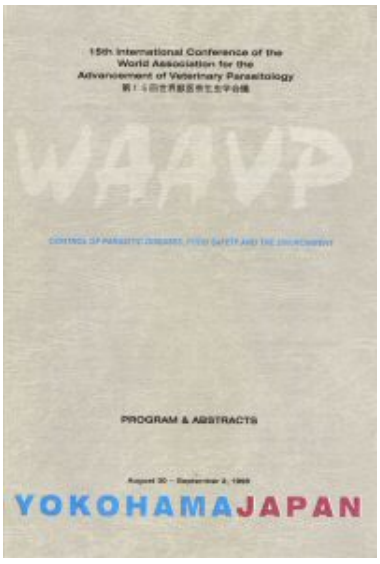
The 15th International Conference of the

WORLD ASSOCIATION FOR THE ADVANCEMENT OF VETERINARY PARASITOLOGY

"Control of Parasitic Diseases, Food Safety and the Environment"

August 30-September 2, 1995

YOKOHAMA, JAPAN



15th International Conference of the World Association for the Advancement of Veterinary Parasitology

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Message from the Chairperson

On behalf of the Organizing Committee, I would like to extend a warm welcome to all of my colleagues and friends who have come here to Yokohama to participate in the 15th International Conference of the World Association for the Advancement of Veterinary Parasitology.

Through the 22 sessions comprising 9 plenary papers, 91 submitted papers, 43 posters, 11 workshops and 2 corporate presentations which are the focus of this Conference, we will have an opportunity to discuss many new and important subjects. I firmly believe that during the course of the Conference, all participants will be able to acquire the latest and most relevant academic knowledge in the field of veterinary parasitology.

In addition to their academic purposes, our International Conference also aims to provide participants with the opportunity to visit somewhere they may have never been before. For most of you, this will be your first visit to Yokohama, the second largest city in Japan with a population of 3,270,000, its nation's major international trading port, thriving commercial and industrial center. I hope that you will all enjoy the fresh seaside and mountain scenery and cosmopolitan atmosphere.

Again, I sincerely hope that you will find the Conference rewarding and interesting and that your time here will be very pleasant and enjoyable.

Nao. Suzuki

Naoyoshi Suzuki
Chairperson

WAAVP Corporate Members:

American Cyanamid Company
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Conference Secretariat:

World Meeting Corporation
1-29-16-201 Shinjuku, Shinjuku-ku
Tokyo 160, Japan
Telephone: 81-3-3350-0363
Telefax: 81-3-3341-1830

GENERAL INFORMATION

DATES:

August 30 (Wed) - September 2 (Sat), 1995

VENUE OF THE CONFERENCE:

The Yokohama Symposia
Sangyo Boeki Center Bldg. (Industry & Trade Center Bldg.) 9th Floor,
No. 2, Yamashita-cho, Naka-ku, Yokohama 231, JAPAN
Telephone: 81-45-671-7151 Telfax: 81-45-671-7187 Telex: 3822844 GREEN J

OFFICIAL LANGUAGE:

The official language of the conference is English; no translation is available.

REGISTRATION:

The Registration and Information desk in the lounge of Yokohama Symposia will be open at the following times:

August 29 (Tuesday)	13:30 - 17:30
August 30 (Wednesday)	08:30 - 17:30
August 31 (Thursday)	08:30 - 17:30
September 1 (Friday)	08:30 - 13:30
September 2 (Saturday)	08:30 - 17:30

Registration fees:

Members	JYE 35,000
Non-members	JYE 40,000
Accompanying persons	JYE 25,000
Students	JYE 20,000
	(JYE: Japanese Yen)

Payment: Payment in JYE only. Cash, Bank Draft and Credit Cards (Visa, Master, Amex and Diners) are accepted. No Personal checks.

Registration fee includes:

Admission to all Scientific Sessions
Exhibition
Opening Ceremony
Welcome Reception
Cultural Event
Banquet
A Copy of the Special Conference Issue
Tea/Coffee

Badges: Participants are kindly requested to wear their badges at all times during the Conference.

SCIENTIFIC PROGRAM

OPENING CEREMONY
PLENARY PAPERS
CORPORATE PRESENTATION

Opening Ceremony

Opening of Conference 08:30 ~ 10:00 Wednesday August 30, 1995

Room A

Dr. O. Slocombe, *Canada*, President WA AVP

Opening lecture Chairperson: **Dr. O. Slocombe**, *Canada*

THE HISTORY OF RESEARCH AND EDUCATION OF VETERINARY PARASITOLOGY IN JAPAN

Dr. N. Suzuki, *Japan*, Chairperson of WA AVP 95

Announcement from Local Organizing Committee

Dr. A. Arakawa, *Japan*, Chairperson of Scientific Program Committee

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Vaccines, Biological control & Technology transfer

10:30 ~ 12:00 Wednesday August 30, 1995
Room A

Chairperson: **Dr. N. Suzuki**, *Japan*

10:30 **VACCINATION AGAINST WORM PARASITES OF ANIMALS**

Emery, D.L., *Australia*

P 1

11:00 **ASPECTS OF BIOLOGICAL CONTROL, - WITH SPECIAL REFERENCE TO ARTHROPODS, PROTOZOANS AND HELMINTHS OF DOMESTICATED ANIMALS**

Grønvold, J., Henriksen, S.Aa., Larsen, M., Nansen, P. and Wolstrup, J., *Denmark*

P 2

11:30 **TECHNOLOGY TRANSFER IN THE DEVELOPED WORLD**

Murrell, K.D., *U.S.A.*

P 3

Marine parasitology, Tropical livestock industry & Technology transfer

10:30 ~ 12:00 Thursday August 31, 1995
Room A

Chairperson: **Dr. R. Prichard**, *Canada*

10:30 **MARINE PARASITOLOGY WITH SPECIAL REFERENCE TO JAPANESE FISHERIES AND MARICULTURE**
Ogawa, K., *Japan*
P 4

11:00 **TROPICAL LIVESTOCK INDUSTRY: PARASITE CONTROL, FOOD SECURITY AND THE ENVIRONMENT**
Hansen, J.W., *Italy*
P 5

11:30 **APPLICATIONS AND TRANSFER OF INFORMATION TECHNOLOGY IN VETERINARY RESEARCH IN DEVELOPING COUNTRIES: THE NIGERIA EXPERIENCE**
Chiejina, S.N., *Nigeria*
P 6

