epg. Both were Thoroughbred studs. Adult horses had been treated 7 times per year with ivermectin or moxidectin, foals 6 times per year (ivermectin until 4 months of age) and yearlings 5 times a year with moxidectin. These results suggest that where MLs have been used intensively resistance to them may be beginning in cyathostomins.

514 **Ivermectin resistant *Parascaris equorum* in Sweden**

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Parascaris equorum (nematode) occurs frequently in foals on studs. Infected foals may show nasal discharge, coughing, ceased growth, inappetence, rough hair coat and in massive infections, intestinal obstruction. This study was initiated because increasing problems associated with *P. equorum* infections have been noticed on many studs with rigorous deworming schedules. The aim of the study was to examine the anthelmintic efficacy of ivermectin, pyrantel and fenbendazole on *P. equorum* by a Faecal Egg Count Reduction Test (FECRT).

Nine big studs involving a total of 165 foals participated in the study, which took place in the autumn after weaning. Foals excreting at least 100 eggs per gram (EPG) faeces were included in the FECRT. On day 0 the foals were dewormed orally with either of ivermectin (6 groups), pyrantel (1 group) or fenbendazole (3 groups). Faecal egg counts were performed on days 0 and 14 following treatment. The efficacies of the drugs were calculated on arithmetic means of EPG.

Sixty-six (48%) of the total number of foals sampled excreted at least 100 EPG of *Parascaris equorum*. In 5 farms out of 6, ivermectin treatment failed to prevent the faecal output of *P. equorum* eggs. The egg reduction in these groups varied from 0-89%. For fenbendazole and pyrantel the reductions of eggs were 100% and 97%, respectively.

According to the results from this study, ivermectin should not be the drug of choice for use against *P. equorum* in foals.

515 **Selection of a benzimidazole-treated *Ostertagia ostertagi* strain**

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Anthelmintic resistance in nematodes is a growing problem in livestock husbandry in many countries. A candidate gene family belonging to the ABC transporter system, the P-glycoproteins are hypothesized to contribute to anthelmintic resistance.

For examining the population genetics and expression profile of P-Glycoprotein in resistant *Ostertagia ostertagi*, two strains are being developed, one under treatment with albendazole. The second, albendazole susceptible strain is being passaged in calves without treatment as a reference

The strain development was initiated using albendazole susceptible *Ostertagia ostertagi* L3 larvae to infect cattle. Starting with a very low subtherapeutic albendazole dosage the anthelmintic was administered to the cattle. The progeny of the survivors were harvested and cultivated to reinfect cattle for the next passage. Continued subtherapeutic treatment was performed using increasing doses. An in vitro selection procedure of larval cultures using thiabendazole was introduced to force selection pressure. The process will be pursued until less than 80% faecal egg count reduction following treatment with 7,5 mg albendazole per kg bodyweight is achieved.

Conventional and degenerate PCR were used to identify different P-Glycoprotein genes in *Ostertagia ostertagi*. Molecular analysis of gene fragments using SSCP (Single Stranded Conformational Polymorphism) is being developed to search for genetic evidence of a possible P-Glycoprotein involvement in benzimidazole resistance. Beta-tubulin codon 167, 198 and 200 polymorphisms are being quantitatively analysed for both strains and after each selection step.

Prospective studies will include RealTime PCR analysis of P-Glycoprotein expression patterns and copy numbers.

516 **Genotyping benzimidazole-resistant *Cylicocyclus nassatus* from an isolated herd of horses from the Ukraine**

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Resistance to benzimidazoles in the gastro-intestinal nematodes of horses is common throughout Europe. We are studying benzimidazole resistance in several species of cyathostomins from a closed population of brood horses established approximately 150 years ago from a herd brought to the Ukraine from Russia. Faecal egg count reduction tests indicate a high degree of resistance to benzimidazoles in these species of cyathostomins. We have begun a molecular analysis of the worm populations by genotyping the known resistance-associated mutations in the isotype 1 beta-tubulin gene in *Cylicocyclus nassatus*. Genotyping by means of pooled-DNA sequencing indicates that the codon 167 mutation is more common than the codon 200 mutation, and most of the alleles carry one or the other mutation. No polymorphism at codon 198 has been detected.

517 **The cyathostominae species resistant to benzimidazole anthelmintics in Ukraine**

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Introduction. Resistance of the horse *Strongylidae* nematodes to benzimidazoles has been registered in Ukraine. However, it was unknown which of the strongyles species are resistant to benzimidazoles. The aim of our study was to identify the strongylid species resistant to anthelmintics.

Methods. The study was carried out on 24 horses from the Voronkov horse farm (Kyiv region) and 45 horses from the Dubrovsky horse farm (Poltava region). Faecal samples were collected a day before and 14 days after treatment. McMaster technique (Herd, 1992) was used to calculate the strongyle eggs (EPG). All horses were treated with “Albendazole-7.5” (Ukraine). The horses with EPG>150 on the 14th day were re-treated with “Ivomec-P” (Germany). Faecal sampling (200 g) was performed at 24, 36, 48 and 60 hours post treatment; all nematodes expelled were collected and identified.

Results. The average EPG in horse faeces from the Voronkov farm was 889 and 311 – from the Dubrovsky farm. Twenty strongylid species were found in horses from the Voronkov; all of them were sensitive to albendazole – no eggs were found in faeces at 14th day after treatment. Nineteen species were found at the Dubrovsky farm. Fourteen horses were re-treated with “Ivomec-P” and 11 cyathostome species were found to be albendazole resistant: *Cylicocyclus nasssatus*, *C.ashworthi*, *C.leptostomus*, *Cyathostomum catinatum*, *C.pateratum*, *Coronocyclus coronatus*, *C.labiatus*, *Cylicostephanus goldi*, *C.longibursatus*, *C.minutus*, *C.calicatus*. These species were also prevalent in the strongylid community.

Conclusion. The results obtained showed that benzimidazole resistance is proper only to the most prevalent strongylid species at some horse farms.

518 **Multiple anthelmintic resistance against mixed gastrointestinal nematodes in dairy goats**

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This paper presents the first report of anthelmintic resistance in dairy goats in desert (Thal) in Pakistan. Three breeds of dairy goats i.e., Dera Din Panah, Pak Angora and Beetal kept at Government Livestock Farm, Rakh Khairwala, district Jhang/Layya, Pakistan were surveyed for gastrointestinal nematodes resistant to commonly used three anthelmintics i.e., benzimidazole, levamisole and ivermectin. Sixty animals of each breed were selected randomly on the basis of their weight and egg count. Three preparations (fendamex, oxazole, systamex) of a drug of benzimidazole group viz., oxfendazole, and levamisole and ivermectin were given at the recommended dose to five groups while one untreated group was kept as control for each breed. Faecal egg counts, faecal egg count reduction test, postmortem worm count and copro-culture were performed to assess the efficacy of selected anthelmintics. Levamisole in two breeds and ivermectin in all the breeds led to reduction ($P < 0.05$) of prevalent species of gastrointestinal nematodes in both flocks. *Haemonchus controtus* and *Trichostrongylus* species were suspected for the presence of resistance against oxfendazole preparations which exhibited low efficacy ($P > 0.05$). Of these oxfendazole preparations, the resistance was more pronounced in oxazole followed by systamex and fendamex in Pak Angora goats. In Beetal and Dera Din Panah goats, systamex and oxazole showed almost same result while fendamex was also leading preparation with high anthelmintic activity. It was concluded that all oxfendazole preparations had low efficacy with suspicion for anthelmintic resistance while levamisole seemed to be losing efficacy in beetal goats and ivermectin had reasonable efficacy.

519 **Anthelmintic resistance in Ireland: current status**

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Introduction : Gastrointestinal parasites are an important constraint in sheep production and broad spectrum anthelmintics have become central to parasite management. In Ireland, anthelmintic resistance was first confirmed in sheep in 1989 and a limited survey carried out in 1991 confirmed evidence for benzimidazole in 20% of farms sampled. However, the extent of anthelmintic resistance in Ireland is unknown.

Methods: Between 2002 and 2006 a number of studies have been carried out examining this issue on lowland farms with a significant sheep enterprise. Methods used included faecal egg count reduction tests and micro agar larval development tests.

Results : Collectively, the data from these studies indicate an alarming situation with respect to benzimidazole resistance. The first evidence of levamisole resistance was also observed. Furthermore a nationwide survey has been completed which has shown a large increase in resistance to benzimidazole and levamisole, 95 % and 48 % of flocks respectively.

Conclusion: As there is only one alternative anthelmintic class available, the results indicate urgency for education / training in best practices that promote the sustainable use of anthelmintics and preserve anthelmintic efficacy.

520 **Preliminary observations on drug resistance in sheep gastrointestinal *Trichostrongyles* in central Italy**

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Drug resistance in sheep gastrointestinal (GI) trichostrongyles is a cosmopolitan major threat to small ruminant production. Despite widespread infection in Italy information on the presence of drug-resistance is scarce. The purpose of this study was to acquire preliminary information on the presence of anthelmintic resistant in sheep GI trichostrongyles in central Italy. Fifty sheep with values of ≤ 150 eggs per gram (EPG) were selected on each of three farms (total n=150 sheep) in central Italy and randomly allocated to 1 of 5 groups. Groups were treated with febantel (5 mg/Kg), levamisole (5 mg/Kg), ivermectin (0.2 mg/Kg) or moxidectin (0.4 mg/Kg) while the fifth group remained untreated. Individual faecal samples were collected prior to dosing and 10 days later for a faecal egg count reduction test (FECRT). The mean FEC of each treatment group (EPGt) was calculated and compared to that of the control group (EPGc) from the same flock. Percentage reduction (R) was determined using the formula $R = [1 - \text{EPGt}/\text{EPGc}] \cdot 100$ and resistance declared when $R < 95\%$. Levamisole (mean $R = 89\%$) and ivermectin (mean $R = 93\%$) resistance was found on all farms and the post-treatment larval cultures showed the presence of *Teladorsagia* (*Ostertagia*) *circumcincta* and *Trichostrongylus* spp. larvae. Febantel (mean $R = 96\%$) and moxidectin (mean $R = 100\%$) were effective on each of the 3 surveyed farms. Even though febantel showed $>95\%$ EPG reduction, GI trichostrongyles seem to be close to becoming resistant to this molecule. This preliminary study suggests that drug resistance in sheep GI trichostrongyles is present in central Italy and a potential problem which would justify a broader nationwide geographical investigation.

521 The efficacies of three macrocyclic lactones against nematodes in sheep with confirmed macrocyclic resistance

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An initial FECRT carried out on a commercial sheep property confirmed moxidectin resistance, with an efficacy of 83.6 %. Examination of a small number of worm burdens (4 per group) 14 days post drenching found that *Teladorsagia circumcincta* burdens were reduced by 70% after moxidectin treatment.

The following season 84 lambs were randomly selected for a FECRT, comprising six anthelmintic treatments plus a control run on pasture. Lambs were randomly allocated to one of seven groups, of which six received one of the following anthelmintic treatments, fenbendazole, levamisole, ivermectin, abamectin, moxidectin oral and injectable. FEC were carried out at day 10 and 14 post treatment. On day 16 post treatment fifty-six animals were sampled for worm burden analysis (8 per group).

Fenbendazole reduced FEC at day 10 and 14 by 87% and 72%. Levamisole, abamectin and both moxidectin treatments reduced FEC by at least 96 % however ivermectin treatment reduced FEC by 90%. Examination of worm burden data revealed a serious situation on this property; with both *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* exhibiting resistance to all three drench families and *Nematodirus spp* also exhibiting resistance to benzimidazoles (81% efficacy). Treatment with either fenbendazole or levamisole failed to achieve any reduction in *Teladorsagia* worm burdens. The efficacies for ivermectin, abamectin and moxidectin against this ML-resistant strain of *Teladorsagia* were of 83%, 62% and 99.7 respectively. For ivermectin to have a higher efficacy than abamectin is contrary to the published data on efficacies of the various ML against ML resistant *Teladorsagia* strains.

522 A field study on the effect of targeted selective treatment (TST) on lamb performance and the phenotypic expression of anthelmintic resistance in nematodes exposed to ivermectin treatment

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The world wide problem of escalating anthelmintic resistance has led to research on refugia based approaches to the management of resistance. Previous studies have shown that targeted selective treatments (TST) based on anaemia (Famacha©) or high milk production in goats can reduce anthelmintic usage whilst maintaining acceptable levels of performance. This paper describes replicated field studies in which TST was applied on the basis of liveweight gain. A variety of parasitological, performance and resistance parameters were compared in four groups given TST, metaphylactic treatments (MT), strategic prophylactic treatments (SPT) and neo suppressive treatments (NST). The performance figures during the first grazing season showed that the NST group, as expected, had the best overall and post-weaning performance figures. Using this group as the comparator, the overall and post-weaning daily weight gain figures showed reductions of 6, 15 % (TST), 13, 31 % (SPT) and 14, 26 % (MT) respectively. Body condition score did not prove to be a sensitive indicator for TST application. The reduction in performance in the TST animals was offset by the reduced number of individual treatments that were administered and beneficial effects on the selection of resistance. The numbers of individual treatments administered to the TST, NST, SPT and MT groups were 76, 144, 92 and 96 respectively. Faecal egg count reduction tests showed no decline in efficacy in the TST and SPT groups, a small decline in efficacy (1%) in the MT group but a 15 % decline in the NST group.

523 **Drug combination against multiple resistant nematodes: albendazole-ivermectin kinetic interaction and clinical efficacy in lambs**

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It has been suggested that drug combinations may be efficacious against resistant nematode strains where the failure of individual drugs is documented. The trials reported here were addressed to compare the clinical efficacy and plasma pharmacokinetic behaviour of albendazole (ABZ) and ivermectin (IVM) given either separately or co-administered to lambs naturally infected with resistant nematodes. Seventy parasitized lambs were involved in the following trials: a) 'Clinical efficacy study': The animals were allocated into 7 groups (n= 10), untreated control and treated either with ABZ iv (Group I), IVM iv (Group II), ABZ iv + IVM iv (Group III), ABZ ir (Group IV), IVM sc (Group V) and ABZ ir + IVM sc (Group VI). The efficacy of the different treatments was estimated by the FECRT. b) 'Pharmacokinetic study': Animals from Groups I to VI (n= 6) were used in this trial. Plasma samples were collected over 15 days post-treatment and drug concentrations measured by HPLC. Parametric and non-parametric statistical tests were applied. The following egg output reduction values were obtained: 67.1% (Group I), 78.3% (Group II), 91.9% (Group III), 44.3% (Group IV), 77.9% (Group V) and 68.6% (Group VI). The drug-drug kinetic interaction accounted for: 1) a higher IVM plasma AUC obtained in the ABZ iv + IVM iv (Group III) compared to the IVM alone treatment (Group II); 2) a higher ABZ-sulphoxide AUC value obtained in the presence of IVM (Group VI) compared to ABZ alone (Group IV). Finally, the co-administration ABZ-IVM results in a non-adverse kinetic interaction, which may facilitate an additive drug effect against resistant nematodes.

524 **Faecal egg count reduction test on a new commercial goat farm in Malaysia**

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Nematode Faecal Egg Count Reduction Test (FECRT) was conducted on a new commercial goat farm near Ipoh, Perak, Malaysia to evaluate the status of resistance to 4 anthelmintics from the major groups of anthelmintics available in Malaysia. The farm had 150 goats of mixed breed and practiced intensive management, whereby animals were not allowed to graze but grass from paddocks were cut and carried. Faeces was used as fertilizer for the grass paddocks.

Faecal samples were subjected to McMaster method for worm burden estimation and larval culture for third stage larvae identification. The mean pre-treatment faecal egg count was 5822 e.p.g. It was found that the nematode population was resistant to benzimidazole, levamisole and closantel as the percentage reduction was between 54%-91%. Only macrocyclic lactones could be used for nematode control in the farm (percentage reduction of 99%).

Third stage larvae collected from the larval culture of the control group were *Haemonchus contortus* (81%), *Oesophagostomum* sp. (12%) and *Trichostrongylus* sp. (7%) *Haemonchus contortus* was found in all post-treatment larval cultures, indicating resistance to all anthelmintics tested. The FECRT is critical for a newly established farm as it will give important information on the worm population and the status of worm resistance as well as the worm species that exists in the farm so that further action can be taken to manage the problem.

525 **Effect of lipids on the active transport of xenobiotics in nematodes**

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The efficacy of anthelmintic treatments of small ruminants against nematodes is compromised by the emergence of resistance. Besides specific mechanisms associated with mutations in anthelmintic cellular targets, we previously showed that anthelmintic resistance also strongly depends on both the presence of membrane pumps such as P-glycoprotein (Pgp), members of the ATP-binding cassette (ABC) transporter family causing accelerated drug efflux, and on the lipid composition of membranes. The relationships between Pgp and lipids are here specified. Pgp activity was significantly correlated to the cholesterol content of nematode eggs, especially to free cholesterol (FC). In addition, Pgp activity and resistance to anthelmintics were significantly correlated with the FC / phospholipids (PLs) ratio, in particular phosphatidylcholine (PC) present in the external leaflet of cell membranes. These results suggested that the respective distribution of cholesterol and PLs within the membranes may influence Pgp conformation and activity. Similar results obtained in vertebrate cells have allowed to identify lipid microdomains in membranes with high cholesterol content and presence of specific lipids (GM1-ganglioside, PC) where part of the Pgp are trapped and acquires the active conformation. Thanks to a fluorescent marker that specifically binds to GM1, we also identified such microdomains in nematode eggs. The comparison of several isolates with different resistance status showed that resistant eggs contain both more active Pgp and more microdomains than the susceptible isolates. The results are to be confirmed in the other stages of the parasites. These observations could improve the understanding of drug resistance in nematodes and anthelmintic therapy.

526 **Effect of the metabolic inhibitor methimazole on the drug susceptibility of a triclabendazole-resistant isolate of *Fasciola hepatica***

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Recent studies have shown that triclabendazole (TCBZ)-resistant isolates of *Fasciola hepatica* have an increased capacity to oxidise TCBZ and triclabendazole sulfoxide (TCBZ.SO) to inert forms, which affects the ability of the drug to reach its target and exert its fasciolicidal effect. In this in vitro study methimazole, a metabolic inhibitor of the flavin-monooxygenase (FMO) system, was used to assess the effect of metabolic inhibition on TCBZ-resistant fluke. The FMO system was inhibited by a 2-h pre-incubation in methimazole (100 µM). The flukes were then incubated for 22h in NCTC medium containing either methimazole; methimazole+NADPH (1nM); methimazole+NADPH +TCBZ (15 µg/ml); or methimazole+NADPH +TCBZ.SO (15 µg/ml). Flukes were then processed for transmission electron microscopy. The results have revealed ultrastructural changes to the tegument and associated tegumental cells, testes and vitelline cells. The changes were more severe in TCBZ-resistant than -susceptible fluke following treatment with TCBZ.SO+methimazole and than that seen with TCBZ.SO on its own. The results suggest that inhibiting drug metabolism can alter the drug sensitivity of TCBZ-resistant flukes. They support previous pharmacological data indicating that altered drug metabolism may contribute to the development of TCBZ resistance.

527 **The efficacy and persistent activity of moxidectin, abamectin and ivermectin against susceptible ML resistant and F1 cross *Ostertagia (Teladorsagia) circumcincta* in sheep**

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The efficacy of moxidectin, abamectin and ivermectin against a susceptible strain of *Ostertagia (Teladorsagia) circumcincta* (SS), a highly selected ML-resistant strain (RR) and an F1 cross between these two isolates (RS) in sheep were compared. Sixty four newly weaned 12-15 week old Romney cross ewe lambs were allocated to 12 treatment groups, 1A, 1B, 1C, 2A, 2B, 2C, 3A, 3B, 3C, 4A, 4B and 4C. On Day -21, lambs in groups designated with an A, B or C were each infected with 10,000 SS larvae, RR larvae or RS larvae respectively. Groups 1, 2 and 3 were treated with commercially available oral formulations of moxidectin, abamectin and ivermectin respectively on Day 0. The lambs were slaughtered on Day 17/18 for worm counting. All 3 compounds were highly effective (99 - 100%, $p < 0.01$) against the SS strain. ABA and MOX were also effective (>95%) against the F1 cross but not so against the RR strain, though reduction in worm burdens was significantly different to the Controls and IVM groups ($p < 0.01$). Both the RR and F1 strains were highly resistant to IVM.

528 **P-gp interfering agents potentiate ivermectin susceptibility in nematode ivermectin-resistant strains**

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Ivermectin resistance has become a major threat to parasite control programmes. The membrane P-glycoproteins (P-gps) are thought to be involved in resistance mechanisms by actively driving the drug out of the parasite. Our study aimed at restoring ivermectin susceptibility in nematode resistant strains by using P-gp inhibitors.

In a first step, valspodar, ketoconazole, pluronic85 were selected in vitro for their ability to induce the rhodamine123 accumulation in P-gp (murine *mdr1a* gene) overexpressing cell line. Then, increasing ivermectin concentrations were tested in first stage larvae of susceptible and resistant strains of *Teladorsagia circumcincta* and *Haemonchus contortus* by using the optimised larvae feeding inhibition assay (LFIA) in presence or not of P-gp inhibitors.

Valspodar, ketoconazole, pluronic85, were able to induce Pgp-substrate accumulation in P-gp overexpressing cells with respective IC50 of 0.1, 5 and 11 μ M.

The respective resistance factors (calculated by dividing the LFI50 values of a resistant isolate by that of a susceptible isolate) for *T. circumcincta* and *H. contortus* were 1.4 and 4.4.

In resistant strains, the LFI50 values were reduced in the presence of inhibitors leading to increases in susceptibility factors (LFI50 anthelmintic alone/LFI50 anthelmintic+inhibitor) of between 3-12 (*T. circumcincta*) and 105-174 (*H. contortus*).

Our data support the idea that P-gp function is one of the key factors involved in ivermectin resistance in nematodes and the use of P-gp reversing agents could represent an attractive strategy to control the spread of ivermectin resistance in nematodes.

529 **Identification of putative QTL for nematode parasite resistance in sheep**

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Gastrointestinal nematode parasites have a profound effect on sheep production. A sheep population segregating for parasite burden was constructed at Louisiana State University utilizing Gulf Coast Native (resistant) and Suffolk (susceptible) crosses. A genome-wide QTL scan has been implemented using samples from 42 grandparents, 3 F1 sires, 97 F1 dam, and 195 F2 offspring, for which fecal egg count (FEC) for *Haemonchus contortus* and packed cell volume (PCV) were measured after natural and experimental challenges. Selective genotyping of the upper and lower 20% of the lambs for FEC after natural challenge identified potential QTL regions on ovine chromosomes (OAR) 1, 6, 9 and 19. The full population, including grandparents, was then genotyped with 14 markers on OAR1, 9 on OAR6, 6 on OAR9, and 5 on OAR19. QTLs significant at chromosome-wide levels ($P < .05$) were identified on OAR9 at 40 cM for FEC after natural challenge and at 95 cM for FEC and PCV after experimental challenge. Another QTL was identified on OAR19 at 20 cM for FEC and PCV after natural challenge. Fine mapping of these regions will more precisely locate the QTL and allow the identification of positional candidate genes.

530 **Reversion of ivermectin resistance in *Haemonchus contortus* by verapamil: in vitro and in vivo studies**

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There are convincing evidences that one of the molecular mechanisms involved with ivermectin resistance in *Haemonchus contortus* is the efflux action of P-glycoprotein (P-gp). This activity can be inhibited by verapamil, a multidrug resistant reversing agent. The aim of this study was to evaluate the efficacy of ivermectin in combination with verapamil against an ivermectin-resistant field isolate of *H. contortus* using a larval migration assay and an experimental infection trial. The in vitro assay used three groups with 8 different concentrations: ivermectin (1 to 128 μ Mol), verapamil (1 to 100mMol); and ivermectin (LD50) plus verapamil. The in vivo trial used twenty-four lambs that were artificially infected with 8.500 *H. contortus* third stage larvae. The animals (n=6) received a subcutaneous treatment of ivermectin (0.2mg/kg), ivermectin plus verapamil (3mg/kg/3x at 12h interval), verapamil and a non-treated group. All animals were necropsied seven days after treatment for parasite burden counts. Verapamil given alone had no anthelmintic effect on both experiments. It was observed a proportional enhancement of ivermectin's efficacy of up to 94.3% when verapamil was used at increasing concentrations of up to 100 mmol, using the in vitro migration assay. For the in vivo test, ivermectin in combination with verapamil had an efficacy of 36.02% compared to 7.75% for ivermectin alone. The data shows a significant increase in ivermectin's efficacy of 465% against a very strong field isolate of ivermectin-resistant *H. contortus*. As proposed in previous studies, the combination of ivermectin and verapamil is a promising treatment management strategy for Veterinary Parasitology.

531 **Induction of resistance in *Haemonchus contortus* to thiabendazole and ivermectin by in vitro exposure**

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Increased use of anthelmintics in livestock has contributed to the development and spread of drug-resistance in parasitic nematodes threatening effective deworming of sheep world-wide. Molecular tools are needed to monitor the emergence of resistance in the field early on. In order to generate starting material for developing molecular markers, fully susceptible *Haemonchus contortus* (Hc) isolates were subjected to a new in vitro selection protocol using thiabendazole (TBZ) and ivermectin (IVM). Eggs were allowed to develop in vitro to L3 larvae at various drug concentrations. The larvae recovered from the dose levels that had eliminated 95% of the population were then used to infect a sheep. After 8 generations, the resistance factor (RF) for the TBZ treated isolate (HcTBZ) had increased 1000-fold, while the isolate exposed to IVM yielded a RF of 22 after 10 generations. RF changes were monitored during each cycle using larval development assays (LDA) to determine the respective LC95 values. Parallel testing in sheep showed that TBZ resistance induced in vitro was relevant to the curative dose levels required in vivo. Sheep inoculated with infective larvae of the 6th generation and treated with albendazole at the recommended dose rate of 3.8 mg/kg bodyweight could not be cured (mean efficacy of 65%). However, IVM resistance induced in Hc was not sufficiently high to allow the survival of adult worms in sheep after treatment with IVM at 0.2 mg/kg. At the molecular level, a mutation at position 198 in the β -tubulin gene of HcTBZ was detected by PCR-RFLP analysis, which has recently been described to be involved in benzimidazole resistance. These results indicate that the selection technique described here is a suitable tool for rapidly generating resistant isolates of Hc under controlled laboratory conditions that requires a smaller number of sheep than conventional selection procedures.

532 **A novel approach for combining the use of in vitro and in vivo data to measure and detect emerging moxidectin resistance in gastrointestinal nematodes of goats**

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Ivermectin and moxidectin are closely related avermectin/milbemycin anthelmintics and available data suggest that side resistance occurs with these two drugs. However, moxidectin remains effective against many species of ivermectin-resistant worms due to its higher potency. The larval development assay (LDA) is routinely used to diagnose ivermectin resistance in *Haemonchus contortus* but laboratory diagnosis of moxidectin resistance is hampered by the lack of any validated in vitro tests. The objective of this study was to measure the relative susceptibility/resistance of *H. contortus* to moxidectin on goat farms in Georgia, and to validate the DrenchRite® LDA for detecting resistance to moxidectin. Fecal egg count reduction tests (FECRT) were performed at five different moxidectin dose levels and DrenchRite® LDAs were performed in duplicate on nine meat goat farms in Georgia, USA. To improve our ability to make inferences on the relative levels of resistance between farms, FECRT data were first analyzed using a linear mixed model, and then Tukey's sequential trend test was used to evaluate the trend in response across dose levels. LDA data were analyzed using log-dose logit-response and probit models. Using these statistical results, we were able to rank the nine farms from the least to the most resistant, and to develop a set of criteria for interpreting DrenchRite® LDA results so that this assay can be used to diagnose both clinically-apparent moxidectin resistance, as well as sub-clinical emerging resistance. These results suggest that our novel approach for examining these types of data provides a method for obtaining an increased amount of information, thus permitting a more sensitive detection of resistance.

Poster group 13: Helminths of Companion Animals

533 Intestinal parasites in shelter dog populations from Northeastern Spain

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Animal shelters provide suitable conditions for the occurrence and spread of parasitic infections. Contamination of the environment with eggs and cysts facilitates re-infection of the animals and poses a risk for humans since zoonotic parasites are commonly found in animal shelters and constitutes a significant problem.

Within the framework of sanitary controls in different canine shelters, the detection of intestinal parasites was investigated. Potential risk factors concerned to the setting and to the management were also evaluated in order to improve shelter protocols with respect to parasite control.

Nine shelters located at the metropolitan area of Barcelona were studied. 251 fecal samples were collected from soil. Coprological analyse was performed by using Zinc Sulphate centrifugation-flotation technique.

Data related to the setting (structure of kennel floor, number of dogs per cage, ventilation, cage material...) and data concerning management (quarantine; antihelmintic protocol; disinfection protocol; removal of faeces from dog's environment; food ...) were recorded.

Protozoa were the most important parasites present in dog shelters, especially *Giardia spp.* that was present in all shelters analysed, affecting 29% of dogs. *Cystoisospora spp.* was present in 8 out of 9 shelters and was detected in 17.9% of samples. The highest prevalence of infection with parasitic nematode was *Trichuris vulpis* (13,5%) and *Toxocara canis* (9.9%). With respect to cestodes, just *Hymenolepis diminuta* was detected. Mixed infection was detected in 20.3% of stool samples.

Concrete soils, high humidity rates, unsuitable materials used in boxes or cages such wood or straw, lack of quarantine measures, administration of broad-spectrum antihelmintic, disinfection based exclusively on bleach were evaluated as potential risk factors.

534 Examinations in endoparasite prevalences in dogs and cats in animal shelters in Lower-Saxony and investigations of anthelmintic resistance

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The incidence of endoparasites was examined in shelter dogs and cats in representatively selected shelters in the State of Lower Saxony. For epidemiological calculations, the dog and cat population size of the State was estimated. Faecal samples were examined using the sedimentation flotation combination method and a commercially available *Giardia* antigen kit (*Giardia* SNAP test, Idexx).

28 shelters were included in the study so far. Each shelter contains approximately 22 dogs and 70 cats. Questionnaires were collected for each sample/animal containing information about age, breed, gender, life style, outdoor access, anthelmintic treatment regimen, type of shelter, anamnesis of shelter. The following hypotheses are addressed: What is the descriptive biased incidence? Are there differences in size, age, breed, gender and presence of parasites? Are there differences between shelters, type of shelters or localisation towards urban or rural location? Is the "prevalence" calculated higher or lower than an estimated "true prevalence"?

At the moment, approximately 650 cat samples with 35% positives, and approximately 300 dog samples with 10% positives are collected. Dominating parasites in cat samples are *T. cati* with 27.5% (mainly in animals >6months of age) and *Isospora spp.* (8.3%). In dog samples the dominating parasites are *T. canis* (3.8%) and *Isospora spp.* (3.5%).

For the faecal egg count reeducation test, so far 17 dogs and 66 cats were tested with either fenbendazole, pyrantel or milbemycinoxime, but so far no reduced efficacy of the anthelmintics used were observed.

535 **Coprological survey of intestinal parasites in dog breeding kennels in Flanders, Belgium**

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A recent study showed a low prevalence of intestinal parasites in household dogs in Flanders, but current data from other dog populations are lacking. The objective of this study was to obtain preliminar data on the prevalence of intestinal parasites in dog breeding kennels in Flanders. A total of $\pm$ 500 dogs will be sampled. Between October 2006 and March 2007 faecal samples were collected from 68 pups and 105 adult dogs in 14 breeding kennels. Samples were examined for the presence of worm eggs and protozoal oocysts using a sedimentation-flotation technique. For the detection of *Giardia* a direct immunofluorescence assay was used. PCR protocols targeting the β -giardin gene and the *tpi* gene were used for the molecular identification of a subset of *Giardia* isolates. One hundred and six faecal samples (61%) were positive for intestinal parasites. Pups were more frequently infected (84%) than adult dogs (47%). *Giardia* was the most prevalent parasite in pups (56%). Both zoonotic *Giardia* species (*G. duodenalis*) and host specific species (*G. canis*) were found. *Cystoisospora* oocysts were present in 32 pups (47%). *Toxocara canis* was the most commonly found helminth species with 37% positive pups, followed by *Toxascaris leonina* (6%). In adult dogs *Giardia* was also the most common parasite (32%), followed by *T. canis* (14%) and *Cystoisospora* spp. (10%). *Trichuris vulpis* and *Uncinaria stenocephala* were detected in 5% and 1% of adult dogs, respectively. Conclusions: The prevalence of intestinal parasites in breeding kennel dogs in Flanders was high. *Giardia* and *Toxocara* were commonly found parasites, and present a zoonotic risk.

537 **Zoonotic potential of the helminths infection of stray dogs from Havana City**

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During a period of 13 months, 461 dog intestines were collected and dissected to evaluate their parasitic load, from the 15 municipalities of Havana City. Dogs were obtained from the Canine Observation and Control Centre. The dogs were selected at random and euthanized. The following data were acquired for each dog: gender, breed, age, place of capture (district), and date of capture. The small intestine was removed from each animal and then transported at 4 °C to the "Helminthology Laboratory. Each intestine was cut longitudinally so as to expose the mucosa. The prevalence of dogs parasitized was analyzed using descriptive statistics. Different indicators were used to evaluate either epidemiological matters as well as the parasite communities structure. We found parasite in 263 (57, 04%) dog intestines during the study. The nematodes collected were: *Ancylostoma caninum* in 97 (36, 88%) intestines, *Toxocara canis* in 91 (34, 60%). Cestode collected was: *Dipylidium caninum* in 75 dogs (28, 51%). By age, *A. caninum* and *D. caninum* were the most prevalent species in older animals, while *T. canis* was more prevalent in young animals ($p < 0.01$). *D. caninum* was most common in the dry season ($p < 0, 01$). Female (57, 2%) were more infested than male (56, 87%) , *T. canis* was more prevalent in female. In the rainy season *A. caninum* was the most founded, 26, 72%, ($p < 0.01$) and in the dry season *D. caninum*, 22, 7% ($p < 0, 01$) was the most common.

538 **Zoonosis in companion animal in Sinaloa, Mexico**

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Information about zoonotic diseases in companion animals does not receive enough distribution, due mostly to the fact that their presence is unknown. That is why it is necessary to assess which zoonosis prevail in each area. In Sinaloa México, the principal health problems in dogs and cats are dermatophaties, gastrointestinal parasites and hematic parasites. In order to assess which of the causative agents of this pathologies are zoonotics, a review was made from skin scrapes, feces and blood samples examined at the parasitological laboratory of the veterinary school of Autonomous University of Sinaloa. From 67 skin scrapes from dogs, dermatofits was detected in 33.54% and *S. scabei* in 8.07%; in cats, dermatofitoses represents 28.6% and scabioses 14.3%. Close to 50% of humans exposed acquire the disease; in 69.6% of all the homes with affected cats, at least one person is infected (Green, 1993). Likewise 30% of the dermatofitosis in humans in urban areas had been associated to direct contact with companion animals (Ettinger, 1995). *Sarcoptes scabei* affects people as an incidental host (Quiroz, 1990; Birchard/Sherding, 1996), in which the symptoms are purities and papular dermatitis (Nesmit & Alkerman, 1996). The highest number of cases in humans have been associated to occupational risk (Quiroz, 1990). Of intestinal parasitoses, the zoonotic agents found in 980 feces samples were *Ancylostoma spp* in 14.9% of dog feces samples, and 23.7% in cat feces samples; *Giardia spp* was found in 2.3% of the dog feces samples, and 1.3% of the cat feces samples; *Dypilidium spp* was found in 1.6% of all samples, *Taenia spp* in 0.40% of dog samples, and *Entamoeba spp* in 0.20% of dog samples. *Ancylostoma canium* is a nematodus that was considered specie specific, but today is accepted that it can also infect humans (Quiroz, 1990; Martinez, 2006). *Dipylidium caninum* and *Taenia spp* are the zoonotic cestodosis of major importance in dogs and cats (Martinez, 2006). *Giardia spp* is an accepted zoonotis and has been widely studied (Abe & col., 2002; Traub & col., 2004; Lalle & col., 2005). Amibiasis, caused by *Entamoeba histolytica*, is more common in humans than in dogs or cats (Green, 1993), and is transmitted through feces. Finally, a prevalence of 70% for ehrlichiosis, a hematic parasite, was observed. The causative agent of ehrlichiosis in humans is *E. chaffensis* (Inokuma H. y col., 2001) and is accepted as a zoonotic disease since it is transmitted by ticks (Rikihisa, 1994); In 1999, *E. ewingii*, which was known as a pathogen for canines was recognized as pathogen for humans as well (Osu, 2001).

539 **A comparative study into the prevalence of intestinal parasites in owned dogs in Grenada, West Indies**

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This study explores the hypotheses that the prevalence of canine intestinal parasites in Grenada is comparable to those found in other countries of similar climate, and that they constitute risk factors for both animal and public health. One hundred owned dogs were used in this study. Group 1 (n = 69) were owned dogs presented to the small animal clinic, for either a medical problem or routine examination, over a five week period from September to October 2006. Group 2 (n = 31) were owned dogs seen as part of a neutering program. With owner permission, fecal samples were collected and analyzed using the centrifugal flotation method (zinc sulfate, specific gravity 1.180) to ensure the maximal recovery of canine intestinal parasite stages. The overall prevalence of intestinal parasites in dogs in this study was 63%. *Ancylostoma spp* were the most common (55% of dogs), followed by *Strongyloides* (12%) and *Toxocara canis* (7%). Owned dogs that had never received veterinary care had a significantly ($p < 0.05$) higher parasite prevalence than dogs seen through a veterinary clinic and there was a significantly greater parasite prevalence in young dogs. Dogs living outside, dogs that associated with other animals and dogs that had not been dewormed were also all at a significantly higher risk of being infected ($p < 0.05$). A multidisciplinary approach involving veterinarians is needed, both to continue to understand the epidemiological situation on the island, and to educate owners, particularly targeting those owners without access to veterinary care.

540 **Toxocariasis in dogs, its zoonotic importance and chemotherapy**

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Toxocariasis in man is traditionally thought to be contracted through ingestion of eggs from contaminated soil. The disease may manifest itself in different syndromes such as ocular larval migrans, visceral larval migrans and covert toxocariasis. The study accesses the evidence for the soil contamination hypothesis and proposes that direct contact with dogs may provide a better explanation of the epidemiology of the disease.

Results are presented in two parts; part-I deals with prevalence of *Toxocara canis* eggs. A total of 600 faecal and hair samples (200 of dog faecal, 200 dogs hair and 200 dog owners faecal samples) were collected and examined for the presence of *Toxocara canis* eggs. *Toxocara canis* eggs were found in 74 (37%) faecal samples, 25 (12.5% in hair samples and in 47 (23.5%) dog owners faecal samples. Prevalence was higher in pups and children than adults. Twenty five eggs were recovered, of which 2.1 % were embryonated, 12.5 percent were embryonating. The maximum densities of the embryonating and embryonated eggs were 180 and 20 EPG per gram of hair respectively, much higher than the densities reported for soil samples. It is suggested that dogs infected with *T. canis* may infect people by direct contact.

Part-II deals with chemotherapeutic trails. A total of 60 dogs positive for

toxocariasis were divided randomly into three groups, A, B and C (control group) each with 20 dogs. Dogs in group A and B were treated with Ivermectin and Levamisole hydrochloride, respectively. The efficacy of the drugs was calculated on the basis of reduction in the number of ova discharged in faeces. Results showed that Ivermectin and Levamisole hydrochloride were 97.3 percent and 97.4% effective respectively. Levamisole hydrochloride is much cheaper than Ivermectin and it was slightly more effective.

541 **Patterns of parasitic infections in faecal samples from stray cat populations in Qatar**

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The parasite fauna of stray cat populations, comprising mainly helminth parasites, is described for the first time from the arid environment of the Qatar peninsula. 824 faecal samples were examined from six sites in Qatar during the winter and summer months of 2005. Up to seven species of parasites were identified, six of which were nematodes and these included *Strongyloides stercoralis* as the most prevalent (18.4%), followed by *Aelurostrongylus abstrusus* (7.5%), *Toxocara cati* (6.1%), *Ancylostoma tubaeforme* (5.9%) and *Physaloptera* sp. (4.8%) and *Toxascaris leonina* (0.7%) and one sporozoan species, *Isospora felis* (0.5%). Unidentified cestode eggs were also recovered from 10.7% of samples examined. The parasite species were found to be highly overdispersed in faecal samples from all sites, whereas the prevalence and intensity of infections were influenced by site and season. Infection levels tended to be higher during the winter season especially in the case of *A. abstrusus* and *A. tubaeforme*, where conditions of temperature and humidity were more favourable for the development of egg and/or larval stages of parasites compared with the extremely hot and dry summer months. The results are discussed in relation to the distribution of the cat population in the vicinity of Doha and its outskirts and the potential threat of parasite transmission to human communities in Qatar.

542 **Study of intestinal helminthes of stray cats in Sari, north of Iran**

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Intrudocion: The population role of stray cats in distribution of helminth parasites and good environment of North of Iran for living theirs, this study achieved with aim of prevalence and intensity of intestinal worms in stray cats of Sari city.

Material and methods: A cross-sectional study from April to October 2004, 100 stray cats studied in 5 area of Sari city. Intestine and feces of cats studied with parasitological methods, then intestinal worms separated and distinguished with taxonomy methods.

Results: Seven genus and species intestinal worms were distinguished with 90% prevalence of intestinal worms that maximum prevalence related to *Ancylostoma tubeaforme* (64%) and *Toxocara cati* (44%) and minimum prevalence related to *Dipilidium caninum* (2%) and *Physaloptera preputialis* (1%) that *Dipilidium caninum* was reported for first times in north of Iran. In feces 3 cats, *Capillaria Spp.* eggs were found for first times in north of Iran. Prevalence of intestinal worms in non-adult stray cats was 100% versus adult stray cats (84.8%) and statistical association between theirs was significant ($P<0.05$). Maximum intensity of infestation related to *Ancylostoma tubeaforme* with 9.20 worm per one cat.

Conclusion: High prevalence and intensity of intestinal worms in Sari cats demonstrates this animal is reservoir and source of this worms and it is effective in distribution of this worms in Sari city.

543 **Prevalence of gastrointestinal nematodes in stray cats from Shiraz, Iran**

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In a survey carried out during the period March 2005 to November 2006 in different area of Shiraz, southern Iran, 114 stray cats were examined for *Toxocara cati* and other intestinal nematoda parasites. All of stray cats were autopsied less than one hour after being euthanized so that all adult worms were still alive prior to fixation in 10% formalin solution. *Toxocara cati* worms were separated from other helminths and the males and females were identified by microscopic examination for future study. The following nematodes, with their respective prevalence were diagnosed in the stray cats: *Toxocara cati* (41.2%), *Toxascaris leonina* (5.3%) and *Physaloptera sp.* (44.7%).

The high prevalence of *Toxocara cati* in stray cats is considered to be critical from the viewpoint of public health importance, some of which are responsible for several zoonotic disease such as Visceral Larva Migrans (VLM) and/or Ocular Larva Migrans (OLM). In conclusion, nematoda infection of stray cats is still very serious problem for public health in Shiraz.

544 **Epidemiological studies on the gastrointestinal helminth parasites of dogs in Ibadan, Nigeria**

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An epidemiological study of gastrointestinal helminths of dogs (*Canis familiaris*) in two veterinary clinics in Ibadan was conducted between January 2001 and December 2002. Faecal samples collected from 959 dogs were processed by modified Kato-Katz technique and then examined for helminth eggs. The results of the study showed that 237(24.7%) of the dogs examined were infected with different types of helminths. The prevalences for the various helminth eggs observed were *Toxocara canis* 9.0%, *Ancylostoma sp.* 17.9%, *Toxascaris leonina* 0.6%, *Trichuris vulpis* 0.5%, *Uncinaria stenocephala* 0.4% and *Dipylidium caninum* 0.2%. The egg intensities of infection, determined as mean egg count per gram of faeces ($\pm$ SEM), were *T. canis* 462.0 ± 100.5 , *Ancylostoma sp.* 54.1 ± 8.6 , *T. leonina* 0.8 ± 0.4 , *T. vulpis* 0.1 ± 0.0 , *U. stenocephala* 1.0 ± 0.7 , and *D. caninum* 0.2 ± 0.1 . Host age was found to be a significant factor with respect to the prevalence and intensity of *T. canis* and *Ancylostoma sp.* infection. The prevalence of helminth infections was significantly higher ($P < 0.05$) in dogs from Mokola Veterinary Clinic (39.8%), a public clinic than at Bodija Veterinary Clinic (12.8%), a private practice. This may be a reflection of the social status of the dog owners because majority of dogs at the Mokola Clinic are local dogs belonging mostly to poor owners while most of the dogs at Bodija are exotic breeds belonging to relatively rich owners who can afford the more expensive but effective care offered at the clinic.

545 **Prevalence and risk factors for *Toxocara canis* infection in dogs in Ile-Ife and Ibadan, southwest Nigeria**

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The prevalence and intensity of *Toxocara canis* was investigated in a sample of 1228 dogs aged 1-120 months between January 2001 and December 2004 from Ile-Ife and Ibadan, Southwest Nigeria. On the basis of stool examination for the helminth eggs, an overall prevalence of 14.4% was recorded for *T. canis* among the dogs. Intensity of infection, determined by the mean number of eggs per gram of faeces, ($\pm$ SEM) was 447.00 ± 80.59 . Relationships were explored between prevalence and intensity and host factors including age, sex, mode of life and other risk factors including dog breed types and socioeconomic status of dog owners. The prevalence (32.1%) and intensity (1650.8 ± 339.9) of *T. canis* in dogs aged 0-6 months were significantly higher ($P < 0.05$) than in dogs of older age groups. Logistic regression analysis identified dogs of age 0-6 months (OR= 0.04, 95% CI 0.01-0.17), local breed (OR=0.11, 95% CI 0.03-0.38) as well as dog owners whose occupation are teaching (OR= 2.57, 95% CI 1.06-6.23) as significantly associated with *T. canis* infection.