Zoonoses, Regulatory affairs & Residues

10:30 - 12:00 Friday September 1, 1995
Room A

Chairperson: **Dr. J. Tharaldsen,** *Norway*

10:30 **STRATEGIES TO REDUCE TRANSMISSION OF TOXOPLASMA GONDII TO ANIMALS AND HUMANS**

Dubey J.P., *U.S.A.*

P 7

11:00 **IRRADIATION AS A COLD PASTEURIZATION PROCESS OF FOOD**

Loaharanu, P., *Italy*

P 8

11:30 **DEPLETION AND BIOAVAILABILITY OF ^{14}C--OXIBENDAZOLE RESIDUES IN SWINE TISSUES**

Gottschall, D.W., *U.S.A.*

P 9

Corporate Presentation

MSD AgVet Presentation 17:30 ~ 19:00 Wednesday August 30, 1995

Room A

17:30 **IVERMECTIN, OTHER AVERMECTINS AND MILBEMYCINS, SCIENCE AND INNOVATION**

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Corporate Presentation

Pfizer Presentation 17:30 ~ 19:00 Thursday August 31, 1995

Room A

17:30 **DORAMECTIN, A NOVEL, LONG-ACTING ENDECTOCIDE FOR USE IN SWINE**

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ABSTRACTS OF PLENARY PAPERS

P 1

VACCINATION AGAINST WORM PARASITES OF ANIMALS

D.L. Emery
CSIRO Division of Animal Health, McMaster Laboratory, Locked Bag 1, Blacktown, NSW 2148, Australia

The 1990s have seen the culmination of decades of painstaking research with the registration and launch of Tickgard® (Hoechst), a recombinant vaccine against *Boophilus microplus*, and the provisional registration of a *Taenia ovis* vaccine. Research continues to hold promise for immunological control of *Echinococcus, Fasciola, Haemonchus, Trichostrongylus* and *Ostertagia*. Blood sucking parasites (eg. ticks and *H. contortus*) are susceptible to control by vaccines containing "novel" or "concealed" antigens where serum antibodies in blood meals attack targets in the gut. Antibodies also provide protection in *Taeniid* models, while the protective response to be sought in *Fasciola* remains unclear. More problematic are formulations and delivery strategies to induce expulsion of gastrointestinal nematodes, using vaccines containing recombinant "conventional" antigens. The use of computer models to simulate vaccine efficacy in worm control, and challenges to the concept of "hypo-responsiveness" of young lambs, will encourage cautious optimism and lively debate as to the prospects for integrated worm control using parasite vaccines. This review covers the aspirations, current success and problems faced by vaccinologists in the parasite arena.

P 2 BIOLOGICAL CONTROL

J.Grønvold[*], S.Aa.Henriksen[°], M.Larsen[*], P.Nansen[*] and J.Wolstrup[*].

*) Royal Veterinary and Agricultural University

°) Danish Veterinary Laboratory Copenhagen, DENMARK.

Biological control describe situations when a living antagonist (a predator, parasite, parasitoide or a pathogen) is distributed by man to lower a pest (parasite) population to acceptable sub-clinical densities or to keep the population on a non-harmful level. Ideally biological control has no negative effects on the environment whereas chemical control is not always so harmless. Laboratory and field observations have revealed many organisms, such as viruses, bacteria, fungi, protozoans, turbellarians, nematodes, tardigrades, insects, copepods and mites as antagonists to parasitic arthropods, protozoans and helminths of domesticated animals. However only very few of these antagonists have shown promising qualities as biological control agents within veterinary science. The lack of success should be linked to the lack of knowledge about complex natural biological systems and the antagonists that may be found here. This situation has restricted the companies interest in developing biological products. In the future however, the industry may become more interested in biological control facing the increasing problems with parasite resistance to drugs in combination with the increasing cost of developing new chemical products and because of increasing public concern about chemical residues in animal products and in the environment.

P 3 COMMUNICATION: TECHNOLOGY TRANSFER IN DEVELOPED COUNTRIES

K. D. Murrell, U.S. Department of Agriculture
Beltsville Agricultural Research Center, Beltsville, Maryland 20705, U.S.A.

Support for research is increasingly dependent upon the results of that research having relevance to society's needs and public benefits. This increases pressure to move research results out of the laboratory and into user's hands. To facilitate this, a variety of technology transfer mechanisms have evolved to facilitate transfer of knowledge and processes. These mechanisms, in addition to their implied benefits for the researchers and the user, often have certain consequences which are unanticipated and cause transfer to fail or not meet expectations. Foremost, cultural change on the part of both private organizations and public laboratories is probably necessary to allow effective partnerships. Coupled with the transfer of knowledge is the opportunity to more effectively explain to society the benefits it receives for its investments in research. Such communication has not been especially successful for veterinary parasitology. However, the revolution in communication (e.g., rise of mass media; computer networks) presents new opportunities to parasitologists to more effectively communicate both technology and knowledge directly to the users, and also to inform supporters, policy makers and the general public of the relevance and importance of veterinary parasitology in improving society's well-being. This review will discuss these new instruments of communication, the need to construct better messages, the benefits of technology transfer and the various means to meet the challenges associated with transferring research innovation to the user and the market place.

P4

MARINE PARASITOLOGY WITH SPECIAL REFERENCE TO JAPANESE FISHERIES AND MARICULTURE

K. Ogawa: Department of Fisheries, Faculty of Agriculture, The University of Tokyo, Tokyo, Japan

Marine parasites with special relation to Japanese fisheries and mariculture include different types of pathogens: those causing mortality, deformity, weight loss etc.; those giving unesthetic appearances to the hosts; and those which are zoonotic. Japanese mariculture typically utilizes net cage culture systems in coastal areas. Parasite invasion in such systems is practically more difficult to control than in freshwater facilities. The limited use of chemicals and drugs for treatment makes the situation even more difficult to handle. About 5 species of parasites from marine organisms have been known to be zoonotic. This is closely associated with the Japanese tradition to eat raw fishes and invertebrates. Infection of maricultured species with larval trematodes, cestodes and nematodes has not been confirmed. On a more positive side, attempts have been made to utilize parasites as biological tags to obtain information on host biology, ecology etc.

Recent trends in Japanese mariculture include technical improvement on seed production and importation of large quantities of various species of culture seedlings. Drastic increase in the supply of seedlings of selected fish species has resulted in changes of culture methods and created parasite problems in much larger scale. International trade of live fishes and shellfishes has introduced parasites hitherto unknown in Japan. An efficient quarantine system to prevent and control introduction and spread of marine parasites needs to be urgently established.

P5 TROPICAL LIVESTOCK INDUSTRY: PARASITE CONTROL, FOOD SECURITY AND THE ENVIRONMENT.