546 **Nation wide survey of intestinal parasites in dogs and cats in Australia**

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Current information on pet intestinal parasites in Australia is based on studies conducted some 20 years ago in high risk animals and or localized geographical areas. These early studies indicated a high risk of helminth infection for pets. The aim of the present study was to investigate the current prevalence of intestinal parasites in pets, and through the use of molecular tools provide additional information on the epidemiological and public health significance of some of the parasites identified.

Method: In total 1400 dog faecal samples were collected from dogs at refuges (590) and pet dogs presented at veterinary clinics (810) for vaccination. A total of 1069 cat faecal samples were also collected from refuges (491) and veterinary clinics (578).

Materials: All samples were screened via microscopy. In addition molecular methods were applied to those samples found positive for Hookworm, *Giardia* and *Cryptosporidium* in order to determine the species or genotype.

Results: The most prevalent parasite in dogs was *Giardia* (9.3%) followed by Hookworm sp. (6.7%). The most common parasites in cats were *Isospora felis* (5.6%) and *Toxocara cati* (3.2%). Significantly, *Ancylostoma ceylanicum* was identified for the first time in Australia. Almost all of the *Giardia* genotyped in the dogs was identified as the dog specific genotypes C and D.

Conclusion: It now appears that the most common parasites in pet dogs and cats are protozoan and that the level of helminth infection is much lower than that reported in previous studies. The presence of *Ancylostoma ceylanicum* is of public health importance since this parasite is capable of causing patent infections in humans. The clinical significance of *Giardia* infection in dogs warrants further investigation, yet the zoonotic importance in this instance seems low.

547 **Design and testing of a natural *Dirofilaria immitis* infection model to evaluate the S-safety of Advocate®/Advantage Multi™ for cats**

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A natural infection model was developed to establish adult *D. immitis* infections in cats for subsequent product safety testing. Eighty cats (40 male/40 female) were each inoculated with 60 *D. immitis* L3. The cats were then maintained for 8 months for the development of adult worms. Antigen/antibody testing and echocardiographic imaging were performed to confirm and estimate adult worm burdens. Thirty five cats determined to have clinically relevant *D. immitis* infections were blocked by infection intensity score and randomized to 1 of 4 treatment groups: 1, placebo [9 cats]; 2, 10% imidacloprid +1% moxidectin [Advocate®/Advantage Multi®] 1X dose (9 cats); 3, 10% imidacloprid +1% moxidectin 5X dose (9 cats); or 4, 6% selamectin [Revolution®] 1X dose (8 cats). Treatments were topically administered on test day (td) 0. Cats in groups 1, 2, and 4 received additional treatments on td 28 and 56. No adverse events attributable to treatment with the test articles were observed during the study. Overall, adult *D. immitis* were recovered from 62 of the 80 cats (34 male / 28 female) and included worm ranges of 1-3 worms (11 cats), 4-11 (20 cats) and > 11 worms (31 cats). Six cats each harbored ≥ 20 worms.

548 **Imidacloprid/Moxidectin (Advocate®) to prevent heartworm infection (*Dirofilaria immitis*) in ferrets**

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Ferrets are susceptible to *D. immitis* infection and develop a disease comparable to that in cats and dogs. Treatment options are limited, thus prevention would be an important alternative. A study was conducted to evaluate the efficacy of a combination of 10% w/v imidacloprid plus 1.0% w/v moxidectin spot-on (Advocate®) to prevent patent *D. immitis* infection in experimentally infected ferrets when administered once 30 days post-infection. Twelve ferrets were acclimated to the study facility for seven days. The ferrets were free of heartworm infection prior to the artificial infection as demonstrated by negative test results on microfilarial and antigen tests. On Study Day -30, the twelve ferrets were artificially infected with 25 infective, third-stage larvae of *D. immitis*. On Study Day -1, the twelve ferrets were randomized and allocated to two study groups so that each group contained six ferrets. On Study Day 0, the animals assigned to Group 1 were treated with a combination of 10% imidacloprid / 1% moxidectin at a dose of 0.4 ml per ferret. The animals assigned to Group 2 served as untreated controls. On Study Day 124, the ferrets in both study groups were euthanized and necropsied to determine the worm burden. Ferrets in the control group (Group 2) were adequately infected with *D. immitis* at necropsy to allow assessment of the treatment group in this study GM = 17.2 (Group 2). The efficacy of a combination of 10% imidacloprid / 1.0% moxidectin (Advocate®) against artificial infections with *D. immitis* in ferrets was 100.0% when administered once at a target dosage of 0.4 ml 30 days post-infection. No adverse events were observed which were considered to be treatment related.

549 **Inhibitory efficacy on mosquito-feeding and experimental infection of *Dirofilaria immitis* by administration of topical imidacloprid-permethrin compound to dogs**

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Topical Imidacloprid-Permethrin compound (Fortreon™, Bayer), consist of Imida-cloprid 100mg and Permethrin 500mg per 1 ml, was evaluated for an inhibitory efficacy on mosquito-feeding and experimental infection of *Dirofilaria immitis* to dogs. Nine experimental female beagle dogs (9 months age each, mean 9.2 kg body weight) were divided into three groups that were the administration on 3 days before mosquito feeding as group 1, the administration on 36 days before mosquito feeding as group 2 and un-administration with mosquito feeding only as control as group 3. In study 1, young-fresh female *Aedes togoi* mosquitoes emerged from larvae were trapped into cage covered with stainless-steel-mesh were contacted for 30 min onto skin of anesthetized dogs. Resulting mosquito-feeding rates were 12.1, 11.1 and 40.6 % in groups 1 to 3, respectively. In study 2, another group of female mosquitoes were feeding a microfilaremia dog and reared for 10 days until infective larvae grew up, then again these were feeding 9 beagles in 3 groups above mentioned. After 141 days, all of the beagles were counted for the worm burden of 5th stage-*D. immitis* in the pulmonary arteries. Resulting number of infected dog and infectious rate in each group were 1 dog(3 worms) and 33%, 1 dog(3 worms) and 33%, and 3 dog(1-4 worms) and 100% in groups 1 to 3, respectively. These indicate that the compound markedly suppress mosquito-feeding and effectively inhibit *D. immitis* infection.

550 **Combined ivermectin and doxycycline treatment has microfilaricidal and adulticide activity against *Dirofilaria immitis* in experimentally infected dogs**

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There is still a pressing need for effective adulticide treatment for human and animal filarial infections. *Dirofilaria immitis* is the causative agent of canine heartworm disease. Like many filarial nematodes, *D. immitis* harbours the bacterial endosymbiont *Wolbachia*, which has been shown to be essential for worm fecundity and survival. Here the authors report the effect of different treatment regimens in dogs experimentally infected by intravenous transplantation of adult *D. immitis* on microfilariemia, antigenemia, worm vitality and *Wolbachia* content. Ivermectin (IVM; 6 mcg/kg per os weekly) combined with doxycycline (DOXY; 10 mg/kg per daily intermittently) resulted in significantly faster decrease of circulating microfilariae and higher adulticide activity when compared to either IVM or DOXY alone. Quantitative PCR analysis of *Wolbachia*/nematode DNA ratios showed significant decrease in *Wolbachia* content in worms recovered from DOXY-treated dogs that was not, however, associated with worm death. Worms from IVM/DOXY-treated dogs, on the other hand, had ratios similar to those of control worms, indicating a loss of both *Wolbachia* and nematode DNA. Histology and TEM of worms recovered from the IVM/DOXY combination group showed complete loss of uterine content in females and immunohistochemistry for *Wolbachia* revealed only a few, degenerating bacteria within lateral chords. Results indicate that combination of these two drugs causes adult worm death. This could have important implications for control of human and animal filarial infection.

551 **The use of imidacloprid / ivermectin spot-on in the treatment of canine angiostrongylosis**

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Canine angiostrongylosis is a snail borne disease caused by the metastrongyloid nematode *Angiostrongylus vasorum* (Baillet, 1866). Domestic dogs, wild foxes and related species can be infected and develop patent disease. The predominant clinical signs are related to the respiratory system and are caused by the presence of the adult worm, eggs and L1-larvae in the pulmonary arteries. Most treatment protocols are based on anecdotal reports or small case series using i.e. fenbendazole, levamisole or macrocyclic lactones.

In a randomised, blinded, controlled field trial study, privately owned dogs naturally infected with *A. vasorum* were examined. Dogs were randomised into two treatment groups receiving either a single dose of 0.1 ml/kg of imidacloprid 10 % / ivermectin 2.5 % spot-on (Advocate®, Bayer HealthCare AG, Germany) or fenbendazole at a dose of 25 mg/kg daily for 20 days. Treatment success was defined as a negative Baermann test 42 days after treatment start. Efficacies were calculated with a 95 % confidence interval (CI) and compared by a Fisher's exact test. P was set at 0.05.

Twenty-seven dogs were treated with imidacloprid / ivermectin and 23 dogs with fenbendazole. The obtained efficacies and 95 % CI for imidacloprid / ivermectin was 85.2 (66.3-95.8) and 91.3 (71.9-98.9) for the fenbendazole group. When comparing the two efficacies a p-value of 0.67 was obtained.

Under the conditions of this study imidacloprid / ivermectin spot-on and fenbendazole p.o. proved to be effective against *A. vasorum* infection and no statistically significant difference could be demonstrated between the two treatment protocols.

552 **Efficacy of an imidacloprid/moxidectin spot-on solution against fleas (*Ctenocephalides felis*) and hookworms (*Ancylostoma spp*) in Brazilian cats and dogs**

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Pharmaceutical industry constantly searches for new formulations for parasite control of companion animals, which are affected by fleas and gastrointestinal nematodes worldwide. Aiming to evaluate the efficacy of a spot-on imidacloprid/moxidectin combination on flea infestation and natural acquired *Ancylostoma spp* infection on dogs and cats, two formulations (imidacloprid 10.0% + moxidectin 2.5% for dogs, and imidacloprid 10.0% + moxidectin 1.0% for cats) were tested in two independent experiments. In the flea efficacy experiment, 21 adult cats and 21 adult dogs were infested artificially with *Ctenocephalides felis* on day -4 and -2 and weekly up to the 64th day post-treatment (DPT) and the 71st DPT on feline and canine tests, respectively. Flea counts started after 48 hours post-treatment and revealed an efficacy higher than 95% till the 36th DPT in cats and the 50th DPT in dogs. For the second experiment, 21 animals of each species, naturally infected with *Ancylostoma spp*, were treated with the imidacloprid/moxidectin combination, following the label instructions. Evaluations revealed that the endectocide reduced 100.0% *Ancylostoma* fecal egg counts (FEC) in dogs one week post-treatment, while in cats the reduction was observed on the 5th day post-treatment. At necropsy, performed at the 7th DPT, the formulation exhibited 100.0% efficacy for *Ancylostoma* in dogs. In cats, efficacy for *Ancylostoma tubaeforme* was 100.0%. Therefore, the spot-on combination of imidacloprid/moxidectin may be used for flea and *Ancylostoma* control in dogs and cats raised under Brazilian conditions.

553 **Anthelmintic efficacy of the emodepside + praziquantel spot-on association against *Ancylostoma braziliense* in experimentally infected cats**

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Ancylostoma braziliense has been reported as a common parasite in cats in Brazil and in other countries. This nematode shows high pathogenicity, with intense hematophagism and high zoonotic potential, due to the possibility of the infective larvae to penetrate the human skin and cause a dermatosis called “Cutaneous larva migrans (CLM)”. For the efficacy evaluation of the emodepside + praziquantel association against this nematode, 16 cats were selected, between two and five-months-old of age, in good nutritional conditions and free from ecto and endoparasites. Each cat was orally infected with 500 *A. braziliense* larvae, obtained from a pure culture maintained in CPPAR/FCAVJ/UNESP. The cats were distributed into two groups (n=8): one treated with the emodepside + praziquantel combination, via spot-on, and the other group was not treated and kept as control. After the patency period (17-21 days), EPG counts were performed on three consecutive days (-3, -2 and -1) before treatment and also on days 1, 3, 5 and 7 post-treatment (DPT). The evaluated drug combination showed a reduction of 100% in the EPG counts. At necropsy, performed at the 7th DPT, it was recovered a total of 2 and 878 *A. braziliense* from treated and control groups, respectively. The new drug association showed high efficacy against *A. braziliense*, eliminating 99.79% of the male and 99.81% of the female parasites, present in the experimentally infected cats. Therefore, the emodepside + praziquantel formulation, in the via and dose used, can be recommended in for the treatment of *A. braziliense* infection in cats.

554 **Tools for exploring pyrantel resistance in *Ancylostoma caninum***

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Evidence has emerged that heavy use of the drug pyrantel has resulted in significant levels of resistance in Australian populations of the hookworm *Ancylostoma caninum*. To address the need for a rapid laboratory test for pyrantel resistance in this species, we have assessed a number of different in-vitro bioassays including larval motility (LMA) and larval feeding inhibition (LFIA) assays, and developed a new assay, the larval arrested morphology assay (LAMA). To date, the LAMA appears to be the most effective assay, showing an IC₅₀ value of 0.043 µg/ml for a resistant *A.caninum* population (drug efficacy in-vivo of 25 %) compared to an IC₅₀ value of 0.0025 µg/ml for an isolate of low resistance status (drug efficacy in vivo of 75 %), representing a resistance ratio of 17.2. Our investigation into pyrantel resistance has continued with full-length sequencing of the nicotinic acetylcholine (nACh) receptor subunit UNC-38 gene. We plan to sequence the UNC-38 gene from pyrantel resistant and susceptible *A.caninum*, and also assess expression levels of UNC-38 mRNA in these populations using quantitative PCR. Additionally, PCR with degenerate primers for conserved regions of nACh receptor subunit genes from other organisms is being used to identify additional subunit genes from *A. caninum*. It is anticipated that this work will provide a foundation for a future understanding of the molecular mechanism of pyrantel resistance in this species.

555 **Efficacy of emodepside + praziquantel topical solution (Profender® Spot on) against immature stages of nematodes (*Ancylostoma sp.* and *Toxocara sp.*) in cats**

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To evaluate the efficacy of emodepside against immature stages (L 4) of *Ancylostoma sp.* 18 healthy kittens less than 6 months of age were orally infected with approximately 300 infective *Ancylostoma sp.* larvae each on two successive days. On day + 7 after the first infection, 10 kittens were dermally treated with emodepside 1.98% w/w plus praziquantel 7.94% w/w at a dose rate of 3,2 mg/kg emodepside. The kittens were necropsied at the end of the prepatent period (21 days), and the intestines examined for *Ancylostoma sp.* The efficacy was calculated by using the average *Ancylostoma sp.* counts against the average of the untreated controls. Untreated kittens had an average count of 254 worms. Treatment on day +7 after the first infestation was 99.7% effective against immature stages (L 4) of *Ancylostoma sp.* In a second experiment to test the efficacy of emodepside against immature stages (L 3) of *Toxocara sp.* 18 healthy kittens less than 6 months of age were orally infected with approximately 100 infective *Toxocara sp.* eggs. On day + 7, 10 kittens were dermally treated with emodepside 1.98% w/v + praziquantel 7.94% w/v at a dose rate of 3,2 mg/kg emodepside. The kittens were necropsied at the end of the prepatent period (42 days) and the intestines examined for *Toxocara sp.* Efficacy was calculated by using the average *Toxocara sp.* counts against the average of the untreated controls. Untreated kittens had an average count of 37 worms. Treatment on day +7 after infection was 100% effective against immature stages (L 3) of *Toxocara sp.*

556 **Comparative plasma disposition of fenbendazole, oxfendazole and albendazole in dogs**

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Introduction: Benzimidazole anthelmintics are used widely to treat gastrointestinal helminthiasis including migrating larvae and lungworm infections in animals, they have generally broad spectrum of activity and low toxicity. The plasma disposition of fenbendazole, oxfendazole, and albendazole; and the enantiospecific disposition of parent drugs and metabolites were investigated following an oral administration in dogs. **Material and methods:** Fifteen adult (3-5-year-old) dog weighting 22-31 kg body weight were allocated into 3 groups of five. The animals in each group received fenbendazole, oxfendazole or albendazole orally as a single bolus dose at 50 mg/kg. Blood samples were collected from 1 to 120 h post-treatment and the plasma samples were analysed by HPLC. **Results:** The plasma concentrations of all parent molecules and respective metabolites displayed a significantly different plasma disposition from each other. The sulphone metabolite of fenbendazole was not detected in any plasma samples and the parent molecule albendazole did not reach quantifiable concentrations following fenbendazole and albendazole administration, respectively. Oxfendazole and its sulphone metabolite attained a significantly higher plasma concentration and remained much longer in plasma compared with fenbendazole and albendazole and their respective metabolites. The C_{max}, AUC and MRT of parent oxfendazole were more than 30, 68 and 2 times those of fenbendazole, respectively. The same parameters for ABZSO were also significantly greater than those of FBZSO. The ratio for total AUCs of both the parent drug and the metabolites were 1:42:7 for following fenbendazole, oxfendazole or albendazole administration, respectively. The AUC of (+) enantiomers of oxfendazole and albendazole sulphoxide was respectively more than 3 and 7 times larger than that of (-) enantiomers of both molecules. **Conclusion:** It is concluded that the plasma concentration of oxfendazole was substantially greater compared with fenbendazole and albendazole. The data on the pharmacokinetic profile of oxfendazole presented here may contribute to evaluate its potential as an anthelmintic drug for parasite control in dogs.

557 **The dog as an erratic intermediate host for *Taenia crassiceps***

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Taenia crassiceps is a cestode found mainly in the intestines of foxes (less often of dogs and cats). Small rodents act as intermediate host for the bladderworm. In the Netherlands, it is a rare infection in muskrats. A pup was presented to the veterinarian after being bitten by another dog. Suspected damage in the inguinal area due to trauma was the reason to perform surgery. Numerous macroscopically visible cysticerci were found in the inguinal region. Some were sent to the Faculty of Veterinary Medicine in Utrecht but definite diagnosis of the *Taenia* species by microscopic examination was impossible as the hooks of the scolices were pleomorphic in size and shape (they were not fully developed).

The pup deteriorated suffering from neurological problems and was euthanized. Necropsy showed large numbers of cysticerci, from 2 to 5 mm in size, subcutaneous in the inguinal region extending to the tarsal area. These cysticerci showed the characteristic budding. PCR and DNA sequence-analysis confirmed an infection with larval stages of *T. crassiceps*. Necropsy was performed on another puppy, from the same litter but from another owner, also showing neurological symptoms. In this dog an infection with the larval stage of *T. crassiceps* was confirmed again. In both pups encephalitis was diagnosed caused by canine distemper. This virus infection may have caused immune-suppression allowing the cestode to use dogs as their intermediate host.

This is the first documented case of a dog with a larval infection with *T. crassiceps* in the Netherlands.

558 **Comparison between four coproparasitological techniques for the efficiency in the diagnosis of eggs or cysts of enteroparasites in dogs**

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In this study we evaluated the frequency of enteroparasites in pet dogs and their association with age, sex and breed, as well as the efficiency of the Willis-Mollay, Faust, Sedimentation and Direct exam methods. By these methods we processed 401 fecal samples. The samples tested positive in the following numbers and percentages, *Ancylostoma spp.* (53.12%), *Toxocara canis* (20.70%), *Cystoisospora ohioensis* (15.71%), *Trichuris vulpis* (3.74%), *Dipylidium caninum* (2.49%) and *Taenia spp.* (1%). *T. canis* (67,3%) and *C. ohioensis* (47,3%) showed higher positivity in the puppies. The Willis-Mollay technique was more efficient in the diagnosis of *Ancylostoma spp.* and *T. canis* eggs. The direct method was the least efficient. It was found that the majority of the cases of *D. caninum* were diagnosed by the Sedimentation method (8=2%), while for *T. vulpis* the Willis-Mollay (12=2,99%) and Sedimentation (13=3.24%) were more efficient. In view of these results, we can recommend the association of the Willis-Mollay and Sedimentation methods for the diagnosis of gastrointestinal helminths. Due to the elevated occurrence of *Ancylostoma spp.* and *T. canis*, which are involved in zoonoses, it becomes necessary to apply more efficient prophylaxis of canine intestinal parasitoses.

559 **Active *Strongyloides stercoralis* infection in dogs from Brittany, France**

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Three female Golden Retriever dogs belonging to the same litter from a breeding located in Brittany were placed after weaning at the Alfort Veterinary School in November 2006. They were kept by veterinary students to follow a 15-month training and socialization period before their adoption by handicapped persons. In December 2006 at the age of 3 months, the three dogs presented diarrhoea, vomit and slight asthenia. These clinical signs were associated with the presence of faecal *Strongyloides stercoralis* L1 larvae.

The dogs were treated with fenbendazole given for 5 consecutive days (oral 50 mg/kg daily dose) leading to the disappearance of symptoms, but *S. stercoralis* larvae were still present in faecal samples processed with the Baermann technique. Fenbendazole treatment was renewed at the same regimen three weeks later but without parasitological cure as L1 larvae were still detected in the faeces one month later for the three dogs. Then, as the animals were tested negative for the MDR1 gene mutation, ivermectin was administered orally at 500 µg/kg during 7 consecutive days which resulted in negative coprological controls at one and two months after treatment (May 2007).

All other dogs of veterinary students expressed no clinical signs and faecal *S. stercoralis* larvae were not detected. A coprological survey conducted in February 2007 among dogs at the breeding in Brittany showed the presence of various parasites and *S. stercoralis* L1 larvae in two female dogs including the mother of the three former dogs. Follow-up of all animals is going on.

560 ***Ancylostoma braziliense* experimental infection in domestic cats:****Clinical and biological aspects**

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Canine and feline ancilostomes, besides their importance in public health as etiologic agents of cutaneous larva migrans, are significant parasites of domestic carnivores. Reports about clinical signs and life cycle of *Ancylostoma braziliense* in cats are scarce, what encouraged this work. Aiming to evaluate the pre-patent period, percentage of fixation on host and clinical aspects, 13 mixed breed three months-old kitten were orally infected with 600 third stage larvae of canine *A. braziliense* strain from pure coprocultures. Pre-patent period ranged from 17 to 20 days, and a total of 1718 adult *A. braziliense* were recovered from small intestine with range of intensity of 19 – 329, representing a percentage of larvae fixation of 22.2%. Anemia, wasting, ocular secretion, dark diarrhea with blood and abdominal dilatation were observed since 2nd week post-infection. The obtained data may facilitate future studies on experimental infection, possibiliting anthelmintic efficacy tests.

561 ***Toxocara cati* infections in a mice model: migratory pattern and pathological responses**

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The nematode *Toxocara canis* and *Toxocara cati* have a tissue-migratory phase in animals and produce the syndrome of larva migrant visceral in human. The objectives of the present research were to study the migratory pattern of *T. cati* and pathological response in mice model. Eggs of *T. cati* were collected from faeces of naturally infected cats, were embryonated and a doses of 1000 eggs were administrated to each mice. Animals were euthanized on days 1, 2, 3, 4, 5, 6, 14, 21, 28 post-inoculation. Samples of tissues were taken for digestion techniques and pathological study.

The highest recoveries of larvae on the different tissues were: in liver days 1 and 2 p.i.; in lungs day 2 p.i., in brain days 3 and 28 p.i. and in muscles there was a significant association between recovery of larvae and time post inoculation ($X^2 = 13,06$; $p < 0,016$), the highest value was on days 21 and 28 p.i. Macroscopic lesions were found in lungs and brain surfaces with hemorrhagic focus. Microscopic lesions were seen in liver, lungs, muscles, heart and kidneys, with oedematous areas and congestion of the parenchyma. Lymphocytes, neutrophils and eosinophils were present and in brain there were congestive areas without inflammatory reaction.

The results of the migratory pattern of *Toxocara cati* show similarities with *Toxocara canis* and the recovery of larvae in tissues shows the potentials to infection of the *T. cati* eggs presents in cats faeces with the possible hazard of transmission to human.

562 **Preliminary result of the natural infection with *Toxocara cati* in cats**

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Toxocara cati is a gastrointestinal nematode parasite of cats, these animals are the definitive hosts. Larval stages of this parasite have a tissue-migratory phase in cats. The objective of this study is to determine the presence of eggs, larvae and adult parasites of the *T. cati* in naturally infected cats. Twelve dead cats obtained from public spaces were analysed by necropsy. They were classified by age and sex, the digestive tract was observed and samples of the faeces were collected to analyse; samples of muscles, lungs, kidneys, liver and brain were taken for digestion techniques.

The results of the positive animals to faecal analysis were 25% of *T. cati*; 17% of *Toxascaris leonine* and 8% of both species. The 50% of the animals had adult forms of *T. cati* and the 20% of them had also *T. leonine*. The relation between the presence of adult form and the age of the animals was statistically significant ($p=0.04$; 95% CI= 24,5 - 108,8), the relation to sex was not significant. Larvae of *T. cati* were not found in tissues.

The prevalence of *T. cati* in faeces is similar to the result of other authors. The results observed in relation to presence of adult form and eggs in faeces may be caused by immature stages of *T. cati* and *T. leonine*, or due to the intermittent elimination of eggs in faeces. The larval stage in tissues of infected cats must be studied with more detail by means of the development of an infection in an experimental study.

563 **Nested PCR assay for the specific detection of *Aelurostrongylus abstrusus* (Nematoda, Strongylida) DNA in faeces and pharyngeal swabs from naturally infected cats**

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Aelurostrongylus abstrusus (Nematoda, Strongylidae) is a cosmopolitan parasite of cats transmitted by the ingestion of terrestrial molluscs and/or paratenic hosts. Adults live in the lungs of infected animals, causing conspicuous respiratory distress. Information on the biology and epidemiology of feline aelurostrongylosis is fragmentary, mainly due to the limits inherent in the classical diagnosis. In this work a nested PCR method has been evaluated for its ability to amplify the *A. abstrusus* DNA in biological samples from naturally infected cats. For each of sixteen cats naturally infected by *A. abstrusus*, four samples (i.e. faeces, floatation supernatant, Baermann sediment and pharyngeal swab) were subjected to a nested PCR specific for the ribosomal second Internal Transcribed Spacer (ITS2) of *A. abstrusus*. Conserved primers amplifying the entire nematode ITS2 were used in a first PCR round, followed by a second round using primer set AabFor-AabRev, specific for a 233bp region internal to the *A. abstrusus* ITS2. Amplicons were produced for 12 faeces (75%), 14 floatation supernatants (88%), 15 Baermann sediments and pharyngeal swabs (94%). Sequencing confirmed that all generated amplicons represented the appropriate species (*A. abstrusus* GenBank™ Accession Number: DQ372965). The present nested PCR assay showed a specificity of 100% and a sensitivity ranging from 75% to 94%. Despite the need for a further critical evaluation, this PCR method is a promising tool for a molecular diagnosis of feline aelurostrongylosis which overcomes the constraints of the classical diagnosis and for bio-epidemiological studies in both intermediate and definitive hosts.

564 **A study on intestinal helminthes of jackals in the western provinces of Iran**

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Since infection of canids with intestinal helminthes could be a leading source of infections in humans, the present work was conducted to study intestinal helminthes of jackals in the western provinces of Iran based on their autopsy data.

In this survey, 10 jackals were collected from the West Azarbaijan, Kordestan and Kermanshah provinces in the west Iran. After autopsy of animals, their intestines were searched for the presence of helminthes. The species, which were recovered, are listed as follows:

T. canis (10%), *T. leonina* (30%), *R. affinis* (50%), *T. hydatigena* (10%), *D. caninum* (20%), *M. lineatus* (70%), *J. pasqualei* (30%), *Alaria canis* (10%), *M. hirudinaceus* (30%) and *Macracanthomynchus* sp. (10%).

565 **A new technique for worm burden assessment of *Angiostrongylus vasorum* in experimentally infected foxes (*Vulpes vulpes*)**

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In a comprehensive study on angiostrongylosis including 28 experimentally infected foxes, a novel technique for reverse perfusion of the cardio-pulmonary vascular system was developed in order to recover a maximum of intact worms. Anaesthetized foxes were given heparin i.v. in order to prevent blood clotting during post mortem. Three minutes later a lethal pentobarbital dose was given, the thorax was opened and the thoracic organs were perfused in situ. The aorta, vena azygos and both venae cavae were clamped off. About three litres of isotonic perfusion liquid (sodium citrate, 15 g/l + NaCl, 8.6 g/l of tap water) were pumped via a 16G needle into the left atrium, through the pulmonary veins, the lung capillaries and the pulmonary arteries to the pulmonary trunc from which it was led via a plastic pipe (5 mm diameter) onto a sieve (200 µm aperture) for collection of worms. This procedure was followed by removal of the lungs, dissection of the pulmonary arteries and subsequent baermannization of the dissected lung tissue in normal saline. By estimating the overall worm burden as the sum of recoveries obtained by perfusion technique, dissection and baermannization, respectively, 49%, 40% and 11% of the total worm burden were recovered by each of these three methods, respectively. In contrast to dissection and baermannization, however, close to all worms recovered by perfusion were intact, indicating the obvious qualitative superiority of the latter technique. Results from a subsequent experimental study comprising 47 infected foxes (ending in May 2007) will also be presented.

566 **Competence of wolf (*Canis lupus*) as host of *Thelazia callipaeda* (Spirurida, Thelaziidae) eyeworms**

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Introduction: *Thelazia callipaeda* infects the eyes of humans, rabbits as well of domestic and wild carnivores. In the present work the first three cases of thelaziosis by *T. callipaeda* in wolves have been reported and the competence of this species of animal as a definitive host is discussed. **Materials and methods:** From March to October 2006, three male adult grey wolves (*Canis lupus*) were retrieved dead in the National Park of Gallipoli Cognato and of Pollino, Basilicata region, Southern Italy. Wolves were necropsized and adult nematodes were retrieved at the inspection of conjunctival sacs. All nematodes were collected and morphologically identified. All specimens were then molecularly processed to amplify and analyse a partial sequence of the mitochondrial cytochrome c oxidase subunit 1 gene (cox1). **Results:** A total number of 114 nematodes were collected from the eyes of the three wolves and morphologically and molecularly identified as *T. callipaeda* haplotype 1 (h1). **Conclusion:** The host competence of wolf was demonstrated by retrieving for the first time adult worms in the eyes of three specimens of *Canis lupus* living in an endemic area for *T. callipaeda* infection. This finding indicate that the spectrum of competent definitive hosts of *T. callipaeda* is much wider than for other species affecting cattle and horses (e.g. *Thelazia gulosa*, *Thelazia rhodesi* and *Thelazia lacrymalis*). Sequence analysis of the partial cox1 confirmed that only one haplotype of *T. callipaeda* is present in that area. The competence of wolves as a definitive host for *T. callipaeda* must be considered in view of the relevance of wild fauna in maintaining and spreading the eyeworm infection in humans and domestic animals.

567 **Important helminthozoonoses circulating in red fox (*Vulpes vulpes*) as wildlife reservoir**

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Zoonoses with a wildlife reservoir are considered to be a major public health problem, affecting all continents. The importance and recognition of wildlife as a reservoir of zoonoses are increasing. As a part of biocenosis, red fox in the wild participates not only in maintaining of the cycle in nature, but also in the spreading of several parasitic diseases. The most important are alveolar echinococcosis caused by small tapeworm *Echinococcus multilocularis*, trichinellosis, and toxocarosis. The systematic long-term investigation of parasitofauna of red fox was carried out during the period of 2000 – 2006 covering the whole area of the Slovak Republic. The aim of the study was to determine the prevalence of parasites in this host and to illuminate their role in the epidemiology of parasitozoonoses in both humans and animals with special regard to zoonotic species. More than 4000 red foxes were inspected for *E. multilocularis* infection, trichinellosis, and toxocarosis using sedimentation and counting method, artificial digestion of muscle samples, and standard coprological methods. The study revealed that 85.6 % of red foxes harboured parasite infection. Regional differences in prevalence and infection intensity are influenced by climatic conditions, landscape use, anthropogenic and other ecological factors. From veterinary point of view, foxes represent source infection for farm and domestic animals, not only in rural, but more frequently in urban areas.

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Poster group 14: Echinococcosis

568 Echinococcosis/hydatidosis prevalence in animal and human in Nepal

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A total of four hundred fifty slaughtered buffaloes and eighty-one slaughtered goats in various localities of Banepa, Panauti and Dhulikhel Municipalities were examined for hydatid cyst during Feb 2002 to Feb 2003. A surveillance study was also carried out with butchers, meat sellers, farmers and meat consumers to study the awareness of hydatidosis /echinococcosis infection. Out of four hundred and fifty slaughtered buffaloes and eighty-one slaughtered goat examined. Seventy-three (16.22%) buffaloes and seventeen (19.33%) goats were found positive hydatidosis. Sexwise prevalence rate of infection was 22.98% male buffaloes, 40.57% female buffaloes and 4.02% calves. The results reveal that females were infected more than males. The hydatidosis infection goat (19.33%) was only in the fatty body. Out of two hundred and eighty five slaughtered buffaloes and forty-seven slaughtered goat examined in Banepa municipalities, fifty five (19.29%) lungs and three(1.5%) livers were found positive in buffaloes and eleven(23.4%) were found positive in fat body of goat. In Panauti Municipality, eighty slaughtered buffaloes and eleven slaughtered goats were examined for the hydatidosis. Ten (12.50%) were found positive hydatidosis in buffaloes and two (18.18%) were found positive hydatidosis in goats. In Dhulikhel Municipality, eighty-five slaughtered buffaloes and twenty-three slaughtered goats were examined for the hydatidosis. Eight (12.50%) were found positive hydatidosis in lungs of buffaloes and four (17.39%) were found in fat body of goats. The patients which were selected in door and out door patients basically sign and symptoms of hydatidosis. Twenty-two patients which were suffered from the helminthes diseases, two (9.09%) patients infected hydatidosis by the biopsy case in the Sheer Memorial Hospital Banepa and Dhulikhel Hospital. The result reveals that the infection rate is higher in fact in uncooked meat consume and contamination of hydatid infected dog faeces with vegetables & fruits. Basically 25 to 35 age groups, which is dynamic and consumed any types of meat whose cause is infected hydatidosis. The findings of the surveillance study reveals that echinococcosis/ hydatidosis are prevalent in the research areas of Banepa, Panauti and Dhulikhel due to various factors, via uncontrolled slaughtering buffaloes and goats, uncontrolled stray dogs, lack of meat inspection program, unawareness of the meat borne diseases, unhygienic condition of slaughtering places and environmental pollution etc.

569 Hydatids in the garden: encroachment of *Echinococcus granulosus* into urban areas in Australia

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Intestines from 117 wild dogs (dingo and dingo/domestic dog hybrids) from coastal Queensland (Fraser Island and an area west of Maroochydore on the Sunshine Coast) were examined for the presence of intestinal helminths. *Echinococcus granulosus* was found only in wild dogs from the Maroochy Shire(43.5%) caught in and around urban areas. The reasons for wild dogs entering urban areas and the public health implications of *E. granulosus*-infected wild dogs infiltrating urban areas are discussed.

570 **Canine alveolar echinococcosis: report on 20 cases in Germany diagnosed between 1995 and 2007**

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Objective: To describe the signalment, clinical and pathological findings in 20 cases of canine alveolar echinococcosis (metacestode stage of *Echinococcus multilocularis*) in Germany.

Material & Methods: Twenty dogs originating from Germany with confirmed alveolar echinococcosis by histopathology, PCR and/or serology between June 1995 and March 2007 were included in this retrospective study.

Results: The age of the dogs at first presentation ranged from 11 months to 10 years (median 3.5 years). Fourteen dogs were female and 6 male. There were 11 breeds including Labradors (n=5), Terriers, German Shepherds, German Spaniels and Münsterländers. All but one dog had not been used for hunting. In most cases a progressive abdominal distension was recorded. Clinical examinations including abdominal palpation, radiography and/or ultrasonography revealed an enlarged liver with multiple cyst structures resulting in the putative diagnosis of a liver tumour. Most of the animals were euthanized at the time of exploratory laparotomy because of unfavourable prognosis; 3 dogs received surgical and subsequent albendazole therapy for months. Main findings at necropsy were peritonitis, ascites and extensive liver infiltration with masses of pinhead- to fist-sized cysts with caseous, jelly or fluid contents. In few cases the spleen or pancreas was involved. Histologically, the cysts consisted of an outer, acellular laminated layer and inner germinal layer and contained calcareous corpuscles. In half of the cases protoscolices were present.

Conclusions: Alveolar echinococcosis has to be included as a possible differential diagnosis in dogs with cavitary liver masses, even in young animals.

571 **Anthelmintic baiting to wild foxes by local residents for the control of alveolar echinococcosis in Hokkaido (Koshimizu and Kutchan), Japan**

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Alveolar echinococcosis endemic area in Japan is restricted to the northern island of Hokkaido. Prevalence of the parasite among wild red foxes, the main natural definitive host in Hokkaido, increased during 1980s and 1990s and was about 40% in the last decade (Hokkaido Government). Since 1998 we distributed anthelmintic baits and evaluated the deworming effect by the coproantigen- and egg-positives rates in field collected fox faeces in a pilot area (Koshimizu-200km²).

In Koshimizu, the coproantigen- and egg-positive rates were 20-30% in 1998 (beginning of the study) and reduced to 0-2% in 2002 after baiting and the bait distribution stopped in the end of 2002. The rates increased to 5% and 9%, in August of 2003 and 2004, respectively. After the increasing of the rate, local residents started the bait distribution at intersections (200 points) between roads, and in windshield forests and the borders of forests during May to December (2,000 baits/time, monthly and bimonthly, in 2005 and 2006, respectively) and collected fox faeces along roadside in August. The rate reduced to 5% and 0%, in 2005 and 2006, respectively.

In a new pilot area (Kutchan-100km²) the coproantigen-positive rates were 21% (55/268) in 2005, local residents distributed the baits along road during May to December in 2006 (1,300-1,500 baits/time, monthly). The coproantigen-positive rates were reduced to 2% (2/104) in October.

In the both area the positive rates were reduced significantly. The current practice of involving local residents is believed to be a driving force for large-scale control in future.

572 ***Echinococcus multilocularis* infection in a Lemurian *Varecia variegata***

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A naturally occurring *Echinococcus* infection in an 4.5 -year-old lemurian Maki vari (*Varecia variegata*) was diagnosed in the clinics of zoo and exotic animals in Veterinary School of Nantes. The lemurian was transferred for a progressive abdominal enlargement and listlessness. Echotomodensitometry revealed a large and poorly delimited intraabdominal mass. A laparotomy was performed and showed an invasive tumor-like lesion including a part of liver and spleen with several nodules 3 to 5 cm in size. Post mortem Histopathology revealed the parasitic origin. The infection was attributed to *Echinococcus multilocularis* based on histopathological examination and the shape and size of hooks of the protoscolices. The lemurian was born in France. It is considered that the infection was most probably acquired from foxes of the area through contaminated branches or foliage. This is the first description of this fox tapeworm infection in this species. It is concluded that this lemur may be a favourable intermediate host for *E. multilocularis*. The animal had changed of park since 2 years. The special epidemiology of this case is discussed.

573 **The carriage of the larval forms of *Echinococcus multilocularis* and other cestodes by the musk rat (*Ondatra zibethicus*) trapped along the river Ourthe and its tributaries (Belgium)**

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In Belgium, the carriage of *E. multilocularis* by the red fox can be very high. This study was designed to evaluate the carriage of the larval form of *E. multilocularis* and other cestodes in a musk rat population trapped along the river Ourthe. Six hundred fifty seven musk rats were necropsied. The larval cestodes of the abdominal and pleural cavities were identified. In the case of *E. multilocularis*, the fertility of the cysts was verified in 58 liver samples. The following species were found : *T. taeniaeformis* (65.75%), *T. martis* (22.22%), *E. multilocularis* (22.07%), *T. polyacantha* (2.59%), *T. crassiceps* (0.91%). Results were analysed according to the place of capture (upper, middle and lower Ourthe). There was a highly significant relationship between the carriage of *E. multilocularis* and the place of capture (the prevalence being higher in the upper side of the river). This difference could be due to the geoclimatic conditions prevailing there. All but one sampled liver lesions were found to contain protoscoleces of *E. multilocularis* (98.8%). The musk rat is probably infected through the consumption of feed contaminated by the fox's faeces. The red fox can occasionally prey on living musk rats but the musk rat cadavers which are left on the river banks by the trappers are probably also consumed. In conclusion the musk rat seems to be highly receptive to *E. multilocularis* and in Belgium could play the role of reservoir; when present this species could represent a cheap and sensitive bioindicator for monitoring this zoonosis.

574 **Hydatidosis: study of the immune response of a promising new orale vaccine in dogs**

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Hydatidosis causes high levels of mortality and morbidity in many part of the word. Dogs are the main source of the disease. A vaccine could be an efficient contribution for control programs in endemic countries. In this study, a new orale vaccine against *E.granulosus* in dogs was prepared using two recombinant proteins from adult worms in a live attenuated strain of *Salmonella enterica serovar Typhimurium*.

This work was conducted parallely in Morocco and in Tunisia. The vaccinated dogs received 2 oral doses of the vaccine 21 days apart, and they were challenged, with controls, 20 days later with 75000 living protoscoleces. All dogs were sacrificed 26-29 days post-challenge.

An Immunostaining study was carried out to identify the immune response in duodenum. The level of immunoglobulins in the serum and the histological response to the vaccine in duodenum were evaluated.

The vaccination induces a strong decrease of parasite burden in vaccinated dogs (70 to 80%) and a slowing of the development for the remaining worms. The number of plasma cells decreased from the base to the top of the, with a clear imbalance in the ratio of B to T lymphocyte. However any immunoglobulins were detected in the serum. The vaccine shows an important local humoral immune response, compared to a absence of a systemic immune response.

The Salmonella vaccine EgA31- EgTrp showed strong efficacy against *E.granulosus*. That points out the interest of this vaccine to reduce the transmission of the parasite to humans and animals.

575 **Assessment of microsatellite polymorphism for improving the spatial distribution tracking of *Echinococcus multilocularis***

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Alveolar Echinococcosis (AE) – caused by the cestode *Echinococcus multilocularis* - is a severe zoonotic disease found in temperate and arctic regions of the Northern Hemisphere. Even though the transmission patterns observed in different geographical areas are heterogeneous, the nuclear and mitochondrial targets usually used for the genotyping of *E. multilocularis* have shown only a marked genetic homogeneity in this species. We used microsatellite sequences, which have distinct typing resolutions, to explore the genetic diversity of *E. multilocularis*. Four microsatellite targets (EmsJ, EmsK and EmsB, specifically designed for the present question, and NAK1, selected from the literature, were tested on a panel of 76 *E. multilocularis* samples (larval and adult stages), obtained from Alaska, Canada, Europe and Asia. Genetic diversity for each target was assessed by size polymorphism analysis. With the EmsJ and EmsK targets, 2 alleles were found for each locus yielding 2 and 3 genotypes, respectively, discriminating European isolates from the other groups. With NAK1, 5 alleles were found, yielding 7 genotypes, including those specific to Tibetan and Alaskan isolates. The EmsB target, a tandem repeated multilocus microsatellite, found 17 alleles showing a complex pattern. Hierarchical clustering analyses were performed with EmsB findings and 29 genotypes were identified. Due to its higher genetic polymorphism, EmsB exhibited a higher discriminatory power than the other targets. The complex EmsB pattern was able to discriminate isolates on a regional and sectoral level, while avoiding over-distinction. EmsB will be used to assess the putative emergence of *E. multilocularis* in Europe.

576 **Sero-diagnostic potency of purified fractions of hydatid fluid and protoscoleces of both camel and donkey origin**

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The taeniid tapeworm *Echinococcus granulosus* is the causative agent of the echinococcal disease, an important zoonosis with worldwide distribution. Accurate immunodiagnosis of the infection requires highly specific and sensitive antigens to be used in immunodiagnostic assays. The specificity and sensitivity of enzyme-linked immunosorbent assays (ELISA) and Western immunoblot assays in detecting antibodies in hyperimmune sera and naturally infected sera from camels and donkeys suffering cystic hydatid disease (*Echinococcus granulosus*) are compared using crude and purified antigen preparations (total hydatid fluid and homogenates of protoscoleces of either camel or donkey origin). Gel filtration was used to purify crude antigens from hydatid fluid (HFC& HFD) and protoscoleces of hydatid cysts (PC & PD) of both camel and donkey). Two purified peaks were obtained from each crude extract (HFCp1, HFCp2, HFDp1, HFDp2 PCp1, PCp2, PDp1 and PDp2). The crude antigens and the purified peaks were resolved by SDS-PAGE which revealed distinct variations among them. ELISA was performed twice; the first one was carried out to evaluate the prepared antigens against their versus hyperimmune rabbit sera. The results demonstrated the sensitivity of ELISA was 100% using PCp1 and HFDp2 in serodiagnosis of hydatidosis in camel and donkey. The second ELISA was performed using the later two purified peaks against sera obtained from naturally infected camels and donkeys with hydatidosis or with other parasites. The specificity of ELISA was 97.6 % and 95.9 % in camels and donkeys respectively. Western blot analysis recognized two polypeptides at 80 and 150 KDa which might be diagnostic bands in the two intermediate hosts. While the epitopes at 21 and 59 KDa might be specific for camel hydatidosis. The common shared reactive band at the level of 138 KDa might be specific for hydatidosis in donkeys.