Jorgen W. HANSEN
FAO Food and Agriculture Organization of the United Nations
AGA/AGAH Animal Production and Health Division
Rome - ITALY

The frame for Livestock production in the tropics broadly consists of three production systems; the crop/livestock system (mixed farming); the pastoral system and the peri-urban system which may be intensive/semi-intensive. Livestock is unfortunately grossly undervalued as a component in food security and there is a need for changing this concept. It is recognized that the greatest potential for increased productivity and production is in the mixed farming systems. However a large untapped potential may be in the pastoral systems where the majority of livestock is to be found. A pre-requisite for harvesting this potential is the recognition of the major constraints preventing the development of the sector. The lack of sufficient and balanced food resulting in under and malnutrition combined with the presence of parasitic and other diseases are still major obstacles. Sufficient accumulated knowledge on epidemiology and control of parasitic diseases and appropriate production technology exists for the tropics to easily increase the output per animal unit considerably. This would require efficient animal health delivery systems, extension, technology transfer and sufficient resources. Ever since man discovered the benefits of farming and animal husbandry he has, continuously and with increasing speed altered his environment to suit his needs, particularly the crop/livestock systems. Changes in the environment which have had an impact on parasite control and parasite control practices which have influenced the environment will be discussed.

P 6

PROFESSOR S.N. CHIEJINA

UNIVERSITY OF NIGERIA, NSUKKA
NIGERIA

An overview and analysis of the current status of information technology (IT) and its role in animal health research and development (R&D) projects in developing countries have been undertaken. The analysis has emphasised the current status and the limited impact of IT on large-scale field projects; the major factors militating against its successful acquisition, application and transfer; short and long term needs and priorities for IT and IT inputs for effective implementation of field projects and exchange of R&D information. Of the numerous factors which have hindered the growth and transfer of IT in much of the developing world the most important are:(1) weak science and technology (S&T) base. (2) shortage of highly skilled frontline scientists, technologists and information professionals. (3) inadequate financial resources. Experience gained from three field projects in Nigeria highlighted the fact that while IT inputs, such as computers, are vital for the efficient management and rapid communication of research information, careful planning of projects, adequate financial and logistic support, availability of skilled manpower and basic infrastructural facilities are indispensable for the effective utilisation of the inputs. A promising and cost-effective method of transfer of IT inputs to those countries which can hardly afford them at present is through the establishment of institutional research links between developed and developing countries. This form of IT transfer is an important step towards increased application of IT inputs in R&D projects, creation of the much needed inventory of databases and information systems on animal health projects in developing countries. Only then can meaningful exchange of R&D information, in an acceptable format and at affordable price, be realised within and between them. A possible role is envisaged for the major international organisations such as the Food and Agriculture Organisation of the United Nations and the United Nations Educational Scientific and Cultural Organisation in ensuring the success and sustainability of these developments.

P 7 STRATEGIES TO REDUCE TRANSMISSION OF *TOXOPLASMA GONDII* TO ANIMALS AND HUMANS

Dubey J P
U.S. Department of Agriculture, Agricultural Research Service, LPSI, Parasite Biology & Epidemiology Laboratory, Beltsville, MD 20705-2350, USA

Infection by the protozoan parasite *Toxoplasma gondii* is widely prevalent in animals and humans throughout the world. The ingestion of food and water contaminated with oocysts from infected cat feces or the ingestion of tissue cysts from infected meat are the 2 major sources of *T. gondii* infection in humans. At present there are no subunit or killed vaccines for immunization of farm animals or humans for toxoplasmosis. A live vaccine containing tissue cysts or bradyzoites of a mutant strain of *T. gondii* (T-263) is being developed for marketing in the U.S. This vaccine, when given orally to cats, prevents the formation of oocysts. Vaccines that reduce the number of tissue cysts in farm animals are needed. Recent studies indicate that vaccination of farm animals with nonpersistent strains of *T. gondii* is possible and could reduce parasite burdens and prevent damage to the fetus. A live vaccine containing tachyzoites to reduce fetal damage in sheep is available commercially in Europe and New Zealand. Another strategy to minimize transmission of *T. gondii* to humans is to kill tissue cysts in meat. Tissue cysts of *T. gondii* in meat are rendered noninfective by cooking to an internal temperature of 66°C, by freezing at -12°C for 1 day, and by exposure to 50 kilorads gamma-irradiation (Cesium-137).

P 8 IRRADIATION AS A COLD PASTEURIZATION PROCESS OF FOOD

Paisan **Loaharanu**
Head, Food Preservation Section, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, International Atomic Energy Agency, P.O.Box 100, Wagramerstrasse 5, A-1400 Vienna, Austria

ABSTRACT

A number of emerging pathogenic microorganisms and parasites in food, the wide publicity of outbreaks of foodborne diseases and the increasing number of immuno-compromised population have resulted in a need to develop proper strategies and technologies to protect health of consumers. Regulations in most countries which are promulgated to ensure safety of foods, are not properly enforced to protect consumers' health especially with regard to food of animal origin. While regulations are in place and properly enforced for liquid foods such as milk and fruit juices, more solid food especially those of animal origin, e.g. poultry, meat and seafood which are often contaminated by pathogenic microorganisms and parasites are not similarly regulated. The number of incidences of foodborne diseases and the subsequent economic impact to the society can be enormous. Recently, increasing number of national and international organizations have recognized the use of irradiation as a method to ensure hygienic quality of more solid food of animal origin in the same manner as thermal pasteurization does for liquid foods. The effectiveness of irradiation as a cold pasteurization method to control foodborne diseases caused by pathogenic microorganisms and parasites, especially in food to be consumed raw or partially processed, is established. Its role in overcoming trade barriers of food of animal origin based on the principle of the Agreement on the Application of Sanitary and Phytosanitary Measures, adopted during the GATT Uruguay Round will be discussed.

1. Foodborne Disease
2. Zoonoses
3. Irradiation

P 9 DEPLETION AND BIOAVAILABILITY OF ^{14}C-OXIBENDAZOLE RESIDUES IN SWINE TISSUES

David W. Gottschall and Richard Wang, Department of Developmental Pharmacokinetics and Drug Metabolism, Pfizer Animal Health, 1600 Paoli Pike, West Chester, PA 19380-1169, U.S.A.

Groups of male swine were administered a single oral dose of ^{14}C-oxibendazole in a gelatin capsule at a level of 15 mg/kg bodyweight and sacrificed after 10-hr, 24-hr, and 7-days withdrawal. Combustion analysis indicated that liver was the only tissue which contained significant residues. Due to the insolubility of oxibendazole, absorption was variable, especially at the early time points. Total residues were highest after 24 hr withdrawal (4 ppm) and depleted relatively slowly to approximately 1.8 ppm after 7 days. Extractable residues decreased from 35% to 11% over this same period. When ^{14}C-oxibendazole was fortified into control, lyophilized, pelleted swine liver and fed to rats utilizing the Gallo-Torres model, bioavailability was >94%. In contrast,, bioavailability was substantially reduced to ca. 40% when liver containing ^{14}C-oxibendazole residues from dosed swine was fed. This percentage remained constant for liver samples from the 24-hr or 7-day withdrawal swine. The data were consistent with the rapid metabolism of oxibendazole and the early formation of a high percentage of bound tissue residues. The significant differences in residue bioavailability and extraction efficiency between the fortified control and dosed tissues indicates that little, if any, parent oxibendazole is present in the dosed tissues, even at early withdrawal times. Since non-bioavailable residues are of no toxicological concern, the 60% reduction in bioavailability is significant from a regulatory perspective. The wider safety margin, relative to the established ADI for oxibendazole, is beneficial to the consumer in terms of human food safety.

SCIENTIFIC PROGRAM

SUBMITTED PAPERS

Chemotherapy and delivery system

13:30 ~ 15:00 Wednesday August 30, 1995

Room A

Chairpersons: **Dr. D.E. Jacobs**, *United Kingdom*
Dr. E. Baba, *Japan*

13:30 **EFFICACY OF THE IVERMECTIN SUSTAINED RELEASE BOLUS IN ANIMALS WEIGHING GREATER THAN 300 KG AT THE TIME OF TREATMENT**

Cramer, L.G., Eagleson, J.S. and Farrington, D.O., *U.S.A.*

A 1

13:45 **SAFETY AND EFFICACY OF IVERMECTIN POUR-ON FORMULATION AGAINST STRONGYLOIDES PAPILLOSUS INFECTION IN CATTLE**

Nagata, T., Roncalli, R.A., Mishiba, T., Yamada, K. and Ura, S., *Japan*

A 2

14:00 **THE PERSISTENT ACTIVITY OF IVERMECTIN AND ABAMECTIN IN CATTLE CHALLENGED DAILY WITH NEMATODES**

Reid, J.F.S., Wallace, D.H., Barth, D., Cox, J.L. and Ericsson, G.F., *Belgium*

A 3

14:15 **IVERMECTIN PROPHYLAXIS IN CATTLE AND THE DEVELOPMENT OF IMMUNITY**

Ryan, W.G., Forbes, A.B. and Grimshaw, W.T.R., *U.S.A.*

A 4

14:30 **EFFICACY OF IVERMECTIN SUSTAINED RELEASE BOLUS FOR CATTLE AGAINST THE TROPICAL WARBLE FLY, *DERMATOBIA HOMINIS***

Benitez Usher, C., Baez Kohn, A., Farrington, D.O. and Barrick, R.A., *Argentina*

A 5

14:45 **EFFICACY OF IVOMEC® PREMIX AGAINST *STRONGYLOIDES RANSOMI***

Reid, J.F.S., Barth, D., Rehbein, S. and Barrick, R.A., *Belgium*

A 6

Epidemiology

13:30 ~ 15:00 Wednesday August 30, 1995
Room B

Chairpersons: **Dr. N. Taira**, *Japan*
Dr. A.L. Willingham, *Denmark*

13:30 **POLYMERASE CHAIN REACTION-BASED MARKER SYSTEM FOR DIFFERENTIATING THEILERIA SERGENTI AND T. BUFFELI**

Kawazu, SI., Kamio, T., Sekizaki, T. and Fujisaki, K., *Japan*

B 1

13:45 **A STUDY OF THE RELATIONSHIP BETWEEN PARASITE COUNTS, LESIONS AND DAILY WEIGHT GAINS IN PSOROPTES OVIS INFESTED CATTLE**

Lonneux, J.F., Bossaert, K., Leclipteux, T., Mignon, B. and Losson, B., *Belgium*

B 2

14:00 **FASCIOLOSIS IN CATTLE: CORRESPONDANCES AND DISCREPANCIES BETWEEN SEVERAL METHODS OF DIAGNOSIS**

Bossaert, K., Leclipteux, T., Protz, M., Lonneux, J.F. and Losson, B.J., *Belgium*

B 3

14:15 **FATAL STRONGYLOIDOSIS IN CALVES IN THE SAWDUST LITTER CONFINEMENT PENS IN JAPAN**

Taira, N., Nakamura, Y., Kakihira, H. and Ura, S., *Japan*

B 4

14:30 **SEROPREVALENCE OF TOXOPLASMOSIS IN FINNISH LYNX (FELIS LYNX)**

Oksanen, A., *Norway*

B 5

Vaccine development

13:30 ~ 15:15 Wednesday August 30, 1995
Room C

Chairpersons: **Dr. U. Cahyaningsih**, *Indonesia*
Dr. K. Shimura, *Japan*
Dr. I. Igarashi, *Japan*

13:30 **DIROFILARIA IMMITIS: ANTIGENIC CROSS-REACTIVITY AMONG NEMATODES**

Hayasaki, M., *Japan*

C 1

13:45 **EFFECTS OF VACCINATION WITH A RECOMBINANT SCHISTOSOMA BOVIS-DERIVED GLUTATHIONE S-TRANSFERASE ON EXPERIMENTAL AND NATURAL S. MATTHEEI INFECTIONS IN CATTLE**

De Bont, J., Vercruysse, J., Meeus, P., Grzych, J.M. and Capron, A., *Belgium*

C 2

14:00 **CHANGES OF T-CELL SUBPOPULATION IN THE PERIPHERAL BLOOD OF CHICKENS INFECTED WITH *LEUCOCYTOZOON CAULLERYI***

Isobe, T., Shimizu, S., Tsuji, N. and Shimura, K., *Japan*

C 3

14:15 **PROTECTIVE IMMUNITY AGAINST CHALLENGE INFECTION WITH *BABESIA MICROTI* IN MICE**

Igarashi, I., Suzuki, R., Omata, Y., Saito, A., Toyoda, Y. and Suzuki, N., *Japan*

C 4

14:30 **THE IMMUNOHISTOCHEMICAL LOCALISATION OF PEPSINOGEN IN THE ABOMASAL MUCOSA OF SHEEP INFECTED WITH HAEMONCHUS CONTORTUS AND IN PARASITE-NAIVE CONTROLS**

McKellar, Q., Scott, I., Irvine, J. and Dick, A., *United Kingdom*

C 5

14:45 **PROTECTIVE EFFECTS OF A STAGE-SPECIFIC, SPECIES CROSS-REACTIVE MONOCLONAL ANTIBODY AGAINST THE MAJOR OOCYST WALL PROTEIN OF EIMERIA TENELLA**