577 **Development of a multiplex PCR to identify carnivore origins of feces for applying to epizootiological surveys of *Echinococcus multilocularis* in Hokkaido, Japan**

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Epizootiological information of *Echinococcus multilocularis* could be obtained from field-collected carnivore (mainly foxes) feces, however, the origins of feces were ambiguously identified in previous studies. To identify carnivore origins of feces collected in Hokkaido, Japan, a multiplex PCR system was constructed. Six forward primers were designed in the D-loop region of mitochondrial DNA, each of which was specific to fox, raccoon dog, dog, cat, raccoon and weasels. A reverse primer used was mammalian common (modified from Gerloff et al., 1999). The sizes of PCR products were ~160bp for fox and cat, ~240bp for raccoon dog and raccoon, and ~330bp for dog and weasels. In order to distinguish animals by the size of PCR products, two primer mixtures, Mix1 (fox, raccoon dog and dog) and Mix2 (cat, raccoon and weasels) were used simultaneously in the system. In field application, fecal DNA was extracted from surface washings of frozen feces using QIAamp DNA Stool Mini Kit (Qiagen) to minimize PCR inhibition due to fecal PCR inhibitors. When 272 field-collected feces were examined, origins of 251 samples (92.3%) were identified by their single products of the target sizes. Overall proportions for positive result in *E. multilocularis* coproantigen and taeniid egg examinations were 33.7% and 11.1%, respectively, whereas those for individual animals were 38.6% and 12.9% in foxes (n=210), 26.3% and 0% in cat (n=19) and 0% and 0% in other animals, respectively. The results suggest that the developed multiplex PCR would be a valuable tool in field surveys for *E. multilocularis*.

578 **Diagnosis of infected dogs with *Echinococcus. granulosus* by Dot-blotting test**

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The definitive hosts of the *Echinococcus. granulosus* parasite, are carnivores such as dog and wolf. So the diagnosis of infection in dog can reduce hydatidosis in intermediate hosts such as human, sheep and cattle.

In the present study, dot-blotting test for *Echinococcus. granulosus*-specific coproantigens has been evaluated in dog. Three puppies of 2-3 months of age of mixed sex were treated by piperazine and a few days later their fecal samples were collected as pre-infection samples. Seven days after treatment, the puppies were fed with hydatid-cystic livers and lungs of sheep. At the end of each week (till 5 weeks) their fecal samples were collected as post-infection samples. Soluble protein of pre and post-infection of fecal samples were prepared and dot-blotting test was conducted. Also for detection of parasite protein bands, polypeptide profile was analyzed by SDS-PAGE.

The results showed that, after the fourth post-infection incremental of spot colors was observed in experimentally infected dogs.

In SDS-PAGE, four bands were observed in fifth- post infection week sample. The molecular weights of the fifth-week sample bands were 14, 22, 36 and 45 kDa respectively. The results in this study indicated that dot-blotting and SDS-PAGE methods are suitable for detection of *Echinococcus granulosus* infection in dog four weeks after infection.

579 **Evaluation of a coproantigen enzyme linked immunosorbent assay for the diagnosis of canine echinococcosis in Iran**

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Cystic echinococcosis has been recognized as a major public health problem in sheep-rising regions of the world. Different studies in Iran have shown that echinococcosis is a main problem among human and domestic livestock in several provinces of the country. Due to this fact that providing a reliable diagnostic method for detection of this infection in definitive host is a critical pre requirement for establishment of appropriate control programs in our country, we designed this project for evaluation of coproantigen ELISA (CA-ELISA).

For performing the study, one hundred and sixteen carnivores including 80 stray dogs , 27 jackals, 8 foxes and one wolf were collected from rural areas of Hamadan, Azarbaijan and Tehran provinces. All the specimens were examined by direct microscopic examination of intestinal contents and CA- ELISA using a standard commercial kit (Echinotest® , Dr. Bommeli Diagnostics , Switzerland).

The overall prevalence of canine echinococcosis using CA-ELISA was 43.1% (50/116) in comparison with microscopic method that showed 37% (43/116). The sensitivity and specificity of the CA-ELISA as referenced by necropsy findings was 72.1% and 74% respectively.

So we found this assay to be a suitable and advantageous method for surveillance of canine population especially in regions with endemic echinococcosis.

580 **Determination of electrophoretic patterns of proteins of hydatid cyst of two strains of *Echinococcus granulosus* in Iran**

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Hydatidosis caused by *Echinococcus granulosus* is one of the most important zoonotic infections, which are of high hygienic and economic importance. The purpose of the present study was to identify electrophoretic pattern of different strains of hydatid cysts endemic in Iran ecosystem.

32, 18, 14, 7, 3 samples from liver and lung were obtained from sheep, camels, buffalos, cattle and goats, respectively, and were prepared for electrophoresis. Furthermore, rabbit antisera were obtained after injection of laminated layer, fluid cyst and protoscolices from hydatid cysts of camels and sheep. Immunogenesis of the different extracted antigens were evaluated using positive sheep antiserum against hydatid cysts.

This study demonstrated that antigens of laminated layer in hydatid cysts from species of sheep, camels, buffalos, cattle and goat have similar bands of 25, 26, 27, 29, 38, 42, 55, 68, 83 and 145 KD. In the laminated layer of camel hydatid cyst there is a thick band of 68-76 KD. This antigen has also a band of 122 KD which was not present in other antigens. A band of 116 KD was found in the hydatid cysts of sheep, buffalos, cattle and goats.

Fluid cyst and protoscolices antigens were very similar in inquired species. Different bands of 200, 160, 116, 98, 83, 72, 54, 50 and 42 KD from laminated layer were immunogenic in reviewed species. Besides, a band of 122 KD from laminated layer of camel cyst was immunogenic which shows that this band is specific.

581 **Survey of appearance of parasite antigen in experimental sheep hydatidosis by coagglutination method**

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Introduction: One of the most important problems in hydatidosis is cyst diagnosis in human and animals. In the present study, for decrease of bias of antibody detection, antigen detection was standardized. Since, evaluation of hydatid antigen detection in human is restricted; Coagglutination test was evaluated in experimental sheep hydatidosis in serum and urine.

Procedure: Hydatid cysts of sheep liver and lung, collected from Ahvaz abattoir, were used. The hydatid fluid and protoscolices were obtained in laboratory. The centrifuged hydatid fluid was injected to rabbits in two steps. The rabbit hyperimmune sera were collected when the antibody titer reached 1/2048 of IHA test. Also, each dog was given 15,000 viable protoscolices. Less than two months later, as soon as the taenid eggs were observed in the dogs stools, the dogs were autopsied, after euthanasia. The small intestines were opened and *Echinococcus granulosus* were collected and placed in physiologic saline. Almost, 2000 eggs were orally administered to each of 13 sheep. The six other sheep were kept as controls. All sheep were bled each week, whereas, their urine samples were collected once a fortnight. All sera and urine samples were then examined with coagglutination test.

Results: The sensitivity of coagglutination test, in detecting the hydatid antigens in the sera of infected sheep, was nil during five weeks of post-infection (p.i.). The sensitivity increased to 23% in sixth week and it was 100% only in 12th and 13th week of p.i. Then it decreased in the following weeks. However, the specificity of the test was 100% by examining the non-infected sheep sera, throughout the experiment. Examination of the infected sheep urine resulted positive reaction only from 6th week of p.i. and the sensitivity gradually reached to 100% only in 12th week of p.i. and the specificity of the test for non-infected sheep urine samples remained 100% too.

Discussion : It seems that the time of appearance of hydatid antigens in serum and urine are approximately equal. Although positive results are very valuable, the negative results are not indicative of hydatid cyst absence in patient, for the test was negative during the first five weeks of p.i. and its sensitivity rate was very variable throughout the following weeks, due to different amounts of hydatid cyst antigens in blood and urine.

Poster group 15: Fasciola and Trematodes

582 The value of pooled sera for the estimation of seroprevalence of liver fluke in a herd of beef cattle

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Introduction : The serology method from the Institute Pourquier (Montpellier, France) allows to perform an early diagnosis of an infection with *Fasciola hepatica*. The purpose of the study was to validate the serological analysis of pooled serum samples for the evaluation of the seroprevalence of fasciolosis in a cattle herd.

Materials & Methods : In a population of 300 beef herds in the Vendée Département (France) analysis of 1 pooled serum sample per herd allowed to select 20 herds assumed to be seropositive and another 20 seronegative herds. In the individual samples of each of these herds, the laboratory selected at random 13 sera from heifers (18-30 months) and 13 sera from cows (> 30 months). In total 1040 individual sera and 40 pooled sera (aliquots of 13 cattle from each group). All sera have been analyzed using the same automated method and the semi-quantitative results expressed as the optical density S/P ratio, the positive threshold being /30%. The average S/P results were 5.8% and 7.9% respectively in the heifers and the cows from the negative groups and they were 179.5 % and 178.4% respectively in the heifers and the cows from the positive groups.

Conclusion : The correlation between the S/P value of pooled sera and the frequency of positive S/P values of individual sera from cattle of the same group is $R^2 = 0.76$ in the groups of heifers and $R^2 = 0.86$ in the groups of cows.

583 Choice of sera in the large Charolais cattle herds for the serological diagnosis of liver fluke - Approach per pasture group

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Introduction : A semi-quantitative expression of the results of the serological Pourquier ELISA method has been validated using pooled serum samples (PSs) to evaluate the prevalence of *Fasciola hepatica* in a beef cattle herd. But, how should one select which samples to pool from a large herd of adult cattle?

Materials & Methods : In the practice of Epinac, blood samples have been collected per pasture group from all Charolais cattle older than 2 years. In the laboratory a pooled sample was prepared starting from 5 individual sera collected from each pasture group with 25 to 35 cattle (cows + bull) each, all rotating on the same pastures. Subsequently 42 pooled samples were assayed for 42 pasture lots spread over 10 herds. The results of the negative pooled samples (PSs) have an average S/P = $12.37 \pm 7.5\%$ and those of the positive PSs have an average S/P of $143.0 \pm 62\%$, with a positive threshold S/P/30%.

Results : The results of 293 PSs from pasture groups coming from 73 Charolais herds confirms a clear differentiation of the test results between seronegative and seropositive groups within the same herds.

Conclusion : The diversity of the parasitic risk, depending from the presence of humid and contaminated zones grazed by each pasture group within a same farm, has to be taken into consideration. This approach seems to be the most appropriate for the serological diagnosis of liver fluke and subsequently the implementation of the most adequate control or treatment measures .

584 **Seroprevalence of bovine fasciolosis in herds of beef breeds in France**

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Introduction: Fasciolosis due to *Fasciola hepatica* is still widespread, although underestimated, especially in the Charolais and Limousin breeds. Because of the low sensitivity of the coprological diagnostic method and the low feedback on rejected livers from the slaughter houses towards the veterinarian and the farmer, Janssen Animal Health France has organized a large serology survey in the major areas of beef cattle covering 19 Départements in France.

Method & Materials: During the 2004-2005 prophylactic brucellosis campaign, 132 veterinarians collected blood from all cattle of more than 24 months old in herds known to be at risk for fluke infection. The local laboratory prepared 1 or 2 pooled serum samples per herd (starting from aliquots of 10 individual randomized sera). All the pooled sera have been assayed using the ELISA method of the Institute Pourquier (Montpellier, France). In total 1331 herds have been involved in the survey. This represents 15 to 155 herds per Département, which was correlated with the number of herd per Département. The average cattle sample represented 5.1% of the cattle population in each Département (range 4 to 12%). Results: With a positivity threshold with a S/P optic density ratio / 30%, the seroprevalence of fasciolosis reached in average 48.8% for the total of the tested herds. A variation between 15 to 88% was noticed in between the Départements. The highest prevalence was observed in the areas with the highest cattle density.

Conclusion: The authors focus on the importance of the presence of the infected snail population in some wet areas or streams and the application of inadequate treatment programs in the control of liver fluke.

585 **Investigations on the role of wildlife in the spread of resistant liver flukes**

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Resistance of liver fluke (*Fasciola hepatica*) to triclabendazole (TCBZ) has been reported from Australia, Ireland, Wales, Scotland and Spain. In the Netherlands, the first case of TCBZ-resistance has been detected on a farm in the province of North Holland in 1999 in cattle and sheep. In the following years, resistance was observed on several farms in the vicinity of the first farm, almost spreading as an oil spot. The most likely cause of spreading of TCBZ-resistance is by introduction of infected animals on other farms. However, some farmers assure that their farm is 100% closed and no other animals have been introduced. A possible other source of spreading is by wildlife. In a three year study (2006 – 2009), the role of wildlife (hares, roe deer and musk rats as most important candidates) will be investigated. In cooperation with the Royal Netherlands Shooting Association, hunters were asked to support this study by collecting livers from hares shot in the 'resistant endemic' area. In this area also trapped musk rats were collected. Livers from hares and musk rats were carefully investigated for the presence of flukes. In 2006, 59 livers from hares and 39 livers from musk rats were examined. Fifteen livers from hares were positive for flukes with a mean number of 6.5 per liver. All musk rat livers were negative for liver fluke. At a later stage, the collected liver flukes will be tested for drug resistance with molecular biological techniques.

586 **The prevalence of fluke intermediate stages in *Lymnaea natalensis* collected from streams around Zaria, North Western Nigeria**

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Numerous studies abound on fasciolosis in North Western Nigeria but little is known about the snail intermediate hosts. A survey was carried out between July 2006 and October 2006 on the prevalence of fluke intermediate stages in *Lymnaea natalensis* snails recovered from various isolated breeding sites in Zaria, Nigeria. Snails were collected, identified and dissected for fluke infections. A total of 326 snails were collected out of which 166 (50.9%) were positive both for *Lepodermis* and *Fasciola cercariae* and 99 (30.3%) for *Fasciola cercariae*. The shell length of the snails ranged from 8mm – 16mm. There was a relationship between size of infected snails and intensity of cercarial infection. The highest prevalence was observed in the month of October and the lowest in July.

587 **Estimating the prevalence of *Fasciola hepatica* infection in dairy cow farms in Schleswig-Holstein, Germany**

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Objective: To estimate the current prevalence of *Fasciola hepatica* infections in dairy cow farms in the federal state of Schleswig-Holstein, northern Germany.

Material & Methods: Two cross-sectional surveys were performed between November 2005 and April 2006. In the first survey bulk-tank milk samples collected from 35% of the approximately 6000 dairy herds of the federal state of Schleswig-Holstein were examined by a commercial ELISA for detecting *F. hepatica*-specific antibodies (Institut Pourquier). The second survey was performed on 131 farms randomly selected from the members of the cattle association of the Dithmarschen district, Schleswig-Holstein. On each farm faecal and milk samples of 7 cows were examined by the sedimentation method for *Fasciola* eggs or the milk ELISA for *Fasciola*-specific antibodies, respectively; one bulk-tank milk sample was additionally examined. Data on farm and individual cow profiles were collected by a questionnaire and exploratively analysed.

Results: In the first survey *F. hepatica*-specific antibodies were detected in 50% of the 2136 bulk-tank milk samples collected throughout Schleswig-Holstein. In the second survey 15% of all cows examined (n=917) and 47% of the farms were positive for *Fasciola* eggs. *Fasciola*-specific antibodies were detected in 45% of the cows, and the herd seroprevalence was 74% in the Dithmarschen district.

Conclusions: Currently the *F. hepatica* infection is highly prevalent in dairy cow farms in Schleswig-Holstein.

588 **A bulk milk epidemiological study of *Fasciola hepatica* infection in dairy cattle in Northern Germany**

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Fasciolosis caused by the trematode *Fasciola hepatica* is a worldwide parasitic disease common in ruminants, especially cattle, sheep and goats. It may, however, affect humans. The parasite is found in regions with temperate climates and once ingested, migrates through the wall of the small intestine and into the liver parenchyma to reach the bile ducts. The subclinical and chronic disease usually results in decreased production of meat, milk and wool, secondary bacterial infections and fertility problems.

The purpose of this study was to determine the prevalence of *Fasciola hepatica* antibodies in dairy herds in the north-western part of Germany and to evaluate factors that may influence the seroprevalence of the disease in this area. 817 bulk milk samples were collected from different farms in January 2006 and again in November 2006 and tested for antibodies against *Fasciola hepatica* by ELISA using the Pourquier® ELISA Serum & Milk kit.

Of the tested herds 53% and 54%, respectively, were positive for *Fasciola hepatica*, with 43% and 44%, respectively, testing strongly positive (>60% of the individual animals are positive in the flock). A questionnaire was sent to the owners of the farms requesting data on the size of the herds, type of housing and pasture, timetables for the period of grazing, number and age of animals grazing together, appearance of parasites in the flock, treatment plans against parasites, usage of liquid manure on the pasture and the type of forage. The evaluation of this data compared to the ELISA results, showing a high prevalence for *Fasciola hepatica* in this region, will be presented.

589 ***Fasciolasis pestholes in the North-West of Russia (Novgorod region)***

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Fasciolasis is a common cattle disease transmitted by lymnaeid molluscs. The most effective strategy against fasciolasis should be based on the control of parasite pestholes, maintained by molluscs. So our aim was to describe snail habitats on cow pastures and to appreciate the roles of different lymnaeids in the pestholes. We investigated some fasciolasis pestholes in Novgorod region, Russia in 2004-2006. Some of caught molluscs were dissected to reveal the parasite. The snails grown up in laboratory were infected by *F. hepatica* miracidia (3-50 per animal).

We have detected 5 *Lymnaea* species, potential hosts of *F. hepatica*: *L. pereger*, *L. truncatula*, *L. palustris*, *L. stagnalis*, *L. glabra*. *L. pereger* and *L. truncatula* appeared to be the most common species on all pastures. The habitats the mentioned lymnaeids are: I. Pools, footprints; II. Drains, ditches and tracks watered constantly; III. Drains watered not constantly; IV. River banks. *L. pereger* has adjusted to all these habitats. *L. truncatula* don't live in ditches and drains deeper than 15 sm, but survive successfully in the rest types of biotopes. The rest species are rare. Wrong exploitation of pastures provides successful reproduction of fasciolan potential hosts.

L. truncatula and *L. pereger* experimentally invaded by *F. hepatica* demonstrated different patterns of infection. In *L. truncatula* the "partenogenetic" part of the cycle takes 30-50 days, while in *L. pereger* it takes 90 days. These host species play complementary roles in *F. hepatica* pestholes. Due to *L. pereger* the parasite survives severe conditions and due to *L. truncatula* it benefits in favourable situations

590 **Prevalence of *Fasciola hepatica* in dairy cattle from an Andean region in Venezuela (Bailadores, Mérida, Venezuela)**

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Introduction Considering the number of livers condemned in the Abattoir of Bailadores (Mérida, Venezuela), *Fasciola hepatica* seems to be a significative problem for cattle in this Andean region. **Objective:** to estimate the prevalence of *Fasciola hepatica* in dairy cattle from Bailadores (Mérida, Venezuela) on the basis of coprological examination.

Materials and Methods: -5 farms (F1 to F5) located at the surroundings of Bailadores (Mérida, Venezuela), at an altitude of 1500-3000 meters over sea level and 104 bovines (Breeds: Jersey, Swiss Brown, Holstein, and mixed breed) representing approximately 50% of the total population of each farm (F1 n=20, all females, 19 adults, one calf. F2 n=24, all female 20 adults, 4 calves; F3 n=20, 17 adults, 1 calf, male and all other females. F4: n=20, all females 19 adults, one calf. F5: n= 20, all females, 17 adults and 3 calves). Coprological samples were taken from the rectum, once from each animal and processed using the Sedimentation technique. The area near of water collection or irrigation system of each farm was checked looking for the intermediate hosts (Lymnaeid snails). **Results:** All farms (100%, 5/5) were positive to *Fasciola* infection. Prevalence was: F1: 50%; F2: 12,5%; F3: 15%; F4: 35%, F5: 15%.. General prevalence was 25,74%. Lymnaeid snails were found in all farms and natural infection with this trematode was detected in snails from two farms (F1 and F2). **Conclusions:** Fasciolosis is a widespread parasitic disease in this zone and an important factor to consider for improving the milk production there.

591 ***Fasciola hepatica* in Brazil: current situation and perspectives**

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Although *Fasciola hepatica* is known to be endemic in some parts of Brazil, little data is available on this parasite in terms of importance and economical impact. The situation is more evident in data from slaughter house surveys where liver damage is evaluated and the level of infection can be measured. Previous data from the Federal Inspection System on 351,902 adult cattle slaughtered from 253 municipalities from the Central, Southeast and Southwest regions of Rio Grande do Sul State revealed that 67 cities (26.5%) had infection rates above 20% (Boa Vista do Incra: 68.2%; Maquine: 84% and Presidente Lucena: 91.3%) and only 14.6% of the cities did not have a positive animal. All the cities with major prevalence were at the lowest topographic levels of the State, with high soil humidity. Enumeration of adult parasites and evaluation of organ damage is also a part of current studies. Data on liver tissue damage and parasite counts was scored from 0 to 5. The overall prevalence of adult worms found in livers was 35.03%, with a relatively low pathogenicity score of 2, with burdens of 6 to 50 adult parasites. Monte Carlo simulation is in progress for analysis of all data (parasite count, number of bovines, region, number of animals slaughtered, age) to estimate the economical impact.

592 **Co-infections with the liver fluke, *Fasciola hepatica* and *Salmonella dublin* infections in dairy heifers**

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Introduction. Experimental data have indicated that infections with the liver fluke, *Fasciola hepatica* may prolong the duration of *Salmonella* infections as well as increase faecal excretion of bacteria. In order to investigate the epidemiological relevance of this interaction within a herd, co-infections of *F. hepatica* and *Salmonella Dublin* were examined in Danish dairy herds.

Materials and method. Seven herds with a known history of both infections were selected. In each farm 50 heifers (age 8-36 months) were sampled in December, January and March following their first grazing season (FGS) (n=182) or second grazing season (SGS) (n=155). At each occasion serum was obtained and analysed for specific antibodies against both infections. Faeces was collected from 10 FGS and 10 SGS heifers in each herd and analysed for *Fasciola* eggs by sedimentation.

Results. The sero-prevalence (positive in minimum 1 of the 3 samples) of *F. hepatica* was 40% with large variation between herds (12-89%). There was a higher prevalence among SGS heifers (61%) than FGS heifers (23%; $P<0.0001$, Fisher exact-test). Based on detection of eggs, the overall prevalence of *F. hepatica* was 28% and thus markedly lower than by serology ($P<0.0001$, McNemar's test). 19 heifers (6%) were sero-positive for *S. Dublin* and only 5 heifers were sero-positive for both infections.

Conclusions. Due to the low *Salmonella Dublin* prevalence levels, it was not possible to demonstrate any interaction between infections. The prevalence of *F. hepatica* was alarmingly high in some herds and consequently, it is likely that levels of production will be markedly affected.

593 **Survey of *Fasciola gigantica* infection in buffalo and cattle by eggs or/and flukes examination in gall bladders**

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In southern China, *Fasciola gigantica* infection in buffalo and cattle is found frequently and causes considerable economic losses. From January 2005 to April 2006, 918 gall bladders of buffalo and 229 gall bladders of cattle were collected from local slaughterhouse (Nanning, Guangxi, China). The bile of each gall bladder was collect to exam the egg of fluke. The gall bladders were then cut to recover the flukes. The flukes recovered were counted and the total number of flukes from each gall bladder was recorded. The gross lesion of the gall bladders cut was also observed. The results indicated that *F. gigantica* infection rates in buffalo and cattle (indicators: eggs or/and flukes in gall bladder) were 53.8% (eggs + fluke: 47.6%; eggs only: 6.2%) and 43.7% (eggs + fluke: 28.9 %; eggs only: 14.8%), respectively. Generally, more flukes were recovered from gall bladder individual of buffalo than from that of cattle (of which 52 flukes were recovered from 1 gall bladder of buffalo). The gross lesion of gall bladder of buffalo was also more severe than that of cattle. Our results suggested that *F. gigantica* infection in buffalo and cattle was really a problem in the region; buffalo was highly susceptible to *F. gigantica*; and eggs or/and flukes examination in gall bladders could be used to estimate the infection rates in the animals.

594 ***Paramphistomum daubneyi* infection in grazing goats: results from an epidemiological study in Saône and Loire, France**

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Infection of goats by *Paramphistomum daubneyi* has been already described in France, in particular in the Saône-et-Loire department. This area is characterized by a high level of grazing goats and pastures are usually shared with beef cattle. The aim of the present study was to describe more precisely the distribution of this parasite in this area and to determine risk factors for this infection. Forty-four goat farms using pasture were selected according to the goat farming density of the department. Each farm was visited once in November-December 2006 : at this occasion, 15 goats were sampled for faeces and the farmer filled in a questionnaire on general characteristics of the farm (number of goats, annual milk yield, other bred species, ...), on the use of pasture (mixed grazing with cattle, pastures with humid areas, kid grazing, ...) and on the management of parasitism (number of treatments per year, drug, dose). 58.1 % of the farms presented at least one animal excreting *P. daubneyi* eggs. No area at particular risk was seen regarding the geographical distribution of *P. daubneyi* infection. Most of the farms (48 %) presented less than 3 animals excreting eggs. 72.5 % of the positive goats excreted less than 100 eggs per gram. The egg excretion increased with the age of the goats. A logistic regression analysis was made to determine risk factors for infection but at the flock level, none of the tested factors was significant. To control this infection, 56 % of the owners used oxclozanide, usually once a year during the dry period. The diversity in dose rates used demonstrate the lack of clear therapeutic scheme for this compound in goats.

595 **An overview of paramphistomosis in ruminants in Punjab, India**

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Paramphistomosis is considered as one of the main pathogenic disease of ruminants specially in low lying areas of Punjab state (India) causing heavy economic losses to the livestock owners . The disease is a major concern in low-lying and paddy growing areas during monsoon and post monsoon season due to the higher rate of breeding of snails which act as intermediate host for the disease and help in its transmission. Pathogenicity of the disease is directly correlated to the worm burden in the small intestine particularly duodenum causing immature or intestinal paramphistomosis which is characterized by persistent fluid fetid diarrhea along with emaciation, dehydration, submandibular oedema resulting into mortality particularly in young ones. The disease was first reported in Punjab as “Gillar rog” by Baldrey in 1906. Later during survey a mortality rate of 35.75 and 53.5% was recorded in sheep and goats respectively by Dutt (1980). Recently in 2004 an overall prevalence rate of 4.25% was recorded among 3877 faecal samples examined parasitologically from different agro climatic zones of Punjab state with the highest incidence in buffaloes (5.49%), followed by cattle (3.46%), sheep (1.32%) and goats (0.89%). Out of 2849 snails (*Indoplanorbis*, *Lymnaea*, *Gyraulus*) screened, 13.40% were found infected with highest infection rate in *Indoplanorbis exustus* (24.85%). The disease was encountered maximum in monsoon, followed by post monsoon and pre monsoon season. The paper overviews the epidemiology, clinical signs, immuno-diagnosis and control strategies of Paramphistomosis.

596 **Prevalence of dicrocoeliasis in slaughtered animals and its histopathological lesions in liver, in Ardebil, Iran**

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Paramphistomosis is considered as one of the main pathogenic disease of ruminants specially in low lying areas of Punjab state (India) causing heavy economic losses to the livestock owners . The disease is a major concern in low-lying and paddy growing areas during monsoon and post monsoon season due to the higher rate of breeding of snails which act as intermediate host for the disease and help in its transmission. Pathogenicity of the disease is directly correlated to the worm burden in the small intestine particularly duodenum causing immature or intestinal paramphistomosis which is characterized by persistent fluid fetid diarrhea along with emaciation, dehydration, submandibular oedema resulting into mortality particularly in young ones. The disease was first reported in Punjab as “Gillar rog” by Baldrey in 1906. Later during survey a mortality rate of 35.75 and 53.5% was recorded in sheep and goats respectively by Dutt (1980). Recently in 2004 an overall prevalence rate of 4.25% was recorded among 3877 faecal samples examined parasitologically from different agro climatic zones of Punjab state with the highest incidence in buffaloes (5.49%), followed by cattle (3.46%), sheep (1.32%) and goats (0.89%). Out of 2849 snails (*Indoplanorbis*, *Lymnaea*, *Gyraulus*) screened, 13.40% were found infected with highest infection rate in *Indoplanorbis exustus* (24.85%). The disease was encountered maximum in monsoon, followed by post monsoon and pre monsoon season. The paper overviews the epidemiology, clinical signs, immuno-diagnosis and control strategies of Paramphistomosis.

597 **Comparative analysis of neuropeptide distributions in the larval stages of the trematode *Fasciola hepatica***

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The liver fluke *Fasciola hepatica* is a major parasite of livestock in temperate regions; the disease, fascioliasis induces severe liver damage to its definitive host. Gross anatomy of muscle and motor innervation of intramolluscan developmental stages of *F. hepatica* have been investigated to ascertain the organisation and the functional correlates of any stage-specific patterns of staining. The technique of indirect immunocytochemistry has been used to demonstrate neuroactive substances combined with phalloidin conjugated TRITC fluorescence for the staining of myofibril F-actin in muscle systems. The immunocytochemistry employed an anti-GYIRFamide primary antiserum and the results were viewed by confocal scanning laser microscopy (CSLM). This work enabled the neuropeptide distributions to be visualised within the larval stages of *F. hepatica*. The neuropeptide-signalling systems of helminths are central to nerve muscle communication and this ultimately plays a role in parasite behaviour and motor function. The techniques revealed complex muscle arrangements within each of the larval stages as well as differing peptidergic nerve elements. The neuronal staining was most well developed in the final larval stage, the cercaria, where innervations of both the oral and ventral sucker were observed as well as an abundance of immunopositive cell bodies seen in the body and tail of the cercariae. The results obtained suggest that the cercaria is the most well developed larval stage and this physiology is essential for development into the juvenile and ultimately the adult fluke.

598 **Peroxioredoxins of the liver fluke, *Fasciola hepatica***

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Cryptosporidiosis is one of the causes of neonatal enteritis on dairy farms worldwide. On affected farms the level of contamination of farm buildings etc with *Cryptosporidium parvum* oocysts increases during the calving season and calves born later are exposed to a greater infectious load than earlier calves. Thus on farms where calving occurs throughout the year it is difficult to break the infectious cycle and to prevent the build up of oocysts in the farm environment.

This study describes the use of the antiparasitic halofuginone lactate (Halocur, Intervet, UK) on a dairy farm with a year round calving programme and with a high prevalence of *C. parvum* infection. The experimental design included four groups of eight newborn calves. Halocur was administered to two groups of calves immediately after being feed colostrum and thereafter for seven consecutive days, according to the manufacturers instructions and two groups were used as untreated controls. Further experimental treatments included treated and untreated calves being either reared in previously washed and disinfected individual calf pens or being mixed together in a larger pen. Oocyst excretion was monitored by microscopic examination of faecal smears or by 18srRNA PCR.

Oocyst excretion occurred earlier in the untreated calves. The amount of oocysts excreted was also greater in the untreated animals than the treated groups. Oocyst excretion was detected by microscopy in some of untreated calves on day 5 and the majority of them were positive by microscopy on day 7. The number of oocysts in the faeces of all the control calves had dropped below the level of detection by microscopy on day 13 although all the calves were positive with the PCR assay. Whereas oocyst excretion was detectable by PCR in all the treated calves it only ever got above the microscopy threshold on day 13 in three animals.

These results will be discussed in terms of controlling on-farm disease and the possible benefits for reduced environmental contamination.

599 **Molecular cloning and bioinformatic analysis of cysteine proteinase (FhPcW1) from adult *Fasciola hepatica***

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Fasciola hepatica, commonly known as liver fluke, is a parasitic flatworm that infects the hepatic bile ducts of sheep and cattle, sometimes also humans. Infections are the cause of serious veterinary and human medicine problems as well as economical.

The new cathepsin L - FhPcW1 cDNA was cloned from *Fasciola hepatica* adult flukes using RACE-PCR method. Adult flukes were subjected to the total RNA isolation. mRNA fraction was reverse transcribed to obtain double stranded mRNA-cDNA hybrid structures with added poly C tail to the 5' end of mRNA strand using Terminal Deoxynucleotidyl Transferase. For first RACE reaction amplifying 3' end polyT and FhPcL (5' ATGCGGTTCTTCGTATTAGCCGTC 3') primers were used. FhPcL primer was designed after analysis of variety of cDNA sequences encoding F. hepatica cathepsin Ls. In second RACE reaction we used polyG and FhPcR (5' ACCGCGTGAGAGTAACTCAGACGAGC 3'), which was designed basing on known 3' end sequence, primers. After sequencing of cloned 5' end we established whole cDNA sequence encoding FhPcW1.

Nucleotide and amino acid sequences were submitted to GenBank under accession number: EF407948. Probably, it is secreted protein containing signal peptide, serine, threonine, tyrosine phosphorylation sites and four tyrosine sulfation sites, three disulfide bonds and does not contain glycosylation sites. The ORF corresponding to FhPcW1 exhibited strong similarity to previously cloned cathepsins L of *Fasciola hepatica* as well as *Fasciola gigantica*. Moreover, secondary structure, three dimensional models and MHC types ligation strength prediction were performed. High ligation strength scores calculated by SYFPEITHI point at probable immunogenicity of the protein.

600 **Molecular cloning and expression of cDNA of a novel gene encoding cathepsin D-like protease from *Fasciola hepatica***

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Fasciola hepatica infections constitute a significant global veterinary problem. Chemotherapy is expensive, harmful for an environment and becomes less and less efficient because of the development of drug resistant populations of the fluke. Therefore, the best way of prophylactics against *F. hepatica* infection seems to be vaccination. In this work we cloned a novel cathepsin D-like protease from adult fluke, no such enzyme has been cloned from *F. hepatica* so far. However, cathepsin D-like proteases from *Schistosoma japonicum* induced a considerable level of protection in vaccination trials. Total RNA was isolated from adult *Fasciola hepatica* and reverse transcribed. First strand cDNA was synthesized using Reverse Transcriptase and the pTBam primer. After analysis of *Schistosoma sp.* cathepsin D-like proteases, cDNA sequences of the conserved regions were found and specific gene primers were designed. The 3' (about 1000 bp) and the 5' (about 750 bp) ends were amplified using poliG or pTBam and internal specific gene primers using the method of RACE-PCR and sequenced.

Sequence analysis showed that both fragments belong to one protease. Further sequence investigations revealed presence of two conserved regions characteristic of cathepsin D-like proteases. VAST program revealed the similarity of cloned protease to other known and crystallized cathepsin Ds. We also obtained recombinant protein and computed its potential biochemical properties, secondary structure, three dimensional models and MHC types ligation strength prediction. The novel protease was submitted in GenBank database under accession number EF000001.

601 **Comparison of ITS-1 and ITS-2 of *Fasciola hepatica* from different hosts and geographical locations in Spain**

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The ITS-1 and ITS-2 sequences from twenty-five worms of *Fasciola hepatica* (Platyhelminthes: Trematoda: Digenea) from different host species and geographical locations in Spain (9 host species and 19 geographical locations) were characterized genetically. The first and the second internal transcribed spacer (ITS-1 and ITS-2) of nuclear ribosomal DNA (rDNA) were amplified from individual liver fluke by polymerase chain reaction (PCR), and the representative amplicons were cloned and sequenced. The length of the ITS-1 and ITS-2 sequences was 946 bp for all Spanish liver fluke specimens sequenced. Sequence difference was detected between specimens from different host species and geographical location in Spain. Based on ITS-1 and ITS-2 sequence data, the results testify that the intraspecific sequence divergence was negligible. Our study should provide a useful tool for studying the epidemiological situation and population genetic structures of *Fasciola hepatica* from Spain.)

602 **Complete mitochondrial genomes for *Fasciolopsis buski*, *Fasciola gigantica*, *Clonorchis sinensis*, *Opisthorchis viverrini*: highly conserved features**

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The entire mitochondrial DNA (mtDNA) for *Fasciolopsis buski*, *Fasciola gigantica*, *Clonorchis sinensis* and *Opisthorchis viverrini* were obtained. The genome size for *F. buski* is 14413 bp; for *F. gigantica* is 13924 bp; for *C. sinensis* is 14452 bp; and for *O. viverrini* is 14046 bp. The organization of these genomes is not strikingly different from those of other eumetazoans, although one gene (atp8) commonly found in other phyla is absent from these flukes (and other flatworms, see Le et al., 2002). The gene order in opisthorchiids/fasciolids has similarities to those seen in platyhelminths except African schistosomes. There are 12 protein-coding, 2 ribosomal and 22 transfer RNA genes. Liver and intestinal flukes have short non-coding region which possesses tandem repeats and hairpin and loop structure. Inter-specific divergent rate between these species is around 20-30%. Ribosomal RNAs (rrn) including large (rrnL) and small (rrnS) and 22 transfer RNAs (trn) generally resemble those of other flatworms. A standard clover-leaf structure can be inferred for most tRNAs. Exceptions include both forms of transfer RNAs for Serine (trnS1; trnS2) from which the paired DHU arm is missing in all liverflukes and other flatworm species (a situation also seen in some other metazoans). These new mitochondrial genomic data for a range of zoonotic flukes provide an extremely rich and valuable source of genetic markers for analysis of species diagnosis/identification, biogeography, phylogenetics, genetic variation/hybridization of genotypes, population genetics and possibly, new insights into mitochondrial function in parasitic liver and giant intestinal flukes. We thank the ICGEB (grant to Le TH), JSPS (to Sato Y and Agatsuma T) for their financial support to this investigation.

603 **Serotonin and FMRFamide immunoreactivity in *Opisthorchis felinus* (Trematoda, Opisthorchiidae) cercaria**

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In the nervous system of parasitic flat worms (trematodes) cholinergic, serotonergic and peptidergic components were revealed (Halton, Maule 2004). These neurotransmitters, as suggested, take part in the muscle activity of the body wall, suckers and reproductive organs of worms. The complicated life cycle of trematodes includes free-living stage – cercariae. Data about neuronal signal systems in trematode larvae is limited.

In the present work neurotransmitters - serotonin (5-HT) and FMRFamide were studied by immunocytochemical technique interfaced with confocal scanning laser microscopy in *Opisthorchis felinus* Rivolta, 1884 (Opisthorchiidae Braun, 1901) cercaria. 5-HT- immunoreactive (IR) nerve cells and fibers were revealed in brain ganglions and along the nerve chords of cercaria. Two 5-HT –IR cells and fibers were seen in the tail. Small FMRFamide –IR cells and fibers were revealed in the region of brain ganglions and in the nerve chords extended along the body, in commissures connecting the long nerve chords. Several 5-HT- and FMRFamide –IR small structures are presented on the border of the tail and the body.

Results received are in general in agreement with data received by Sebelova et al., (2004) and Terenina et al. (2006), as to cercariae of *Echinostoma caproni* and *Chinoparyphium aconiatum*. The difference is concerned to the number and prevalence of investigated neuronal signal compounds in cercariae.

The study was partly supported by the MI project of Siberian Department of RAS 19 (2).

604 **Role of tryptaminergic agents in regulating the motility of gastrothylax crumenifer, a rumen amphistome**

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Tryptaminergic system plays an important role in different animals. Its presence has also been established in different trematodes. In the present study, affect of different tryptaminergic agents on spontaneous muscular activity of *Gastrothylax crumenifer*. 5-hydroxytryptamine caused concentration-dependent (10^{-7} - 10^{-3} M) increase in amplitude, baseline tension and frequency of spontaneous muscular activity of rumen fluke whereas its precursor, 5-hydroxytryptophan also induced a significant increase in amplitude at 10^{-5} and 10^{-4} M concentrations and baseline tension at 10^{-4} and 10^{-3} M concentrations without affecting the frequency of spontaneous contractions of rumen fluke. Cyproheptadine, a 5-HT receptor antagonist (10^{-7} - 10^{-4} M) elicited significant decrease in amplitude and baseline tension, as compared to control amplitude (0.49 ± 0.06 g) and baseline tension (0.13 ± 0.01 g) and did not affect frequency of spontaneous muscular activity of rumen fluke. These specific precursor and antagonist also had a differential influence on muscular activity indicating the presence of well developed tryptaminergic system and its excitatory role in regulating spontaneous muscular activity of rumen fluke.

605 **Flow cytometric analysis of the early local cellular response in rats infected with *Fasciola hepatica***

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The liver fluke, *Fasciola hepatica* is a parasite of great significance in livestock production. For this reason many attempts are made to develop an efficient vaccine against liver fluke disease. Newly excysted juvenile (NEJ) flukes, which penetrate the gut wall and migrate through the peritoneal cavity, are essential for establishing the infection in the definitive host. Termination of infection at pre-hepatic stages would minimize damages inferred by the fluke. The immune mechanism involved in the killing of juvenile flukes still remains unknown, making it more difficult to analyze the performance of vaccines - whether they activate the necessary immune response. Furthermore, the parasite has been shown to have an immunomodulating influence on the host and can accommodate many immunoevasive mechanisms.

The aim of this study was to analyze the dynamics of cell populations in the peritoneal fluid of rats at an early stage of infection with *Fasciola hepatica*.

Male Sprague-Dawley rats, infected with 50 metacercariae of *F. hepatica* and non-infected controls were euthanized 15 hours and 4 days post infection (pi). The peritoneal fluid of experimental animals was analyzed by flow cytometry to estimate cell phenotypes.

In infected rats there was a distinct increase in the percentage of neutrophils accompanied by a decrease in lymphocyte values compared to non-infected animals. Lymphocytes exhibiting CD4 markers and MHC class II receptors dominated in the peritoneal fluid of infected rats, as early as 15 h pi but most markedly at 4 days pi. In contrast to infected rats, control animals showed no activation of T-cells.

These findings suggest that the local immune response in rats is shifted towards a Th2 type very early in the course of *Fasciola hepatica* infection.

606 **Serum biomarkers of fasciolosis in sheep: insights into host-parasite interactions**

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Fasciola hepatica is the causative agent of fasciolosis in sheep, cattle and humans. We are interested in understanding the pathogenesis of fasciolosis in ruminants and the biology of the host-parasite interaction during infection. We generated profiles of serum proteins from sheep infected with *F. hepatica* over the first twelve weeks of infection using SELDI-TOF mass spectrometry. From a total of 2302 clusters, 100 clusters showed significant differences in intensity between control and infected animals at different times during infection. Of these clusters, 26 were defined as biomarkers in the m/z range 1832-89823 Da. Six biomarkers were significantly increased at weeks 3 and 9 post infection (pi), 16 biomarkers were increased only at week 9 pi and 4 biomarkers were significantly decreased at week 9 pi. Certain biomarkers were purified using chromatographic separation and identified using tandem mass spectrometry and subsequent searches of the MASCOT database. The identity of certain markers was then confirmed by Western blotting. These markers will provide insights into the course of pathology induced during fasciolosis and may provide potential candidate antigens for early detection of the disease.

607 **Characterization of a 2.9 kDa-*Fasciola hepatica* recombinant protein**

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Production of recombinant proteins offer the possibility for obtaining homogeneous antigens, so these proteins have been widely used for the immunoprophylaxis of several parasitic diseases, and for their detection also. In the current investigation the immunological characterization of a 2.9 kDa-*Fasciola hepatica* recombinant protein was done by measuring the antibody response (IgG1, IgG2a, IgG2b, and IgG2c idiotypes) in rats experimentally infected with metacercariae by using an ELISA. Animals of group 1 (G-1) remained infected throughout 14 weeks, and rats of group 2 (G-2) were left intact as uninfected controls. The functional activity was assessed by determining the enzymatic activity. IgG2b and IgG2c values increased significantly from the 1st to the 5th weeks after infection, when began to decrease. The curves of IgG2a and IgG1 increased from the 3rd w.a.i. to the end of the study. The highest absorbance values were reached in the IgG2a response. The infected rats reached higher titres of antibodies than the rats of G-2, and these differences were statistically significant ($p < 0.05$) in all the idiotypes evaluated. A positive significant correlation between the IgG2b and IgG2c curves ($r^2 = 0.882$, $p = 0.001$) and between the IgG1 and IgG2a ones ($r^2 = 0.878$, $p = 0.001$) was obtained. The recombinant protein showed serine-protease activity.

608 **Evaluation of protection induced by recombinant cathepsin L1 in goats challenged with *Fasciola hepatica***

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Introduction: Immunisation with recombinant Cathepsin L1 (CL1) has induced partial protection in sheep and cattle, but studies have not been conducted in goats. The present study evaluates protection induced by immunization with recombinant CL1 in goats challenged with *F. hepatica*.

Material and Methods: Fourteen Florida-breed goats were used for the study, group 1 (n=7) was immunized with two doses of CL1 on week 0 and 3; group 2 (n= 7) was inoculated with adjuvant (Quil A) on the same weeks. Goats of both groups were orally infected with 200 mc on week 10, and sacrificed on 17 week post-infection (wpi).

Results and Conclusion: Hepatic fluke burdens revealed reduction of 38.7% in the CL1 group; however, this reduction was not significant due to the high individual variability. Similar results were found for the parasite egg counts. Serum levels of glutamate dehydrogenase (GLDH) increased by 6 wpi and decreased by 12 wpi (group 1) or 15 wpi (group 2). Gross and microscopical hepatic changes (fibrous perihepatitis, fibrous tracts containing pigment laden macrophages, hyperplasia of bile ducts and infiltration of lymphocytes, plasma cells were variable in both groups, but in general more severe in group 2, whereas infiltration of eosinophils was more severe in group 1. Hepatic lymph nodes showed severe hyperplasia of lymphoid follicles and medullary cords. Results of the present study suggest that CL1 induced a limited protection against *F. hepatica* in goats. Further studies should investigate antigens combinations and new vaccine formulations.

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609 **The effect of adjuvants on the immune response to *Fasciola hepatica* in sheep**

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Fasciola hepatica infection serious clinical disease and economic losses in ruminants. Anthelmintic treatment as a means of control is problematic because of increasing drug resistance. We are interested in the possibility of immunoprophylaxis and one strand of research involves characterizing the effect of adjuvants on the immune response of ruminants to infection. We intend in particular to investigate the “adjuvant effect” that we have seen in several vaccination trials.