Karim, M.J., Basak, S.C. and Trees, A.J., *United Kingdom*

C 6

15:00 **DETECTION OF IMMUNE RESPONSE IN CHICKEN INFECTED WITH LOCAL ISOLATE OF ATTENUATION EIMERIA TENELLA THROUGH SELECTION FOR PRECOCIOUSNESS**

Cahyaningsih, U., *Indonesia*

C 7

Zoonoses & Biological control 13:30 ~ 15:00 Wednesday August 30, 1995

Room D

Chairpersons: **Dr. H. Nagasawa**, *Japan*
Dr. I. Scott, *United Kingdom*

13:30 **SELECTIVE PERFUSION OF PIGS INFECTED WITH SCHISTOSOMA JAPONICUM. A METHODOLOGICAL STUDY**

Bøgh, H.O., Willingham, A.L. and Johansen, M.V., *Denmark*

D 1

13:45 **EXPERIMENTAL SECONDARY PULMONARY ALVEOLAR ECHINOCOCCOSIS IN RATS**

Ito, A., Osawa, Y., Hashimoto, A., Okamoto, M. and Nakano, M., *Japan*

D 2

14:00 **COPROANTIGEN DETECTION FOR DIAGNOSIS OF *ECHINOCOCCUS MULTILOCULARIS* IN FOXES**

Deplazes, P., Alther, P., Mathis, A., Skaggs, J. and Eckert, J., *Switzerland*

D 3

14:15 **BIOLOGICAL CONTROL OF NEMATODES OF FREE-RANGED PIGS BY MEANS OF A PREDACIOUS MICROFUNGUS**

Nansen, P., Larsen, M., Roepstorff, A., Grønvold, J., Wolstrup, J. and Henriksen, S.A., *Denmark*

D 4

14:30 **THE EFFECTS OF EXCRETORY/SECRETORY PRODUCTS OF OSTERTAGIA CIRCUMCINCTA ON PEPSINOGEN SECRETION AND SMOOTH MUSCLE CONTRACTION IN ABOMASAL TISSUES DERIVED FROM PREVIOUSLY INFECTED SHEEP AND IN PARASITE-NAIVE ANIMALS**

Scott, I. and McKellar, Q., *United Kingdom*

D 5

Chemotherapy and delivery system

09:00 ~ 10:00 Thursday August 31, 1995

Room A

Chairpersons: **Dr. M. Hayasaki**, *Japan*
Dr. C.P.F. Genchi, *Italy*

09:00 **CAT HEARTWORM (DIROFILARIA IMMITIS) INFECTION IN ITALY: SPREAD AND PROPHYLACTIC TREATMENT**

Genchi, C., Venco, G. and Di Sacco, B., *Italy*

A 7

09:15 **EFFICACY AND SAFETY OF TOPICAL IVERMECTIN IN RED DEER**

Reid, J.F.S., Cox J.L., Gogolewski, R., Fulton, R.K., Barth, D., Barrick, R.A., *Belgium*

A 8

09:30 **THE EFFICACY OF DORAMECTIN AGAINST FIELD NEMATODE INFECTIONS OF CATTLE IN TROPICAL AND TEMPERATE REGIONS IN LATIN AMERICA**

Muniz, R.A., Steffan, P., Divino-Lima, J., Errecalde, O. and Goncalves, L.C.B., *U.S.A.*

A 9

09:45 **EFFICACY OF DORAMECTIN AGAINST *MECISTOCIRRUS DIGITATUS* AND OTHER ABOMASAL PARASITES OF CATTLE IN VENEZUELA**

Rew, R.S., de Moreno, L.G., Muniz, R.A. and Moreno, J., *U.S.A.*

A 10

Chemotherapy and delivery system

13:30 ~ 15:00 Thursday August 31, 1995

Room A

Chairpersons: **Dr. Y. Matsumoto**, *Japan*
Dr. V.J. Theodorides, *U.S.A.*

13:30 **EFFICACY OF DORAMECTIN AGAINST NEMATODE INFECTIONS OF CATTLE IN AUSTRALIA**

Rew, R.S., Anderson, N., Hooke, F. and Pope, M., *U.S.A.*

A 11

13:45 **FIELD TRIALS WITH DORAMECTIN USED IN CATTLE IN THE NETHERLANDS**

Borgsteede, F.H.M., Gaasenbeek, C.P.H., Linden, J.N.v.d. and Rijkenhulzen, Th.A.A., *The Netherlands*

A 12

14:00 **ACTIVITY OF DORAMECTIN AGAINST INDUCED AND NATURAL INFESTATIONS OF *DERMATOBIA HOMINIS* IN CATTLE**

Muniz, R.A., Soracl, O., Moya-Borja, G.E., Goncalves, L.C.B. and Errecalde, O., *U.S.A.*

A 13

14:15 **FULL SEASON CONTROL OF *DICTYOCAULUS VIVIPARUS* INFECTIONS OF CATTLE IN IRELAND WITH 2 TREATMENTS OF DORAMECTIN: IMMUNE CONSEQUENCES**

Taylor, S.M., Kenny, J. and Edgar, H., *United Kingdom*

A 14

14:30 **RESPONSES OF WEANER BULLS DURING ADMINISTRATION OF DORAMECTIN OR IVERMECTIN USING 4- OR 8- WEEK TREATMENT INTERVALS**

Watson, T.G., Hosking, B.C., Hooke, F.G. and McKee, P.F., *New Zealand*

A 15

14:45 **EFFICACY OF DORAMECTIN UNDER FIELD USE CONDITIONS IN NEW ZEALAND: COMPARISON WITH MOXIDECTIN, IVERMECTIN AND OXFENDAZOLE**

Dell'Osa, D., Hooke, F., Clement, P., Dell'Osa, D., Porter, R.M.and McCall, D., *Australia*

A 16

Epidemiology

09:00 ~ 10:00 Thursday August 31, 1995
Room B

Chairpersons: **Dr. T. Fukata**, *Japan*
Dr. J.B. Malone, *U.S.A.*

09:00 **PORCINE SCHISTOSOMOSIS JAPONICA: THE HOST-PARASITE RELATIONSHIP IN RELATION TO INTENSITY AND CHRONICITY OF INFECTION**

Willingham, A.L. and Bøgh, H.O., *Denmark*

B 6

09:15 **CROSS-TRANSMISSION OF *CRYPTOSPORIDIUM MURIS***

Zhang,X., Yang, J. and Yin, Jigang, *P.R. of China*

B 7

09:30 **GASTROINTESTINAL NEMATODE INFECTION LEVELS ON DAIRY FARMS IN THE NETHERLANDS AFTER THE FIRST GRAZING SEASON**

Eysker, M., Poot, J. and Lam, T.J.G.M., *The Netherlands*

B 8

09:45 **EPIDEMIOLOGY OF *DIROFILARIA IMMITIS AND SPIROCERCA LUPI* IN GUANGDONG PROVINCE**

Xie, M., Wu, H., Zhang J., Zhang, F. and Wen, L., *P.R. of China*

B 9

Epidemiology

13:30 ~ 15:00 Thursday August 31, 1995
Room B

Chairpersons: **Dr. C. Sugimoto**, *Japan*
Dr. J-F Lonneux, *Belgium*

13:30 **EPIDEMIOLOGY OF FELINE HEARTWORM INFECTION: LABORATORY STUDIES ON TRANSMISSION AND ON HOST PREFERENCE OF MOSQUITO VECTORS**