We carried out a study in which cross-breed sheep were injected with one of three adjuvants – Quil A, TiterMax or FICA (Freund’s incomplete adjuvant), or a PBS control. Flow cytometry analysis to determine CD4 and CD8 ratio and other changes in lymphocyte cell subsets, ELISA analysis of specific antibody responses, cytokine assays and other immunological parameters were correlated with faecal egg output, fluke burdens and degree of liver damage. Quil A proved to be significantly different from the remaining two adjuvants in terms of kinetics and quality of both antibody and cellular responses

610 **The cellular and humoral response of cattle vaccinated intranasally with a recombinant cysteine protease of *Fasciola hepatica* in the form of inclusion bodies**

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Fasciolosis is a widespread parasitic infection leading to severe economical losses in meat and milk production worldwide. There is a strong need for developing an efficient vaccine against the liver fluke due to increasing drug-resistance and tendencies to decrease overall drug use in food-producing animals. The aim of research was to evaluate the cellular and humoral response of male and female cattle vaccinated intranasally with a recombinant cysteine protease of *Fasciola hepatica* (CPFhw) expressed in the form of inclusion bodies. Animals were immunised twice with 500ug of protein and challenged with 400 metacercariae at monthly intervals. Specific antibody titres against CPFhw were measured by the ELISA method. T-cell phenotypes in the peripheral blood were investigated through flow cytometric analysis. Postmortem examinations were conducted 12 weeks post infection (wai) for estimating fluke burdens in the livers of vaccinated and control animals. The results of this experiment showed a distinct difference in the cellular response and protection levels between animals of different sexes. Females exhibited a stronger cellular response up to the 8th wai and higher protection levels than males. However the humoral response did not vary as clearly between the sexes and no correlation between antibody titres and protection was observed. Females presented higher levels of CD2+, CD4+, CD8+ and most markedly NK cells, compared to males. These findings may indicate a cellular mechanism conferring immunity in experimental animals. The presented research suggests that it is possible to induce partial protection of cattle against liver fluke infection with a recombinant cysteine protease using the mucosal route of delivery.

611 **Evaluation of protection induced by recombinant thioredoxin peroxidase in goats challenged with *Fasciola hepatica***

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Introduction: An international increasing interest in the development of protective vaccines against *F. hepatica* in ruminants has risen during recent years. The present study evaluates the protection induced by immunization with recombinant thioredoxin peroxidase (Trx) in goats challenged with *F. hepatica*.

Material and Methods: Fourteen Florida-breed goats were used for the study, group 1 (n=7) was immunized with two doses of Trx on week 0 and 3; group 2 (n= 7) was inoculated with the adjuvant (Quil A) on the same weeks. Goats of both groups were orally infected with 200 mc on week 10, and sacrificed on week 27.

Results and Conclusion: Hepatic fluke burdens revealed a non-statistically significant reduction of 33.1% in the immunized. Parasite egg counts were similar in both groups thorough the infection. Serum levels of glutamate dehydrogenase (GLDH) were similar in both groups during pre- and post-infection stages. Gross and microscopical hepatic changes (fibrous perihepatitis, fibrous tracts containing pigment laden macrophages, portal fibrosis, hyperplasia of bile ducts and infiltration of lymphocytes and plasma cells showed high individual variability, but in general were more severe in group 2. Hepatic lymph nodes showed severe hyperplasia of lymphoid follicles and medullary cords, except in some goats of the immunized group with a very low number of flukes. Results of the present study suggest that Trx induced a limited protection against *F. hepatica* in goats. New vaccine formulations and/or antigens combinations should be evaluated in further studies.

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612 **Immune response in *Fasciola gigantica* experimentally infected rabbits treated with either carnosine or mirazid**

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The aim of the present study was to determine and compare the antibody (Ab) response against *Fasciola gigantica* in experimentally infected rabbits treated with mirazid or carnosine by ELISA. Six groups of five rabbits each, were examined (negative control, negative treated with carnosine, negative treated with mirazid, infected positive control, infected treated with carnosine and infected treated with mirazid). Each infected rabbit was infected with 20 *Fasciola metacercariae*. Anti-*Fasciola* IgG were weekly evaluated by ELISA, in each animal serum, along the period of experiment (14 weeks). After sacrificing the all animals, each liver slices were examined for the determination of worm burden. Results showed highly significant difference between the numbers of *Fasciola* flukes in liver of infected treated and control positive rabbits. A reduction of 54.5% in worm burden was detected in the carnosine treated group. While, results of mirazid treated group presented complete eradication of flukes. IgG response in experimentally infected and treated groups, either with carnosine or mirazid, was significantly higher than that of control infected group. Carnosine infected group showed the highest Ab response of all groups. However carnosine action was not as potent as mirazid although it raised the general defence of the animal (Ab) plus moderate eradication effect on the worm.

613 **Molecular identification of *Schistosoma bovis* isolated from intermediate snail hosts in Senegal and Uganda**

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Schistosoma bovis, which can be a serious veterinary problem in cattle, has a wide distribution across northern, western and eastern parts of Africa and extends in to the Mediterranean region and Middle East. *S. bovis* develops naturally in a range of *Bulinus* species and transmission of *S. bovis* often overlaps with *S. haematobium*, which is responsible for urinary schistosomiasis in man. The cercariae of both species are morphologically very similar and the identification of parasites emerging from *Bulinus* species collected from natural water bodies is best achieved using molecular markers. Mitochondrial gene sequences, especially Cytochrome Oxidase 1 (COI), allow the differentiation of species within the *S. haematobium* group. Isolates of *S. bovis* have been identified and recovered recently from naturally infected *B. truncatus* in Senegal and *B. nasutus* (productus) in Uganda. Variation has been observed in COI sequences from *S. bovis*, across this large geographical range, but the sequence is of value for species identification and may be used for the differentiation of *S. bovis* cercariae from *S. haematobium*. Molecular markers (barcoding) promise to be of value for mapping the distribution and intermediate host range of this important parasite of cattle.

614 **Proteins expressed on the tegument surface of *Schistosoma bovis*. Which of them play as plasminogen receptors?**

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Schistosoma bovis is a pathogenic blood fluke of ruminants that lives for years in its vertebrate host. Due to its localization

in blood vessels, the parasite must neutralize the immune response and the haemostatic mechanisms of its host. We have observed that adult *S. bovis* worms bind plasminogen (a central component in the host fibrinolytic system) as a likely way to prevent blood clotting around them. We have identified a range of plasminogen binding proteins in an extract of worm tegument. We do not know, however, which of these proteins could act in vivo as true receptors for plasminogen, which would require that the potential receptors be exposed to blood on the outer tegument surface of the worm.

Our purpose was to identify the tegumental proteins that are exposed to host in the tegument surface and to verify whether the major plasminogen-binding proteins already identified (enolase, actin and glyceraldehyde 3-phosphate dehydrogenase (GAPDH)) are expressed on the worm surface.

Intact adult worms were subjected to a soft digestion with trypsin, and the tryptic peptides released were analyzed by LC-MS/MS to identify surface proteins. Immunofluorescence and confocal microscopy studies using anti-actin, anti-enolase and anti-GAPDH antibodies were also performed on intact worms.

Mass spectrometry analyses identified a set of tegumental proteins including actin, enolase and GAPDH. Immunofluorescence assays confirmed the expression of actin and GAPDH on the worm surface. The surface expression of enolase remains controversial. Further investigation on these proteins is required to establish the physiological role of their interaction with plasminogen.

615 **Congenital transmission of *Schistosoma japonicum* and dynamic of serum antibodies**

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Schistosomiasis japonicum is one of the important human parasitic diseases prevalent in Far East, including China, P. Philippine and Indonesia, where an estimated two million people were infected. More than forty mammals, such as cattle, buffaloes, mouse, pig and rabbit etc, act as its nature definitive host. When illustrating the life circle of *S. japonicum*, the percutaneous route of infection is always shown as the only way to infect the definitive host. However, congenital transmission has been demonstrated as an alternative in early and recent studies, and these studies demonstrated that the rate of congenital infection was associated with both the gestation period of infection and the infection dose of cercariae. Further research on congenital transmission of *S. japonicum* will be needed to eventually prevent and control this severe parasitic disease. To elaborate further on the possibility and the consequences of congenital infection, the present study was designed to elucidate the parasitological and immunological effects of a prenatal *S. japonicum* infection on litter mice and newborn rabbits respectively. One part of the experiment is to construct animal model of congenital transmission of *S. japonicum* in mice and observe whether congenital infection exists in litter mice. In the other part of the experiment, ELISA is applied to detect the specific antibodies of IgG and IgM in serum samples of congenitally infected new born rabbits, and the potential mechanisms of congenital transmission of *S. japonicum* is explored in the perspective of immunology. Part one: Experimental Studies on Congenital Transmission of *S. japonicum* in Mice. The present study therefore didn't support the previous positive result of congenital infection of *S. japonicum* in litter mice and suggested that mice might not take significantly important role in the epidemiology of *S. japonicum*. Further studies are needed to determine if the occurrence of congenital infection is associated with the experimental animal species. Part two: Immunological studies on congenital transmission of *S. japonicum* in rabbits. The results revealed that congenital infection of *S. japonicum* can decrease the kittens' specific effective humoral immune response, and immunologic tolerance might exist in congenitally infected newborn rabbits. In contrast, serum antibodies of both IgG (anti-SEA, anti-AWA) and IgM (anti-SEA, anti-AWA) in almost all kittens born to Control group were detected to be positive in the 95th day after birth. The amount of kittens with positive IgG and IgM was much more than that of kittens in G1 and G2, and the mean OD values of IgG and IgM in Control group were significantly higher than those in G1 and G2 as well in the same time of detection. The results suggested that kitten rabbits congenitally infected with *S. japonicum* showed relatively low immune response to the stimulation of SEA and AWA, which might be indicative of immunological tolerance. No protective immunity was induced in congenitally infected kittens with challenging infection. The fact that the low immune response to AWA is more obvious than to SEA was supposed to be the result of much more exposure of kittens to AWA before delivery.

616 ***Fasciola gigantica* (Trematoda: Fasciolidae): morphological observations using scanning electron microscopy**

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Fasciola gigantica Cobbold, 1885 is the common liver fluke of domestic stock in Africa; it occurs frequently in Asia, the Pacific islands, the Philippines, southern USA, southern Europe, European Russia and the Middle East. A profound anaemia and changes in the serum proteins are found in sheep. The debility, emaciation and diarrhea are other clinical signs. The most important intermediate host is *Lymnaea auricularia*. Scanning electron microscopy (SEM) was used to study the morphological aspects of adult worm of this trematode in detail. A total number of seven adult *F. gigantica* were removed from the liver of infected sheep. The worms were washed in 2% sodium cacodylate buffer. The worms were next placed in 4% glutaraldehyde. The trematodes were put into fresh 4% glutaraldehyde for 2 h, post fixed in 2% osmium tetroxide in cacodylate buffer at 4°C for 2 h, and dehydrated through a series of increasing ethanol concentrations. They were then dried with liquid CO₂ in a critical point drier. Each worm was placed on a numbered aluminium stab, which was coated with gold in an ion coating and viewed with a JEOL JSM-840 Scanning Electron Microscope. *F. gigantica* was readily recognized by its larger size, being 25-27 mm in length and up to 12 mm in breadth. The anterior cone is smaller than that *F. hepatica*, the shoulders were not as prominent and the body was more transparent. The tegument was armed with sharp spines. Shape, size and arrangement of tegumental spines were described. Furthermore, details of excretory pore and their tegumental spines were studied. Three types of tegumental papillae including sensory ciliated papillae, domed papillae and sensory structures were observed on suckers and body surface. Finally, the pattern of the tegument of the cirrus was discussed.

617 **Modification of dipstick as a field applicable technique for multiple diagnosis of some parasitic infections**

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This work evaluates the use of Nitrocellulose Cassettes (NCC) as a qualitative serological test for rapid diagnosis of some parasitic infections mainly human schistosomiasis and fascioliasis. The NCC technique was compared with c.ELISA for detection of antigens and/or antibodies in serum samples. Based on the detection of circulating schistosomal antigens (CSA) using anti-*S.mansoni* SEA monoclonal antibody, the sensitivity was 97.5 % and 100 % for c.ELISA and NCC respectively while the specificity was 95.1 % and 100 % for c.ELISA and NCC respectively. On the other hand, when c.ELISA was used for detection of species specific antibodies in sera of each parasitic group, the sensitivity of the technique was 94.7 % and 91.6 % while the specificity was 92.8 % and 97.5 % for schistosomiasis and fascioliasis respectively. However, the use of NCC technique improved both sensitivity and specificity of specific antibodies detection. The sensitivity was reached to 97.3 % and 95.8 % while the specificity was increased to 96.2 % and 98.7 % for schistosomiasis and fascioliasis respectively. The qualitative results of NCC are easy to read and are determined in 10-15 minutes and could be promising to use as a field applicable technique for multiple diagnosis of parasitic infections.

618 Seroprevalence of fasciolosis and rheumatoid factor in patients with eosinophilia

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Fasciolosis is a widespread zoonosis acquired by ingestion of *Fasciola hepatica*-metacercariae in infected raw vegetables. Although livestock ruminants (sheep or cattle) are the usual hosts for this trematode, this helminthosis has been considered by the WHO as an emerging human disease in many countries. Inflammatory diseases constitute a significant health burden to the community, in terms of loss of duration and quality of life, and of direct and indirect costs. A case-control study was carried out to investigate the possible relationship between the presence of IgG antibodies against *F. hepatica* and that of IgM-rheumatoid factors (IgM-RF) in patients with eosinophilia. According to the values of eosinophils, patients were divided into three groups: G-I (0.4-0.9 10⁹ l⁻¹), G-II (1-3 10⁹ l⁻¹) and G-III (>3 10⁹ l⁻¹). Antibodies against *F. hepatica* were analyzed in 909 individuals by modified enzyme-linked immunosorbent assay (ELISA) employing, as antigen, excretory/secretory products of the trematode, and IgM-RF was detected by the latex agglutination test. Seropositivity was determined in 23% (207/909) cases, with the greatest values in women from rural areas. IgM-RF was found in 14% (130/909) individuals, and the highest values were reached in women from the countryside also. The presence of IgM-RF was detected in 24% (49/207) ELISA-positive patients and 11% (81/702) ELISA-negative ones. The risk for developing IgM-RF was higher in patients with elevated eosinophilia and to the presence of antibodies against *F. hepatica* also. We concluded that people living in rural regions have a higher risk for fasciolosis and RF autoantibodies.

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619 Paragonimus in Zhrjiang, China: morphology and molecular biology

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China is the endemic area of paragonimiasis. In order to better understand the background of *Paragonimus* species, the metacercariae were collected from Zhejiang Province for studying on Morphology and molecular biology of *P. westermani* and *P. harinasutai*. *P. westermani*: Adult worms were collected from 10 cities in Zhejiang. The specimen were fix by bless and dyed by carmin, then measured under the microscope. Worm size was 8.98±1.38-12.96±0.86 X 4.69±1.04-6.92±0.58mm. The ratio of wide to long was 1: 1.77-1:2.1. The oral sucker was 0.57±0.07-0.71±0.09 X 0.84±0.14-0.93±0.09mm in size. The ventral sucker was smaller than the oral, measuring 0.55±0.08-0.72±0.06 X 0.60±0.07-0.75±0.07mm. Single spines and a few double spines besides acetabulum and between two tests. The ovary was like fan in shape with 4-7 lobes. The ratio of ovary to body is 0.11-0.15. The testes were changeable in shape with 4-7 branches. The ratio of the tests to body was 0.10-0.15. Although the morphology variations existed, all specimen accorded with the basic characters of *Paragonimus westermani*. The metacercariae were collected from Xiaoting, Xixi and Yushan villages in Ninghai County, Zhejiang, for ITS2 and CO1 sequence analysis. 363 bp of ITS2 sequence were the same in three populations, two types of *P. westermani* in Ninghai (China). Significant difference of ITS2 sequence was found between the populations from Zhejiang and Nakorn Nayok (AF159604, Thailand) with nucleotide identity 97.5%. 390 bp of CO1 sequence were different with 3 nucleotide code (No.49,178,331) from Xiaoting and Yushan villages in Ninghai County. *P. westermani* in Zhejiang belonged to North-east group in Asia; The molecular markers for identification of pathogenetic type of *Paragonimus westermani* would be further studied by COI analysis. *P. harinasutai*: The metacercaria of *P. harinasutai* were collected from Xixi village in Ninghai County, Zhejiang. The ratio of wide to long for adult worm is 1:2.838±0.228. The ventral sucker is larger than the oral, ratio of 1.26±0.116:1. The ovary and the testes are 4-6 and 4-5 branches respectively. The spines are mixed. The metacercaria are pinkish in color with 2 cyst walls and 525.16±38.61 X 500.19±31.7lum in size. The variations were found between the specimens from China and Thailand. The molecular biology study indicated: Although the fingerprintings of endonuclease BsaHI and StuI (PCR-RFLP) were the same, 366 bp of ITS2 sequence and 390 bp of CO1 sequence were significant difference between the populations from China (Zhejiang) and Thailand with the nucleotide identity 95.6% (AF159609) and 89.7% (AF159600) respectively. The study further confirmed *Paragonimus harinasutai* from Ninghai, Zhejiang, with significant variation on morphology and molecular biology. This study Supported by Chinese Ministry of Sciences and Technology, Chinese-Thai cooperation Programm (17-603J), Zhejiang Ministry of Sciences and Technology (2005F13023); Japanese-Chinese Medical Association (No.117).

Poster group 16: Arthropods of Large Animals

620 **HOBO micro stations and the report of data from Romania in frame of the East BTNET Project**

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This paper contains the data collected from the six HOBO Micro Stations placed on the Rumanian territory, in frame of the East BTNET project and processed by using the BoxCarPRO4 programme.

The used Micro Stations are provided with sensors to register information about: air temperature, soil temperature, relative humidity of the air, soil moisture, wind velocity, factors that influence the development and activity of the culicoide insects, vectors for the bluetongue disease.

In the same time, besides the monitoring of the environment parameters were performed captures of insects in order to establish the correlation between the environment parameters and the collected culicoide insects populations.

The six HOBO Micro Stations were placed in locations with different environmental and geographic conditions (seaside, mountain, plateau and plain) in order to determine the regions favorable for the development of some culicoide species, especially the complex of *Culicoides pulicaris*, *Culicoides obsoletus* and *Culicoides nubeculosus*.

Three Micro Stations worked adequate (in the North-East, Center. South-West), the other three did not work properly due to objective or subjective reasons; totally those in the South-East and West, partially in the Center.

The data collection about the environment is important for establishing the biology and size of vector population in a particular area. The HOBO Micro Stations were very useful for this purpose.

621 **Study of Tabanidae (Diptera) in the Shadegan and Hovayzeh marshland in Khoozestan province - Southwest Iran**

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The family Tabanidae includes approximately 4290 species world wide and it is the biggest bloodsucking fly that is mechanical transport some pathogenic agents to human and animals , and as well as Causing decreases in meat and milk in domestic animal and important in terms of health and economic .

Studies of Abbassian - lintzen (1964) showed that 67 species and 12 sub species from Tabanidae flies in Iran , and 19 species and 5 genus from Khoozestan province .

At present study 74 speciemens (adult fly) were collected randomly from Shadegan and Hovayzeh marshlands during the spring to autumn 2006 .

Results showed that 4 genus and 8 species of Tabanidae from these area .

In this study *Tabanus tergstinus* and *T. gratus* are recorded for first time from Khoozestan .

622 **Epidemiological study of ruminant anaplasmosis: Seroprevalence and associated ticks in North Center of Morocco**

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A seroepidemiological survey on anaplasmosis in ruminants was carried out in 3 regions of Morocco (Gharb, Saïs and Middle Atlas) throughout a transect of about 250 Km from Atlantic coast to Atlas Mountains.

A total of 803 sera was collected; 451 in cattle, 244 in sheep and 135 in goats. The seroprevalence was assessed by a cELISA test using specific recombinant antigen. Ticks were also collected and identified to determine their relative importance.

The results showed that seroprevalence of *Anaplasma marginale* in cattle was estimated to 26.16% and 15.01% for *Anaplasma ovis* in sheep and 28.43% in goats.

Ten tick species belonging to 3 genus were identified. *Rhipicephalus. spp* (44.1%) and *Hyalomma.sp* (43.6%) were dominant species in the 3 sites, followed by *Boophilus annulatus*. (12.3%).

623 ***Amblyomma triste* (Ixodidae) in Uruguay: Distribution, hosts and seasonality**

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This study is devoted to summarize the information obtained through active sampling of the the tick *Amblyomma triste* in Uruguay, in the years 2003-2006, from vegetation, wild and domestic hosts, and humans. A total of 125 collections of ticks were obtained from hosts, 79 (443 specimens) of adult ticks, and 46 lots (547) of immatures. Furthermore, 806 ticks were collected from the vegetation. The tick is restricted to zones close to Atlantic ocean and Rio de la Plata: Colonia, San José, Montevideo, Canelones, Maldonado y Rocha. Domestic dogs are the most important hosts for adults (50.6% of collections) with an average of 8.9 ticks/dog (maximum: 100). Humans are very important hosts for the adults (39.2% of collections). Other hosts for adults are cattle, goats and horses (2.5% each), and *Ozotoceros bezoarticus* (1.3%). Immatures were mainly collected on rodents: *Scapteromys tumidus* (39.1%), *Oxymycterus nasutus* (34.8%), *Cavia aperea*, *Necomys obscurus* y *Oligoryzomys flavescens* (2.1% each); the marsupial *Monodelphis dimidiata* (15.2%), the bat *Myotis albescens* (2.1%) and the dog (2.1%). Concerning seasonality, adults were collected on vegetation mainly between August and February, with a marked peak in September-October (41 and 24% of the total ticks collected from vegetation). This is the same pattern of adult ticks on hosts. Immatures were collected on hosts in November and January to May, being the maximum concentrated around January-March.

624 **Ecological variables affecting the tick *Ixodes ricinus* abundance in north-eastern Italy**

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Vector-borne diseases (VBD) are emerging in Europe and monitoring ticks dynamic is a basic tool for VBD predictive models.

M&M - During 2005/06, ticks were collected by dragging in north-eastern Italy (provinces of Verona, Vicenza, Treviso, Udine, Pordenone). Dragging was performed with a white flannel cloth (1sm) along 100m of transect. Temperature, relative humidity, vegetation cover, day length, altitude and presence of wild ungulates were recorded for each dragging. From March to November, 20 fixed transects were visited each month to evaluate variation of tick's abundance. Log linear models were employed to estimate ticks density (number of ticks/100ms) in each transect and negative binomial error was used to consider overdispersion of the distribution of host-seeking ticks. Multivariate negative binomial regression models were used to evaluate relationship between ticks and ecological factors.

Results - 3473 ticks (2052 larvae, 1379 nymphs, 42 adults) were collected and identified as *Ixodes ricinus*. Mean temperature was 19.4°C (range=5-34°C), mean relative humidity 66.4% (range=39-95%) and mean altitude 564 masl (range=143-1010). Day length varied from 9.27 to 15.48 hours. The lowest density (mean=0.2; 95%CI=0.02-1.7) was observed in Verona province, the highest (mean=88.9; 95%CI=46-171.5) in Udine province. Multivariate regression highlighted that nymph's abundance was positively associated with day length and negatively with temperature and altitude above 750 masl. Relative humidity and type of vegetation did not influence nymph's abundance. Abundance of larvae was positively associated with day length and relative humidity, negatively associated with altitude above 750 masl and meadow as type of vegetation.

625 **Mites as newly emerging disease pathogens in rodents and human beings**

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Different mite species, generally found on animals, may temporarily also infest man and consequently, such arthropods may be responsible for pruritic skin reactions which are often misdiagnosed. Mite dermatitis caused by the Tropical Rat Mite *Ornithonyssus bacoti* occurs in several small mammals and rodents under tropical and temperate climatic conditions. According to various observations in Germany, *O. bacoti* appears in wild rodents more frequently than it seems. In most cases, symptoms of mites are recognized only when they attack humans, but the diagnosis of a rat mite dermatitis requires identification of the parasite, which however is more likely to be found in the environment of its host than on the hosts' skin itself. Here, 30 different outbreaks from Germany are reported. As a clinical example is presented the case of a 23-year-old medical student and several other residents inhabiting a rat- and mice-infested house in Munich. Mites were found in large number in the students' flat. The patient was suffering from severe itching and papular urticaria. He consulted a dermatology clinic complaining of a pruritic dermatitis. It was misdiagnosed as allergy, treatment with an antiphlogistic agent remained unsuccessful. In another case three children were infested by Tropical Rat Mites from two gerbils and showed severe excoriations on the body surface. Furthermore Tropical Rat Mites were found in a home of disabled persons. *O. bacoti* is a periodic hematophagous parasite and spends a relatively short time on a host. Considerably epidemiological data about population dynamics of *O. bacoti* in Germany are presented.

626 **The prevalence of nymphal stage of *Linguatula serrata* in camels (*Camelus dromedarius*) in Najafabad, Iran**

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Linguatula serrata well known as “tongue worm” is an aberrant cosmopolitan arthropod parasite, which infests the nasal sinuses and nasopharynx of carnivorous mammals belonging to the families of Canidae, Hyaenidae and Felidae. Eggs of *L. serrata* are infective to a range of mammal intermediate hosts, particularly large grazing herbivores. *Linguatula serrata* infection has been reported in most domestic and wild animals including cattle, sheep, goats, pigs, camels, lion, leopard, giraffe and kudu, and rodents. The objective of this study was to determine the prevalence rate of *L. serrata* nymphs in liver and MLNs of camels that were processed in the Najafabad, Iran abattoir. Over a 3-month period (June, July, August 2006), 200 slaughtered camels were selected randomly. Age was determined on the basis of eruption of permanent incisor teeth. Five jejunal MLNs were collected from each camel. The samples were then examined for the presence of nymphs using a stereomicroscope. Liver samples were examined to observe the nymphs. The data were analyzed using chi-square and Fisher’s exact tests. Seventy out of 200 (35%) MLNs and 23 out of 200 (11.5%) liver samples were found positive ($P < 0.001$). The nymphs were about 4–6mm long and were covered with spines. The mouth flanked by two pairs of hooks, was located on the cephalothorax. The posterior part of the parasite exhibited annulations and thorns. No difference of prevalence was found between the age groups ($P > 0.05$). The number of nymphs from MLNs and from livers ranged 1–30 and 1–4, respectively.

627 **Population dynamics of the parasitic state of *Boophilus microplus* in bovines of the municipality of Culiacán, Sinaloa, Mexico**

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The objective of the study was to determine the population dynamics of *Boophilus microplus* in cattle naturally infested in the municipality of Culiacán, Sinaloa, Mexico, and its relation with the temperature, pluvial precipitation and evaporation. The infestation was determined weekly over a period of two years by means of individual counts of *B. microplus* larvae, nymphs and adult females in the bovines. I determined the linear correlation between the climatic variables with the parasitic states and the best model of multiple regression under the criterion of number of parameters was considered. The infestation by *B. microplus* showed different peaks of activity for each one of the parasitic stages during the mentioned period. The larvae predominated from April to July, with small peaks of activity in the last week of autumn. The nymphs predominated from March to May, showing their highest peak in the second week of May. Finally, the adult ticks showed their highest activity during the summer months. It was observed that the effect of temperature and evaporation were the climatic factors of greater relevance ($P < 0.01$) in the presence of larvae and nymphs, whereas for the adult stage, the temperature and environmental precipitation showed to be the more important climatic variables ($p < 0.01$). These results allow us to establish strategic treatments for their control, with recommendations to establish a program to apply ixodicidas at the beginning of the month of March and September.

628 **Over-wintering of *Lucilia cuprina* in south-eastern Australia**

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In south-eastern Australia, *L.cuprina* over-winters as larvae in the soil, yet the termination of arrested development, and subsequent emergence of flies in spring, remains unclear. Consequently, a 2-year study was undertaken in Western Victoria during 2005- 2006. Post-feeding larvae were deposited regularly, and daily emergence of flies and meteorological observations recorded. The data were used to validate existing models of *L.cuprina* development. Flies from larvae deposited in summer and early autumn emerged rapidly, 10-19 days after deposition. Larvae deposited in mid-autumn yielded some flies within 30-44 days, whilst others entered developmental arrest and emerged as flies the following spring. Monitoring of larvae showed that their development recommenced after a sequence of changes in average daily soil temperature, including a 1.5oC rise, a 1.5oC drop and then a subsequent rise. Spring emergence of flies was synchronous for deposits made in autumn and winter, with mortality of these deposits 2-3 fold higher than for deposits made in summer and early autumn. Existing models use soil temperatures, estimated from air temperatures, and varying development thresholds. Using actual and estimated soil temperatures, the predicted spring emergence of flies was a poor fit with the actual dates. One model required soil temperature to remain above 16oC until pre-pupae accumulated sufficient development for pupation. A reasonable fit was found by changing this threshold to a 5-day rolling average of 9-10oC. In conclusion, absolute temperature, as well as a pattern of temperature changes, may be important cues that terminate over-wintering and should be incorporated in future models.

629 **An outbreak of severe chorioptic mange in dairy cows in south-west France**

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In February 2007, chorioptic mange was diagnosed in a dairy herd in the Ariège Département in South-west France. The disease was unusually severe in that the majority (82%) of animals in the herd were affected and the lesions were extensive, extending from the classical location around the tail-head to the escutcheon, the hind limbs, the back and shoulders. The only mite identified was *Chorioptes bovis*. The herd comprised 77 adults, including heifers and bulls, most of which were housed in tie-stalls, bedded on straw. Because of the unusual presentation of the disease, it was decided to monitor the animals closely following herd treatment with eprinomectin and to collect individual milk samples in order to evaluate an ELISA for mite antibodies. Monitoring comprised clinical examination, serial photography of the animals and scoring of the mange lesions, plus additional skin scraping as required. The response to the initial treatment was generally good with a marked reduction in pruritus and the severity and extent of the lesions, even in severe cases, over the subsequent 6 weeks. Nevertheless, some animals showed incomplete resolution of the lesions and, in some of these live mites could still be found in the skin scrapings. A repeat herd treatment with eprinomectin, 7 weeks after the first, was administered in an attempt to further reduce chorioptic mite populations on the farm, prior to turn-out. The mean ELISA result of this farm (OD 0.8) was 2 times higher than the mean ELISA result (OD 0.4) of a negative control farm.

630 **A report of ovine acariasis due to mixed *Sarcoptes scabii* and *Psoroptes ovis* in a sheep flock**

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On winter of 2005, an acariasis with clinical signs such as, a sever itching dermatitis, loss of wool and hyperkeratosis was occurred in small flock with 75 sheep associated the farm of Agriculture faculty of Ferdowsi University of Mashhad. In order to diagnose the causative mites, we referred to farm and scarping skin samples were prepared from head and abdomen parts of 10 sheep with sever acariasis. The scarping samples of each parts of body were collected in seprated pockets. Because, the infested sheep were suffered from moderate anemia, Blood samples were collected in tubes with EDTA anticoagulant. The skin scraping samples were digested in 10% potassium hydroxide and prepared several wet mounting and examined by using light microscope with 100 and 400 magnifications. Microscopic examination was shown that head area of sheep were infested to *Sarcoptes scabii* and abdomen area to *Psoroptes ovis*. In this study, fungal mycelium and spore were not observed. The results of hematological examination were shown that the PCV level of infested sheep were lower the normal range, Also moderated eosinophilia were observed in some blood samples of sheep.

631 **Generalized demodectic mange in a free-ranging mule deer (*Odocoileus hemionus*) from Colorado, USA**

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Generalized demodectic mange in North American cervids has rarely been reported. In November, 2006, a hunter-harvested adult male mule deer (*Odocoileus hemionus*) was submitted for evaluation of hair loss. Grossly, there was generalized thickening of the skin over the entire body with patchy alopecia. Moist crusts were found clinging to matted hair. On cut section of the skin, the hair follicles were markedly dilated and filled with whitish semisolid material. Wet mounts of this material exhibited high numbers of demodectic mites. Markedly dilated follicular structures packed with mites were also apparent on histologic examination of the skin. Moderate mixed chronic inflammatory cell infiltrates encircled many of the affected follicles and abundant keratin debris and inflammatory crusts covered the epidermal surfaces. A few mite fragments were present in the dermis surrounded by granulomatous inflammation. Special stains demonstrated scattered bacterial colonies in the surface crusts but no fungal organisms were in surface or follicular structures. Morphologically, mites did not conform to descriptions of any species of *Demodex* described from cervids, including the only North American species described thus far, *Demodex odocoilei* of white-tailed deer (*Odocoileus virginianus*).

632 ***Melophagus ovinus*: Dynamics of sheep ked populations on an infected flock and its effect on weight gains and wool production**

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We studied the dynamics of a population of *Melophagus ovinus* on a moderately infected and properly managed merino flock, during a 2 year long period (2005-2006) in Patagonia, and determined its effects on sheep body weight and wool production by comparison with a ked controlled lot. Individual ked counts, body weight and wool analysis were examined. Although infestation of keds was moderate at the start of the trial, ked population increased during winter and was larger during the first year. Peak densities were reached in August (mean 182, range 120-366) when lambs were 10 months old. As a result of shearing and summer temperatures, ked numbers drastically declined both the first (mean 3, range 0-12), and second (mean 1, range 0-6) year of observations. There were no significant differences in body weight gains and wool production between ked controlled and non controlled lots, but differences in colour and percentage yield of scoured wool were observed. Under the studied conditions there is little evidence that an economic profit could be improved by controlling keds. We conclude that sheep ked control may be enhanced by good management practices.

633 **Efficacy of moxidectin 2% long acting for sheep against natural infections of nasal bots (*Oestrus ovis*) in sheep in Spain**

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Ovine oestrosis is a cavitary myiasis caused by larvae of *Oestrus ovis*. This infestation produces important losses in sheep production. In the central and southern provinces of Spain, the incidence of oestrosis is high. Studies in those areas demonstrate that 98% of the flocks are parasitized, the intensity of infestation being widespread within sheep population, with a high prevalence (71%). Moxidectin is an endectocide of macrocyclic lactone compound highly active against nasal bots. Thus, the objective of this study was to evaluate the efficacy of a single treatment with moxidectin 2% LA injectable (Fort Dodge) at 1 mg/kg bodyweight against naturally acquired infections by nasal bots in sheep under field conditions in Spain. A total of 24 sheep showing evident symptoms of oestrosis were ranked in ascending order of live weight to form 12 blocks of two animals each. Within each block, animals were randomly assigned to a treatment group and a control group. Moxidectin was administered as a single subcutaneous injection at the base of the ear. Animals were slaughtered on day 26 post-treatment. The head was cut off and all larvae present in different sites were collected, differentiated live and dead, counted and identified as 1st, 2nd and 3rd instars stages of *O. ovis*. Efficacy was calculated as the % of the reduction in the geometric means of each instar stages of *O. ovis* in treated group compared with control group. Only first instar stage efficacy (94.4%) could be calculated due to the lack of the second and third stages in the animals.

Acknowledgement We acknowledge Fort-Dodge Animal Health for the funding of this trial.

634 **Evaluation of three solvents as vehicle of crude methanolic plant extracts in the larval immersion test using *Boophilus microplus* ticks**

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The larval immersion test (LIT) has been used to evaluate the acaricide efficacy of both synthetic compounds and plant crude extracts. This technique requires innocuous solvents for larvae and complete dissolution of samples. To measure the effect of three different solvents in the LIT, crude methanolic plant extracts obtained from root, leaves and stem bark of *Diospyros anisandra* collected in Yucatán, México were evaluated. Larvae of *Boophilus microplus* were exposed for 30 seconds to crude extracts of each plant part previously dissolved in Tween 20 (1% and 2%), Ethanol (95%) and Dimethylsulphoxide (DMSO, 2.5%) respectively. Three replicates were done for each treatment and three solvent controls were used. Larval mortality with crude-extracts of root was: Tween-20 1% (35%) Tween-20 2% (52%), Ethanol (77%) and DMSO (14%). For leaf: Tween-20 1% (82%), Tween-20 2% (88%), Ethanol (77%) and DMSO (70 %). For stem bark: Tween-20 1% (60%), Tween-20 2% (99%), Ethanol (55%) and DMSO (73%). No significant differences were found in larval mortality using the different solvents in each control test (Kruskal Wallis test, $p>0.05$). It is concluded that the three solvents can be used to dissolve crude plant extracts in the LIT; however, Tween-20 (no reported in literature) has the advantage since it is less toxic to larvae and safe to handle by the operators.

635 **Efficacy of a 20% clorpiriphos + 50% amitraz * formulation against *Rhipicephalus (Boophilus) microplus* in naturally and artificially infested bovines**

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Two studies were done to assess the therapeutic efficacy of a 20% clorpiriphos + 50% amitraz formulation in bovines artificially (Assay I) and naturally (Assay II) infested with *R. (Boophilus) microplus*. After an adaptation period, in experiment I bovines were infested with 5,000 (0,25g) 14 to 21 day old *R. (Boophilus) microplus* larvae. By assessment of mean number of female *R. (Boophilus) microplus* ticks collected from the bovine hosts on days -3, -2 and -1, two experimental groups of five animals each were formed. For the second experiment, 20 Holstein bovines were selected (10 per group) by three consecutive counts (days -3, -2 and -1) of female *R. (Boophilus) microplus*, between 4.5 and 8.0 mm of length, found on the left side of each host. In the first experiment, results showed that the treatment attained more than 90% efficacy from the 3rd to the 35th day post-treatment (DPT). On 18 observed dates the anti-ixodidic efficacy for the formulation was higher than 95%. Therapeutic action decreased after the 35th DPT (< 90%), becoming almost ineffective on the 38th DPT. In the second experiment (natural infestation) the 20% clorpiriphos + 50% amitraz formulation attained more than 95% efficacy from the first to the 21st DPT, showing its highest efficacy (99.65%) on the 21st DPT. A decrease in efficacy values was observed on the 28th (81.05%) and the 35th (27.78%) DPT. Both experiments showed clearly that the 20% clorpiriphos + 50% amitraz formulation is a promising alternative for treatment and control of bovine tick infestations.

636 **Efficacy of a long acting moxidectin formulation to protect against ovine *Psoroptic mange* (sheep scab)**

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Forty full fleeced, close woolled, yearling sheep of mixed sex were divided into two study groups of 20 according to bodyweight. Each study group was further divided (according to bodyweight) into 4 sub-groups of 5. One group of 20 was treated with Cydectin 2% LA for Sheep at the recommended dosage of 1.0 mg moxidectin/kg bodyweight (0.5ml/5kg) administered once subcutaneously at the base of the ear. The other group of 20 acted as untreated controls. Five sheep from each study group were challenged with *P. ovis* mites 30, 60, 75 or 90 days post-treatment (PT). Study sheep were examined on days 7, 14, 21 and 28 after challenge, for the presence of any scab lesions and live *P. ovis*. Live *P. ovis* were not observed on any treated sheep following challenge 30, 60, 75 or 90 days PT and no active lesions were recorded. In contrast 4/5, 4/5, 5/5 and 5/5 sheep in the untreated control group presented with scab lesions following the 30, 60, 75 and 90 -day challenges, respectively. The mean number of live mites observed 28 days following each challenge was 13.6, 10.4, 12.4 and 6.8, respectively. No adverse reactions and no swellings at the injection site were recorded. The results of this laboratory study demonstrate that a single injection with Cydectin 2% LA for Sheep protected sheep from infestation by *Psoroptes ovis* for 90 days.

637 **Efficacy of a long acting moxidectin formulation to cure ovine *Psoroptic mange* (sheep scab) under field conditions in the UK**

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Fifty three clinically infested sheep were selected from a flock naturally infested with *Psoroptes ovis* (sheep scab) and treated with Cydectin 2% LA for Sheep at the recommended dosage of 1.0 mg moxidectin/kg bodyweight (0.5ml/5kg) administered once subcutaneously at the base of the ear. Assessment of lesion area, mite counts, touch hypersensitivity score, and resolution of clinical signs were carried before treatment (Day -1) and on Days 21, 35, 56 and 70 post-treatment (PT). All but one of the 53 sheep had scab lesions (range 1.0 to 12,296 cm²), 37/53 had live mites (<10 to >50), 23/53 presented with a touch sensitivity and 10/53 were seen to be rubbing and scratching at rest pre-treatment. On Day 70 PT, all lesions were dry and lifting away from the skin apart from in one animal, also seen rubbing and scratching, however, no live *P. ovis* were observed in skin scrapings. No other animal was seen rubbing and scratching or presenting with a touch sensitivity following treatment. Animals were observed for adverse reactions 3 and 24 hours PT. The base of the ear was also examined for local reactions/swelling at 24 hours PT. No adverse reactions following treatment and no swellings at the injection site were recorded. The results of this study demonstrated that a single injection with Cydectin 2% LA for Sheep was 100% effective in curing a natural outbreak of the sheep scab mite (*Psoroptes ovis*) under field conditions in the UK.

638 **Pathogenicity and thermotolerance of various species and strains of entomopathogenic fungi against *Psoroptes ovis*, the cattle scab mite**

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In order to control psoroptic mange in cattle and sheep several studies were conducted using biological agents, e.g. entomopathogenic fungi. Whereas in vitro bioassays gave promising results, showing interesting activity of some fungal species, in vivo trials revealed several problems. In fact, in vitro assays were conducted at 27°C whereas the temperature measured in sheep and cattle at the skin surface reached 35°C and 37°C respectively. The purpose of this study was to compare the virulence and the thermotolerance of 6 species (12 strains) of entomopathogenic fungi originating from temperate and tropical areas. The comparison of the pathogenicity of these fungi showed that differences exist between species but also within species, indicating that the selection of a particular strain is crucial. The comparison of the thermotolerance showed that this parameter varied between species and that, as expected, tropical strains were able to grow at higher temperatures than temperate ones. Two strains (*Metarhizium anisopliae* IHEM18027 and *Paecilomyces farinosus* MUCL18885) were highly pathogenic at 27°C killing 50% of the mite population within 0.5 and 1.5 days at the dose of 108 conidia ml⁻¹ respectively and were able to grow at a temperature of 35°C. These fungi could be considered as good candidates in the frame of the control scheme of psoroptic mange in cattle.

639 **Comparative efficacy of ivermectin and cypermethrin against *Hyalomma* Ticks (Acari: Ixodidae)**

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The objective of this paper is to describe the current status of the in vitro and in vivo effectiveness of the two acaricides viz; Ivermectin and Cypermethrin against prevalent tick species (*Hyalomma anatolicum*) of bovines. To this end, four hundred and eighty live engorged female *Hyalomma anatolicum* ticks were collected from the animals and separated into two equal groups viz; I & II for the efficacy trial of Ivermectin (Ivomec, Merial, France) and Cypermethrin pour-on (Cipermetriven, Ivan Labs, Spain), respectively. Each group was further divided into four sub-groups (A through D) to administer various concentrations of each drug. The ticks were counted after an immersion period in order to compare the efficacy of the two compounds. For an in vivo trial, three hundred and sixty bovines were selected on the basis of criteria as (i) having tick burden at the rate of >100 ticks per animal (ii) age of animals is more than 1 year (iii) No history of application of acaricide to any of infested animals. The comparison of the two groups was done on day 0, 5, 10, 15 and 20 after treatment of the two test acaricides. The results of the study indicated better in vitro and in vivo efficacy of cypermethrin pour-on as compared to ivermectin against *Hyalomma* ticks. The results, thus obtained provide some useful tools in decision making of tick control providing the basis for testing the findings on provincial and national levels in future studies.

640 **Does topical insecticides have behavioural effects of lice? An example with imidaclopride and biting lice: *Damalinia (Bovicola) bovis***

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Studies on behavioural activities of insecticides have been focused on fleas or biting dipterans for prevention rather on the effects induced on the body surface. Little is known on such activities on lice. Sucking lice have limited motility and can be used as local markers of insecticidal activity/spreading whereas biting lice are mobile and could be influenced by repellency. We used as a model a young beef highly infested by both types of lice and treated on day 0 with Imidacloprid (ADVANTAGE). Lice were collected by combing and clipping areas (25 cm²) symmetrically to the dorsal line. Four areas were defined (1 to 4) at respectively 5, 40, 75, 110 cm. caudally to the site of application. Lice, dead or alive, were identified and counted on the surfaces on days D0, D5, D7 and D9.

A 100% insecticidal effect on sucking lice was obtained at D5 for area 1, D7 for area 2 ; but only respectively 96 - 92% on areas 3 - 4 at D9. As compared to initial level, a strong reduction in the total number of biting lice was obtained on areas 1 and 2 (-76 to - 93%) but comparatively lower on area 3 (-70% D5 ; - 60% D7). On area 4 the evolution was opposite with a considerable increase + 45% D5 ; + 352% D9. These results suggest : a) A very progressive diffusion on skin surface on the 9 days period and b) A repellent effect on biting lice.

641 **Infestation of horses by flies - Interest of a treatment with deltamethrine diluted in water**

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Objective : Evaluation of the significance of deltamethrine in aqueous dilution for treating and preventing infestations by flies on horses during the summer pasture period.

Materials and methods : During summer 2005, 42 horses of various breeds were kept in pasture at two different sites in the Rhône area. Using a randomization process which guaranteed equality in the average number of flies between the groups, the horses were allocated either to control, or to treatment group. 60mL of Butox 7.5 Pour On (7.5 % aqueous suspension of deltamethrine) diluted in 180mL of tap water was applied by sponge on the whole body of the treated horses. At day 1, 2, 3, 4, 7, 14, 21 and 28 standardized pictures of the head, barrel, and perineo-inguinal region were taken, allowing counting and identification of the flies.

Results :

-Countings in untreated groups remained stable or increased steadily depending on the site.

-*Musca* and *Simulidae* flies were found on the barrel, and *Hippobosca* flies in the perineo-inguinal region. From day 1 to 28, the number of those flies was reduced by 90-95 % ($p < 0.05$) in the treated groups at both sites.

-Only *Musca* flies were found on the head. A likewise significant reduction in infestation by those flies was observed from day 1 to 7.

-No intolerance reactions were noticed.

Conclusion: Application by sponge on the entire body of 60 mL Butox 7,5 Pour On diluted in 180 ml water provides an immediately effective, well-tolerated treatment of flies infestation in horses.