McCall, J.W., Abdelmoneim, E. and Mansour, *U.S.A.*

B 10

13:45 **UTILITY OF ELISA-BASED ANTIGEN AND ANTIBODY TESTS FOR DETECTION OF HEARTWORM INFECTION IN CATS**

McCall, J.W., Supakorndej, N and Ryan, W., *U.S.A.*

B 11

14:00 **RESISTIBILITY TO *THEILERIA SERGENTI* INFECTION IN HOLSTEIN AND JAPANESE BLACK CATTLE**

Terada, Y., Ishida, M. and Yamanaka, H., *Japan*

B 12

14:15 **GEOGRAPHIC INFORMATION SYSTEMS AND CONTROL OF FASCIOLOSIS IN THE SOUTHERN USA**

Malone, J.B., *U.S.A.*

B 13

14:30 **CORRELATION BETWEEN THE NUMBER OF *Boophilus microplus*, *Babesia* spp. AND *Anaplasma marginale* PARASITAEMIAS IN CROSSBRED-DAIRY CATTLE. IN CHARQUEADA DISTRICT, SOUTHERN BRAZIL**

Kasai, N., Dell'Porto, A., Louvandini, H., Penna, H.F.J. and Gennari, S.M., *Brazil*

B 14

Marine parasitology & Tropical livestock industry

09:00 ~ 10:15 Thursday August 31, 1995

Room C

Chairpersons: **Dr. S. Imai**, *Japan*
Dr. Y. Tsutsumi, *Japan*

09:00 **BENEDENIINE (MONOGENEA: CAPSALIDAE) PARASITES OF CULTURED MARINE FISH IN JAPAN**

Bondad-Reantaso, M.G., Ogawa, K. and Wakabayashi, H., *Philippines*

C 8

09:15 **IMMUNE RESPONSE OF JAPANESE FLOUNDER (PARALICHTHYS OLIVACEUS) AGAINST NEOBENEDENIA GIRELLAE, A SKIN PARASITE OF CULTURED MARINE FISH IN JAPAN**

Bondad-Reantaso, M.G., Ogawa, K., Yoshinaga, T. and Wakabayashi, H., *Philippines*

C 9

09:30 **AN EPOZOOTIC OF ANGUILLICOLOSIS IN AQUACULTURED AMERICAN EEL, ANGUILLA ROSTRATA, IN TAIWAN**

Ooi, H.K., Wang, W.S., Chang, H.Y., Wu, C.H., Lin, C.C. and Hsieh, M.T., *Taiwan*

C 10

09:45 **SERUM ENZYMES, HEMATOCHEMICAL PROFILE IN TRYPANOSOMIASIS INFECTED CAMEL**

Rahman, Z-U., Butt, A.A., Haq, I.U. and Ahmad, A., *Pakistan*

C 11

10:00 **EFFECT OF COCCIDIOSIS ON THE HEMATOCHEMICAL PROFILE IN CAMEL**

Rahman, Z-U., Ahmed, A., Fatima, N. and Haq, I.U., *Pakistan*

C 12

Host genetics & Others 13:30 ~ 15:00 Thursday August 31, 1995

Room C

Chairpersons: **Dr. Q.A. McKellar**, *United Kingdom*
Dr. Y. Oku, *Japan*
Dr. K. Sasai, *Japan*

13:30 **THE PHYLOGENY OF THE SARCOCYSTIDAE DEDUCED FROM 18S RIBOSOMAL DNA SEQUENCE COMPARISONS**

Ellis, J. and Morrison, D., *Australia*

C 13

13:45 **IDENTIFICATION OF A COMMON CONOIDAL DETERMINANT AMONG DIFFERENT *EIMERIA* SPECIES WITH A CHICKEN MONOCLONAL ANTIBODY TO *EIMERIA ACERVULINA***

Sasai, K., Lillehoj, H.S., Matsuda, H., Hanioka, Y., Fukata, T., Baba, E. and Arakawa, A., *Japan*

C 14

14:00 **EFFECTS OF TESTOSTERONE ON THE MUCOSAL DEFENCE AGAINST INTESTINAL HELMINTHS IN INDIAN SOFT-FURRED RATS, *MILLARDIA MELTADA* WITH REFERENCE TO GOBLET AND MAST CELL RESPONSES**

Iiuria, R., Horii, Y., Makimura, S. and Nawa, Y., *Japan*

C 15

14:15 **THE ROLE OF THE MUCOSAL GROWTH FACTORS,TRANSFORMING GROWTH FACTOR-ALPHA AND EPIDERMAL GROWTH FACTOR IN THE PATHOGENESIS OF INFECTION OF SHEEP WITH OSTERTAGIA CIRCUMCINCTA**

Scott, I., McKellar, Q., Irvine, J. and Dick, A., *United Kingdom*

C 16

14:30 **GOBLET CELL MUCINS AS THE SELECTIVE BARRIER FOR THE INTESTINAL HELMINTHS**

Horii, Y., Ishikawa, N. and Nawa, Y., *Japan*

C 17

14:45 **HYPERGASTRINEMIA AND GASTRIC ACID SECRETION IN RATS INFECTED WITH LARVAL *TAENIA TAENIAEFORMIS***

Oku, Y., Yamanouchi, T., Matsuda, K., Ooi, H.K. and Kamiya, M., *Japan*

C 18

Ecology & Others

09:00 ~ 10:15 Thursday August 31, 1995
Room D

Chairpersons: **Dr. T. Isobe**, *Japan*
Dr. R.A. Roncalli, *U.S.A.*

09:00 **SOME ECOLOGICAL ASPECTS OF HYALOMMA ANATOLICUM ANATOLICUM IN BANGLADESH**

Mondal, M.M.H., Islam, M.K. and Kibria, A.K.M.G., *Bangladesh*

D 6

09:15 **THE HISTORY OF VETERINARY PARASITOLOGY IN JAPAN**

Roncalli, R.A., *U.S.A.*

D 7

09:30 ***EIMERIA HANGANI* ISOLATED FROM JAVAN JUNGLEFOWL (*GALLUS VARIUS*)**

Shimura, K., Isobe, T., Tsuji, N., Koyama, K., Watanabe, Y. and Shu Min, Z., *Japan*

D 8

09:45 **SCANNING ELECTRON MICROSCOPY STUDY OF *RHINOESTRUS USBEKISTANICUS* (GAN 1947) LARVAE AND IMAGO**

Dorchies, P. and Guitton, C., *France*

D 9

10:00 **KINETICS OF *OESTRUS OVIS* (LINNE 1758) LARVAE DEVELOPMENT IN NAIVE LAMBS AFTER NATURAL INFECTION IN FIELD**

Dorchies, P., Duranton, C. and Bergeaud, J.P., *France*

D 10

Technology transfer & Zoonoses 13:30 ~ 15:00 Thursday August 31, 1995

Room D

Chairpersons: **Dr. H. Hirumi**, *Japan*
Dr. J. Eckert, *Switzerland*

13:30 **SUSCEPTIBILITY OF IMMUNODEFICIENCY (SCID AND NUDE) MICE TO HIGH AND LOW VIRULENT STRAINS OF *TRYPANOSOMA BRUCEI GAMBIENSE***

Narumi, D., Inoue, N., Saito, A., Suzuki, N. and Hirumi, H., *Japan*

D 11

13:45 ***TRYPANOSOMA BRUCEI GAMBIENSE* : IN VITRO CULTIVATION OF BLOODSTREAM TRYPOMASTIGOTES OF A LOW VIRULENT STRAIN (IL 3253)**

Inoue, N., Hirumi, K., Narumi, D., Saito, A., Suzuki, N. and Hirumi, H., *Japan*

D 12

14:00 **THE CULTIVATION OF *EIMERIA NECATRIX IN VITRO***

Xie, H., Xie, M., Wu, H., Peng, X., Wei, W. and Wen, L., *P.R. of China*

D 13

14:15 **NEMATODE CONTROL USED BY DAIRY FARMERS IN SOUTHEASTERN BRAZIL**

Charles, T.P., and Furlong J., *Brazil*

D 14

14:30 **A RANDOM AMPLIFIED POLYMORPHIC DNA MARKER ASSOCIATED WITH MURINE VIRULENCE OF *TOXOPLASMA* GONDII**

Guo, Z. and Johnson, A.M., *Australia*

D 15

14:45 **NEW MOLECULAR EVIDENCE FOR ZOONOTIC INFECTIONS WITH MICROSPORIDIA (ENCEPHALITOZOON CUNICULI)**

Deplazes, P., Mathis, A., Müller, Ch., Kuster, H., Akerstedt, J. and Weber, R., *Switzerland*

D 16

Chemotherapy and delivery system 09:00 ~ 10:00 Saturday September 2, 1995

Room A

Chairpersons: **Dr. K. Fujisaki**, *Japan*
Dr. K.J. Krieger, *Germany*

09:00 **MODEL FOR EVALUATING STRATEGIC PROGRAMMES FOR FLEA CONTROL ON CATS**

Jacobs, D.E., Fisher, M.A. and Hutchinson, *United Kingdom*

A 17

09:15 **CLINICAL TRIALS OF LUFENURON (PROGRAM®), FLEA CONTROL AGENT IN JAPAN**

Arakawa, A., Tagawa, M., Hara, Y, Kagota, K., Oda, K., Ura, S., Toshida, T. and Nakano, M., *Japan*

A 18

09:30 **FLEA AND TICK CONTOL IN DOGS AND CATS WITH KILTIX A NEW INSECTICIDAL AND ACARICIDAL COLLAR**

Krieger, K.J. and Dorn, H., *Germany*

A 19

09:45 **THE EFFICACY OF A PHYTO-AROMATIC EAR GEL AGAINST AURICULAR MANGE IN RABBITS AND CARNIVORES**

Mignon, B.R., and Losson, B.J., *Belgium*

A 20

Chemotherapy and delivery system 10:30 ~ 11:00 Saturday September 2, 1995

Room A

Chairperson: **Dr. M. Kamiya**, *Japan*

10:30 **PRAZIQUANTEL TREATMENT OF FREE-RANGING FOXES AGAINST ECHINOCOCCUS MUTILOCULARIS**

Romling, T. and Richard, L., *Germany* Sponsored by Bayer

A 21

Chemotherapy and delivery system

09:00 ~ 10:00 Saturday September 2, 1995

Room B

Chairpersons: **Dr. C. Benitez-Usher**, *Argentina*
Dr. E. Papadopoulos, *Greece*

09:00 **FIELD EFFICACY OF POUR-ON AND INJECTABLE FORMULATIONS OF MOXIDECTIN AND IVERMECTIN IN PSOROPTES OVIS INFESTED CATTLE: PARASITOLOGICAL, CLINICAL AND SEROLOGICAL DATA**

Losson, B.J., Lonneux, J.F., Mignon, B., Bossaert, K. and Leclipteux, T., *Belgium*

B 15

09:15 **KINETICS OF SPECIFIC ANTIBODY RESPONSE AFTER SUCCESSFUL TREATMENT IN NATURALLY PSOROPTES OVIS INFESTED CATTLE**

Lonneux, J.F., Bossaert, K., Leclipteux, T., Mignon, B. and Losson, B.J., *Belgium*

B 16

09:30 **PERSISTENT ACTIVITY OF MOXIDECTIN AGAINST *OSTERTAGIA OSTERTAGI* AND *DICTYOCAULUS VIVIPARUS***

Vercruysse, J., Claerebout, E., Demeulenaere, D., Hilderson, H., Meeus, P. and Deroover, E., *Belgium*

B 17

09:45 **EFFICACY AND PERSISTENCY OF MOXIDECTIN 2% EQUINE GEL AGAINST SMALL STRONGYLES (CYATHOSTOMINAE) IN NATURALLY INFECTED HORSES**

Genchi, C., Basano Solari, F. and Nogara, B., *Italy*

B 18

Chemotherapy and delivery system 10:30 ~ 12:00 Saturday September 2, 1995

Room B

Chairpersons: **Dr. G.T. Wang**, *U.S.A.*
Dr. J. Vercruysse, *Belgium*

10:30 **PREVALENCE OF BENZIMIDAZOLE RESISTANT NEMATODES IN DAIRY GOAT FARMS IN WESTERN FRANCE**

Chartier, C., Pors, I., Hubert, J., Benoit, C., Rocheteau, D. and Bernard, N., *France*