642 **Oestrosis diagnosis in sheep by ELISA test using salivary gland antigens of *Oestrus ovis* (Diptera:Oestridae)**

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Oestrus ovis L. is a parasite of upper respiratory tract of sheep and goats. The most important antigens are in the salivary gland and their diagnostic value has not been investigated in naturally exposed sheep. The objective was to determine the diagnostic value of *O. ovis* saliva antigens by iELISA test to detect the oestrosis in sheep. *O. ovis* larvae were collected from heads of sheep and goats. Somatic antigens of second instar (SAL2) and salivary gland antigens of L2 (SGAL2) and of third instar (SGAL3) were prepared. The sera and nasal mucus samples of necropsied sheep (n=166) naturally exposed to *O. ovis* infection were used, and an experimental infection was carried out in 12 lambs. It was determine the specific IgA and IgG. Data of necropsied sheep (no. larvae, category, weight and size) were recorded. The mean number of larvae in naturally infected sheep was 3.54 ± 2.9 (0.56 ± 1.3 for L1, 1.41 ± 1.8 for L2, and 1.57 ± 2.2 for L3). The weights for L2 and L3 were 71.7 ± 114 and 611.8 ± 780 μg , respectively, while the sizes were 1.1 ± 0.3 (L1), 8.3 ± 3.6 (L2), 17.7 ± 3.4 (L3). The sensibility and specificity of the test with serum IgG were, respectively, 96.1 and 46.9 with SAL2 and, 99 and 25 with SGAL2. In contrast, the sensibility and specificity with SGAL3 were 96.1 and 48.4, respectively. The IgA in serum and in mucus were very low. This study confirmed the potential of *O. ovis* salivary gland antigens to detect the oestrosis in sheep by iELISA test.

643 **Diffrentional study of morphology and Elisa test in diagnosis of Hypoderma of goat of Iran (Khoozestan province)**

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Hypodermosis is a parasitic disease of goat caused by *Przhevalskiana* sp. It is an important health and welfare problem of infested goat a cause of considerable economic loss. In live animals detection can be either by direct parpation of second and third stage larvae in the back and presence of larvae in the back and presence of larvae under the skin of goat carcase. During the period of march 2004. to march 2005, 92 negative sample serum and 100 positive sample serum (infested goat) for Elisa test and 1500 adult larvae (L3 satage) for morphological study were collected. To evaluate the applicability of Elisa rechnique different dilutions of serum and conjugate were tested and the results were compared with observation technique. Sensitivity and specifity calculated from this panel were 86.66% and 98.87% in three first month of disease started.

The best results were obtained with a serum dilution 1/40 and 1/9000 conjugated serum.

In the prevalence study 36.56% of khoozestan goats were infested. Morphological studies of larvae showed that.

Przhevalskiana silenus 13.66%

Przhevalskiana crossi 59%

Przhevalskiana agageri 27.34%

In view of the simplicity and rapidity of the assay, the Elisa test can be considered as a useful tool for the diaynosis of Goat Warble Fly infestation specially in the first stage of disease (L1 stage).

644 **Development and evaluation of an ELISA for diagnosis of *Sarcoptes scabiei*-infection in sheep**

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Sarcoptes scabiei infection in sheep is mainly restricted to the head, causing pruritus and crust formation. The disease has adverse effects on milk and meat production, decreases the reproductive performance and has animal welfare implications. Accurate and early treatment of affected flocks detected by using a serological test could be advantageous in control programmes. The test could also be a reliable tool for epidemiological surveys. Here, we have developed and valued an indirect ELISA for the diagnosis of sarcoptic mange in sheep. Sera from naturally and experimentally *S. scabiei*-infected sheep, sera from clinically unaffected sheep and sera from sheep with other cutaneous diseases were tested, and the results compared with those of skin scrapings and existence of clinical signs. We found that the sensitivity of the test was greater than skin scrapings and %OD had a positive relationship with lesion extension. The test also detected around 50% of clinically normal sheep from affected flocks. In addition, several ewes from suspect flocks, where sheep with compatible signs were present but *S. scabiei* mites were not found, were seropositive. Seroconversion of experimental infected sheep took place after two to five weeks. The specificity of the test was good, since only less than 10% of animals from flocks without sarcoptic mange were seropositive. Furthermore, the cross reaction of anti-*Psoroptes ovis* sera was negligible. The antibodies declined after successful treatment with ivermectin, and at three months about half of the animals were seronegative. The ELISA described here can be a useful tool in control programmes and epidemiological surveys of ovine sarcoptic mange.

645 **Comparison of clinical signs, ear swabbing and ELISA for the diagnosis of sub-clinical ovine *Psoroptic otacariasis***

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A field case of 24 breeding rams presenting with headshaking, ear rubbing and aural damage was investigated to compare the relative efficacies of clinical signs, ear swabbing and ELISA to diagnose sub-clinical, ovine, psoroptic otacariasis. Aural damage (haematomae or fibrosis) was recorded and both ears of each ram were swabbed using 7.5 cm long swabs with a 1.0 to 1.5 cm cotton head ("Cotton Buds"). Swabs were inserted into the external auditory canal (EAC) until resistance was met and gently twisted and removed. Swabs were examined under a dissecting microscope with overhead lighting (x40) for the presence of live or dead mites. Once examined the swabs were digested in boiling potassium hydroxide and the presence of mites recorded. Blood samples were also taken from 21 of the 24 rams and the processed sera examined by an improved ELISA. Results demonstrated that 4/24 (17%) of the rams presented with mild to severe aural damage (unilateral or bilateral haematomae/fibrosis). Ear swabbing demonstrated *Psoroptes* mites from 10/24 of the rams, representing 42% of the group. ELISA demonstrated anti-*Psoroptes* antibodies in 12/21 (57%) of the rams. Results show that aural damage is not indicative of infestation and that ELISA appeared to be more effective in diagnosing psoroptic otacariasis compared to ear swabbing. However, none-parasitic forage mites (Acaridae) were isolated from 2/24 (8 %) of the rams (not identified as infested with *Psoroptes* mites), and their presence may produce false positive ELISA results.

646 **Efficacy of an improved ELISA to diagnose sub-clinical ovine *Psoroptic mange* (Sheep Scab)**

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Forty, six month old, full fleeced, pure-bred Suffolk lambs of mixed sex were divided into four Study Groups, each comprising of 10 sheep. Prior to challenge with *Psoroptes ovis* all animals were examined for scab and other ectoparasites, ear swabbed and blood samples taken twice, one week apart. One study group was challenged per week over a four week period. 35 days after challenge of Group 1 all sheep were treated with a licenced product. Thus at the time of treatment infestations were 35 (Group 1), 28 (Group 2), 21 (Group 3) and 14 (Group 4) days old. Blood samples were taken weekly over the 35 day infestation period. Prior to challenge all sheep were confirmed negative for *Psoroptes* and other ectoparasites by clinical examination and no sheep (0/80) was sero-positive for *Psoroptes* prior to challenge. The improved ELISA demonstrated that 2.5% (1/40) were sero-positive for *Psoroptes* 7 days post-challenge (PC); no sheep was sero-positive 14 days PC; 7.5% (3/40) were sero-positive 21 days PC; 10% (2/20) were sero-positive 28 days PC and 50% (5/10) were sero-positive 35 days PC. Sheep presenting with no clinically detectable lesion were recorded at 50/140 clinical examinations and these were correspondingly sero-negative by ELISA. Active scab lesions were clinically recorded at 90/140 examinations, with areas ranging from 0.04 cm² (0.001% body cover) to 252 cm² (5% BC). Sheep presented with lesions < 10 cm² (below 0.2% body cover) at 67/140 examinations, only 1/67 (1.5%) of these lesions were sero-positive. Sheep presented with lesions 10 to 50 cm² (0.2 to 1.0% BC) at 15/140 examinations, 5/15 (33%) of these lesions were sero-positive. Sheep presented with lesions 50 to 100 cm² (1.0 to 2.0% BC) on 5/140 examinations, 4/5 (80%) of these lesions were sero-positive. Sheep presented with lesions > 100 cm² (above 2.0 % BC) on 3/140 examinations, 3/3 (100%) of these lesions were sero-positive. All ELISA sero-positives were weak (< 15 AU).

647 ***Melophagus ovinus*: Experimental infection of sheep and its survival outside the host**

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Sheep ked (*Melophagus ovinus*) is a parasite of frequent appearance in sheep of different countries. Although the dissemination of *M. ovinus* is related to the climatic conditions, other factors that could affect their dissemination, has not been studied. By means of 2 controlled tests, the colonization capacity of *M. ovinus* in Corriedale ovines and its survival outside the host under different environmental conditions were recorded. Colonization ability in susceptible hosts was measured through artificial inoculation of 205 adult parasites in 4 susceptible ovines. 45.8% of inoculated *M. ovinus* adapted themselves and continued in their new hosts, with individual variations ranging from 23.8% to 73.7%. Concerning parasite survival outside the host, out of 1489 subjects, 100% died before the 9th day, under temperature conditions varying from 15 to 27°C, which allowed less than 1% to live up to days 7 and 8. The described observations would contribute to previous work made on the potential dissemination of *M. ovinus*, where it was demonstrated that the dissemination took place at slow or moderate rate through the time

648 **Analysis of world strains of *Anaplasma marginale* using major surface protein 1a repeat sequences**

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Anaplasma marginale is a tick-borne pathogen of cattle that causes the disease bovine anaplasmosis worldwide. Major surface proteins (MSPs) are involved in host–pathogen and tick–pathogen interactions and have been used as markers for the genetic characterization of *A. marginale* strains and phylogenetic studies. MSP1a is involved in the adhesion and transmission of *A. marginale* by ticks and varies among geographic strains in the number and sequence of amino-terminal tandem repeats. The aim of this study was to characterize the genetic diversity of *A. marginale* strains collected from countries in North and South America, Europe, Asia, Africa and Australia, inclusive of all continents. In this study, we characterized 131 strains of *A. marginale* using 79 MSP1a repeat sequences. These results corroborated the genetic heterogeneity of *A. marginale* strains in endemic regions worldwide. The phylogenetic analyses of MSP1a repeat sequences did not result in clusters according to the geographic origin of *A. marginale* strains but provided phylogeographic information. Seventy-eight percent of the MSP1a repeat sequences were present in strains from a single geographic region. Strong ($\leq 80\%$) support was found for clusters containing sequences from Italian, Spanish, Chinese, Argentinean and South American strains. The phylogenetic analyses of MSP1a repeat sequences suggested tick–pathogen co-evolution and provided evidence of multiple introductions of *A. marginale* strains from various geographic locations worldwide. These results contribute to the understanding of the genetic diversity and evolution of *A. marginale* and tick–pathogen interactions.

649 **Effect of hypodermins A, B and C on the secretion of gamma interferon (IFN-gamma) by bovine lymphocytes stimulated with two mitogens**

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The gamma interferon is a cytokine produced in animals upon challenge with specific antigens or in lymphocyte-macrophage cultures by stimulation with polyclonal T-cell mitogens. The main objective of this study was to determine the modulatory effect of the three fractions, called hypodermins A (HA), B (HB) and C (HC) and purified from *Hypoderma lineatum* larval excretory /secretory products, on the secretion of IFN-gamma by T-cells. Lymphocytes were isolated by a gradient of Ficoll from infested and non infested cattle, then they were stimulated with the HA, HB and HC and 10 µg/ml of the mitogens concanavalin A (Con A) and phytohemagglutinin A (PHA) and incubated at 37°C in 5% CO₂ during 24, 48 and 72 h, respectively. Finally, IFN-gamma levels were determined by a commercial ELISA test (Biosource).

The presence of the different antigens in absence of mitogen did not stimulate the secretion of IFN-gamma; by T-cells from infested and non infested animals. In naïve-cattle lymphocytes the presence of any hypodermin (A, B or C) inhibited the effect of Con A diminishing the secretion of IFN-gamma; however, they did not modify considerably the effect of PHA on the secretion of this cytokine. On the contrary, in infested-cattle lymphocytes the incubation with HA and con A stimulated the production of IFN-gamma, whereas the HB and C decreased the effect of both mitogens, especially at 48h of incubation. Those results suggest that the three hypodermins could be involved, especially in naïve cattle, in the immunosuppression that would allow larvae to avoid the host's immune response.

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650 **Immunogold localization of serine proteinases in midgut of *Hypoderma lineatum***

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First instar *Hypoderma spp.* have a blind gut necessitating regular regurgitation of its contents into the adjacent host tissues. Enzymes secreted by salivary glands and presumably by midgut epithelium pre-digest host tissues in front of the larvae and provide a rich nutrient 'soup' that is subsequently imbibed. Immunogold localization was used to determine the presence and location of the major proteinases known to be excreted/secreted by first instars of the common cattle grub *Hypoderma lineatum*. The location of these enzymes in midgut cells of both newly hatched larvae and six-seven month old, parasitic larvae were determined. Hypodermin B (HyB) and hypodermin C (HyC) were localized in midgut epithelial cells of newly hatched larvae. No immunolabelled hypodermin A (HyA) was evident in these larvae. All three hypodermins were localized in midgut epithelium of the parasitic larvae recovered from artificially infested cattle. HyA was evident at very low levels in these larvae. HyB was abundant and localized primarily in association with the microvilli. HyC, also abundant, was localized in association with cytoplasmic vesicles as well as with microvilli. HyC was also evident in the gut lumen. The electron microscopy results were confirmed by SDS -PAGE separation of products from selective isolation of midgut membrane.

651 **Evaluation of crude and purified gut extract antigens for protection against camel tick *Hyalomma dromedarii* (Acari: Ixodidae)**

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Gel filtration was used to purify gut extract antigen (GEA) of *Hyalomma dromedarii* ticks. Two purified peaks were obtained from crude extract (GEAP1 & GEAP2). Crude extract and the two purified peaks were used as immunogens for rabbits with Freund's adjuvant. The six immunized and two control groups were challenged with adult and larvae of *H. dromedarii* (3 immunized and 1 control for each stage). The efficacy of immunogens against adult (E%) showed that the peak1 fraction of gut extract antigen (GEAP1) recorded highest immune effect on the adults (64.97%) followed by the crude gut extract antigen (GEA) which recorded (30.97.9%) and lowest protection with peak2 fraction of gut extract antigen (GEAP1), 21.57%. The rejection percentages of larvae increased to the highest value (84.5%) in immunized group GAP than that in control one. Also, it increased (80.6%) in group GEAP1 and to (70.1%) in group GEAP2. The antibodies in sera of rabbits challenged with both adults and larvae increased gradually reaching their highest level at 5th week post immunization by using GEA and GEAP1, while by using GEAP2 the antibody levels reach eth the highest value at 6th week till 6 weeks post-immunization. The antibody responses in sera of rabbits challenged with larvae were significantly higher in group SGA and SGAP2 and lower in group SGAP-1. A direct correlation between antibody response and protection against infestation was observed. Western blot analysis detected two polypeptide bands of 32 and 29 kDa in the sera of immunized animals.

652 **Novel salivary proteins of the argasid ticks *Ornithodoros erraticus* and *Ornithodoros moubata* identified by a proteomic approach based on de novo sequencing**

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Introduction: Tick saliva contains pharmacologically active proteins that allow ticks to obtain a blood meal from the host and help tick-borne pathogens to infect the vertebrate host more efficiently. This makes the salivary proteins attractive targets to control ticks and tick-borne pathogens. Although *Ornithodoros moubata* and *O. erraticus* are important argasid ticks that transmit severe diseases such as human relapsing fever and African swine fever, only a few of their salivary proteins have been already identified.

Methods: To identify novel salivary proteins of these argasids, their salivary gland extracts were analyzed by 2D-electrophoresis, MALDI-MS, MALDI-MS/MS, nLC-MS/MS and de novo sequencing.

Results and conclusions: 2D maps of the salivary proteome and immunome of both ticks were constructed and a high number of their spots were analysed. Despite the good general quality of the peptide mass maps obtained, any identification was achieved on the only basis of MS/MS data. This made it necessary to obtain peptide sequences by de novo sequencing. We obtained 22 peptide sequences from 10 proteins, which allowed us to identify 2 proteins of *O. moubata* and 6 proteins of *O. erraticus*. The roles of these proteins in tick saliva are discussed. The limited number of acarine sequences currently available in public databases was a serious drawback to the identification of tick salivary proteins using proteomic approaches based on MS(/MS) data and was also a limitation when conducting sequence similarity searches.

653 **Molecular and reverse genetic characterization of a hemolytic serine proteinase in the midgut of the Ixodid tick *Haemaphysalis longicornis***

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Ixodid ticks are obligatory haematophagous arthropods that live solely on blood-meals throughout their life. Hemolysis is one of the key physiological processes in the tick digestive system, because it is the first phase of degrading the host's erythrocytes to make hemoglobin available. Enzyme-induced hemolysis has been shown to occur in the midgut of ticks; however, little is known about the molecular basis for hemolytic activity. We report here the molecular and reverse genetic characterization of a hemolytic midgut serine proteinase, HISP, recently identified from the babesial parasite vector *Haemaphysalis longicornis*. Endogenous HISP was found in the midgut lumen and its contents, indicating that HISP is extracellularly secreted. Recombinant *H. longicornis* serine proteinase (rHISP) showed dose-dependant hemolytic activity towards rabbit erythrocytes, with a maximum hemolysis of 94.5 % within 1 h in vitro. Tests of pH dependency showed that rHISP displayed optimal activity at pH 6.0. Affinity binding assays demonstrated that rHISP binds strongly to band 3, which shares major erythrocyte membrane protein. Disruption of HISP-specific mRNA by RNA interference resulted in inhibition of the degradation of host erythrocyte membranes by endogenous HISP in the knock-down ticks, indicating that HISP plays a crucial role in the hemolysis in the midgut of haematophagous ticks. Our results suggest that HISP may be essential for initiating the proteolytic cascade for the degradation of the host blood-meal.

Poster group 17: Arthropods, miscellaneous

654 **Relevant Diptera frequency in public health and *Enterobacteriaceae* associated from Baixada Fluminense area, RJ, Brazil**

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Some Diptera *Muscidae*, *Calliphoridae* and *Sarcophagidae* have importance in public health, because they are mechanical and biological vectors for many pathogens including humans parasites and domestic animals. The objective of this study was to verify the frequency adults species of Diptera (*Muscidae*., *Calliphoridae* and *Sarcophagidae*), and some *Enterobacteriaceae* associated. The experiment was carried at the Animal Parasitology Laboratory and Microbiology at the Veterinary School of Universidade do Grande Rio- Unigranrio, Duque de Caxias, RJ, during November 2005 to October 2006. The flies were captured with Pet plastic bottle. The adult insects were collected from natural breeding grounds as urban sanitary landfill and near by restaurants from Caxias and New Iguaçu municipalities. After the capture, the insects were touching on selective culture media and the cultures were maintained for twenty four hours at 37°C. The development of purple colonies for *Enterobacteriaceae*, brilliant green for *Escherichia coli* and in some cases characteristic colonies of mushrooms, were observed and after this phase were transferred for other selective medias McConkey, Agar SS, Manitol Salt for identification. The *Enterobacteriaceae* isolated were *Salmonella sp*, *Shigella sp*. and *Escherichia coli*. The culture media contacted by *Musca domestica* had growth 100% for *Enterobacteriaceae*, however to *Chrysomya sp* occurred development for mushroom, as well as for *Sarcophagidae* that just presented mushroom and *Escherichia coli*. The seasonality of the Diptera presented variation in the different seasons of the year. This experiment demonstrated the dissemination of *Enterobacteriaceae* by sinantropic Diptera.

655 **Poultry mite (*Dermanyssus gallinae*) dermatitis in a horse**

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This case report describes for the first time an uncommon poultry mite (*Dermanyssus gallinae*) dermatitis in a 16-year-old horse in contact with domestic hens. Major clinical signs consisted of self-induced hair loss located mainly on the head. In spite of multiple skin scrapings performed on the horse including at night, mites were only isolated from in contact poultry and from the environment of the horse. The animal was cured by spraying a 2% permethrin solution on the entire body once a week for 4 weeks and by decontaminating the horse-stable by vacuuming, cleaning and spraying with amitraz (0.125% solution). Despite the persistence of mites in the environment and the further contacts between the horse and poultry, the recurrence of dermatitis was prevented using regular topical permethrin applications and biannual decontamination of the horse-stable.

656 **Finding of *Megninia gynglimura* and *M. crinita* mites as cause of dermatitis and fall of feathers oin egg layer hens of commercial type coming from a farm in Colima State on Mexico**

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The present paper has as objective to communicate for the first time in the State of Colima Mexico the isolation of mites that know themselves like mites of the feathers of the birds and that also can be cause of fall of as well as of same dermatitis and the pruritus in them. The mites of *Megninia* genus have been reported for Mexico since 1987, where the presence of three species in hens of different states from the Mexican Republica were communicated, these have been *Megninia gynglymura*, *M. cubitalis* and *M. ortari* the material consisted of collected dust of different places from the ship of the farm: of deep litter ; of the bed of the nests and 5 birds by sampling. Of the dust were diverse organisms and among them mites that were clarified in Kono, liquid the mites were mounted between slides and coverslides using Hoyer liquid of when observing the slides could be corroborated that it was determined that the mites corresponded to the *Megninia* genus , species:*Megninia.ginglymura* and *M. crinita*. The importance of this communication is in the fact that this is the first time that communicates the presence of *Megninia* mites in the State of Colima, Mexico as cause of dermatitis and fall of feathers reason why will have to put attention on these mites since there will be to determine the fenology of the different phases of development in the hens and thus to propose the method of suitable control more for this mites in this locality.

657 **Acaricidal activity of *Melaleuca alternifolia* (tea tree) oil and ivermectin in patients with different skin lesions due to *Demodex follicullorum* infestation**

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Tea-tree oil (an essential oil of the native tree *Melaleuca alternifolia*) has long been regarded as a useful topical antiseptic agent and has been shown to have a variety of antimicrobial activities; however, few evidences exist for its efficacy as an acaricidal agent for treatment of various skin conditions. Patients in the present study were chosen after clinical and microscopical confirmation of of *Demodex follicullorum* infestation by non invasive skin surface biopsy .The patients were given 50% tea-tree oil (TTO) alone , oral ivermectin therapy alone or combination of both.. To evaluate the efficacy of treatment, cases were followed up every week after treatment for four visits by counting the follicle mites and by clinical evaluation .The results of this study showed that 50% tea-tree oil had a significant effect in ameliorating the patients' manifestations and reducing the mite densities. However its effect was augmented when it was combined with a single dose of ivermectin . So skin scrubbing with TTO when accompanied by oral ivermectin, can be a safe and effective medicament for cases with different skin lesion due to D.folliculorum infestation

658 **Effects of 4 different chemical compounds at control and treatment of varroasis in *Apis mellifera*, a comparative study**

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Honey bees for producing of honey, royal jelly, poison, pollination of flowers and ...are very important. *Varroa jacobsoni* is the most pathogen in this useful insects. This ectoparasites by nutrition via larvae and adult bees in beehives cause a serious damages and bees population would be weakened.

In this study we had 5 experimental group and used 4 chemical compounds including : Formic acid, Flumethrin (Bayvarrol), Thymol (Apiguard) and Fluvalinate (Apistan). In each group we had selected 7 beehives with same origin and population. These beehives had not received chemical treatment at 1 year ago.

Then primary infestation by *Varroa jacobsoni* in larvae and adults bees were determined in each beehives of groups. Primary infestation had not significant difference between all groups ($P < 0.05$). In next step, chemical compounds were applied in considered group and then the killed mites were quantitated at 48,96 and 144 hours after drug application. In 6 months later, mite infestation in larvae and adults bees in each group were determined. Then analytical process were applied by ANOVA and Post Hoc tests.

Results showed, all 4 chemical compounds had varroacidal effects that Formic acid had the least and Flumethrin the most effects. Also, comparison between mite infestation at 6 months after applying drugs in all groups (in larvae and adults bees) were shown that in Flumethrin group, larve infestation decrease from 13% to 1% and infestation in adults from 16.91% to 1.01% which this decreasing is significant ($P < 0.001$) and this effects was more better than other chemical compounds. In fact, Flumethrin has have more controlling effect on Varroasis in comparison with 3 other used compounds. This compound had the most varroacidal effects at time 96 after drug applying (by killing 1447 mites). Controlling effects of Flumethrin in comparison with groups Control and Formic acid were shown a significant increasing but this increasing in comparison with Apiguard and Apistan groups was not significant in Dunnet C Post Hoc analytical test. (confidence interval=95%)

Poster group 18: Coccidia

659 **Coccidiosis of calves in Hungarian dairy farms: prevalence and *Eimeria*-species**

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Coccidiosis is considered to be a problem in young calves around the world. However, no reliable data had been available on the prevalence and the species of this parasitic infection in Hungary. Faecal samples of 743 calves aged between 2 and 6 months old were collected in 88 dairy farms located in different geographical regions of the country and examined for the occurrence of *Eimeria* oocysts between September 2005 and February 2007. Species identification was done by measurement and morphological characteristic of each species. The number of examined animals in a dairy farm varied from 2 to 17.

In most farms (76; 86.3%) 10%-100% of animals were found be infested. The faecal samples of 219 calves (29.5%) contained coccidian oocysts. In total seven *Eimeria* species were identified. *E. bovis* and *E. zuernii* occurred in 111 calves of 41 (46.6%) and 45 calves of 21 (23.8%) farms, respectively. Both pathogenic species were found in 18 calves of 10 (11.4%) farms. *E. auburnensis* (52), *E. ellipsoidalis* (48), *E. cylindrica* (15), *E. pellita* (6) and *E. subsphaerica* (6) were also detected in decreasing number of the infected farms. Diarrhoeic calves had been in 12 farms but pathogenic *Eimeria* species was found only in 4 of these farms.

660 **The epidemiology of calve coccidiosis (*Eimeria spp.*) in Poland**

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The aim of the study was to estimate the prevalence of *Eimeria spp.* infections in calves in Poland.

Material and methods: During the studies the total number of 579 fecal samples was obtained from 86 Polish farms. The number of animals on the farms varied from 8 to 1000. The age of calves was from 1 to 18 months. The calves were sampled from spring to autumn 2006. The total number of 579 fecal samples was examined.

Results: Farms were allocated into 2 groups: small <100, big farms >100 animals.

It was found that *Eimeria spp.* occurred in 93% of farms. The highly pathogenic coccidia were found in 88,4% of farms. The prevalence of highly pathogenic *Eimeria* was 92,3% at big farms, and 85,1% at small farms. The low pathogenic coccidia (alone) were found in 5,1% of big and in 4,3% of small farms. Only 2,6% of big and 10,6% of small farms were found free of *Eimeria* infection.

Eimeria spp. were present in 73,1% of fecal samples: highly pathogenic were noticed in 55,8%, low pathogenic (alone) - in 17,3%, respectively. Only 26,9% of calves were free of infection.

The intensity of *E. bovis* as well as *E. zuernii* infection remained in most cases low (75,4 and 79,8%) or moderate (21,3 and 18,5%), respectively. The intensity of those infections was as well 3.1x and 1.9x higher in big farms.

Conclusion:

1. *Eimeria spp.* are very common pathogens in cattle farms in Poland.
2. The highly pathogenic *Eimeria* occurred more frequently on big then on small farms.

661 **Early interactions of peripheral blood mononuclear cells with *Eimeria bovis* infected host cells**

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Eimeria bovis is an important coccidian parasite of cattle causing severe haemorrhagic diarrhoea in primary infected calves. The first schizogony of *E. bovis* takes place in lymphatic endothelial cells of the ileum, resulting in the formation of macroschizonts within 2-3 weeks. Here, we analyse early cellular immune responses of experimentally *E. bovis* infected calves to infected host cells on the basis of peripheral blood mononuclear cells (PBMC) adhesion on and transmigration through infected bovine umbilical vein endothelial cell (BUVEC) monolayers. Adhesion of PBMC was enhanced in infected monolayers. Most marked effects were observed 1 day p. i.; thereafter PBMC adhesion declined reaching control levels from day 8 p. i. onwards. CD8+ T cells adhered more frequently to infected BUVEC (42 %) than CD4+ T cells (25 %). About one third of attached PBMC were represented by gamma delta-TCR+ T cells. Adhesion of T cells was not restricted to parasitised host cells, but occurred almost equally on non-infected BUVEC within the same monolayer. Furthermore, we found moderately enhanced PBMC transmigration through infected BUVEC monolayers, in particular on day 2 p. i. The data presented here suggest that *E. bovis* infection of BUVEC induces endothelial cell-derived, proinflammatory reactions which appear suitable for the initiation of both, adaptive and innate immune responses.

662 **Diclazuril for coccidiosis in ruminants: safe for the environment?**

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Introduction: Diclazuril is an anticoccidial for use in broiler chickens, turkeys, lambs and calves. Extensive environmental impact information became available. This contribution will present a comprehensive summary.

Methods: Environmental exposure was calculated on the basis of animal treatment patterns, data on metabolism and excretion, current agricultural practices and mathematical models. Environmental impact was explored in laboratory protocols compliant with regulatory guidance from EU, USA and OECD.

Results: Diclazuril is not metabolized in lambs and calves and is excreted completely and unchanged. Only parent substance is of environmental relevance. Dung from animals treated on pasture contains about 5.4 and 7.5 mg diclazuril per kg in lambs and calves, respectively. Manure from calves treated in house results in soil concentrations of about 0.005 mg/kg. Concentrations in ground water are below 10-5 mg/l. Diclazuril strongly absorbs to soil. It biodegrades in soil with DT50 < 60 days and DT90 < 1 year. Diclazuril will not accumulate after repeated manure applications. Diclazuril is devoid of insecticidal properties. It does not affect survival or body weight of earthworms and it does not bioaccumulate in their body. Diclazuril is not toxic to plants, although some bioaccumulation can occur. Soil respiration or soil nitrification are not affected. Toxicity to aquatic organisms can only be provoked at concentrations above the intrinsic aqueous solubility of diclazuril.

Conclusion: Environmental exposure to diclazuril from coccidiosis treatments in calves and lambs is low. Diclazuril is immobile but biodegradable in soil. It is safe for earthworms, plants, soil micro organisms and aquatic organisms.

663 **Control of coccidiosis due to *Eimeria bovis* and *Eimeria zuernii* in calves with toltrazuril (baycox 5%) under field conditions in comparison with diclazuril and untreated controls**

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The efficacy of a prophylactic treatment with toltrazuril (Baycox 5% susp.) was investigated against natural infections with *Eimeria bovis* and / or *E. zuernii* in calves in comparison with diclazuril and untreated controls. The study was conducted as a multi-centred, blinded, controlled and randomised field study with 164 calves on 4 centres (farms) in northern, eastern and southern Germany. All participating farms had a known history of coccidiosis. Animals were randomised and treated 14 days after stabling into the respective facility: group I (57 animals) received 15 mg toltrazuril/kg bw, group II (54 animals) 1 mg diclazuril/kg body weight, group III (53 animals) served as sham-treated control. Assessment of efficacy was based on oocyst excretion of *E. bovis* and *E. zuernii* (opg) investigated throughout the study (3 to 4 times per week; study duration 56 days).

Coccidiosis due to *E. bovis* and / or *E. zuernii* occurred in all centres. However, extent and time course were variable. Duration and rate of oocyst excretion of *E. bovis* or *E. zuernii* were significantly lower in the toltrazuril-treated group compared to groups II and III. It is concluded that a single prophylactic treatment with toltrazuril on day 14 after stabling in provides effective and sustainable control of stable coccidiosis under various field conditions.

664 **Efficacy of diclazuril against coccidiosis in suckling calves**

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Coccidiosis causes significant economic losses in cattle. The parasites responsible of this disease are members of the species *Eimeria*, and the most important of this species for causing disease in cattle are *Eimeria bovis* and *E. zuernii*. The severity of the disease is directly related to the dose of infective oocysts that are ingested. Coccidiosis occurs mainly in calves and is usually accompanied by diarrhoea varying in severity from watery manure to one containing blood. Dehydration, weight loss, depression, loss of appetite and occasionally death may also be observed.

The main goal in this research was to analyze the efficacy and tolerance of diclazuril for preventing coccidiosis in suckling calves. One herd of 78 1-month old infected calves was divided into two groups: G-1 received a milk substitute added with 1 mg/kg diclazuril (4 ml/10 kg Vecoxan®, Esteve, Barcelona, Spain), and G-2 remained without treatment as control. Faecal samples were collected during 23 days (D0, D2, D7, D15, D22), and all the animals were weighed at the same time for determining the variations on the weight.

No clinical symptoms associated to coccidiosis were observed in the two groups. A negative significant correlation between the oocyst-output and the weight gaining was recorded ($R^2 = -0.278$; $p = .013$). Diclazuril reduced significantly the oocyst-output in G-1, and a greater weight-increment than in G-2 was observed (25.43 ± 0.27 vs 23.77 ± 0.31). These results corroborate the efficacy of Vecoxan® administered together with the milk substitute to prevent the coccidiosis in suckling calves.

This work was partly supported by a Scientific Collaboration with Dr. Esteve Laboratories, Barcelona, Spain.

665 **The differentiation of *Eimeria* species using PCR-RFLP to analyze the infected caeca of chicken coccidiosis**

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Introduction: Coccidiosis, a major cause of enteric disease in bird species, was responsible by at least 4 species of the genus, *Eimeria*. There were *Eimeria acervulina*, *E. maxima*, *E. necatrix* and *E. tenella*. This study is to detect the high prevalent species of *Eimeria* in the infected caeca of the chicken experimentally infected with the laboratory isolates.

Materials and methods: The caeca isolated *Eimeria* were collected from the coccidiosis outbreak occurred in the industrial layers in 1995. It was established as the laboratory isolates by the Unit of Parasitology, Department of Pathology, Chulalongkorn University, Thailand. Ten experimental 9-day-old chicks were fed with 10,000 oocysts. Seven days after infection, total DNA was extracted from caeca and subsequently subjected to PCR-RFLP. The 422bp of 18S rRNA gene fragment specific to *Eimeria* were amplified. The species specific pattern of RFLP was analyzed by 4 restriction endonucleases.

Results and conclusion: The PCR-RFLP was successfully produced giving the species specific DNA pattern of *Eimeria* in the gel electrophoresis. At least 3 species of *Eimeria* were found in caeca of the experimental chicken infected with the lab isolate. There were *E. maxima*, *E. necatrix* and *E. tenella*. The method of species differentiation of *Eimeria* is needed for the molecular epidemiological study of coccidiosis in poultry of Thailand. This method is not only useful for the coccidiosis occurred in the commercial poultry but also for the chicken raised as the backyard industry of the country. However, the method should be scaled up to use in the massive samples and a more accurateness.

666 **Immuno-characterization of some *Eimeria* spp. infecting chicken in Egypt**

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Monospecific isolate of *E. tenella* and *E. necatrix* were obtained successfully using rectal inoculation of the merozoites. The isolate has been confirmed through the demonstration of the characteristic lesion at particular sites of infection in chicken intestine, consideration of the pre-patent period, size of oocysts as well as the morphology of the intracellular stages. Electrophoretic analysis of the prepared antigen of *E. necatrix* sporulated oocyst gave 2 bands, while the *E. tenella* sp. oocyst antigen gave 10 bands. *E. necatrix* merozoites antigen revealed 15 bands, while *E. tenella* merozoites antigen gave 9 bands. *E. necatrix* gametocyte antigen gave 10 bands, while *E. tenella* gametocyte antigen gave 12 bands. Western immunoblotting using homologous and heterologous antisera revealed only one specific band of 49.3 kDa for *E. necatrix* spor. oocyst, while it revealed 3 specific bands of 42.9, 30.5 and 25.7 kDa in case of *E. tenella*. *E. necatrix* second-generation merozoites gave 3 specific bands of 79, 38.1 and 23.3 kDa, while *E. tenella* gave 7 specific bands of 95.5, 82.5, 80.8, 78.2, 70.3, 31.4 and 20.3 kDa. *E. necatrix* gametocyte antigen gave 4 specific bands of 69.2, 65, 60.7 and 47.1 kDa. *E. tenella* gametocyte antigen didn't reveal any specific band. Partial cross-reactivity has been noticed in the merozoites of the two *Eimeria* species when reacted with antisera of *E. tenella*.

667 **Immune enhancement of bacterial CpG DNA on Immucox against *Eimeria tenella* infection in chicken**

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As it has the multi-function in immunity, bacterial CpG DNA has been studied extensively and deeply. Nowadays, in many vaccines against parasite infection, the protective efficacy isn't still satisfied for their weak immunogenicity. Hence bacterial CpG DNA was applied as adjuvant of Immucox of attenuated vaccine to examine its protective effect against *Eimeria tenella* infection in the study. New-hatched broilers were randomly divided into six groups. The first and second immunization was made on day 7 and 14, at the dosage of 100 µg/mL CpG DNA per chicken by i.m. in Immucox+CpG. Every chicken was challenged with 1·10⁵ sporulated oocysts on day 21. The oocysts per gram (OPG) and lesion score and weight gain were investigated. The anticoccidial index (ACI) was used as a standard to compare the efficacy of each treatment. The result showed that Immucox and Immucox+CpG and Immucox+FCA had definitive protective effect against challenge. Comparatively, chickens vaccinated with Immucox+CpG DNA were higher than those with Immucox, CpG DNA and the challenged unvaccinated with marked difference ($p < 0.05$) in relative weight gain, less than them in OPG and lesion score with marked difference ($p < 0.05$). Its ACI value was bigger than that of them, reaching 172. It was concluded that bacterial CpG DNA can improve the efficacy of Immucox against coccidiosis.

668 **The effect of administration of anti-coccidial drugs on oocyst shedding and performance in experimental coccidiosis in broiler chickens**

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To compare the effects of coccidiostate drugs on oocysts shedding and performance of coccidi-infected broiler chickens, 180 Ross 308 broiler chickens were randomly assigned to four treatments. Each treatment contained 3 replicates of 15 chickens. Treatment 1, 2 fed diets supplemented with Diclazuril and Salinomycin, respectively. Treatment 3, 4 were designated as positive and negative control received no coccidiostates. Chickens in treatments 1, 2 and 3 were inoculated with a suspension containing four *Eimeria* species. Frequency of excreted oocysts of feces sample from 7-13 day post-challenge was carried out. Body weight, body weight gain, feed conversion ratio and mortality rate were determined weekly. The results revealed that coccidiostate drugs decreased oocysts per gram of feces significantly in 7-13 day post inoculation ($p < 0.05$). The highest mean of Body weight was related to negative control followed by chickens treated with diclazuril. The lowest FCR was belonged to negative control followed by chickens treated with diclazuril. It could be concluded that coccidiostate drugs could decrease the oocysts output significantly and improve production performance in coccidi-infected broiler chicks.

669 **A comparative study on effects of experimental coccidial challenge by increasing *E.maxima* oocysts doses on serum carotenoids in chicken broilers**

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Coccidiosis is one of the most important diseases of poultry that because life cycle of its protozoan agent in intestinal epithelial cells and damage establishing, make disorder in intestinal digestion and absorption so cause a lot of economic losses to world poultry industry.

At now, routine diagnostic methods such as quantitation of OPG give not a concise evaluation from condition of poultry flocks involved by subclinical coccidiosis so using of other diagnostic methods is necessary.

As we know, there is a decreasing in intestinal nutrients intake in coccidiosis cases, so was anticipate that carotenoids should be decrease, absorption and serum carotenoids of chicken, also.

Therefore, in this study, main aim is quantitation of serum carotenoids of chickens following experimental challenge of them by increasing doses of *Eimeria maxima* oocysts. In this survey, 40 one day old chicken (*Arbor Acres B.*) were divided to 4 groups so group:1 as control, group:2 by 50,000, group:3 by 100,000 and group:4 by 150,000 sporulated oocysts of *Eimeria maxima* were challenged at 10 days old then, blood samples were taken via superficial wing vein from each chicken at 17 days old. In next step, serum of samples was separated and then serum carotenoids were quantitated by spectrophotometry method (in 453nm L.W)

Results indicates that serum carotenoids are shown a decreasing process from control to challenged groups 2-4, so this difference was highly significant ($P < 0.001$).

Therefore it seems, quantitation of serum carotenoids is suitable method for evaluation of poultry in flocks involved by subclinical coccidiosis.

670 **A field study assessing control of broiler coccidiosis by paracox vaccination or by baycox (toltrazuril) stand-alone treatment**

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Baycox® (toltrazuril) stand alone treatment and Paracox™ 5 (live attenuated vaccine) were assessed in a field trial for broiler coccidiosis control. The feed did not contain coccidiostats or antimicrobial performance enhancers. Fifteen farms encompassing 90 flocks were followed over three trial episodes (Paracox-1, Baycox and Paracox-2). Each episode included 2 successive flocks/grow-outs. Daily weight gain, feed conversion ratio, production number and mortality rate were assessed as performance parameters, indicative of coccidiosis control. No cases of clinical coccidiosis were diagnosed during the course of the trial. In general, there was a wide variability in production number within most of the farms, with only a few showing a more consistent performance. Although descriptive statistics showed small differences between the treatments, an analysis of covariance revealed no significant ($P < 0.005$) treatment effect on any of the four efficacy parameters. Age at processing and farm significantly affected feed conversion ratio, daily weight gain and production number, but not mortality. Within treatment groups, feed conversion ratio and daily weight gain was significantly influenced by the month of hatch. It was concluded that a single treatment with toltrazuril is a valuable alternative to vaccination for coccidiosis control in a rotation program.

671 **COCCIMORPH: a real-time diagnostic tool based on automatic image recognition of protozoan parasites of the genus *Eimeria***

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Species discrimination of chicken coccidiosis can be obtained through manual microscopic inspection or using PCR-based assays. Here we describe COCCIMORPH, a fully implemented system for the automatic feature extraction and shape characterization of *Eimeria* species. We used digital images of oocysts and extracted three groups of features: curvature characteristics, size/symmetry, and internal structure quantification. A database comprising 3,891 micrographs was constructed and employed as a training set. Using a Bayesian classifier for the species discrimination, we obtained an overall correct classification rate of 85.75% for the seven *Eimeria* species. A standalone program was implemented in C++ language and installed on a web server, thus allowing remote users to access the web page, upload images, and obtain a real-time electronic diagnosis. We foresee that such a system would allow for a reliable diagnosis, with no need of biological sample transportation between the farms and a reference laboratory. This represents an important achievement, since live sample traffic may represent a potential threat of disease dissemination. The incorporation of other parasites to the system may increase the scope of applicability of this tool. Coccidian protozoa and helminth eggs, by sharing a similar morphology, are the next obvious candidates. The proposed system is relatively cheap, since it only requires a microscope coupled to a digital camera through an adapter tube. The system is fully implemented and freely accessible at <http://puma.icb.usp.br/coccimorph/>. We believe that it demonstrates the feasibility of using computer-assisted systems to provide an interesting alternative for the rapid diagnosis of parasites.

Support: CAPES and CNPq

672 **Immune response to coccidiosis depends on the age of rabbits**

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We attempted to infect suckling rabbits at 14, 16, 19, 22, 25, 29 and 34 days of age (DA) with rabbit coccidia *Eimeria intestinalis* and *E. flavescens*. The immune response was investigated in these as well as eight-week-old animals 7, 14 and 21 days after inoculation. The infection was unsuccessful at 14 and 16 DA. The oocyst production was negligible at 19 DA, but since 22 DA the rabbits became infected and excreted large number of oocysts. The humoral response to infection with both parasite species evaluated by ELISA was weak in all sucklings compared to eight-week-old rabbits. Enlargement of mesenteric lymph nodes (MLN) was observed only in the older infected animals. The in vitro proliferation was stimulated by oocyst and sporozoite antigens in lymphocytes from MLN of rabbits infected at 22 DA and later, which corresponds to the age at which a patent infection was observed. The most important difference between two coccidia was markedly increased percentage of CD8+ lymphocytes in the specific site of parasite development in highly immunogenic coccidium *E. intestinalis*, but not in weakly immunogenic *E. flavescens*. Percentage of CD8+ lymphocytes in IEL from rabbits infected with *E. intestinalis* was lower in young infected as well as uninfected animals compared to their eight-week-old counterparts, but since 22 DA a difference between infected and control animals was noted. In conclusion, when the animals were infected, an immune reaction was detected, but it was less conspicuous in sucklings than in rabbits with mature immune system.

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673 **Prevalence and impact of *Isospora suis* in suckling piglets in Ontario, Canada**

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Current information on the prevalence and impact of *Isospora suis* in North American pigs is not available. The objectives of this study were to determine the prevalence of *I. suis* infections, and their association with diarrhea and growth rates, in suckling piglets in Ontario, Canada. Fifty representative herds were identified and, depending on herd size, up to 10 litters were randomly selected on each farm in each of two age groups: 7-15 days of age and 16-21 days of age. Piglets aged 16-21 days were individually weighed and a composite fecal sample was collected from 3-5 piglets per litter. A composite fecal sample was similarly collected from litters aged 7-15 days. All weaning weights were standardized to 21 days of age. All fecal samples were scored for consistency and examined for coccidia oocysts using the Cornell-Wisconsin method. Fisher's exact test and a two-sample t-test were used for significance testing. Farms ranged in size from 30 to 1700 sows (median=270). *Isospora suis* was detected on 70% of farms. On *I. suis*-positive farms, the proportion of infected litters ranged from 5% to 100% (median=21%). Litters that had *I. suis* were 4-times more likely to have diarrhea than negative litters (95% CI: 2.8-5.8; P<0.001). On coccidia-positive farms, the average standardized weaning weight was 6.3 kg, while the average standardized weaning weight on coccidia-negative farms was 6.7 kg (P=0.196). Collectively, these data indicate that *I. suis* is a common infection in Ontario swine herds and associated with diarrhea in piglets at 7-to-21 days of age.

674 **Control of *Isospora suis* in piglets: toltrazuril versus improved hygiene**

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In Denmark, the large majority of suckling piglets receive prophylactic treatment with toltrazuril to prevent clinical coccidiosis. As a result, little or no information exists on the actual prevalence of *Isospora suis* in piglets, and it is likely that in some sow herds treatment with toltrazuril may no longer be needed. The aim of our study was therefore to investigate whether improved hygiene standards in the farrowing pen could be an alternative control measure to toltrazuril treatment. Two sow herds with a history of coccidiosis were selected. In two or three farrowing units per herd the pens were allocated to either (1) standard cleaning (cold water pressure hose) + treatment with toltrazuril (Baycox ®, Bayer Health Care), (2) standard cleaning without toltrazuril treatment, or (3) improved cleaning (cold water pressure hose + detergent + desiccation) without toltrazuril treatment. The effect on *I. suis* occurrence was measured using oocyst excretion (pooled rectal fecal samples from 5 piglets/pen collected on day 18-20 after birth), average litter weight at weaning, and mortality. Preliminary results showed oocyst excretion in 8.3% of piglets treated with toltrazuril, 36.6% in untreated piglets, and 26.1% in untreated piglets from pens with improved hygiene. Weight at weaning was not significantly influenced in one herd, while in the other herd average weaning weights were slightly higher litters treated with toltrazuril and litters from pens with improved hygiene. Mortality rates were highest in pens without toltrazuril treatment and with standard cleaning.

675 **Characterisation of mucosal lymphocyte populations during infection with *Isospora suis***

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Isospora suis, the causative organism of neonatal coccidiosis, is a widespread problem in piglet production worldwide. The parasite affects young animals and is characterised by high morbidity with diarrhoea and poor weight gain which can cause great economic losses in affected breeding facilities. Destruction of host cells leads to pathological alterations like fibroncrotic membranes lining the small intestines. Histologically necrotic enteritis can be diagnosed, with fusion and atrophy of the villi as well as crypt hyperplasia as the most prominent features. The main focus of our project is on innate and adaptive immune system with its response mechanisms to infections especially locally in the small intestine. Thereby lamina propria and lamina epithelialis of the jejunum are two compartments of special interest which are screened for changes in leukocyte populations with respect to infections with *I. suis*. The distribution patterns of leukocytes involved are evaluated in situ by immunohistochemistry with specific markers directed against clusters of differentiation on T lymphocytes (CD3), B lymphocytes (CD79) and monocytes/ macrophages (SWC3) as well as a subpopulation of T lymphocytes, the T-cell receptor –gamma delta positive T-cells. First results indicate an increase of CD3+ cells in infected animals especially in the lamina epithelialis, whereas CD79+ cells prevail in the early stage in uninfected animals. SWC3+ and CD79+ cells could exclusively be found in the lamina epithelialis, CD3+ cells in the lamina propria as well as in the lamina epithelialis. Results suggest that especially intraepithelial T lymphocytes are of particular importance in immune responses to *I. suis*.

676 **Anticoccidial efficacy of diclazuril (Clinacox®) in rabbits: three extensive field trials**

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Introduction: Diclazuril, a benzene-acetonitrile derivative, has shown efficacy against intestinal coccidiosis in rabbits (*Eimeria intestinalis*, *E. magna*, *E. perforans*, *E. flavescens*, *E. irresidua*, *E. piriformis* and *E. media*) under laboratory conditions. To investigate the efficacy of diclazuril against hepatic coccidiosis under practical use conditions at industrial level, a comparison with robenidine was made in 3 extended field studies.

Methods: Comparative field efficacy studies on coccidiosis control are conducted in Germany (5.360 rabbits), Spain (2.469 rabbits) and Belgium (2.469 rabbits) on a total of 10.525 rabbits. In each study, animals were divided in 3 groups being: one group receiving in feed medication of 1 ppm diclazuril, another group receiving in feed medication of 66 ppm robenidine and a third group not receiving coccidiostats. The following parameters are investigated: mortality, body weight, oocyst excretion.

Results: What concerns oocyst excretion, better coccidiosis control in the study sites in Germany and Belgium was found for animals receiving in feed medication of diclazuril at 1 ppm compared to robenidine at 66 ppm. There was no statistical difference between the 2 products in the site in Spain, although diclazuril demonstrated a superior control of oocyst excretion by *E. magna* and/or *E. media*.

Over the 3 study sites there was no statistical difference of mortality and body weight gain between rabbits receiving in feed medication of 1 ppm diclazuril or 66 ppm robenidine.

Conclusion: Diclazuril in the feed of growing rabbits and reproduction animals at 1 ppm feedingstuff is an effective anticoccidial additive for the control of *Eimeria* infections.

There was no negative influence on zootechnical performance.

677 **Safety of 5% ponazuril (totrazuril sulphone) oral suspension and efficacy against naturally acquired *Isospora ohioensis* infection in puppies**

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The safety of 5% ponazuril oral suspension, 250 mg/kg administered once daily for three consecutive days, was evaluated in 2-week-old Beagle puppies. Clinical observations, physical examinations and clinical pathology were evaluated throughout the study. All puppies were necropsied at study conclusion (seven days following the last treatment). Results indicated no treatment related effects on hematology, clinical chemistry, urinalysis, gross or histopathology and organ weights. Two studies to evaluate the efficacy of the product were conducted in Beagle puppies with naturally acquired *I. ohioensis* infections. In both studies, puppies were randomly allocated to treatment or control groups based on oocyst counts. General health observations, body weights and oocyst counts were conducted for 14 days post treatment. In Study 1, puppies were allocated to five groups: Ponazuril 20 or 30 mg/kg administered once or twice (Days 0 and 7) or placebo given twice. A single ponazuril treatment at 30 mg/kg significantly reduced oocyst counts ($P<0.019$), and a second treatment with either dosage also significantly reduced counts ($P<0.05$). In Study 2, 30 puppies were allocated to 6 groups: Ponazuril 20, 30, 40 or 50 mg/kg administered once or twice (30 mg/kg: Days 0 and 7). Oocyst counts were significantly reduced ($P<0.02$) in puppies given 30 mg/kg or 50 mg/kg ($P<0.002$). Dosages >40 mg/kg showed marked reduction in oocyst counts by Day 2 post-treatment. In both efficacy studies, no adverse effects due to ponazuril were observed and no differences in clinical signs of coccidiosis were observed among treatment groups.

678 **A new anticoccidia drug BA3540**

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A technology and a new drug have emerged in the treatment of parasites. The Lipophilization Technology creates new drug entities by adding a lipid tail to a Pharmacore and encapsulates in triglycerides and fatty acids. The creative molecular design allows for a new pathway, lymphatic delivery. An example of this new construct is BA3540*, a powerful new anticoccidia drug. The Pharmacore is 3-amino-5-nitrothiazole and conjugated to a C12 long chain fatty acid. The construct takes on new characteristics.

Unlike the parent drug NTZ (nitazoxanide), it is not toxic. It is more like a saturated lipid, with melting point of 136C that is 67C lower than that NTZ. It imparts no side effects, is palatable and safe in mammals and avian. Pilot safety studies showed that it has no effect on rats and rabbits at 5-10g/kg. Pilot clinical trials showed that the therapeutic efficacy of the new drug is towards the treatment of EPM in horses and anti-coccidia in goats and anti-coccidia in chickens.

*US Patent #7125568B2

Poster group 19: Toxoplasmosis

679 **Occurrence of *Toxoplasma gondii* and *Hammondia hammondi* oocysts in the faeces of cats from Germany and other European countries**

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Little is known on the prevalence of cats shedding *Toxoplasma gondii* and *Hammondia hammondi* oocysts. To this end, faecal samples of 25106 cats from Germany and other European countries were examined coproscopically in a veterinary laboratory in Germany between October 2004 and November 2006. In 74 (0.31%) of the samples, oocysts of 11-14 μ m size were found. Their morphology was similar to that of *H. hammondi* and *T. gondii*. A total number of 53 samples were further characterized to achieve a species-diagnosis and to determine the genotype of *T. gondii* isolates by PCR and PCR-RFLP. From these samples, 47 isolates were obtained: 26 (0.11%) of which were finally identified as *T. gondii* and 21 (0.09%) as *H. hammondi*. *T. gondii* positive samples came from Germany, Austria, France and Switzerland while *H. hammondi* was observed in samples from Germany, Austria and Italy. For the remaining six isolates no species-diagnosis could be achieved due to the low number of isolated oocysts. In two isolates (one *T. gondii* and one *H. hammondi*) PCR indicated the presence of *H. heydorni*. No *N. caninum* DNA could be detected in the cat faecal samples. Genotyping was achieved in 22 of 26 *T. gondii* isolates. A standard PCR-RFLP analysis for the genes of SAG2, SAG3, GRA6 and β -tubulin revealed in all cases *T. gondii* genotype II.

680 **Prevalence of *Toxoplasma gondii* in Belgian house cats**

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Few studies are available on the prevalence of *Toxoplasma gondii* in house cats. House cats live in close proximity with their owners and are a potential source of oocyst infection in humans. Three-hundred and seventy-nine sera of house cats, originating from different parts of Belgium and collected between 2004 and 2006, were examined. The age of the cats varied from 3 months to 7 years. Per age group of one year, 43 to 50 samples were tested. Sera were examined for the presence of IgM and IgG antibodies with an indirect immunofluorescence assay using whole tachyzoites as antigen.

Twenty-six percent of cats tested positive for IgG and/or IgM. Seroprevalence increased with age: merely 2% below one year, 18-22% at 1-3 years, 28% at 4-5 years, and 42-47% at 6-7 years. Infection rates, calculated as the increase in positive cats compared to the previous age group, were highest at 1 year (18% increase) and, remarkably, also at 6 years (14% increase). Infection rates in other age groups varied from 0 to 6%. Six percent of cats were positive for IgM and most of these were also positive for IgG. IgM prevalence peaked at 7 years (23%), indicating a relative high number of recent infections at this age.

Toxoplasma gondii infections appear to be common in house cats and occur at both young and older ages. Most cats are infected between the age of 12 to 24 months, but relatively high infection rates are also detected at 6 and 7 years. Older cats might thus also present a significant infection risk to their owners.

681 **Prevalence of anti-*Toxoplasma gondii* antibodies in serum samples from Marsupials In zoological parks in France**

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Toxoplasma gondii is recognized as a major pathogen for all marsupial species. It causes both chronic and acutely fatal infections in wild as well as captive animals. In zoological parks, the transmission probably occurs horizontally, due to the contamination of feed by oocysts excreted by stray cats. The purpose of the present study was to evaluate the prevalence of anti-*T. gondii* antibodies in sera from marsupials in 7 zoological parks in France. A non-representative sample of 71 herbivorous marsupials sera was tested using the HSDA method (High Sensibility Agglutination Test). These sera were collected from 57 Bennett's wallabies (*Macropus rufogriseus*) and 14 red kangaroos (*Macropus rufus*). The mean seroprevalence was 55%. Positive titres were higher to 12800 UI/mL. Despite of different husbandry conditions, there was no significant difference in seroprevalence according to the origine of the serum samples. However, the proportion of seropositive animals was lower for zoological parks in which the stray cat population was limited. Juvenile marsupials seemed more frequently infected than adults (60% vs. 54%) suggesting that toxoplasmosis occurred early in life. The sex ratio was 1:1 and seroprevalence was 60% in males and 57% in females. Seroprevalences of *Toxoplasma gondii* infection were similar before 2006 (frozen sera) and in 2006 (fresh and frozen sera). The seroprevalence values evaluated in the present study were much higher than those reported in previous investigations (8-10% in herbivorous marsupials and 23% in carnivorous marsupials).

682 **Serological survey of *Toxoplasma gondii* in naturally infected cattle in the Netherlands**

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Toxoplasma gondii is an intestinal coccidium of felids with an unusual wide range of intermediate hosts. It has been suggested that cattle are resistant to *Toxoplasma* infection and are able to clear infection. This implies a low risk for *Toxoplasma* infection from the consumption of beef. In contrast, Cook et al. (2000) reports eating raw beef as one of the risk factors that most strongly predicted acute infection in pregnant women. It is our aim to gain further insight into natural *Toxoplasma* infections in cattle.

Twenty-seven calves kept under natural conditions were followed during 17 months. Serum samples were collected on a regular basis and tested for anti-*Toxoplasma* antibodies by indirect ELISA. Twenty calves had increased titers at the first or second sampling after being put out to the pasture. Sampling was continued for five months after the start of grazing, and although most animals (23) were still positive at the last sampling date, a rapid decline in titer was observed in 10 calves. In addition, a sample of the Dutch cattle population was tested by ELISA to estimate seroprevalence of *Toxoplasma gondii*. Preliminary results show regional differences, but, overall, a high seroprevalence.

These results show that calves can contract a *Toxoplasma* infection, and the large proportion of calves that do contrasts with suggested resistance. The risk of infection appears to be higher on the pasture than in the stable. The high seroprevalence in adult cattle might indicate that infection persists or reinfection occurs.

683 **The prevalence of serological diagnosed toxoplasmosis cases in animals from Romania between 2006 - 2007 years**

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Toxoplasma gondii is an obligate intracellular protozoar parasite that may cause abortion in sheep, goats and pigs, the toxoplasmosis representing a widespread zoonosis. Domestic and wild feline, are the definite and intermediate host, and are a primary reservoir of infection. Presence of antibodies against *T. gondii* is an indicative of exposure to the parasite. The positive serological results obtained are useful for diagnosis when they are associate with clinical signs. The aim of this study was the opportunity to registering in our country some commercial kits for serological diagnosis of toxoplasmosis; the comparison of serological results for same samples using different diagnosis kits; the comparative study between serological results for toxoplasmosis and chlamydiosis using the same diagnosis kits. Between 2006 – 2007 we examined in our laboratory, the following samples: 94 blood samples taken from pigs, 148 from sheep and 94 from cats. 94 samples taken from pigs were examined by indirect ELISA and from those, 72 were positive and 22 were negative. 148 samples were examined by ImmunoComb and from those, 105 were positive and 43 were negative. 91 of them were examined by ELISA and 67 samples were positive, 5 doubtful and 19 were negative. We made a comparative study between the results obtained by ELISA and ImmunoComb, and we found a correlation of 94,5%.

From 94 samples taken from cats and examined by ImmunoComb, 42 samples were positive and 52 negative. The samples that were examined by ImmunoComb also we examined for *Chlamydomphila abortus*, and we calculate a correlation between serological results for two abortive diseases in sheep and cats, that was 44% for sheep and 59,5% for cats. We could done serological differentiation for 37 aborted sheep also, that were positive for toxoplasmosis and negative for chlamydiosis in ImmunoComb and also in CFT and ELISA. In order to register and validate the results of ELISA kit, were calculated: the detection limit, repeatability, reproducibility, variation coefficient of the diagnosis kit. The large number of positive samples obtained, 76,6% from pigs, 71% from sheep and 45% positive samples taken from cats, demonstrated the importance to detect anti-*Toxoplasma gondii* antibodies and the necessity to use these kits in diagnosis laboratories from our country.

684 **Prevalence of *Toxoplasma gondii* in wild game meat from French Guyana**

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Introduction: Toxoplasmosis, the most common parasitic zoonoses world-wide, is caused by the protozoan parasite *Toxoplasma gondii*. The major route of transmission to humans is via the consumption of contaminated food, in particular undercooked meat containing bradyzoite cysts. The infection is usually asymptomatic in immunocompetent patients however severe toxoplasmosis cases have recently been identified in French Guyana. These cases are suspected to be due to potentially new strains of *T. gondii* involving sylvatic tropical fauna in their transmission cycle. Consumption of wild mammal meat is common in French Guyana and could be a major source of human contamination.

Material and methods: Ninety-three muscle samples from six species of wild mammals, including 30 Capybaras (*Hydrochoerus hydrochaeris*), 47 Pecarrys (*Tayassu sp.*), 7 Tapirs (*Tapirus terrestris*), 1 Acouchis (*Myoprocta acouchy*) and 5 Nine-banded Armadillos (*Dasypus novemcinctus*), were collected by veterinarians in slaughterhouses between February and September 2006. Muscular fluids were analysed by ELISA and Modified Agglutination Tests (MAT). Seropositive samples were partially digested in acid pepsin solution and DNA was extracted for real-time PCR detection of *T. gondii* DNA.

Results and conclusions: A good correlation was found between ELISA and MAT with 30% of the samples being positive for *T. gondii* including 49% of Pecarrys, Armadillos (40%) and Capybaras (0.6%). This shows the potential of wild game to play a role as a source of human toxoplasmosis in French Guyana. The Molecular detection of the parasite DNA is currently being processed and will enable characterisation of the parasite strain involved in this tropical cycle.

685 **Seroprevalence of *Toxoplasma* infection in food animals of Fars Province, Southern Iran**

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Toxoplasmosis is a widespread zoonotic disease that causes significant morbidity and mortality in human fetus and in Immunocompromised patients. Since food animals' meat is considered as major source of human infection in Iran, this study was undertaken to determine the prevalence of toxoplasmosis among the animals. Serum samples from 603 sheep of 18 cities, 599 goats of 5 cities and 588 cattle from abattoirs of five geographical districts of Fars province were randomly collected and tested for toxoplasmosis by IFA during the period 2003-2006. Also 203 serum samples from free-ranging chickens of sub-urban districts, 50 serum samples of semi-industrial chickens which rearing in restricted location and 444 serum samples of industrial chickens from slaughters were collected and tested. The total prevalence of toxoplasmosis among sheep, goat and cattle was 26.5%, 14.02% and 20.24% respectively. The prevalence among free-ranging chickens was 27.1% but the rate in semi-industrial and industrial chickens was 12% and 2.02% respectively.

Considering the high prevalence of toxoplasmosis in food animals, in the regions under study, control measures should be taken to prevent transmission of the infection to the animals and humans by Health and Veterinary organizations.

686 **Survey on seroprevalence of anti-*Toxoplasma gondii* in cattle from Tabriz (Iran) by IFAT**

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Toxoplasmosis is a zoonotic disease in various species of animals caused by a sporozoan parasite, *Toxoplasma gondii*, distributed throughout the world including Iran. Toxoplasmosis is a common infection of sheep, goat and cattle. It is recognized as one of infectious reproductive failure. This survey was carried out to show the prevalence of this disease in cattle of Tabriz (Iran). 490 Serum samples from both domestic and industrial cattle from Tabriz in the west north region of Iran, were tested to determine the prevalence of antibodies against *Toxoplasma gondii* using the indirect fluorescent antibody test (IFAT). The animals were divided into two groups based on their age: <1 year, and >1 year. The results indicated that the antibody to *T. gondii* were observed in 15.91% of the samples. Prevalence of antibody to *T. gondii* in the >1 year old was higher than <1 years old cattle. An association between seroprevalence and age was observed ($P < 0.05$). Also a significant difference was observed between rate of seroprevalence and sex in cattle ($P < 0.01$). 36 (28.34%) of male cattle and 32 (12.16%) of female cattle were seropositive for *T. gondii*. The results of the present study together with the previously recorded low seroprevalence of *Toxoplasma gondii* in dog and cattle support the impression that infestation to *T. gondii* is not widespread as a latent infection in animals in Iran.

687 **Seroepidemiology of sheep toxoplasmosis in Babol northern Iran 2004**

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Toxoplasmosis is one of the most important infections in living animals. Because of the wide spreading of the contamination, complication and future risks in humans and domestic animals must be taken into consideration. Meanwhile this study is carried-out with the aim of determining the prevalence rate of toxoplasmosis in sheep at Babol (Bandpei part).

In this study, 285 sheep of Bandpei area in Babol was selected. The samples studied with indirect immunofluorescence antibody. The results showed that 31.2 % of sheep in this study were affected by toxoplasmosis. In positive cases, there was not any statistically significant difference between male and female sheep. The most positive cases observed by 1:200 titer. According to the researches in other parts of this country, the prevalence rate in this area is more than the others.

688 **Epidemic population structure of *Toxoplasma gondii* in Brazil revealed by multilocus pcr-rflp genotyping**

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Recent studies found that the isolates of *Toxoplasma gondii* from Brazil are biologically and genetically different from those in North America and Europe. In the present study, DNA samples of 42 *T. gondii* isolates from cats of 11 counties in São Paulo state, Brazil were genetically characterized using 11 PCR-RFLP markers including SAG1, SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, SAG2 (new) and Apico. An additional marker CS3 which is on chromosome VIIa and was previously shown linked to acute virulence of *T. gondii* was also used. Genotyping of these 42 isolates revealed a high genetic diversity with 18 genotypes and no clonal types I, II and III lineages were found. Of the 18 genotypes identified, five have multiple isolates and 13 have only one isolate each. Two isolates had mixed infections. Marker CS3 is strongly associated with virulence in mice. Comparing genotyping data in this study with recent reported typing results of *T. gondii* isolates from chickens, dogs and cats in Brazil revealed that a few genotypes were widespread, some genotypes were prevalent in one area and most genotypes were unique and only found in one location. Taken together, it is clear that *T. gondii* population in Brazil is more diverse than any other regions in the world studied so far. In parallel to the archetypal Type I, II and III lineages that are clonal and predominate in North American and Europe, a few widespread *T. gondii* genotypes are clonal in Brazil and the parasite has an epidemic population structure in this region.

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689 **Prevalence and risk factor studies on *Toxoplasma gondii* in ruminants of Punjab (India)**

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Toxoplasmosis is a widespread zoonosis caused by the coccidian protozoan *Toxoplasma gondii*. A random survey was therefore conducted to study the epidemiology of the toxoplasmosis in Punjab (India) using sampling software 'survey toolbox'. Two stage sampling procedure was adopted; in the first step, villages were selected randomly from sampling frame of all the villages of Punjab followed by selection of owners, and animals in the second step then animals from the individual farm were identified using systematic random sampling. The serum samples collected were screened for *T. gondii* antibodies employing indirect ELISA. The overall apparent prevalence of toxoplasmosis was found to be quite low compared to earlier studies viz. 3.76 and 2.69% in sheep and bovine populations, respectively (true prevalence – 2.82 and 1.72%, respectively). Based on titer of *T. gondii* antibodies, the animals were classified as negative, doubtful, positive and positive with clinical signs. In cattle (n=83), only two animals were found to be positive; in buffaloes (n=103), two animals were positive and one was doubtful; whereas in sheep (n=186), five animals were positive with clinical signs and two animals were doubtful. The prevalence was found to be non-significantly higher in sheep in comparison to bovine population and in female animals. Univariate and final logistic regression model revealed age and agro-climatic zone factors to be significantly associated with *T. gondii* seroprevalence. There was four-fold increased risk of infection in animals above two year age ($P < 0.10$) and two-fold increased risk in animals in central zone ($P = 0.42$) and nine fold higher risk in animals of sub mountain zone ($P = 0.05$) in comparison to arid zone.

690 **Occurrence of *Toxoplasma gondii* in urban rodents (*Rattus rattus*, *Rattus norvegicus* and *Mus musculus*) captured in São Paulo, SP, Brazil. Preliminary results**

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Toxoplasmosis is a parasitic zoonosis caused by *Toxoplasma gondii*, that can infect humans and warm blooded animals. Cats are infected mainly by ingestion of tissue cysts in raw meat or by predation. Mice, rats and small birds seem to be the most important preys to domestic cats, due to living close to homes, making it easy the establishment of prey-predator relationship, and can become *T. gondii* reservoirs to domestic felines. In order to determine the occurrence of *T. gondii* in urban rodents and its role as toxoplasmosis reservoirs to cats, rodents are being captured with cages in different parts of the city of São Paulo since April 2005. All rodents were bioassayed in mice in an attempt to isolate *T. gondii* from cysts present in their tissues. Until this moment 135 rodents were examined (119 *Rattus rattus*, 13 *Rattus norvegicus* and 03 *Mus musculus*). All bioassayed mice were seronegative. All mice had their brains fresh examined under microscope but it wasn't found any tissue cyst. Tissues from 10 bioassayed mice had their DNA extracted in order to check for possible presence of *T. gondii* DNA by PCR. None of samples were positive to *T. gondii* DNA, and it was concluded that none of the 135 rodents examined so far were infected with *T. gondii*. More rodents are being captured to continuing of this study.

691 **Seroepidemiological study of toxoplasmosis infection among high school girls Jolfa city using indirect immunofluorescence of assay**

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Introduction: Toxoplasmosis is a world wide zoonotic disease caused by toxoplasma parasite. Acquired infection occur by eating of oocyst which they passes through cat feces or by eating of oocyst which they passes through cat feces or by eating the infect meat. Congenital infection may cause death, pathological of embryo. Acquired infection with toxoplasmosis in immunocompetent persons is generally a symptomatic infection. Or is flu – like illness. Immunodeficient patients often have central nervous system (CNS) disease but may have chorioretinitis, or pneumonitis.

Congenital toxoplasmosis results from an acute primary infection acquired by the mother during pregnancy. The disease agent transfer to the fetus through the placenta. The incidence and severity of congenital toxoplasmosis vary with the trimester during which infection was acquired.

Materials and Methods: Blood samples were collect from 1000 school girls in Jolfa city and Examined by indirect Immuno fluorescence Test (IFAT), and SPSS statistical software was used to analyze the data.

Results: Anti toxoplasma antibodies were detected among 21.8% of the study populations. The number of the people with antibody titer with the amount of 1/20 to 1/100 were 191 (19/1%) and the number of the people who had Ab titer with the among households who kept cat (%37.5), and symptomatic individuals (%37.7).

Discussion: The study Objective was to determination of the Toxoplasmosis infections situation in Jolfa region, and recommendation of the control procedures. In contrast with other studies in Iran the infection rate of %22 was too low, this is may be due to the yearly climate variation, social behaviors and life style of the residents. However highest prevalence of infection found among household with cat which is proved ($p<0.001$) the role of cat on transmission of disease in the area.

There is a meaningful relationship between the disease causal and different parameters such as having contact with cats, symptom and eating of raw liver ($p<0.001$). It became clear that 30%. Avoid from contact with cats will significantly reduce the risk of the infection and disease.

692 ***Toxoplasma gondii* in healthy lambs: which lambs are infected and why?**

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Sheep can acquire the apicomplexan parasite *Toxoplasma gondii* by horizontal (faecal-oral) transmission from cats, and by vertical (transplacental) transmission to the foetal lamb. Transplacental transmission may lead to abortion or perinatal death. In order to investigate the relative importance of horizontal and vertical transmission routes in unvaccinated flocks, one Charollais cohort and one Swaledale cohort of four month old lambs in Northern England were tested for *T. gondii* infection using the modified agglutination test (MAT). 50/411 (12.2%) of Charollais lambs were seropositive: 18 at 1:25, 3 at 1:50, 3 at 1:100, 5 at 1:200, 4 at 1:400, 5 at 1:800, 7 at 1:1600 and 5 at 1:3200. 10/335 (3.0%) of Swaledale lambs were seropositive: 6 at 1:25, 1 at 1:50 and 3 at 1:200. In Charollais, seropositive lambs were overdispersed between litters. The result indicates that for this cohort, horizontal transmission resulting from random field exposure to *T. gondii* from cat faeces did not fully explain the pattern of infection.

693 **Development of an ELISA for the detection of *Toxoplasma gondii* antibodies in macropod marsupials**

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Toxoplasmosis is a devastating disease in Australian marsupials. A commercially available MAT is widely used to detect *T. gondii* antibodies in marsupials, but the cost of this test restricts its use in large numbers. Thus, an ELISA was developed for the detection of *T. gondii* antibodies in three different macropod species. Antigen for the ELISA consisted of the soluble fraction of sonicated, whole *T. gondii* tachyzoites (RH strain). The secondary antibody consisted of rabbit anti-kangaroo IgG. HRP-conjugated donkey anti-rabbit IgG was used as the tertiary antibody. The optimum concentration of the antigen, antibodies and reagents was determined using a checker board system with antigen titrated in one direction and a series of different antibody and reagent concentrations titrated in the opposite direction. Once optimised and validated, the ELISA was in high agreement with the MAT, yielding a kappa value of 0.98. The ELISA is to be used in preference to the MAT in subsequent studies examining the seroprevalence of *T. gondii* in macropods.

694 **Comparison of commercial elisa and modified direct agglutination tests for detection of *Toxoplasma gondii* antibodies in aborted and non-aborted ewes in Ahvaz, Iran**

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Toxoplasmosis is a globally distributed zoonosis with a clinical impact in the unborn fetus of human and sheep and in the immunosuppressed individuals. A commercially available enzyme-linked immunosorbent assay (ELISA) and the modified direct agglutination test (MAT) were compared for detection of antibodies to *Toxoplasma gondii* in naturally infected aborted and non-aborted ewes. For this reason 100 serum samples of ewes with early abortion history and 50 serum samples of ewes without any abortion history (as control group) were taken for serological studies. Results of total samples (150 serum samples) showed 76% (114 of 150) of infection by ELISA and 72.6% (109 of 150) by MAT respectively. The ELISA showed anti-*Toxoplasma gondii* antibodies in 85% (85 of 100) of ewes with abortion history and 58% (29 of 50) in ewes without abortion history, whereas MAT detected anti-*Toxoplasma gondii* antibodies in 80% (80 of 100) of ewes with abortion history and 58% (29 of 50) in ewes without any abortion history. We concluded that *Toxoplasma gondii* may be an important cause of abortion in ewes of Ahvaz, Iran. A good correlation was seen between ELISA and MAT so the results suggested that both ELISA and MAT were good tools for seroepidemiological studies of *Toxoplasma gondii* infection in sheep.

695 **Clinical, obstetric and immunological observations on pregnant queens experimentally infected with different isolates of *Toxoplasma gondii* (Nicolle & Manceaux, 1909)**

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Twelve queens, which were serologically negative to *Toxoplasma gondii*, were distributed into three groups of four animals each and infected from tissue cysts of *T. gondii* through oral via, having them thirty days of gestation. Group 1 - isolate type I; group 2 - isolate type III not virulent; group 3 - control (placebo). Seven uninterrupted days before and during the whole experimental period it was done clinical, obstetric and ultrasonography profiles. Blood samples of the queens were taken in the following days, 0, 15 and 30 pos infection days in order to carry out the hemograms and for the antibodies detection against *T. gondii* by the indirect immunofluorescent assay (IFA). Emesis, diarrhea, hyporexia, purulent nose and ocular secretions, metrorrhagia, abortion and premature birth were the main clinical and obstetric alteration observed. The hemograms showed leukocytosis, eosinophilia and monocytosis. The higher serological titers by IFA were 1:512 and 1:2048 concerned the groups 1 and 2 respectively. Maximum titers of 1:64 were detected in the offsprings. The isolated of *T. gondii* of the types I and III have produced reproductive disturbance such as metrorrhagia, abortion and premature birth in primoinfected pregnant queens.

696 **Development of a sensitive specific PCR assay for the detection of *Toxoplasma gondii* using the repetitive 529bp DNA fragment as genetic marker**

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The objective of the present study was to examine sequence variation in the repetitive 529 bp DNA fragment among 9 *T. gondii* strains from different hosts and geographical locations in China, to compare the sequences with that of the RH reference strain, and to develop a sensitive PCR assay for *T. gondii* using this repetitive 529 bp DNA fragment as genetic marker. The 529 bp fragment was amplified by PCR from the 10 *T. gondii* strains, and the amplicons were then sequenced. Sequence variation in the repetitive 529 bp DNA fragment ranged between 0.8-2.9% among the 10 *T. gondii* strains, with sequence positions of 32-55 nucleotides being the most variable region. But the sequence variation was not related to virulence. The specific PCR assay was highly sensitive which could detect 1 *T. gondii* tachyzoite, and no PCR products were amplified from other related parasites. On day 3 after experimental infection, specific PCR products were amplified from mouse tissues. The findings of the present study showed that the intraspecific variation in the repetitive DNA fragment was low and this repetitive DNA fragment provides an ideal genetic marker for the differentiation of *T. gondii* from other parasites, but it is not suitable for the studies of genetic variability within *T. gondii* or its virulence. Project support was provided by grants from the Natural Science Foundation of Guangdong Province (Team Project, grant no. 5200638), and the Scientific and Technological Programs of Guangdong Province (Grant No. 2004B20201008).

697 **A GRA1-GRA7 cocktail DNA vaccine primes anti-*Toxoplasma* immune responses in mice and pigs**

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The protozoan parasite *Toxoplasma gondii* is the causative agent of a worldwide zoonosis and it is estimated that one third of the human world population is infected with this parasite. An important source of human contamination with *T. gondii* is the consumption of raw or undercooked meat products. In humans and mice, protection against this parasite is associated with strong and persistent cellular immune responses. In mice vaccinated with the GRA1-GRA7 DNA vaccine mixture, high IgG2a levels were elicited against these antigens and against *T. gondii*, as well as a high amount of IFN-gamma producing cells. A reduction in parasite burden of 89% was obtained after challenge with *T. gondii*. For vaccination of pigs, the GRA1-GRA7 DNA vaccine mixture was optimized by codon optimization and addition of heat labile enterotoxin expressing vectors as genetic adjuvant. The sites of injection were stimulated with plasmid encoding GM-CSF one week prior intradermal vaccination. Pigs vaccinated intradermally with this optimized GRA1-GRA7 DNA vaccine mixture developed high antibody levels against GRA1, GRA7 and *T. gondii*, and a stronger cellular immune response could be detected in these animals after challenge with the parasite. These results indicate that pigs can be efficiently primed against *T. gondii* by means of an optimized DNA vaccine. It is of interest to determine whether this DNA vaccine elicited immune responses that can lower the parasite burden in large animals.

698 **Isolation of *Toxoplasma gondii* from goat semen**

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The present study attempted to isolate *T. gondii* from semen samples of goats. Six male goats of reproductive age, free of disease, were randomly separated into three experimental groups. Two goats were inoculated with 1.0×10^6 *T. gondii* tachyzoites subcutaneously, and two others received 5.0×10^5 sporulated *T. gondii* oocysts administered orally. Two animals were maintained as controls and received, orally and subcutaneously, a placebo. Clinical parameters were assessed two days prior to the inoculation and, afterwards, once weekly until the 70th day post-inoculation (DPI). Hyperthermia was detected on the 5th DPI in the goats inoculated with oocysts. Semen samples from the animals were obtained using an electrode to promote ejaculation and were assessed two days prior to inoculation and on days 3, 5, 7, and then once weekly, to the 70th DPI. No alterations that could be related to the *T. gondii* infection were detected in the semen. For parasite isolation, portions of the semen sample from each animal (infected and controls) were inoculated into the peritoneum of mice. Positive serology was detected in mice inoculated with semen collected from two goats that received tachyzoites, on the 5th, 7th, 28th, 49th, 63rd and 70th DPI, and from the two that received oocysts, on the 56th and 70th DPI. Moreover, for confirmation of these results, the presence of *T. gondii* cysts in tissue from the mice's brains was detected. In all, the obtained results make it possible to infer that *T. gondii* could be isolated from seminal ejaculate of goats.

699 **Aspects of toxoplasmatic infection in reproductive organs of artificially infected male ovines (*Ovis aries*)**

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Eight male ovine breeders, free of *Toxoplasma gondii* and other reproductive disorder infections, were distributed into three groups for artificial toxoplasmic infection: GI - three animals (2.0×10^5 oocysts from P strain), GII - three animals (1.0×10^6 tachyzoites from RH strain) and GIII - two animals (control). Clinical parameters of all animals were assessed. Parasitic and serum (IFI) evaluations were done. Sperm quality was assessed and the presence of the parasite in the sperm was investigated by bioassay and PCR. Presence of the parasite in tissue was investigated by bioassay, PCR and immunohistochemistry. The animals infected with *Toxoplasma gondii* oocysts presented hyperthermia and apathy. Parasite infection was determined in five animals. All the inoculated animals responded to antigenic stimulation, producing antibodies against *T. gondii* five days after inoculation, presenting 1:4096 and 1:8192 titer titles for animals from groups I and II respectively. The presence of the parasite was determined in the semen of these same groups by bioassay and PCR. The PCR detected *T. gondii* DNA in the semen from animals 02 and 09 (from GI oocysts) and 07, 48 and 52 (from GII tachyzoites). Tissue parasitism by *T. gondii* (bioassay and PCR) was diagnosed in animals 09, 16 (oocysts) and 07 (tachyzoites). The seminal vesicle and the prostate gland tissue were the most infected by *T. gondii* (immunohistochemistry) in the reproductive system of artificially infected sheep. The results found in the present study suggest that venereal transmission of this coccidia parasite is viable to happen.

700 **Histopathologic evaluation of reproductive organs from male dogs inoculated with *Toxoplasma gondii***

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Six male dogs of reproductive age were inoculated with *Toxoplasma gondii*, three with oocysts (orally) and the other three with tachyzoites (subcutaneously), from “P” and “RH” strains respectively. Four other dogs were maintained as controls and were inoculated with a placebo solution by the same procedures as used for the treated dogs. 77 days after inoculation all the animals had their testicles removed. Fragments of the testis and epididymes were then fixed in 10% buffered formaldehyde for a 24 hour period. Afterwards, tissue fragments were transferred to a 70% alcohol solution and were then imbedded in histological paraffin. The histological blocks were then sliced into 3µm thick sections, positioned on glass silanized slides, and incubated for six hours at 55°C for fixation. The tissue sections were then stained with H&E (hematoxylin and eosin). The tissue sections from the control group dogs did not present any histopathologic alterations, while those from all the inoculated (with oocysts as well as tachyzoites) dogs presented signs of local inflammatory reaction in the epididymis (chronic interstitial epididymitis) and cellular edema in the tissue sections of the testis and epididymes. These results suggest that chronic interstitial epididymitis and cellular edema are histological alterations that could be important for diagnosis of canine toxoplasmosis.

Poster group 20: *Cryptosporidium* and *Giardia*

701 **Survey for the incidence of *Cryptosporidium* spp. oocysts from pet samples submitted to the laboratory**

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Faecal samples from pets are regularly submitted to the laboratory for routine parasitological screening. Following observations in another study that faeces from cats and dogs sometimes contained *Cryptosporidium* spp. oocysts, samples were randomly selected and examined for the presence of oocysts.

The samples were submitted from all geographical areas of the UK, and were from animals in a wide range of ages, although this information was only obtained after testing. This information was provided by a third person so no other details were divulged about the animal (eg. owner, animals name, address etc).

The faecal samples were applied into a well on a glass microscope slide, fixed in methanol, stained with anti-*Cryptosporidium* Immunofluorescent antibody, and examined under a UV microscope. *Cryptosporidium* was identified as apple-green epifluorescing, slightly ovoid or spherical oocysts.

The incidence of *Cryptosporidium*, number of oocysts present, and geographical distribution were examined with the following findings:

1. The incidence of *Cryptosporidium* oocysts was 6% for both dogs and cats with generally low numbers of oocysts present, except in one dog and one cat where up to 500,000 oocysts per gram of faeces were observed.
2. There were no differences when age or geographical distribution were examined.

702 ***Cryptosporidium* infection in Swedish dairy herds**

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Introduction: *Cryptosporidium* spp. are apicomplexan parasites infecting a wide range of animal species. Here we estimated the prevalence of *Cryptosporidium* infection in Swedish dairy herds and identified risk factors for infection.

Materials and methods: Faeces were collected from 10 calves, 10 young stock and 5 cows in 50 dairy herds. Blood was drawn from 1-8 day old calves and farmers were interviewed about herd management. Faeces were concentrated and cleaned by saturated NaCl-flotation, stained with FITC-conjugated mAb against *Cryptosporidium* and examined by epifluorescence microscopy. Blood was analysed for Total Protein (TP). Multivariable regression models were built at herd level (negative binomial) and calf level (logistic).

Results: *Cryptosporidium parvum* was found in 96% of herds and 52% of calves. Youngest positive animals were 2 days old. TP was lower in positive calves. Seven animals were positive for *C. andersoni*. At herd level; “year of sampling”, “routines for cleaning calf pens”, “placing of young stock”, “age at weaning” and “routines for moving young stock” were significant. At calf level; “age”, “prevalence in young stock” and “routines for moving young stock” were significant factors.

Conclusions: *C. parvum* was very common and *C. andersoni* was present in the herds. Several factors influenced how many of sampled animals were positive in a herd and whether an individual calf was positive or not. No association found between *C. parvum*-infection and diarrhoea in calves.

703 **Efficacy of halofuginone lactate on the prevention of cryptosporidiosis in Portugal dairy C-calves**

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Cryptosporidium parvum infection was detected in 137 (74,8%) of 183 dairy calves up to 12 weeks of age on a convenience sample of 30 farms with a history of calf diarrhea. The prevalence was determined by using a formol-ether concentration and acid fast staining method (Ziehl Neelsen). All farms studied had infected calves at the time of sampling and within farm prevalence ranged from 25 to 100%. The higher percentages of oocysts excreted were observed in calves with 7 to 14 days (89%) and 15 to 21 days old (90%). Eighty-four (46%) of the calves were diarrheic, and 74 (88%) of these calves were shedding *C. parvum* oocysts. Cryptosporidiosis appears to be an important cause of dairy calf scours in this region. A second study was performed on 79 newborn calves from the latter farms to assess the efficacy of halofuginone lactate (Halocur) in the prevention of cryptosporidiosis. Halocur was administered at 120g /Kg/ day for 7 days to 39 calves and the other 40 calves did not receive any antiprotozoal treatment. Over a period of 3 weeks the animals were examined four times for shedding of *C. parvum* oocysts and diarrhea. The intensity of infection was determined by a semi-quantitative score of oocyst numbers in faeces. Oocyst excretion peaked on Day 7 and Day 14 in the control group (100% and 94%) being at the same time lower in the treated halofuginone-group (30,5% and 55,6%). During that period intensity of shedding was significantly lower ($p<0,0001$) in the treated group, thus contributing to decrease environmental contamination and zoonotic risks. Halocur reduced significantly the number of calves presenting diarrhea on Day 14 ($p<0,001$). In spite of the low intensity of shedding on Day 7, the high prevalence of diarrhea of the treated group (58%) may suggest the presence of other enteropathogens as the cause of scouring as *Escherichia coli* K99, rotavirus and coronavirus.

704 **Prevalence of bovine cryptosporidiosis in Sardinia (Italy)**

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Cryptosporidiosis is a very important protozoonosis caused by Apicomplexa parasites affecting a wide range of animals (mammals, reptiles, birds, fishes and humans). This is a parasitic disease responsible for neonatal diarrhoea in calves, causing great economical losses especially in farms with inadequate hygiene management. The main objective in this research was to study the distribution of bovine cryptosporidiosis in milk cattle farms from Sardinia (Italy). For this purpose, between October 2004 and July 2005, faecal samples from 468 Friesian and Swiss Brown 0-40 days old animals belonging to 67 herds were collected. A modification of the Dimethyl Sulfoxide Fast Staining (DSFS-m) technique was used to analyze the samples. Results were evaluated according to the age groups: 0-7, 8-14, 15-21, 22-28, 29-34 and >35 days-old, and by the management regime, individual box or grouped. To facilitate the analysis of the results, three areas were considered in the Island.

An individual prevalence of cryptosporidiosis of 33% was observed, and 64% of the farms were positive. The highest percentage of infected-calves was recorded in that of 8-14 d (53%). The percentage of positive-animals was 31% located in individual boxes, and 37% in grouped calves. The Swiss Brown animals reached a higher prevalence (40%) than Friesian ones (29%). By considering age and management regime together, the highest prevalence was achieved in 8-14 d also, with independence of the location. No significant difference in respect to the area was noted.

705 **Genetic identity of *Cryptosporidium* species in captive Palaearctic European tortoise of the genus *Testudo***

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Parasites of the genus *Cryptosporidium* affect majority of vertebrate groups causing debilitating diarrhoeal disease cryptosporidiosis. The information about the epidemiology and the host specificity of the recognized species and characterized genotypes is still incomplete. Terrestrial chelonians are common pets in many American as well as European homes. Oocysts of *Cryptosporidium* species were previously reported in several captive reptiles, including chelonians. Nonetheless, information on the genetic identity of *Cryptosporidium* species/genotypes is almost absent. In the present study we surveyed and genetically characterized *Cryptosporidium* isolates in captive Palaearctic European chelonians. Faecal samples were collected from 15 *Testudo hermanni*, 5 *Testudo marginata* and 1 *Testudo graeca*, all individually owned in Italy. *Cryptosporidium* spp. were detected using a Ziehl-Neelsen staining as well as a two-step semi-nested PCR specific for a 360bp fragment internal to the gene encoding for the *Cryptosporidium* oocyst wall protein (COWP). Obtained sequences were phylogenetically characterised and their species/genotype established. In 1 *T. graeca* and 2 *T. marginata* the sequence revealed 100% identity with the *Cryptosporidium parvum* "zoonotic bovine genotype" (= *Cryptosporidium pestis*), while a sequence from 1 *T. marginata* was a unique uncharacterized genotype (= *Cryptosporidium* sp. ex *Testudo marginata*). This is the first report of *Cryptosporidium* spp. in tortoises of the genus *Testudo*. The presence of potentially zoonotic genotype and of an unidentified genotype undoubtedly gives food for thoughts for further studies aiming to evaluate whether the isolates are: 1) of parasitic or pseudoparasitic nature; 2) a cause of any pathology; and 3) a public risk for immunocompetent and immunocompromised humans.

706 **Epidemiology and incidence of *Cryptosporidium* in dogs in Iran**

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One of the important enteric coccidian, is *Cryptosporidium*, a common enteropathogen of birds and mammals including human particularly in immunocompromised individuals. In this survey rectal swabs from 191 dogs that referred to Small Animal Teaching Hospital of Veterinary Faculty in Tehran (101 household & 90 stray dogs) and from 100 household dogs that referred to Small Animal Teaching Hospital of Veterinary Faculty in Isfahan and from 150 dogs that referred to a Small Animal Hospital in Sari (120 household & 30 stray dogs) were collected from three aged groups (0-6 months, 6 months to 4 years and over 4 years).

The samples were collected From May 2003 to November 2004. Two smears were prepared from each swab sample. One smears underwent Fluorescent Auramine and the other Modified Zeihl-Neelsen technique.

Oocysts were detected by both staining methods in one male household dog with clinical signs of diarrhea (aged 4 months) and in one male stray dog without diarrhea (aged 8 months). There was no significant difference between the household and the stray dogs in Tehran. Oocysts were not detected in any dog in Isfahan and Sari.

707 **Infection by *Cryptosporidium* spp. in dogs from Brazil: Comparison between diagnostic methods and clinical and epidemiological analysis**

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Fecal samples from 420 dogs were examined in order to detect *Cryptosporidium* spp. oocysts by means of Kinyoun acid-fast stain and Sheather techniques and also *Cryptosporidium* spp. antigens using direct ELISA technique. Out of the 420 samples, 10 (2.4%) were positive by the ELISA technique and 4 (1.0%) by the Kinyoun acid-fast stain and Sheather techniques. There was no significant association between *Cryptosporidium* infection and fecal hydration degree, breed, sex, age and dehydration degree of the animals ($P > 0.05$). McNemar's test, revealed a significant difference ($P = 0.0313$) between the occurrence of ELISA (2.4%) and Kinyoun acid-fast stain (1.0%) positive results, with a moderate agreement between both methods (Kappa = 0.5655). The same result was observed when comparing ELISA to Sheather techniques.

708 **Occurrence of *Cryptosporidium* spp. in domestic and captive wild birds in Brazil**

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There are many *Cryptosporidium* genotypes and three accepted *Cryptosporidium* species in birds: *C. baileyi*, *C. galli* and *C. meleagridis*. The occurrence of *Cryptosporidium* spp. in Brazil was carried out through the analyses of 608 fecal samples from domestic and wild birds and four samples from the proventriculus of two canaries (*Serinus canaria*) and two lesser seed-finches (*Oryzoborus angolensis*). *Cryptosporidium* spp. oocysts screening were accomplished using sucrose flotation and malachite green negative stain. Molecular analyses using nested PCR targeting the actin gene and 18S rRNA gene and sequencing of amplified fragments were carried out in 49 (8.05%) samples positive for *Cryptosporidium* oocysts. Proventricular samples were additionally stained with Kinyoun acid-fast technique and examined by histopathology. *Cryptosporidium* evolutionary stages were observed in stained smears and in the proventricular epithelium in the absence of inflammation. Molecular identification of 36 isolates was not carried out since microscopy revealed just one or two oocysts per slide in virtually all positive samples, resulting in false negative PCR or in faint bands observed in electrophoresis. It was possible the identification of *Cryptosporidium* species or genotypes in all proventricular samples and in 13 fecal samples (2.14%): *Cryptosporidium* avian genotype I in one peacock (*Pavo cristatus*), *Cryptosporidium* avian genotype II in four ostriches (*Struthio camelus*), *C. galli* in one cockatiel (*Nymphicus hollandicus*), two canaries and two lesser seed-finches, and *C. meleagridis* (1), *C. baileyi* (5) and *C. galli* (1) in chickens (*Gallus domesticus*). The present results reinforce the conception that avian *Cryptosporidium* have a wide range of hosts.

709 **Risk assessment of *Cryptosporidium* sp. in watershed associated with wildlife**

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Cryptosporidium is a water and foodborne pathogen with a broad host spectrum. Some genotypes of this protozoan are known to be zoonotic. The combination of these characteristics, and others related to this organism have posed a challenge to public health official concerned with the control of water and food borne pathogens. Several gastrointestinal outbreaks associated with exposure to contaminated water and food products have been attributed to the exposure to this pathogen. We carried out a quantitative risk assessment study to shed light on the potential pathways through which zoonotic genotypes of *Cryptosporidium* may be exchanged between wild and domestic species in the watershed ecosystem, and perhaps leading to contamination of the water supply systems. Scenario pathways models were developed to elucidate these pathways to be explored through quantitative risk assessment models. Inputs for the model were obtained from our observational epidemiologic studies on two watersheds in New York State. Complementary data and appropriate mathematical assumptions were obtained from the literature. Uncertainty about the parameters values and model structure were assessed using sensitivity analysis.

The majority of the *Cryptosporidium* genotypes recovered from wildlife identified in this study were not the zoonotic types. This indicated that wildlife is not as a major threat to environmental contamination in watersheds in comparison to that of domestic animals such as cattle.

710 **Natural infection with zoonotic subtype of *Cryptosporidium parvum* in Capybara (*Hydrochoerus hydrochaeris*) from Brazil**

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A total of 145 capybara (*Hydrochoerus hydrochaeris*) fecal samples from the state of São Paulo, Brazil, were screened for *Cryptosporidium* spp. oocysts using the malachite green method. Eight samples (5.52%) showed positive results and were further submitted to nested PCR reaction for amplification of fragments of 18S rRNA gene and 60-kDa glycoprotein gene for determination of species, alleles and subtypes of *Cryptosporidium*. Sequencing of the PCR products of the 18S rRNA gene fragments and 60-kDa glycoprotein gene fragments showed that for both genes all *Cryptosporidium* isolates from capybara were respectively 100% genetically similar to a bovine isolate of *C. parvum* and to *C. parvum* subtype IIaA15G2R1. To the best of our knowledge this is the first report of *Cryptosporidium* infection in this rodent. The finding of zoonotic *C. parvum* infection in a semi-aquatic mammal that inhabits anthroponotic habitats raises the concern that human water supplies may be contaminated with zoonotic *Cryptosporidium* oocysts from wildlife.

711 **Molecular identification of *Cryptosporidium* spp. isolates from fecal samples of felines, canines and bovines in the State of São Paulo, Brazil**

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Cryptosporidium is a coccidian which infects fish, reptiles, amphibians, birds and mammals. The occurrence in the animal population is cause for concern to public health due to its zoonotic potential, even though the role of each animal species in the transmission of this protozoan to humans is not well known. The objective of this study was to obtain information of epidemiological nature through molecular identification of *Cryptosporidium* isolates shed by dogs, cats and bovines from the São Paulo State, Brazil. Oocysts from seven cats, nine dogs and nine bovines were tested by nested PCR using primers directed to the small subunit RNA coding gene, followed by DNA sequencing of PCR products. The nucleotide sequences of the resulting amplicons were multiple aligned against each other and against genetic sequences representatives of *C. bovis*, *C. parvum*, *C. felis* and *C. canis*. After that, the p-distance between each pair of sequences was calculated and the species of the isolates were assigned after each sequence was paired compared to each standard sequence. The sequence analyses of the resulting amplicons revealed seven *C. felis* among samples from cats and nine *C. canis* among samples from dogs. The genotypic analysis of samples from cattle revealed *C. parvum* in eight and *C. bovis* in one sample. The results indicate that the molecular epidemiology of *Cryptosporidium* spp. infection in São Paulo might be similar to that of other countries. *Cryptosporidium felis* in cats and *C. bovis* in cattle were detected for the first time in Brazil.

712 **Molecular characterisation of *Cryptosporidium* isolates from pet reptiles**

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Cryptosporidium infections are common in reptiles in which the disease has been described in at least 57 different species. Even though a high prevalence of infection by *Cryptosporidium* has been described in captive reptiles, few studies have attempted to identify the species of the parasite involved. To date, only two species, *Cryptosporidium serpentis* and *Cryptosporidium saurophilum*, have been described in these animals although morphological and molecular analyses may indicate the presence of other species or genotypes of the parasite.

The use of reptiles as pets is on the increase and little is known about cryptosporidiosis in this particular population which is at particular risk due to the special care and maintenance they need.

In this study, we investigated the presence of *Cryptosporidium* in 171 faecal samples from reptiles commonly used as pet animals. These include lizards belonging to the genera *Eublepharis*, *Pogona*, *Chlamydosaurus*, *Hemiteconyx*, *Teratoscincus*, *Tilicua*, *Iguana*, and *Chamaeleo*, snakes of the genera *Lampropeltis*, *Elaphe*, *Python*, *Boa* and *Corallus*, and tortoises belonging to the genera *Testudo* and *Kinixys*. *Cryptosporidium* oocysts were detected by immunofluorescence using a commercially available kit and cryptosporidial DNA by amplification of a fragment of the 18S rDNA.

Cryptosporidium was detected by either method in 29% of the samples analysed. Molecular characterisation of the isolates confirmed that *Cryptosporidium serpentis* and *Cryptosporidium saurophilum* are the main species involved in infection in pet reptiles but also showed the presence of up to 5 new species or genotypes of this parasite.

The significance of these findings will be discussed.

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713 **Neonatal foal cryptosporidiosis in New Zealand**

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Introduction: Foal cryptosporidiosis is considered a rare disease despite co-existence of foals and ruminants on the same farm being common in New Zealand.

Methods: A survey of *Cryptosporidium* in diarrheic faeces of foals submitted to veterinary diagnostic laboratories was performed in New Zealand in 2006 using a modified Ziehl-Neelson stain and direct immunofluorescence which detected oocysts in 2 of 15 specimens. One originated from a broodmare facility which was subsequently visited and subjected to a thorough investigation.

Results: On this farm about 90% of the newborn foals (n=45) born on the facility during the four-month foaling season had diarrhoea and one foal died. On the day of the first farm visit, three newborns were affected with diarrhoea. Specimens from these foals were negative for *Salmonella*, rotavirus, and helminth ova, but contained >1 million *Cryptosporidium* oocysts/g. No oocysts were seen in the faeces of 3 mares. *C. parvum* and a *Cryptosporidium* 18S rRNA gene sequence similar to *C. parvum* were identified in DNA. *C. parvum* DNA was also detected from soil. Management practices that were considered to have contributed to the high incidence of disease included: close proximity to beef and dairy farms with common drainage; sick foals kept in the same stable block as the newborn foals.

Conclusion: This case together with a previously documented outbreak in New Zealand suggests foal cryptosporidiosis caused by *C. parvum* is underdiagnosed in New Zealand and may be part of the 'foal heat diarrhoea' complex. The zoonotic impact of foal *C. parvum* is unknown.

714 **Comparison of three halofuginone treatment regimens in the control of ovine cryptosporidiosis**

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Objective: To compare 3 halofuginone lactate (Halocur®) treatment regimens, the only product registered in Europe for the prevention of calf cryptosporidiosis since 1999.

Materials and methods: 40 lambs were separated from their mothers 24 hours after colostrum intake (D1). They were weighted, then allocated into 4 treated groups: [A] with a Placebo (10 ml water/day from D2 to D8), [B] with 100 µg/kg/day halofuginone for 7 days from D2 to D8, [C] and [D] with respectively 250 and 500 µg/kg/day halofuginone on D2, D4 and D6.

All lambs were orally inoculated with 5x10⁶ *Cryptosporidium parvum* oocysts (D2). They were raised under controlled conditions until D23.

Results: In control group A, oocyst excretion began on D5, was maximal on D6 and D7, then decreased to zero on D19. Excretion was delayed and 70.8, 15.7 and 9.8 times lower in the 3 treated groups B, C, D, respectively, compared to group A. Treatment B had a better and longer impact on diarrhea than treatments C and D. From D2 to D23, compared to group A, weight gain was improved, not significantly ($p < 0.05$) by 5.1% (B), 20.9% (D) and significantly ($p = 0.04$) by 30.3% (C).

Conclusion: The registered 7-day treatment can be reduced to 3 days (which is more practical for farmers), by increasing the dosage from 100 to 250 µg/kg/day and administering the dose every 2 days. This treatment regimen was well tolerated and resulted in a significant increase in the weight of treated lambs. The 500 µg/kg/day dosage did not give any additional improvement.

715 **Control of cryptosporidiosis in neonatal calves: the use of halofuginone lactate**

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Cryptosporidiosis is one of the causes of neonatal enteritis on dairy farms worldwide. On affected farms the level of contamination of farm buildings etc with *Cryptosporidium parvum* oocysts increases during the calving season and calves born later are exposed to a greater infectious load than earlier calves. Thus on farms where calving occurs throughout the year it is difficult to break the infectious cycle and to prevent the build up of oocysts in the farm environment.

This study describes the use of the antiparasitic halofuginone lactate (Halocur, Intervet, UK) on a dairy farm with a year round calving programme and with a high prevalence of *C. parvum* infection. The experimental design included four groups of eight newborn calves. Halocur was administered to two groups of calves immediately after being feed colostrum and thereafter for seven consecutive days, according to the manufacturers instructions and two groups were used as untreated controls. Further experimental treatments included treated and untreated calves being either reared in previously washed and disinfected individual calf pens or being mixed together in a larger pen. Oocyst excretion was monitored by microscopic examination of faecal smears or by 18srRNA PCR.

Oocyst excretion occurred earlier in the untreated calves. The amount of oocysts excreted was also greater in the untreated animals than the treated groups. Oocyst excretion was detected by microscopy in some of untreated calves on day 5 and the majority of them were positive by microscopy on day 7. The number of oocysts in the faeces of all the control calves had dropped below the level of detection by microscopy on day 13 although all the calves were positive with the PCR assay. Whereas oocyst excretion was detectable by PCR in all the treated calves it only ever got above the microscopy threshold on day 13 in three animals.

These results will be discussed in terms of controlling on-farm disease and the possible benefits for reduced environmental contamination.

716 **Prophylactic and therapeutic use of nitazoxanide against cryptosporidiosis in calves**

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Nitazoxanide (NTZ) is an antimicrobial agent approved by the FDA for the treatment in humans of cryptosporidiosis and giardiasis in children and adults. Diarrhoea caused by *Cryptosporidium parvum* also represents a major problem in calves younger than 4 weeks. In a preliminary experimental study, three groups of three calves (1-3 days old) each were inoculated with 10 millions oocysts of *C. parvum* (cattle genotype): the prophylactic group received 15 mg/kg BW NTZ orally with the milk one day before up to 8 days post inoculation (dpi). The therapeutic group received the same dosage of NTZ from the beginning of manifestation of diarrhoea (between 1-5 dpi) for 10 days and the control group was left untreated. The mean daily number of oocysts per gram faeces (OPG) was similar in the prophylactic and control group, while the therapeutic group showed clearly lower values (201'000, 229'000 and 43'900, respectively), similarly to the maximum number of excreted OPG (3.32 and 3.1 millions and 581'000 respectively). One chronic carrier was noted in each the prophylactic and in the control group, but none in the therapeutic group. Diarrhoea was first observed in all calves within 5 dpi, however, during 28 dpi, calves of the prophylactic and therapeutic group showed a longer diarrheic episode of 6 and 5 days, respectively, compared to 1.67 days of the control group, with strong altered faecal consistence. Neither prophylactic nor therapeutic use of NTZ reduced clinical appearance in this preliminary experiment.

717 **Efficacy of nitazoxanide against experimental cryptosporidiosis in goat neonates**

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Preliminary results obtained in mice, rats and piglets experimentally infected with *Cryptosporidium sp* have indicated a partial prophylactic or curative efficacy of nitazoxanide with administered between 50 and 250 mg/kg BW. In this study, the efficacy of nitazoxanide was evaluated in goat neonates experimentally infected with *Cryptosporidium sp* oocysts. Fourty seven 2- to 4-day-old kids were experimentally infected once on day 2 with 106 *Cryptosporidium* oocysts isolated from a dead kid and allocated in 3 groups. The group 1 acted as control untreated group, the group 2 received nitazoxanide for 8 days from day 1 to day 8 at a daily dose rate of 200 mg/kg BW, the group 3 received nitazoxanide for 7 days from day 4 to day 10 at 100 mg/kg BW. Individual oocyst shedding was monitored by daily examination of faecal smears stained by carbol fuschin and scored semi-quantitatively (0 to 4). The other criteria included for nitazoxanide evaluation were diarrhoea, weight gain and mortality. In the control group, oocyst shedding started 3 days PI, was maximal 6-7 days PI (mean scores ranging from 1.7 to 1.9) and became undetectable from day 16 PI. In group 2, oocyst shedding started 1 day later, peaked 9-11 days PI (1.3 to 1.5) and vanished day 18 PI. In group 3, results were similar to those of group 1 except for the mean scores ranging from 1.0 to 1.6. No significant differences were seen for diarrhoea and weight gains between groups. Five kids died in group 1 as well as in group 3 (31%) whereas 7 kids died in group 2. An acute toxicity of nitazoxanide is suspected during the 2 first days of treatment.

718 **Stability of *Cryptosporidium parvum* oocysts against chlorine for treatment of water**
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Cryptosporidium is a single-cell pathogen parasite that lives in the intestines of animals and human. This causes a disease called cryptosporidiosis. The inactive form of *Cryptosporidium*, called an oocyst with tough-wall which survive under a wide range of environmental conditions.

Ecology of *C. parvum* in calves and in humans and the existing scientific evidence that addresses the claim that calves put humans at significant risk for water-borne infection of *C. parvum*.

In this study oocysts were extracted from the feces of experimentally infected calves and cleaned from all organic fecal debris and other microorganisms. Two concentrations of chlorine (1.0 and 8.0 ppm. HOCl in demineralized water) at two temperatures (15 oC or 25 oC) for 6, 12, 24, 48, 72, 96, 120, 144 and 168 hours, were exposed to oocysts and oocysts then administered orally to four neonatal mice by gastric intubations. Each mouse received 100,000 oocysts. Mice were euthanized by CO₂ overexposure 96 hours after intubations. To assess infectivity, hematoxylin- and eosin-stained histologic sections of ileum from each mouse were examined by brightfield microscopy for developmental stages of *C. parvum*.

At concentration of 1 and 8 ppm chlorine in 15 oC water 144 and 24 hours contact times were necessary for killing oocysts and also at concentration of 1 and 8 ppm chlorine in 25 oC water 72 and 12 hours contact time killed all oocysts.

719 **Avoiding codon usage bias for better expression of the *Cryptosporidium parvum* protein P23 in prokaryote systems**

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C. parvum is a protozoan parasite that invades the host cell to complete its life cycle. P23 is a micronemal protein involved in gliding motility. For further study, the protein was expressed using different vectors and expression hosts. P23 was cloned into the pBAD-TOPO and pET-15b vector and expressed respectively in TOP 10 and Rosetta-gami (DE3) cells. Subsequently the His-tagged recombinant protein was purified by Immobilized Metal Affinity Chromatography (IMAC), separated by SDS-PAGE and visualised by Coomassie and silver staining. After expression with pBAD-TOPO followed by IMAC, the His-tagged recombinant P23 could only be visualised on a SDS-PAGE gel by silver staining. After purification of P23 expressed in pET-15b in Rosetta-gami, an intense band was visible on the Coomassie stained gel. The pET-system has two major advantages that can contribute to the higher expression yields of *C. parvum* proteins. The expression is more stringently regulated in pET than pBAD-TOPO, so there's less basal expression of possible toxic recombinant proteins. Moreover the Rosetta-gami strain encodes for six tRNAs that are low abundant in *E. coli* but often used in eukaryote systems like *Cryptosporidium*. This codon usage bias often stalls the expression of recombinant eukaryote proteins. Conclusion: The *C. parvum* protein P23 was cloned and expressed with two different systems. The pET system resulted in higher expression yield of the recombinant antigen than the pBAD system. Choice of promoter and host strain show to have an important influence on the expression of eukaryote proteins.

720 ***Giardia duodenalis*: novel proteins from an ancient eukaryote**

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Introduction: The protozoan parasite *Giardia duodenalis* is thought to be one of the earliest branching eukaryotic organisms. It is a single cellular organism lacking mitochondria and other organelles seen in higher eukaryotes. The *G. duodenalis* genome is currently being completed. The majority of the genes identified in the genome project are based on computer modelling and the transcriptional activity of these genes is not known. Methods: Whole trophozoite proteins from the isolate BAH 2c2 (assemblage A) were separated using two dimensional polyacrylamide gel electrophoresis. Protein spots were excised from gels, digested with trypsin and submitted for mass spectrometric analysis using an ABI 4800 Q-ToF. Mass spectra were searched using the MASCOT search engine. Results: Several proteins were identified that show no homology to any known eukaryotic proteins. These proteins represent novel classes of protein specific to *G. duodenalis*. A protein with homology to a general stress protein from *Bacteroides sp.* was also identified from the gels. This protein is either a remnant from before the eubacterial/eukaryotic split or has been acquired after the divide via lateral gene transfer. Conclusion: We have identified novel proteins from the intestinal parasite *Giardia duodenalis* confirming the work conducted by the *Giardia* genome project. These proteins need to be characterised further to understand their role in the parasite and the likelihood of their use as specific drug targets for infection by *G. duodenalis*. The protein resembling a bacterial protein may give further insight into the evolutionary biology of this parasite.

721 **Molecular identification of *Giardia duodenalis* isolates from humans, dogs, cats and cattle from São Paulo state, Brazil by sequence analysis of fragments of glutamate dehydrogenase coding gene**

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The nucleotide sequence of *gdh* coding genes were obtained from cysts of *G. duodenalis* isolated from feces of naturally infected cats (n=20), dogs (n=31), humans (n=37) and cattle (n=5). The samples were from São Paulo, state, Brazil. The *gdh* nucleotide sequences were multiple aligned against each other and against homologous sequences representatives of each *G. duodenalis* assemblage retrieved from Genbank. The following sequences were downloaded: isolate AD1 (AY178735, assemblage AI), isolate Bris136 (AY178737, assemblage AII), isolate AD28 (AY178738, assemblage B), isolate AD136 (U60982, assemblage C), isolate AD148 (U60986, assemblage D), isolate P15 (AY178741, assemblage E) and isolate AD23 (AF069057, assemblage F). After that, the p-distance between each pair of sequences was calculated and the species of the isolates were assigned after each sequence was paired compared to each standard sequence. Sequences analysis of the 37 specimens recovered from humans revealed 29 *G. duodenalis* assemblage AII and eight *G. duodenalis* assemblage BIV. Among samples from cats, 11 were classified into assemblage F and nine into assemblages AI. Only the host-adapted assemblages C and D were detected in samples from dogs (eight and 23, respectively). Among the samples from cattle, the genotype livestock was found in four samples and the assemblage AI was detected in one sample. The molecular identification of assemblages of *Giardia spp.* isolates from different hosts is imperative for better knowledge of the epidemiology of Giardiasis in animals and humans in Brazil and the typing results of our analysis seem to agree with those of other laboratories from other parts of the world.

722 **Prevalence, and molecular biologic characterization of *Giardia* in household dogs in Hungary**

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In the genus *Giardia*, *G. intestinalis* is the only species found in humans as well as in other mammals including domestic and farm animals. Molecular characterization from different hosts has revealed the existence of seven major genotypic assemblages. Assemblages A and B isolates have been recovered from a broad range of hosts, including humans, livestock, cat, dog, beaver, and guinea pig. Infection and potential cyst shedding contaminates the environment for all mammals, including humans.

The prevalence of *Giardia* infection in household pets is lesser-known in Hungary. In a preliminary investigation, we studied the prevalence of *Giardia* infection in 187 faecal samples of dogs originated from kennels by microscopical examinations, and using a *Giardia lamblia* Ag-specific ELISA coproantigen test (ProSpecT Remel, USA). There was a great difference in the sensitivity of the microscopic examination (7.5 % positive) and the ELISA test (58.8 % positive) for detection of *Giardia* infection. In order to investigate the genotypes of *Giardia*, a nested PCR specific for the *Giardia* 18S-rDNA was introduced. Most of the isolates displays the sequence reported for the dog specific Assemblage D genotype with high confidence. But 2 isolates have an unidentified nucleotide at the genotype determining locus 167 preventing the accurate classification of these isolates.

The preliminary investigations indicate that *Giardia* is a common parasite in dogs in Hungary. Therefore, there is a significant public health need in Hungary to determine the *Giardia* infection rate in dogs, especially in pets, living in close proximity to humans, and other animals.

723 ***Giardia* spp. in dogs: Genotyping of 75 samples from randomly selected dogs presented at local veterinary clinics**

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Recent studies have shown that dogs in Germany harbor different genotypic assemblages, predominantly C and D, but also the assemblage A, which has been confirmed as a pathogenic human genotype. To determine the prevalence of assemblage A, a first study was conducted in dogs which showed clinical signs of diarrhea or other symptoms indicating a possible infection with *Giardia*. 92 dogs were identified positive for *Giardia* by a coproantigen test of which 65 samples were positive for cysts of *Giardia* using MIFC technique. These samples were genotyped, 50 *Giardia* isolates were identified as assemblage D, 33 as assemblage C, and 8 as a mixture of both. One dog harbored a mixture of assemblage D and A.

It was concluded that the predominant dog specific assemblages C and D in this first study might be more commonly associated with GI disorders than the zoonotic genotype A. Alternatively the coproantigen test used in this study might select assemblages C + D in dogs. Therefore in a second study randomly selected dogs presented at local veterinary clinics were examined for cysts of *Giardia* using the MIFC technique. 58 samples of *Giardia* positive dogs were genotyped: 57 % belonged to assemblage D, 36 % to assemblage C and 7 % could be identified as the zoonotic genotype assemblage A. Comparing the 65 MIFC positive samples from the first study with the 58 samples of the second study a significantly higher prevalence of assemblage A ($p=0.0467$) was found in randomly selected dogs.

724 ***Giardia* prevalence in household dogs and cats and validation of a commercial ELISA test**

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The aim of this study was to investigate the prevalence of *Giardia* infection in household dogs and cats and to validate a commercial ELISA test for the detection of *Giardia spp.* cysts from faeces (i.e. Idexx Snap® *Giardia*). A total number of 100 owned dogs and 54 cats were enrolled in the study without bias to age, sex, breed or presence/absence of gastrointestinal clinical signs. Three faecal samples, collected at 1-2 days interval, from each animal were processed for *Giardia* cysts using zinc sulphate (ZnSO₄) flotation/centrifugation. At the meantime, faecal samples from a subset of 60 dogs and 40 cats were also processed using ELISA technique. *Giardia* cysts were retrieved in 25/100 (25%) of sampled dogs and in 13/54 (24.1%) of sampled cats. The ELISA test was unable to detect *Giardia* cysts from 6/21 positive dogs and 1/11 positive cats thus resulting in a final sensitivity of 71.4% and 90.9% for dog and cat samples respectively. Specificity of the ELISA test was of 100% for both dog and cat samples. Cyst shedding was more common in young (< 1 year) animals than in adults and 57.9% of positive animals were asymptomatic. However, diarrhea was the most frequent clinical feature observed in positive symptomatic animals (39.5%). The results from the present study suggest a wide circulation of *Giardia* infection among pets of the sampled population and confirm that, although ZnSO₄ flotation/centrifugation is time-consuming and requires specific technical skills as for the cyst identification, it must be considered as a high sensitivity tool for the diagnosis of *Giardia* infection.

725 **Prevalence of *Giardia intestinalis* infection in Punjab (India) and its association with diarrhea in dairy calves**

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Introduction: The intestinal flagellate *Giardia intestinalis* has a global distribution and is a common protozoan parasite in many animal species and in man. In calves it can cause diarrhea, malabsorption and growth retardation. Cattle harbour a number of protozoan parasites but there is an emerging concern about the role cattle play in human giardiasis & cryptosporidiosis. A prevalence study was contemplated to determine the prevalence of *Giardia spp.* in dairy farms in Punjab, India.

Method: Fresh fecal samples were collected from 187 calves and adult cattle. *Giardia intestinalis* was enumerated in each sample by wet mount and trichrome staining technique. Material (s): Trichrome stain, Lugol's iodine, fecal samples

Results: Prevalence of *Giardia intestinalis* in relation to age and sex were also investigated. The parasite was detected from 16.04 and 8.64% from 106 diarrheic and 81 non-diarrheic animals, respectively. The shedding was significant in calves with diarrhea. The prevalence of the infection peaked in young calves between 1-6 months of age in the diarrheic and 6month -1 year of age in the non-diarrheic groups (34.78 and 18.18% respectively).

Conclusion: The *Giardia spp.* appears to be highly prevalent in dairy calves and should be considered in animals with diarrhea or failure to thrive, in the Punjab province.

726 **Prevalence and genotyping characterization of *Giardia duodenalis* infecting water buffaloe calves (*Bubalus bubalis*) from Egypt**

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The prevalence of *G. duodenalis* infection was determined among 530 water buffalo calves (1-16 weeks (w) old) from 4 localities belonging to 3 Governorates of Egypt. Faecal samples were examined for the presence of *Giardia* cysts using zinc sulphate solution for concentration floatation. Genotyping characterization was done for the isolated *Giardia*. SDS PAGE was performed for trophozoites and cysts of *Giardia sp.* obtained from 3 different Governorates of Egypt. DNA was extracted from *Giardia sp.* cysts obtained from 3 positive calves from 3 different Governorates of Egypt. A PCR based method was employed to amplify and sequence a 293 bp region of the small subunit ribosomal RNA (16S-rRNA gene). The obtained sequence was compared with the same amplified fragments of human, dog and cat, dog isolates (Hopkins et al., 1997) in Australia, Western Australian and Western Canadian dairy calves (O'Handely et al., 2000). The overall prevalence of *Giardia* was 12.8%. Among the 4 age groups (1-4 w, 5-8w, 9-12w and 13-16w) the prevalence was 16.8%, 13.8%, 12.1% and 8.3% respectively.

The SDS PAGE of cysts and trophozoites of *Giardia sp.* obtained from water buffalo calves from 3 different governorates revealed the homogeneity of antigen of the 3 isolates. The cysts showed 5 bands (of molecular weights 110.5, 62.5, 52.5, 29.0 and 22.3 KDa) while 11 bands were reported for the trophozoites (60.5, 47.2, 40.5, 36.7, 34.1, 30.8, 27.3, 26.6, 24.8, 22.3 and 21 KDa).

Our isolate showed little degree of divergence from the fore mentioned previous studies. The difference was only concerned with 10 nucleotides from 293bp (3.4%) while 96.6% had similar sequences.

This molecular study revealed that all these isolates were a mixture of animal and human isolates, which are geographically widespread and belonging to *G. duodenalis* of subgroup AI.

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Abstract Number

Abad T.	354	Alvarez L.	350, 523
Abbas R.	256	Álvarez-García G.	042, 402, 406
Abdalla I.M.	418	Álvarez-Sánchez M.A.	345, 633
Abdalla M.E.	311	Alvenerie M.	076, 117, 134, 320, 528
Abdeen Z.	195	Alzieu J.P.	186, 629
Abdel-Gawad A.	666	Amarante A.F.T.	275, 292, 329, 395, 558, 695, 707
Abdulkareem M.	586	Amoo O.	139
Abdyjaparov T.	025	Amoughli Tabrizi B.	420
Abdykerimov K.	025	Anderson L.	057
Abolins S.	144	Anderson N.	628
Abou-EL Dobal S.	612	Andrada M.	290
Abu-Madi M.A.	541	Andrews J.J.	631
Abuseir S.	029	Angulo C.E.	642
Acosta D.	606	Anjos D.H.	491
Acosta-García I.	608	Anthony R.M.	002
Adamu S.	586	Antolová D.	567
Adhikari B.B.	274	Antoniou S.	364
Adnan M.	264, 524	Antunes R.	708
Aduriz G.	394, 409	Aragão W.R.	258
Afonso S.M.S.	303, 322	Araneda M.	301
Afonso-Roque M.M.	498, 499	Arantes T.P.	321, 700
Afsharnasab M.	455, 456	Araque H.	467
Agatsuma T.	062, 201, 602	Araújo J.V.	275
Aguado E.	572	Arble B.L.	255
Aguado-Martínez A.	042, 406	Arboix M.	322
Aguilar-Caballero A.J.	018, 251, 262, 277, 634	Arcangeli G.	459
Ahadi M.T.	596	Arias M.	335, 500, 607, 664, 704
Ahmad I.	302	Armas S.	590
Ahmed I.	287	Armua M.T.	577
Ahmed K.H.	586	Árnaiz I.	394
Ahoussou S.	024	Arndt N.	161
Airas N.	368	Arné P.	681
Ait Hamou S.	447, 622	Arshad M.	256
Ajdarnejad Sh.	596	Arteaga N.G.	316
Akbarein H.	706	Arther R.	378, 547, 548
Akbarneshad F.	694	Arvidsson S.	142
Al Anazi A.	489	Asadian F.	312
Alasaad S.	601	Asaolu S.O.	544, 545
Alavi S.	596	Ascencio F.	642
Alban L.	469	Asgari Q.	685
Alberti E.	081	Ashmawy K.I.	429
Alcala C.Y.	349	Athanasiadou S.	123
Aldavood S.J.	706	Atkins C.	547
Alexopoulos C.	181	Atkinson L.	021
Alhassan A.	047	Attakpa E.	257
Aliaga-Leyton A.	673	Aubert D.	681
Alim M.A.	653	Aulakh R.S.	725
Alipour-espehkolaei M.	542	Avila R.	354
Allaoui R.	269	Awais A.	540
Allen L.	569	Ayala-Burgos A.	277
Aller-Gancedo J.M.	644	Ayaz E.	356
Almeida F.	254	Azade Ranjbar S.	669
Alonso-Díaz M.A.	262	Azando E.	257
Altea A.	490	Azhar M.	540
Altreuther G.	131, 555	Azizi H.R.	312, 452, 626
Álvarez Carrión B.	712	Azzouz S.	574

Baadsgaard N.P.	674	Bergamin L.K.S.	321, 553, 560
Babar W.	518	Berge T.	426
Bacciarini L.	055	Bergeaud J.P.	093, 289
Bacelar J.	703	Berkvens D.	116
Bach Knudsen K.E.	016, 017	Berrag B.	033, 269
Badie J.	572	Berrilli F.	210
Baeton L.	631	Berriman M.	243, 314, 315
Bahuon C.	366	Berthelemy M.	067
Bailey J.	234	Beschin A.	001
Bain O.	064, 067, 353	Besier R.B.	163, 192, 206, 234
Bakker J.	150	Besson B.	507
Bal M.S.	689	Bethencourt A.	467
Balkaya I.	137	Betschart B.	151
Ball G.	020, 313, 315	Bettini C.M.	695
Ballent M.	336, 340	Beveridge I.	248
Ballweber L.R.	631	Bevilaqua C.M.L.	258, 260
Bandi C.	179, 353	Bezerra S.Q.	491
Banga H.S.	725	Bhoora R.	086
Bangoura B.	663	Bhushan C.	323
Bannach E.	254	Bichuette M.A.	695
Bapst B.	384, 386	Bickel T.	559
Baradaran H.R.	312	Bieñ J.	396
Baranenko J.	467	Bilgili A.	556
Barbuto M.	353	Billingsley P.F.	140
Bareille S.	714	Bingham G.	023, 482
Barfield R.	183	Binos S.	245
Barkema H.W.	211	Birkett M.	081
Barnett S.	383, 384, 386	Biryomumaisho S.	197
Barraza T.C.	433, 538	Bishop R.	229
Barreira J.D.	654	Bishop S.	529
Barrell B.	243	Bista P.R.	112, 568
Bart J.M.	575	Björkman C.	702
Bartley D.	023, 194, 263, 266, 522, 528	Blackhall W.	515, 516
Bartley D.J.	076	Blaga R.	364, 365, 366, 367, 506
Bartley P.M.	106	Blair D.	062, 174, 602
Barufi F.	635	Bleyen N.	204
Barutzki D.	723	Blond-Riou F.	225, 381, 382
Barzegar M.	461, 462	Boes J.	016, 469, 470, 674
Basciu M.	704	Bogoyavlenskiy A.	183
Bates P.G.	480, 482, 636, 637, 645, 646	Bohórquez A.	504, 505
Baudry C.	594	Boireau P.	364, 365, 366, 367, 506, 684
Bauer C.	026, 306, 393, 570, 587	Boldbaatar D.	226
Bautista O.J.	359	Bolln M.	587
Bavia M.	101	Bond E.	305
Bazarusanga T.	088	Bonello F.	710
Bazzocchi C.	309, 400, 408, 550	Bonnet S.	414
Beaurin D.	435	Booth M.	004
Beck R.	239	Borbolla J.	433, 627
Beck W.	372, 625	Borda B.	523
Bédé O.	466	Borges F.A.	323, 530, 560
Beelitz P.	176, 434	Borges R.	634
Behrendt J.H.	108	Borges- Ribeiro R.S.	654
Bélèm A.	091	Borghetti P.	399, 400
Bendall H.	342	Borgsteede F.	343, 585
Bénéré E.	212	Bork-Mimm S.	050
Beninati T.	179	Bornstein S.	217
Bennett H.	606	Botelho A.	403
Berezin V.	183	Bouix J.	093

Bourdeau P.	370, 371, 373, 448, 449, 475, 572, 640	Cantacessi C.	061, 154, 358
Bousquet E.	501, 504, 505, 507, 509	Cantó A.G.J.	349, 359
Boussarie D.	435	Cantoni A.M.	397
Boutet M.	584	Canul-Ku H.L.	018
Bowman D.D.	155	Capece B.P.S.	303, 322
Boyacioglu M.	297	Capelli G.	154, 443, 459, 624
Boykin D.	407	Capetillo-Leal C.	251
Bradley J.E.	005	Carbajal-González M.T.	644
Braga F.R.	275	Carbone S.	369
Braga R.C.	258	Càrcamo N.	433
Braga R.R.	260	Carcy B.	049
Brandt H.	146	Cardillo N.	561, 562
Brar A.P.S.	484	Cardoso J.M.S.	401
Brar R.S.	484	Cardoso L.	423, 424
Brasil-Sato M.C.	485	Cardoso M.	265
Brennan G.P.	060, 121, 166, 172, 173, 242, 341, 347, 348, 526, 597	Carels B.	535
Bresciani K.D.S.	395, 552, 558, 695, 707	Carmona C.	606
Bretagne S.	684	Caron Y.	237
Brianti E.	358, 724	Carrasco A.O.T.	391
Bricarello P.A.	292, 329	Carrijo Mauad J.R.	275
Briciu R.	478	Carrillo E.	422
Brigui N.	441	Carrington M.	090
Britton C.	314	Carrique-Mas J.	480
Bruet V.	370, 371, 373, 640	Carslake H.	713
Brum E.	265	Casaert S.	535
Brun R.	407	Casiraghi M.	353
Brunet S.	015, 251, 261	Cassini R.	624
Bruzinskaite R.	027	Castañon C.A.B.	671
Buffoni L.	608, 611	Castellà J.	533
Buge S.	641	Castells G.	322
Buggelsheim M.	109, 675	Castillo B.R.	348, 359
Bünger L.	361	Castro A.	011
Burden F.	495	Castro C.N.	538
Burden J.	342	Castro N.	433
Burgess I.	380	Castro O.	623
Burke J.M.	019, 259	Catania S.	443
Buxton D.	106	Cavalcanti W.	265
Buzzulini C.	321, 530, 635	Cavaliero T.	386
Cabaj W.	396	Cavallo P.	210
Cabaret J.	033, 079, 269	Ceballos L.	350
Cabezas I.	301	Cedeño V.	215
Cabrera R.	632, 647	Cepeda R.	642
Cacciò S.M.	059	Cerca F.	403
Caceres M.	634	Cerutti M.C.	309
Calderon R.R.	279	Chabalgoity A.	574
Caldon M.	459	Chan S.	136
Callaghan K.	481	Chandrawathani P.	264, 524
Calvo E.	335	Charles S.D.	054, 548, 677
Cámara-Sarmiento R.	018	Charlier J.	010, 012, 034, 104, 629
Camargo G.	323, 552	Chartier C.	167, 268, 594, 717
Campbell B.E.	059, 248	Chaudary F.R.	287, 310
Campbell C.	513	Chauvin A.	013, 414
Campos R.T.	465	Cheah T.S.	417
Camurça-Vasconcelos A.L.F.	258, 260	Cheat Sophal	486
Camuset Ph.	085	Chen N.	246
Cañavate C.	422	Cheraghi E.	107
Cannas da Silva J.	031, 703	Chermette R.	559
		Chianini F.	106

Chiapparrone M.L.	041	Correwyn S.	159
Chicharro C.	422	Cortes H.C.E.	105, 185
Chiers K.	184	Cortiñas J.	704
Chimbwanda T.M.	337	Cos P.	212
Chin P.L.	417	Costa A.J.	321, 323, 530, 552, 553, 560, 635, 654, 695, 698, 699, 700
Chiniforoush M.	596	Costa C.T.C.	258, 260
Chinikar S.	177	Costa I.	011
Chirico J.	142, 479	Costa T.P.	558
Chiumiento L.	366	Costa-Junior L.M.	432, 446
Cho K.O.	389	Costiou P	475
Cho M.H.	411	Cota-Guajardo S.	433, 538
Choi K.J.	436, 437	Coughlan A.	243
Choosaksaengthong S.	665	Couper A.	343, 344
Chopade H.	677	Courouble R.	583
Christensson D.	307, 514	Cousquer G.	175
Christiansen S.	469, 470	Coutinho L.L.	292, 329
Chromá V.	672	Couto M.C.M.	491
Chungpivat S.	665	Cox E.	697
Cid-Del Prado I.	282	Cox J.	298
Cienfuegos S.	649	Cozma V.	364, 365, 375, 478, 506
Cirak V.Y.	137, 511	Craig B.H.	231, 233
Cirillo R.	210	Cremers H.J.W.M.	557
Cissé M.	466	Cretu C.M.	506
Ciszewski D.K.	547, 677	Cringoli G.	008, 033, 072, 100, 278, 339, 369, 503, 513
Citterio C.V.	309	Cristel S.	024
Claerebout E.	012, 065, 104, 125, 134, 330, 331, 332, 512, 535	Cristófol C.	303, 322
Claeskens M.	670	Cromie L.	343, 344
Clark H.	077	Cruz C.	627
Clifford D.	636, 646	Cruz M.	215
Cob-Galera L.A.	018, 277	Cunha T.R.	553, 560
Cockett N.	529	Cunningham A.	215
Coelho M.M.S.	253	Czirjak G.A.	375
Coetzer J.A.W.	158	D'Amelio S.	309
Coklin T.	211	D'Herde K.	184
Colditz I.G.	206	D'Souza S.	680
Coleman G.T.	554	Dacal V.	649, 664
Coles G.C.	033, 034, 035, 190, 270, 503, 510, 513	Dahlgren S.S.	427
Collantes-Fernández E.	390, 394, 404	Dalemans A.-C.	535
Collett M.G.	040	Dalimi A.	564
Collins N.E.	046, 086, 087	Damriyasa I.M.	306, 393
Collins N.E.	416	Danesi P.	459, 624
Colwell D.D.	502, 650	Dankers I.	334
Conceição M.A.P.	011	Dao B.	091
Conn D.A.	362	Darabi S.	312, 626
Conn D.B.	352, 362	Dargantes A.P.	198
Conrath K.	115	Daryani A.	542
Conraths F.J.	679	Das S.	050
Cook C.	213	Dascalu M.L.	620
Corba J.	165	Dashtian Nasab A.	456
Cordeiro-da-Silva A.	423	Daudet A.	681
Cordero-Pérez C.	345, 633	Dauguschies A.	426, 663
Coronado A.	656	Davies A.J.	213
Corrain C.	459	Davies G.	529
Corrain R.	624	Davies P.	503
Corrêa D.	254	Davies R.H.	480
Correia A.	403	Davis W.	378, 547
Correia da Costa	011	Davoust B.	069, 684

Dawson H.D.	002	Divina B.P.	274
De Angelis E.	400	Dixon B.R.	211
De Baetselier P.	001	Dobson R.J.	163, 198
de Boer M.W.	392	Doherty P.	383, 386
de Broucker C.A.	684	Domingos A.	303
de Caprariis D.	161	Donnan A.A.	097, 168, 266, 512, 522
De Cat S.	628	Doorn D.C.K.	150, 318
De Craeye S.	680	Doosti A.	464
De Craeye S.	697	Dorantes A.	634
De Croock F.	535	Dorchies P.	013, 289, 642
De Deken R.	089	Dorny P.	034, 065, 112, 113, 115, 116, 174, 325, 568
de Graaf D.C.	719	Dou L.	367
De Gussem J.	204	Dowdall S.	077
De Gussem K.	204	Downie-Ngini K.	111
de la Fuente J.	138, 648	Doyle J.	387
De la Rocque S.	091	Drigo M.	624
de Lahunta A.	493	du Plessis A.	388
De Meulemeester L.	012	Dubey J.P.	688, 692
de Souza C.G.	623	Ducatelle R.	184
de Vries A.	208	Ducheyne E.	102, 104
de Vries E.	138, 292, 329	Dupree-Olcott D.	095
De Waal D.T.	143, 271, 273, 276, 519, 238	Dupuy J.	076, 320, 528
de Waard I.	159	Duran M.	552, 553
de Waele V.	715	Durand B.	364
De Wilde N.	535	Dussaulx G.	641
de Yaniz M.G.	041	Dyachenko V.	026, 570
Dean S.P.	130	Dzimianski M.T.	056
DeAngelis E.	399	Eckersall P.D.	285, 286
Dear P.	243	Edelhofer R.	048
Deckers N.	115	Edgar H.W.J.	166, 348
Deeg C.	151	El Jirari L.	447
Del Aguila C.	440	El-Abdellati A.	512
Del Bue M.	397	El-Attar G.	617
DeLay R.	224, 225, 382, 381	El-Behairy A.	666
Delbac F.	435	El-Herawy A.Z.	488
Delespaulx V.	199	El-Massry A.A.	576
Dell'Porto A.	253	El-Seify M.A.	488
Delmotte Ch.	252	El-Tayar A.A.	429
Delprat S.	381	Eleno V.A.	656
Demeler J.	034, 190	Elias D.	003
Demonceau T.	507	Ellison S.	348
Den Toom M.	334	Emanuelson U.	702
Denwood M.J.	296	Engström A.	333
Deplazes P.	025, 027, 030, 048, 055, 070, 071, 074, 645, 646, 716	Ennulat D.	493
Depner R.	283	Entrocasso C.	523
Derbala A.A.	576	Epe C.	029, 074, 180, 187, 534
Desouky A.Y.	488, 497	Epis S.	179
Devine C.	172, 526	Erhardt G.	146
Dewey C.	673	Erskine A.	539
Di Lecce R.	397	Esfandiari B.	542
Di Lucente A.	205	Eslami A.	328, 580
Díaz Huerta M.D.	354	Esmail Zadeh S.	581, 685
Díaz J.	354	Esposito M.	105
Díaz P.	500, 607, 704	Estrada-Peña A.	103, 501, 623
Díaz R.	354	Estuningsih E.	169
Díez C.	618	Eysker M.	133, 150, 318, 334
Díez-Baños P.	335, 500, 618, 649	Fahmy M.	666
		Fahrion A.	071

Fairweather I.	037, 060, 121, 166, 172, 173, 242, 341, 346, 347, 348, 350, 526,	Franssen L.	086
Fallah E.	691	Fredholm M.	147, 468
Farkas R.	377, 413, 659	Fregeneda-Grandes J.M.	644
Farshine A.	658	Frey C.	045, 363
Fasshauer C.	267	Friendship R.	673
Fazendeiro M.I.	498, 499	Froyman R.	670
Feenstra P.B.	084	Fthenakis G.C.	299, 300
Félix M.L.	623	Fu B.	366, 367
Fenoy S.	440	Fuchs M.-A.	346
Fenwick S.	218	Fujisaki K.	226, 228, 653
Ferlazzo M.	724	Fukui D.	577
Fernandes K.R.	415	Fumi C.	176
Fernández S.	305, 671	Funada M.R.	401, 721
Fernández-García A.	042, 406	Furuta P.I.	391
Fernández-Pato N.	345, 504, 505, 633	Gaasenbeek C.	585
Ferrantelli V.	443	Gaglio G.	724
Ferre I.	409, 460	Gai W.	367
Ferri E.	353, 399, 400, 408	Gajadhar A.	152, 351, 442
Feseha G.	492	Galke D.	176, 434
Fetterer R.	183	Gallidis E.	299
Fiedor C.	009, 588	Gamble H.R.	152
Field C.	539	Gandji A.	308
Filimonov N.	589	Ganzorig S.	571
Fischer N.	118, 317	Garate T.	354
Fisher M.A.	074, 129	Garate-Gallardo L.	018
Fitton L.	288	García C.	601
Fitzpatrick R.	273, 276	García H.	590
Flanagan A.	166, 348	García M.E.	590
Fletcher H.L.	060	García Z.	627
Fleury A.	354	García-Moreno F.T.	460
Florijancic T.	239	García-Palencia P.	438, 439, 440
Flütsch F.	030	Garcia-Vazquez Z.	634
Flynn R.J.	006	Garippa G.	490
Focant C.	638	Garnier M.	525
Földvári G.	413	Garramuño J.	632
Fonfara S.	216	Garrido-Bailón E.	438, 439, 440
Fontbonne A.	559	Gasanova T.A.	430
Fontoura L.F.	671	Gasbarre L.C.	127, 135
Fonville M.T.M.	682	Gasser R.B.	059, 248
Forbes A.B.	009, 010, 238, 629	Gatti A.	563
Forbes L.	152, 351	Gaully M.	146, 267, 486
Fouquet A.	252	Gause W.C.	002
Fourie L.	388	Gauta J.G.	590
Fournié G.	215	Gavier-Widén D.	217
Fournier E.	594	Gawlowska S.	026
Fournier R.	714	Gaxiola S.	433, 538, 627
Fourqueaux I.	289	GBrandonisio O.	210
Fox M.T.	175	Ge K.	667
Fraga J.S.	671	Geary T.	249
Fragkou I.A.	299, 300	Geerts S.	089, 199
Fragoso	354	Geldhof P.	065, 096, 125, 134, 330, 331, 332, 512
Francart A.	680	Genchi C.	056, 072, 074, 179, 353, 399, 400, 408, 490
Francisco I.	335, 500	Genchi M.	490, 550
Franco L.G.	395	Généreux C.	402
Franco-Navarro F.	282	Gennari S.M.	395, 398, 401, 412, 688, 690, 695, 708, 710, 711, 721
François D.	289	George D.R.	481
Franque M.P.	415	Germann T.	377

Gerner W.	109, 675	Greenwood K.	020
Gersão S.	498	Greenwood S.J.	211
Getachew M.	492, 496	Greer A.W.	124, 266, 522
Geurden T.	065, 325, 535	Greif G.	044
Geysen D.	087, 088	Grifaldo A.F.	656
Ghaednia B.	455	Grillo T.	058
Gherman C.	364, 365, 506	Grimm F.	645, 646
Gholami Sh.	542	Grinberg A.	713
Ghorbanpour M.	428, 581, 694	Grisez C.	093, 289
Giadinis N.D.	032	Gruber A.	671
Giangaspero A.	210	Gruner L.	093
Giannetto S.	358, 724	Guegnard F.	525
Giao H.K.	325	Guerrero J.	056
Gibbs B.	606	Guilliams M.	001
Gibney E.H.	043	Guillot J.	067, 074, 215, 435, 559, 681
Gibson W.	090	Gull K.	090
Gill J.P.S.	473, 725	Gurgis S.	726
Gilleard J.	020, 058, 164, 243, 313, 314, 315	Gustafsson M.	603
Gilles J.	053, 178	Guthrie A.J.	086
Gindri J.	283	Gutiérrez Q.M.	465
Girisgin O.	137	Gutu E.	683
Githiori J.B.	280, 281	Guy C.S.	043
Gjerde B.	427	Guy J.H.	481
Glemarec A.	365, 366	Haçariz O.	609
Globokar M.	026, 679	Haddad N.	215, 435
Gluez E.	090	Haddadzadeh H.R.	706
Gnayoro M.	466	Hadfield T.	529
Goater C.P.	502	Haering D.	014
Godarzi M.A.	621	Hajikhani R.	669
Goddeeris B.M.	204	Halajian A.	328, 457
Godoy G.S.	391	Halferty L.	121, 347
Goff W.	447	Hall M.J.R.	175
Gokbulut C.	297, 511, 556	Halliday A.M.	122
Golinsky A.A.	415	Halos L.	684
Gomes L.	494, 499	Hamarshah O.	195
Gomes T.	494, 498	Hamidinejat H.	428, 694
Gómez-Bautista M.	394, 410, 460, 712	Han H.J.	389
Gómeza E.	354	Hanedan B.	556
González C.	467	Hang Z.Z.	615, 619
González F.	422	Hanna B.	344
González J.F.	290	Hanna R.W.	166
Gonzalez R.J.L.	279	Hanosset R.	573
Good B.	143, 238, 271, 273, 276, 519	Hanrahan J.P.	143, 271, 273, 276, 519
Goodman S.	215	Harder A.	118, 317, 360
Goossens L.	110, 342	Hasan S.M.	617
Goraninezhad S.	694	Hasanpoor A.	420
Gordon Y.	023, 076, 263, 266, 522	Hashemitabar G.R.	578
Gottstein B.	044, 045, 070, 185, 363, 575	Hatta T.	653
Gou H.	367	Havn K.T.	469
Gougoulis D.A.	299, 300	Hayasaki M.	549
Goullet M.	569	Heaney K.	224
Gourène G.	466	Heck I.	283
Gozdzik K.	396	Heckendorn F.	014
Grade J.T.	255	Heesen H.	670
Granados J.-E.	601	Heine J.	141, 387, 548, 551, 563
Granato A.	459	Heinzmann D.	030
Grandi G.	397, 399, 400, 408, 550	Helbig R.	385
Gray J.	238	Hellmann K.	053, 225

Hemphill A.	044, 105, 119, 185, 407, 716	Huntley J.F.	123, 124, 477, 644
Hénaff M.	441	Hurníková Z.	567
Hendawy M.A.M.	617	Hussain A.	639
Hendrickx G.	068, 091, 102, 104	Huyse T.	613
Hepp S.	426	Ibarra V.F.	338, 349, 359
Hermosilla C.	108, 661	Igarashi I.	047, 050
Hernández A.	290	Iluyemi A.	159
Hernández C.A.	359	Im K.I.	436, 437
Hernández M.	354	Imbuga M.	139
Hernández R.	537	Imperiale F.	024
Hernández-Campos A.	348	Innes E.A.	106
Hertzberg H.	014, 030	Innocent G.T.	296, 492
Hestvik G.	217	Inocêncio da Luz R.	212
Higes M.	438, 439, 440	Inokuma H.	047
Hilali M.	726	Inoue T.	571, 577
Hill D.E.	152	Ionescu A.	620
Hillyer G.V.	607	Iorio R.	205, 563, 705
Hillyer M.H.	510	Iqbal Z.	256, 518, 639
Hirzmann J.	432, 446	Iseki H.	047
Hlásková L.	672	Ishizaki M.N.	558, 707
Hoby S.	048	Islam M.K.	653
Hodgkinson J.E.	077, 082	Ismail A.A.	418
Hoey E.	172, 346, 526	Isomursu M.	368
Hoey E.M.	060, 172, 341, 346, 526	Ivanova E.	064
Hoey L.	242	Ivens A.	313
Hogg G.	059	Iwaki T.	571
Hoghooghi Rad N.	581	Iweala O.I.	002
Höglund J.	034, 035, 190, 307, 324, 333	Jabbar A.	256, 518
Hol W.	222	Jackson E.	078, 097, 263, 266, 522
Holder Z.	631	Jackson F.	015, 023, 033, 035, 058, 076, 078, 097, 123, 164, 168, 194, 263, 266, 522, 528
Holmdahl J.	307	Jackson V.	144
Holzhauer M.	084	Jacobs D.	539
Holzmer S.	224	Jacobs F.J.	719
Homedes J.	664	Jacobs J.	342, 469, 470
Hong J.K.	436	Jacquiet P.	013, 093, 289, 642
Hood G.M.	198	Jahadi Hosseini S.H.	543
Hoorens P.R.	332	Jahani Fard E.	643
Hopkins T.	555	Jalali B.	457, 458, 461, 462
Hormaeche C.	574	Jalali M.	428
Hosseini Fard M.	463	Jamnah O.	264, 524
Hosseini H.	718	Janbaz M.	450
Hossieni S.H.	291	Janecka E.	660
Hoste H.	015, 251, 257, 261, 262	Janssen L.	196
Houdijk J.G.M.	357, 361	Januszkiewicz K.	170, 599
Houf K.	184	Jaros S.	170, 599, 600
Houffschmitt P.	641	Jarzabkowski M.	600
Hounzangbe-Adote M.S.	257	Jashin V.	603
Howe L.J.	040	Javanmard A.	451
Howell S.B.	532	Jedlina-Panasiuk L.	605, 610
Hromadová I.	567	Jefferies R.	051
Hu M.	248	Jenkins D.	569
Huang C.Q.	246, 601	Jenkins M.	183
Huang W.Y.	247, 593	Jensen A.L.	551
Huang X.	653	Jeong I.S.	389
Huby F.	442	Jex A.R.	059
Huerta M.	354	Jiménez H.H.	354
Huesca G.A.	349	Jimenez R.	648
Hunt P.W.	057		

Joachim A.	109, 474, 675	Kidman E.R.	380
Johansen M.V.	114, 241, 565	Kim C.	047
Johnston G.M.	286	Kim H.J.	437
Jones I.	380	Kim J.H.	411, 436
Jones J.T.	244	Kipar A.	043
Jones L.A.	357	Kirkwood D.	242
Jongejan F.	049, 086, 138	Kirschvink N.	252
Jongert E.	680, 697	Kiyan C.	159
Jorge R.	011	Klei T.R.	205
Jørgensen C.	147, 468	Klein G.	029
José L.	322	Kleinschmidt N.	190
Joshi D.D.	112, 568	Kliemt D.	426
Joshi S.D.	355	Klockiewicz M.	660
Jouglin M.	414	Kluiters G.	175
Juarez V.G.	656	Knapp J.	575
Jungersen G.	471, 472	Knowles D.	229
Just F.T.	053	Knox D.P.	096, 140, 244, 357
Juyal P.D.	473, 595	Knox M.R.	057
Kaba J.	660	Kocan K.M.	138
Kahn L.	234	Koch C.	525
Kalantari M.	685	Koch J.	551
Kalidari G.A.	476	Kocisova A.	379
Kaliniak M.	610	Koets A.P.	682
Kaminsky R.	188, 531	Köhler K.	570
Kamiya M.	028, 571	Kohler L.	716
Kanan A.	146	Kohn B.	176, 434
Kandil O.	293	Kojouri G.A.	444
Kanobana K.	115	Kok D.	388
Kapel C.M.O	073, 506	Kokotovic B.	471
Kaplan R.M.	077, 136, 189, 203, 532	Koning F.	334
Karabacak A.	137	Koopmann R.	190
Karademir U.	297	Kooyman F.N.J.	292, 329, 334
Karanis, P.	209	Kopacek P.	151
Karatzias H.	032	Kopp S.R.	554
Karlsen M.	592	Kortbeek L.M.	208
Kasenene J.	067	Kosik M.	610
Kassuku A.A.	083	Kostoulas P.	181
Katunguka-Rwakishaya E.	197	Kotze A.C.	554
Kawazu S.	047	Kouakou B.	019
Keiser J.	339, 347	Kozak-Cieszczyk M.	605, 610
Kelly P.	143, 273, 276	Krajewski M.	052
Kempf V.A.J.	153	Králová-Hromadová I.	171
Kennedy T.	131	Kramer L.	007, 056, 399, 400, 550
Kenyon F.	168, 194, 266, 522	Kramer M.	152
Kenyon P.R.	040	Krecek R.C	063
Kerboeuf D.	035, 167, 525	Kreienbrock L.	180, 534
Ketzis J.K.	132	Kreshchenko N.	603
Keywanloo M.	578	Krief S.	067
Keyyu J.D.	083	Kringel H.	472
Khabiri A.	425	Kristensen A.T.	551
Khadijah S.	264, 524	Kucsera I.	722
Khadje G.H.	428	Kudinov A.B.	430
Khalil E.I.	654	Kühne M.	029
Khan M.N.	639	Kumar D.	604
Kharchenko V.A.	496	Kumar H.	689, 725
Khatri P.S.	568	Kumar N.	002
Khosravi M.	626	Kushavar H.	691
Kidd E.	021	Kuticic V.	239

Kutima H.L.	139	Lesaffre E.	116, 157
Kutima P.M.	139	Lespine A.	076, 117, 528
Kuzmina T.A.	496, 516, 517	Letkova V.	379
Kwan D.	436	Levecke B.	065
Kwan E.	713	Lewis J.W.	541
Kyriazakis I.	357	Li G.	445, 667
Kyvsgaard N.C.	083	Li H.	367
L'Hostis M.	067, 414	Li P.	667
Labuda M.	227	Li Q-Y.	601
Lacasella V.	210	Li W.	367, 445
Lacoste E.	381, 382	Li Y.	367
Lacroux C.	093	Lia R.P.	154, 161
Lafi S.Q.	032	Lia R.P.	358
Lagapa J.T.	028	Liao S.Q.	696
Lahmar S.	574	Liebano H.E.	279, 316
Laing R.	313, 315	Liébano-Hernández E.	282
Laing S.	313, 315	Lifschitz A.	024, 336, 340, 523
Lake S.	077	Lightowlers M.	250
Lalonde L.	442	Lim S.	389
Lam T.T.H.	483	Lima R.C.A.	323, 635, 698
Lan Anh N.T.	113, 241	Lima V.M.F.	707
Lanfranchi P.	309	Lin R.Q.	246, 696
Langelaar M.F.M.	682	Lindberg R.	217
Langhans W.	014	Lindsay D.S.	054
Lanusse C.	024, 336, 340, 350, 523	Linh B.K.	113
Larionov S.V.	430	Lipscombe R.	720
Larroza M.	647	Lischinky T.	041
Larsen J.	628	Liu M.Y.	366, 367
Lasbleiz M.	448	Ljunggren E.L.	295
Lasri S.	237	Ljungström B.	324
Le Jambre L.F.	057, 094, 206	Llena J.	664
Le Rhun D.	366	Lo N.	179
Le Roux I.	680	Lonigro A.	210
Le T.H.	062, 174, 602	Lopes W.D.Z.	698, 699, 700
Le Vern Y.	525	Lopez A.M.E.	279, 316
Leal R.	265	López C.	561, 649
Lean I.J.	162	López-Arellano Ma.E.	282
Leandro A.	283	López-Martínez V.	282
Learmonth J.J.	713	López-Pérez I.C.	390, 712
Learmount J.	231	Losson B.	237, 402, 447, 573, 622, 638, 655
Leblond A.	641	Louineau J.	662
Leboeuf A.	305	Loukas A.	248
Lee Y.J.	437	Louvandini H.	253, 304
Lee Y.L.	436	Love S.	296, 492
Leepin A.	407	Lubega G.	197
Leggett F.	650	Lucena G.	494, 498
Lehnert K.	216	Lucinger S.	239
Leisewitz A.L.	049	Ludwig R.	509
Leitao A.	185	Luempert L.	383, 384, 386
Leite C.	283	Lukács Z.	413, 722
Lekimme M.	638	Luke B.	144
Lemos R.	254	Lukesova D.	379
Lems S.	133	Luminet S.	261
Lengauer H.	053	Lundén A.	405
Lens S.	676	Luzón M.	504, 505
Leon R.	590	Maarouf O.A.	576
Leonard V.	252	Máca J.	061
Leontides L.	181	Macedo F.R.	253

Macedo I.T.F.	258	Martinez-Ortiz de Montellano C.	251
Machado R.F.	700	Martínez-Valladares M.	294
Machado R.Z.	391	Martins R.F.S.	253
Maciel M.V.	258, 260	Martins S.	031, 498, 508, 703
MacKellar A.	124, 477	Marton S.	722
Mackenstedt U.	426	Marugán-Hernández V.	406
MacLean C.	175	Marzatico G.	072
Madani A.	621	Marzok M.A.	497
Madder M.	158, 421	Mason S.	692
Madeira de Carvalho L.M.	031, 494, 498, 499, 508, 703	Masood S.	518
Maes L.	212	Massard C.L.	415
Magdas C.	478	Massawe S.	111
Magez S.	001	Masumu J.	089
Magwisha H.B.	421	Masuzawa T.	047
Mahdipourzareh N.	691	Matekwe N.	337
Mahmoud M.S.	576, 612, 651	Mateos-Sanz A.	409
Maia F.	424	Matheeussen A.	212
Maillard S.	575	Mathis A.	025, 027, 030, 048, 105
Maillot A.	681	Mathy A.	573
Maiorka P.	398, 412	Matjila P.T.	049
Majidian F.	453	Matthews J.B.	077, 126, 244, 284
Majoros G.	413, 659	Mattiucci S.	156
Makhmudova N.	183	Mattsson J.G.	120, 142, 333, 405
Makungu L.S.	421	Mattsson R.	217
Malacrida F.	055	Maule A.G.	021, 597
Malecki-Tepicht J.	660	Maurelli M.P.	369
Mallet M.	449	Maurer V.	014
Malone J.	101	Mauro A.	205
Mamona O.A.	517	Mavrogianni V.S.	299, 300
Manazza J.	523	May-Martínez M.	018
Mangold A.J.	648	Mazaheri Y.	621
Manizan P.	466	Mbwambo H.A.	421
Mank T.	208	McAllister H.	076
Manolaraki F.	261	McAllister T.A.	207
Manouchehri Naeini K.	308	McAnulty R.W.	124, 521
Marangi M.	210	McBean D.	078, 097, 266, 522
Marchal T.	574	McCall J.W.	056, 548, 550
Marchand A.	448, 449, 475	McCall S.C.	056
Marcinkute A.	027	McCann C.M.	039
Márialigeti M.	413	McCarthy J.S.	554
Marié J.L.	069, 684	McConvery F.P.	173
Marin P.	505	McConville M.	348
Marinculic A.	239	McCoy M.A.	348
Marks N.J.	021, 597	McFarlane H.	149
Marrero P.Y.	349	McGarry J.W.	039
Mars J.	208	McKellar Q.A.	511, 556
Marshall J.A.	182, 701	McKibben J.	539
Marshall R.N.	182, 701	McManus C.M.	253, 304
Martin C.	353	McManus D.P.	062, 602
Martin J.	717	McMurtry L.	527
Martin R.	022	McNair C.M.	140
Martin S.W.	149	McNally J.	057
Martín-Hernández R.	438, 439, 440	McNeal-Hutchinson P.	063
Martínez G.M.N.	465	Meana A.	345, 438, 439, 440, 504, 505, 633
Martinez J.J.	354	Meaney M.M.	173, 341
Martínez-Cruz S.	611	Mederos A.	305
Martinez-Moreno A.	608, 611	Medley G.F.	280, 281
Martínez-Moreno F.J.	608, 611	Meeusen E.N.T.	092, 169, 290

Mehmood S.	287, 302	Moore D.P.	041
Meikle L.I.	284	Moore G.	023, 482
Meireles M.V.	707, 708, 710, 711	Moors E.	146, 267, 486
Meissonnier E.	582, 583, 584	Morais S.M.	258, 260
Mejer H.	017	Moran C.	384
Melkebeek V.	697	Moreno G.	350
Melville S.E.	197	Moreno J.	422
Mencie N.	154	Moreno L.	350
Mencke N.	161, 546, 552, 553	Morgan C.	231
Mendes R.E.	608, 611	Morgan E.R.	051, 052, 202, 232, 270, 374, 431
Mendez M.	634	Morgoglione M.E.	008
Mendonça R.P.	323, 552, 698	Mörner T.	307
Mendoza-de-Gives P.	277, 279, 282, 316	Morris I.	098
Mengel H.	663	Morris S.T.	040
Menzies P.	305	Morrison D.	142
Mercado P.	198	Morrison D.	217
Mercier P.	509	Morrison W.I.	122
Merza M.	034	Morrondo P.	335, 500, 607, 618, 649, 664
Meshgi B.	291, 580	Mortarino M.	399, 408, 550
Messasalma T.	382	Mortazavi Tabrizi J.	451
Meyer-Kühling B.	141	Morton D.B.	128
Meyvis Y.	125, 330	Moshiri F.	564
Mignon B.	655	Mosjidis J.A.	019, 259
Mikkonen T.	368	Moskwa B.	396
Miller H.R.P.	122, 123	Mota R.A.	409
Miller J.E.	019, 095, 259, 287, 302, 310, 520, 529	Motamedi Gh.	579
Millins C.	215	Motazedian M.H.	685
Mineo T.W.P.	391	Motobu M.	653
Mirbakhsh M.	455, 456	Mottier L.	523
Mircean M.	375	Mottier M.L.	080
Mircean V.	375	Mousa W.	666
Miret J.	422	Mousley A.	021, 597
Miro G.	074	Mtshali M.S.	648
Mishra A.K.	200	Mueller N.	044, 045
Miska K.	183	Muggli L.	045
Mitchell B.	063	Muhammad G.	639
Mitchell S.M.	054	Mukaratirwa S.	337
Miterpáková M.	567	Mula P.	490
Mitreva M.	099	Mulcahy G.	006, 038, 143, 238, 271, 519, 598, 609, 715
Miyoshi T.	653	Muliari R.	443
Moaveni M.	476	Müller J.	105
Moazeni M.	685	Müller N.	105, 185, 363
Mobedi I.	542	Müller-Lindloff J.	141
Modry D.	705	Mundt H.C.	054, 663
Mofya S.	539	Mungall K.	243
Mohajeri F.A.	685	Munguía X.J.	338
Mohamed A.M.	651	Munir W.	287, 302
Mohammed H.	709	Munn E.	096
Molento M.	235, 254, 265, 283, 530, 591	Muradian V.	690
Molin E.U.	120	Murphy L.	285, 288
Molina J.M.	290	Murphy T.	238, 715
Monrad J.M.	073, 083, 565	Murrell K.D.	016, 241
Montarsi F.	624	Musella V.	072, 100
Monteiro A.	494	Musoke A.	229
Monteiro C.M.	485	Mutua F.	111
Montenegro V.M.	180	Muyldermans S.	115
Moore D.	144	Nabavi L.	428, 581, 694
Moore D.A.	019	Naciri N.	714

Naem S.	327, 487, 616	Ogier de Baulny M.	582
Nagaraj S.	248	Oglesbee M.	493
Nagataki M.	201	Ojeda-Robertos N.F.	277
Naghash L.A.H.	669	Okazaki K.	571
Nagler C.R.	002	Oksanen A.	368
Naguleswaran A.	119	Oku Y.	571, 577
Najjari H.	658	Okubo K.	050
Nakamura A.	708	Olaechea F.	632, 647
Naranjo V.	648	Olazaran J.S.	279
Nascetti G.	156	Oleaga A.	614, 652
Nascimento A.A.	553, 560	Oliveira G.P.	323, 552, 635, 698, 700
Nassar A.	726	Oliveira L.M.B.	258
Navarro A.M.C.	258	Oliveira L.N.	690
Navarro V.	712	Olounlade P.	257
Navarro-Lozano V.	042	Olson M.E.	207
Navazesh R.	691	Ong B.	248
Navidpour S.H.	621, 643	Oommen A.	112
Nazemnia M.	658	Oosthuizen M.C.	046, 086, 087, 416
Ndao M.	606	Oppliger A.	045
Neiss J.M.	532	Opsteegh M.	682
Nejsun P.	147, 468	Orfanou D.C.	299, 300
Nekouie H.	425	Ørnbjerg N.	114
Nematollahi A.	452, 453, 686	Orosz E.	722
Nene V.	229	Ortega-Mora L.M.	042, 390, 394, 402, 404, 406, 409, 410, 460, 712
Neveceralová M.	672	Ortuño A.	533
Neves L.	303, 322	Orunc O.	356
Ngo T.H.	062	Oshaghi M.A.	177
Nguyen N.B.T.	602	Osterman Lind E.	307, 514
Nguyen N.T.T.	602	Otranto D.	055, 061, 154, 161, 358, 566, 724
Nguyen T.G.T.	062, 174	Ouattara A.	466
Nguyen V.D.	062, 174, 602	Ouattara M.	466
Nieguitsila A.	067	Ouhelli H.	574
Nielsen L.R.	592	Oyarzún M.P.	081
Nielsen M.K.	203	Ozbek H.	356
Nieto J.	422	Płucienniczak A.	610
Nieto P.	101	Pachabet J.	243
Nijhof A.M.	138	Pachnicke S.	515
Nijse E.R.	557	Packard E.	058
Nikpour S.	453	Painceira A.	500
Nisbet A.	126	Pakandl M.	672
Nisbet A.J.	126, 140, 244, 284, 477	Paller V.G.V.	240
Nishi S.M.	398, 412	Palma C.	298, 301
Noeckler K.	506	Palma R.	081
Nokhahi I.	691	Palmer C.S.	546
Noland G.S.	002	Palmer D.G.	392
Nonaka N.	571, 577	Paludo G.R.	253
Normanton H.	357	Pampurini F.	563
Noronha M.L.M.	485	Panadero R.	649
Norozian S.M.B.	687	Panday K.	355
Nouran A.A.	657	Panousis N.K.	032
Núñez F.	537	Pantchev N.	026, 679
Núñez J.	301	Paoletti B.	459, 520, 563
Núñez M.J.	298	Papadopoulos E.	032, 033, 299, 300
Nurulaini R.	264, 524	Papazahariadou M.	032
O'Handley R.M.	211, 218, 392, 693, 720	Paradies P.	154, 161
O'Neill J.	347	Paraud C.	268, 594
Ocak M.	663	Parkar U.	066
Odongo D.	229		

Parker L.D.	225, 299, 300, 636, 637	Pinitkiatisakul S.	405
Passos L.M.F.	432, 446	Pinto A.A.	391
Pastusiak K.	295	Pinto V.M.R.	700
Patten T.	271, 519	Pirali K.H.	453
Pattison R.S.	040	Pirali-Kheirabadi Kh.	145, 668
Paula A.I.	635	Pires C.	235
Paz-Silva A.	335, 500, 607, 618, 664, 704	Pistoni C.D.N.	698
Pazooki J.	451	Playford M.	162
Peacock L.	090	Pleasance J.	169
Pearl D.L.	149	Ploeger H.W.	084, 133, 334
Pebody R.	039	Poff G.	521
Pedraza-Díaz S.	394, 410, 460, 712	Polack B.	441, 559
Pedreira J.	607	Pomroy W.E.	040, 193, 311, 713
Peelaers I.	330, 331	Ponce M.M.	465
Pelayo L.	537	Pontes J.	265
Pellikka J.	368	Poot J.	196
Pemberton A.D.	286	Poplstein M.	672
Pemberton A.M.	123	Porchet M.-J.	045
Pemberton J.M.	233	Pors I.	167, 268, 594
Pena H.F.J.	688, 695, 711, 721	Portillo J.	627
Pennacchio S.	008	Posso M.A.	041
Penzhorn B.L.	046, 049, 086, 416	Pourjafar M.	312, 626
Peregrine A.S.	149, 305, 673	Pozio E.	365, 506
Pereira-Bueno J.	390	Pozzato N.	443
Perera B.V.P.	214	Pradel I.	053
Pérez A.M.	467, 590	Praet N.	116
Pérez H.	467	Prenafeta A.	404
Pérez J.	608, 611	Presber W.	195
Pérez J.-M.	601	Presidente P.J.A.	245
Pérez R.	298, 301	Prette N.	560
Pérez-Écija A.	608, 611	Prevot F.	093, 289, 642
Pérez-Pérez V.	390	Prichard R.K.	075, 080, 134
Pérez-Sánchez R.	614, 652	Prodöhl P.A.	060
Pérez-Zaballos F.	404	Ptochos S.	300
Perret C.	684	Püllen U.	486
Perri S.H.V.	558	Qayyum M.	287, 302, 310
Peschke R.	474	Qian B.Z.	615, 619
Pessoa-Filho P.	265	Quail M.	243
Petavy A.F.	574	Quezada A.	383, 386
Peterson D.S.	203	Quijada J.	467, 590
Pethick D.	207	Quillet J.M.	582
Petit T.	681	Quinnell R.J.	692
Petkevicius S.	016	Quintero M.T.	656
Petriceanu G.	683	Quintero T.	433, 627
Pfalzer S.	053	Quinton J.F.	435
Pfister K.	050, 053, 141, 151, 176, 178, 432, 434, 446	Quiroz A.	081
Pfleghaar S.	570	Raadsma H.W.	169, 290
Phiri I.K.	114	Rabelo E.M.L.	432
Phuong T.N.	241	Radfar M.H.	450
Physick-Sheard P.	149	Radulescu R.A.	683
Piarroux R.	575	Raes G.	001
Piedrafita D.	092, 169, 290	Raffo F.	632, 647
Pieroni J.S.P.	700	Ragalie A.	683
Pierson L.	597	Rahali T.	447, 622
Pilkington J.G.	233	Rahimi E.	462
Pilo C.	490	Raina R.	604
Pinckney R.	539	Raissy M.	462, 463, 464
Pinheiro D.	254	Rajandream M.-A.	243

Rajapakse R.P.V.J.	201, 214	Rodrigues D.C.	321
Rajshekhar V.	112	Rodrigues J.D.F.	699
Ralston B.J.	207	Rodrigues M.L.A.	485, 491, 654
Ramajo- Martín V.	614	Rodrigues R.L.	485
Ramajo-Hernández A.	614, 652	Rodríguez F.	290
Ramajo-Martín V.	652	Rodríguez S.D.	648
Ramírez A.G.	338	Rodríguez-Cadenas F.	644
Ramirez V.G.	316	Rodríguez-Esparza J.R.	282
Ramos F.	537	Rodríguez-Marcos S.	404
Ranga E.E.	421	Rodriguez-Vivas R.I.	082, 634
Ranganathan S.	248	Roelfstra L.	151
Rankin M.R.	480, 636, 646	Roepstorff A.	016, 017, 147, 468, 471, 472
Rao J.R.	200	Rofina J.E.	557
Rassi Y.	177	Rohen M.	534
Rathbone M.J.	319	Rojo-Montejo S.	404
Razavi M.R.	425	Rojo-Vázquez F.A.	294, 345, 633, 644
Razi Jalali M.H.	581	Rollinson D.	613
Razmi G.R.	476, 578, 630	Roman Y.	681
Rebordosa-Trigueros X.	042	Romasanta A.	618
Redman L.	058, 243, 313, 315	Rondán Z.A.	465
Redmond D.L.	244	Rosa A.	562
Rees R.	546	Rosa F.	424
Refa M.	420	Rosado-Aguilar J.A.	082, 634
Regidor-Cerrillo J.	394, 410	Rosario R.	627
Rehby L.	167	Rosbottom A.	043
Reid S.A.	198	Rosiles M.R.	359
Reid S.J.W.	492	Rossanese W.M.	699
Reinemeyer C.R.	054, 677	Rossini J.	530
Reiner G.	426	Roulet A.	075
Reis Y.	185	Rubilar L.	301
Relf V.	238	Rubino R.	008
Rembeck K.	432	Rufener L.	531
Rennie G.	319	Rugg D.	224, 225, 381, 382, 388
Rennie K.	288	Ruheta M.R.	421
Rennie S.	182	Rüncos L.	254
Reza H.S.	580	Ruttkowski B.	474
Rhalem A.	447, 622	Ruybal P.	648
Riaz F.Ch.	302	Ryan L.	346
Ribeiro M.F.B.	432, 446	Ryan U.M.	392
Ribeiro S.	424	Rydzik A.	324
Ribicih M.	561	Rypula K.	660
Ribot X.	507	Ryser M-P.	048
Richtzenhain L.J.	721	Rysmuhambetova A.	025
Ricklin M.	640	Saalmüller A.	109, 675
Rinaldi L.	008, 072, 100, 339, 369	Saari S.	368, 376
Riou M.	525	Sabiqaa M.	540
Rioux M-C.	606	Sacchi L.	179
Risco-Castillo V.	042, 390, 406	Sachero D.	632
Risi E.	572	Saddiqi H.A.	518
Robergeot V.	594	Sadeghi Z.	452
Robertson I.D.	546	Sadjjadi S.M.	543
Robinson M.T.	202, 374, 431	Sadzikowski A.B.	660
Robinson N.	092	Saeed M.	518
Rocha R.A.	292, 329	Saegerman C.	116
Rock D.	224	Saeki H.	549
Rocques H.	717	Safia M. A.	657
Rödder F.	663	Sager H.	045, 070
Rodrigues A.A.R.	395	Sahibi H.	447, 622

Saijonmaa-Koulumies L.	376	Schnieder T.	009, 029, 118, 180, 360, 588
Sajid M.S.	639	Schnyder M.	055, 645, 646, 716
Sakamoto C.A.M.	553, 560, 695	Schönian G.	195
Sakha M.	450	Schuele G.	383, 384, 386
Salát J.	672	Schunack B.	588
Salem A.	013	Schuppers M.E.	363
Salem G.	726	Schürmann S.	360
Salgado V.L.	223	Sciutto E.	354
Salichs M.	664	Scoles D.	229
Sallovitz J.	024, 336, 340	Sefidgar S.A.A.	687
Salvador A.	459	Sekiya M.	598
Sampaio I.M.	491	Senlik B.	511
Sánchez-Andrade A.	618	Senos J.C.	011
Sánchez-Andrade R.	335, 500, 607, 618, 704	Seo H.S.	411
Sánchez-del Pino M.M.	614	Serbina E.	603
Sanchez-Sanz N.	501	Sereno F.	304
Sandhu B.S.	484, 689	Serrano A.C.M.	395, 558, 707
Sandoval-Castro C.A.	018, 251, 262	Settje T.	054, 378, 547, 677
Sangster N.C.	489	Shaik S.A.	019
Sani R.A.	417	Shakeela A.	540
Sano T.	577	Shakya K.	095
Sanquer A.	501, 504, 505, 507, 509	Sharif M.	542
Santana A.E.	321	Sharifah-Salmah S.H.	417
Santana L.F.	698, 699	Sharifi K.	630
Santaniello M.	008	Sharma D.K.	473
Santarém N.	423	Sharma J.K.	473, 725
Santos C.	494	Sharma R.	090, 473, 725
Santos E.	321, 415, 635	Sharma S.	689
Santos H.A.	415	Shaw E.	121
Santos M.D.	485	Shaw L.	121, 242, 350
Santos R.S.	699	Shaw S.	051, 052, 202, 374, 431
Santos T.R.	321, 323, 698	Shayan P.	580
Santos V.R.V.	304	Shea-Donohue T.	002
Saqib M.	639	Shemshadi B.	718
Sarciron M.E.	574	Shin H.J.	436, 437
Sargison N.D.	164, 194	Shin S.S.	389
Sarkari B.	543	Shojaei S.S.R.	658, 669
Sarkunas M.	027	Shubber A.	510
Sasanelli M.	161	Shuqing L.	648
Sassera D.	179, 408	Siavashi M.	579
Sato Y.	602	Sibeko K.P.	087
Sattari A.	564	Sidibé I.	091
Sattor N.A.	488	Siebert U.	216
Saunders G.	314	Sikasunge C.S.	114
Savage J.	341	Silaghi C.	178
Saverwyns H.	331	Silva B.F.	275
Sayers G.	191, 272, 609	Silva E.	011
Scala A.	490, 642, 704	Silva G.V.O.	265, 415
Scandrett B.	351, 442	Silva H.C.	530, 552, 553, 560
Scarcella S.	336	Silva R.A.	258, 260
Schaap T.	196	Silverlås C.	702
Schallig H.D.F.H.	424	Silvestre A.	079
Schaper R.	548, 555, 723	Silvestre R.	423
Schares G.	393, 679	Simões D.	708
Schettters T.	049	Simukoko H.	089
Schioppi M.	369	Singh B.B.	725
Schmid N.	048	Singh D.P.	419
Schmidt P.	109, 675	Singla L.D.	484

Sissay M.M.	236	Starovir O.	148
Skampardonis V.	181	Stear M.J.	285, 288, 296
Skilton R.	229	Steenhard N.R.	002, 471, 472
Skotarek S.L.	502	Stefan N.	364
Skuce P.J.	097, 168, 512	Stephan R.	030
Slapeta J.	705	Steuart R.	720
Slivinska K.A.	517	Stevens J.R.	061
Smaga-Kozłowska K.	170	Stevens M.	059
Smit N.J.	213	Stijlemans B.	001
Smith D.	095	Stoichici A.	364
Smith J.	058	Stothard J.R.	613
Smith J.E.	692	Strehlau G.	383, 384, 386
Smith L.L.	135	Strohbusch M.	044
Smith R.F.	043	Strubbe B.M.	719
Smith S.	126	Strube C.	009
Smith S.K.	284	Stubbings L.	231
Smith W.D.	094, 122, 286	Stüdl A.	407
Smits R.A.J.	557	Studzinska M.	660
Snibson K.	092	Su C.	688
Soares R.M.	398, 401, 412, 708, 710, 711, 721	Suárez J.L.	335, 607, 618
Soares V.E.	530, 635	Suarez V.	024
Sobhy A.	651	Sudeman R. 2	305
Sohn H.J.	437	Sugiyama H.	615, 619
Solano-Aguilar G.I.	002	Sukura A.	368
Soliman K.	612	Summers B.	493
Solymosi N.	413	Sung M.	678
Somers R.	113, 325	Sutra J.F.	320
Sommerfelt I.	561, 562	Sweeney T.	191, 272
Sondermeijer P.	196	Sykes A.R.	124
Song A.R.	437	Syvak V.	148
Song H.Q.	246, 389, 696	Szeidemann Zs.	377, 659, 722
Song K.J.	437	Szénási Z. 1	722
Sonmez F.	137	Tanasuica R.	683
Sood N.K.	484	Tánczos B.	722
Sosa C.	433	Tanda B.	704
Sosa G.	538	Tandan S.K.	604
Sotiraki S.	181, 261	Taoufik A.	138
Sousa P.D.	303	Táparo C.V.	395, 558, 707
Sousa S.	498, 508, 703	Tas Z.	356
Southgate V.R.	613	Taubert A.	108, 661
Souza F.A.	699	Tavakkol S.	328, 457, 458
Souza M.	283	Taylor F.G.R.	510
Souza P.C.A.	485	Taylor M.A.	144, 231
Souza S.L.P.	721	Taylor S.	343, 344
Souza T.M.	491	Teixeira L.	403
Sowemimo O.A.	544, 545	Tempest L.J.	233
Spakulová M.	171	Terefe G.	093, 289, 642
Sparagano O.A.E.	205, 481	Terenina N.	603
Spera G.	369	Terrill T.H.	019, 259, 532
Speybroeck N.	116	Testini G.	061
Spicher M.	119	Tewari A.K.	200
Spiridonov S.	064	Thamsborg S.M.	017, 073, 074, 147, 241, 468, 471, 472, 565, 592
Spithill T.W.	169, 606	Thanh N.T.G.	325
Srivastava A.K.	604	Thind B.	144
Stäbler S.	071	Thomas M.	684
Stafford K.A.	270	Thomaz A.	711
Stankiewicz M.	124	Thompson R.C.A.	066, 207, 218, 546, 693, 720, 723
Stanneck D.	154, 387		

Thomsen L.E.	016	Várady M.	033, 035, 165
Thu T.T.	241	Varejão E.	424
Tighi S.	177	Vashist U.	415
Tolmacheva V.	183	Vatandoost H.	177
Tolstenkov O.	603	Vatta A.F.	280, 281
Tombeux S.	638	Vaziriyani Zadeh B.	643
Tomczuk K.	660	Vázquez C.	627
Tome R.O.	707	Vázquez L.	649
Toner E.M.	121, 173	Vázquez L.	664
Torgerson P.R.	025	Vazquez P.V.M.	279
Torres A.	670	Vázquez T.O.	465
Torres S.E.F.A.	304	Vazquez-Moreno E.	394
Torres-Acosta J.F.J.	018, 251, 262, 277	Vedrine B.	475
Trachsel D.	025	Vega M.V.	279
Traub R.J.	066, 546, 554	Velludo P.	530
Travers F.	370, 371, 373	Veneziano V.	008, 339, 369
Traversa D.	205, 520, 563, 705	Vennerstrom J.	339, 347
Trawford A.F.	492, 495, 496	Venter L.	158
Trees A.J.	039, 043, 082	Venzal J.M.	623
Troncoso C.	319	Venzi C.	397
Trotz-Williams L.	149	Vera Montenegro Y.	338, 349, 359
Trudgett A.	060, 172, 242, 341, 346, 526	Vercammen F.	065
Tsuji N.	228, 653	Vercruysse J.	010, 012, 034, 035, 065, 088, 104, 112, 113, 115, 125, 134, 174, 184, 190, 325, 330, 331, 332, 512, 515, 535, 613, 629
Turceková L.	171	Verdonck W.	670
Uehlinger F.D.	211	Verma P.K.	604
Ueno T.	395	Verma S.	689
Uga S.	240	Vermeersch M.	212
Ugarte-Garagalza C.	409	Vermeij P.	196
Uggla A.	236	Vermeulen A.	196
Uhlhorn H.	307	Vermeulen B.	067
Ulayi B.M.	586	Verzhikhovskiy O.	148
Uni S.	353	Vestergaard-Nielsen K.	469, 470
Urban J.	002, 471	Veys P.	110, 342
Utzinger J.	339	Viana A.B.	011
Valcárcel F.	633	Viana P.G.	253
Valero M.L.	614	Vicari D.	443
Van Biervliet J.	493	Victor B.	115
van den Berg M.	150	Vidyashankar A.N.	136, 532
Van den Bossche P.	089, 199	Vieira L.S.	258
Van den Broeck W.	330	Viel H.	717
van der Giessen J.W.B.	208, 557, 682	Vien L.V.	325
Van der Kuur A.	334	Viero L.M.	398, 412
van Dijk J.	232	Vilaça K.	673
van Doorn D.C.K.	133	Vilanova M.	403
Van Gucht S.	680	Villegas P.S.L.	082
Van Impe G.	447, 622	Villena I.	681
van Knapen F.	074	Viney M.	051
Van Leemput L.	662	Virkel G.	336, 340, 523
Van Lerberghe J.	676	Virtala A.-M.	368
van Ploeger H.W.	150, 318	Viseshakul N.	665
Van V.K.	062	Visser A.	330
van Wyk J.A.	033, 160	Vitali S	066
Van Zeveren A.M.	134, 190, 330, 331, 512, 515	Vivarès C.P.	435
Vangeel L.	184	Vivas I.	467
Vanguru M.	019	Vlassoff A.	527
VanLeeuwen J.	305		
Vara-Del Río M.P.	294		

Vodicka T.	672	Wohlsein P.	216
von Samson-Himmelstjerna G.	009, 034, 035, 118, 190, 216, 317, 360, 515, 516, 588,	Wolstenholme A.	022, 035, 097, 512
Vos J.H.	557	Woods D.	020, 313, 315
Vu T.T	602	Worliczek H.L.	109, 675
Vyse A.J.	039	Wright H.W.	477
Wade S.	709	Wright S.E.	106
Waikagul J.	615, 619	Wu C.	529
Walkden-Brown S.	234	Wunderlich M.	319
Walker C.L.	245	Xiao S.	445
Walker D.	243	Xu Q.	667
Walker S.M.	060	Xu W.	667
Wall R.	144, 175, 221	Yakhchali M.	107
Waller P.J.	236, 264, 280, 281, 524	Yang A.H.	436
Walsh T.	022, 097	Yatawara L.	201
Wanderley E.	265	Yatsuda A.P.	329
Wang D.Y.	247, 593	Yildirim F.	511
Wang Y.	367	Yilmaz H.	356
Ward B.	606	Yokoyama N.	047, 050
Ward P.	052	Youn H.J.	411
Watkins C.	244	Young D.R.	384, 386
Wawrzyniak I.	435	Youssefi M.R.	687
Webster B.	613	Zafra R.	608, 611
Webster E.	673	Zahler-Rinder M.	217, 432, 446
Webster P.	073, 565	Zahner H.	108, 426, 587, 661
Wedrychowicz H.	170, 599, 600, 605	Zaini C.M.	264, 524
Weeks B.	095	Zaman M.	256
Weigend S.	146	Zamani- Moghadam A.	668
Weinstock J.	002	Zaragoza A.D.	465
Weller K.M.	319	Zarlenga D.S.	127
Wells L.	503	Zaros L.G.	292, 329
Welz C.	118, 317	Zavattiero S.	397
Weng Y.B.	696	Zawadzki J.L.	245
Wesolowska A.	170	Zebardast N.	308
West D.M.	040	Zeeh F.	363
Weteling A.	133	Zelmadarraiy Z.	177
Whipp M.	059	Zhang D.	367, 696
White B.	243	Zhang W.Y.	247, 593
Wickramasinghe S.	201	Zhou R.	445
Widmer G.	713	Zhu X.Q.	246, 454, 601, 696
Wielinga C.	723	Ziadinov I.	025
Wielinga P.	208, 557	Ziaei H.	542
Willems H.	426	Ziapour S.-P.	542
Willesen J.L.	551	Zibaei M.	543
Williams D.J.L.	012, 036, 039, 043, 104	Ziegler P.	709
Williams T.	022, 202, 374, 431	Zimmermann W.	363
Williamson L.H.	532	Zolfo M.	159
Williamson S.	022	Zou F.C.	246
Willingham A.L.	111, 114	Zweygarth E.	046, 086
Winton R.G.	094	Zygner W.	170