B 19

10:45 **ISOLATION AND DROUGHT IN THE DEVELOPMENT OF ANTHELMINTIC RESISTANCE IN NEMATODES**

Papadopoulos, E., Himonas, C. and Coles, G., *Greece*

B 20

11:00 **THE RATE OF RESISTANCE DEVELOPMENT BY H. CONTORTUS TO ENDECTOCIDES**

Wang, G.T., Berger, H., Simkins, K. and Rock, D., *U.S.A.*

B 21

11:15 **ANTHELMINTIC RESISTANCE IN SHEEP IN THE NETHERLANDS**

Borgsteede, F.H.M., Pekelder, J.J., Dercksen, D.P., Sol, J., Vellema, P., Gaasenbeek, C.P.H. and Linden, J.N.v.d., *The Netherlands*

B 22

11:30 **ISOLATION OF A FIELD STRAIN OF *HAEMONCHUS CONTORTUS* RESISTANT TO DORAMECTIN, IVERMWCTIN AND MOXIDECTIN**

Benitez-Usher, C., Santos, C.M., Bridi, A.A., Carvalho, L.A. and Cruz, J.B., *Argentina*

B 23

Others & Tropical livestock industry 09:00 ~ 10:00 Saturday September 2, 1995
Room C

Chairpersons: **Dr. Z-U Rahman**, *Pakistan*
Dr. M.G. Bondad-Reantaso, *Philippines*

09:00 **IMMUNOHISTOSHEMICAL OBSERVATION ON AVIAN STRONGYLOIDOSIS IN CHICKEN**

Sakamoto, T. and Aoki, M., *Japan*

C 19

09:15 **DYNAMICS OF ANTIBODY TITER AND FECAL EGG OUTPUT IN CATTLE AND BUFFALO FOLLOWING INFECTION WITH 500 AND 1,000 F. *GIGANTICA* METACERCARIAE**

Nithiuthai, S., Prasittirat, P., Thammasart, S., Chompoochan, T. and Taira, N., *Thailand*

C 20

09:30 **DYNAMICS OF ELISA TITER IN CATTLE, BUFFALO AND SHEEP INFECTED WITH 500 F. GIGANTICA METACERCARIAE**

Nithiuthai, S., Prasittirat, P., Thammasart, S., Chompoochan, T. and Taira, N., *Thailand*

C 21

09:45 **ANTHELMINTIC EFFECTS OF LEVACID AGAINST SHEEP EXPERIMENTALLY INFECTED WITH *FASCIOLA GIGANTICA***

Chompoochan, T., Prasittirat, P., Thammasart, S., Nithiuthai, S. and Taira, N., *Thailand*

C 22

Biological control & Zoonoses 09:00 ~ 10:00 Saturday September 2, 1995

Room D

Chairpersons: **Dr. A. Ito**, *Japan*
Dr. P. Nansen, *Denmark*

09:00 **HORSE STRONGYLES - PROSPECTS FOR NEMATODE-TRAPPING FUNGI AS BIOLOGICAL CONTROL AGENTS**

Larsen M., Nansen P., Henriksen S.A., Grøndal C., Thamsborg S.M., Grønvold J. & Wolstrup J., *Denmark*

D 17

09:15 **THE EFFECT OF NEMATOPHAGOUS FUNGI FED TO CATTLE, SHEEP AND HORSES ON THE DEVELOPMENT OF INFECTIVE LARVAE**

Bird, J. and Herd, R.P., *U.S.A.*

D 18

09:30 **SPECIFICITY OF HOST-PARASITE RELATIONSHIP. WHAT ABOUT THE DEVELOPMENT IN MAN OF PARASITES OF ANIMAL ORIGIN?**

Euzeby, J., *France*

D 19

09:45 **RODENT ATERNATIVE DEFINITIVE HOST MODEL FOR ECHINOCOCCUS MULTILOCULARIS IMMUNODIAGNOSIS AND PROPHYLAXIS**

Kamiya, M., Oku, Y., Inohara, J., Nonaka, N., Otubo, R., Kondoh, Y. and Ooi, H-K., *Japan*

D 20

Chemotherapy and delivery system

10:30 ~ 12:00 Saturday September 2, 1995

Room C

Chairpersons: **Dr. T. Yoshihara**, *Japan*
Dr. M. Xie, *P.R. of China*

10:30 **EFFICACY OF BITHIONOL PASTE AGAINST ANOPLOCEPHALA PERFOLIATA IN NATURALLY INFECTED HORSES**

Yoshihara, T., Toguchi, M., Komazawa, H. and Ohwa, Y., *Japan*

C 23

10:45 **EFFECTIVENESS OF STRATEGIC USE OF CLOSANTEL AND ALBENDAZOLE IN CONTROLLING GASTROINTESTINAL NEMATODES OF SHEEP IN KENYA**

Maingi, N., Gichohi, V.M., Thamsborg, S.M., Munyua, W.K., Gathuma, J.M. and Nansen, P., *Kenya*

C 24

11:00 **THE EFFECTS OF ANTHELMINTICS ON NATURALLY INFECTED SHEEP AND SUGGESTION OF ALTERNATIVES OF TREATMENT PROGRAMES**

Asegede, G., *Ethiopia*

C 25

11:15 **SCREENING OF ANTICOCCIDIAL EFFECTS OF HERB EXTRACTS TO *EIMERIA TENELLA***

Youn, H-J., Hong, G-O. and Kang, Y-B., *Korea*

C 26

11:30 **EFFECT OF NEMATOCIDES ON *ORIENTOSTRONGYLUS* EZOENSIS (NEMATODA; HELIGMONELLIDAE) IN RATS**

Fukumoto, S.-I., *Japan*

C 27

ABSTRACTS OF SUBMITTED PAPERS

A 1 EFFICACY OF THE IVERMECTIN SUSTAINED RELEASE BOLUS IN ANIMALS WEIGHING GREATER THAN 300 KG AT THE TIME OF TREATMENT

J.S. Eagleson, L.G. Cramer, D.O. Farrington

Merck Research Laboratories, P.O. Box 2000, Rahway, NJ 07065, USA

The ivermectin sustained release bolus (IVOMEC® SR Bolus) is recommended for use in cattle weighing 100-300 kg at the time of administration. The device, which delivers ivermectin at 12 mg per day for 135 days, has therapeutic and prophylactic efficacy against gastrointestinal and pulmonary nematodes and also provides efficacy against mange mites, sucking lice and *Hypoderma* spp grubs. Calves, particularly those of the Continental breeds, are often brought to their first grazing season when they have already attained a body weight greater than 300 kg. Six studies were undertaken to confirm the efficacy of the SR Bolus in cattle weighing more than 300 kg at treatment. Each study included at least one untreated control group and a group in which each animal received a single SR Bolus. Three studies investigated therapeutic and prophylactic efficacy against gastrointestinal and pulmonary nematodes with animals weighing 296-459 kg at treatment. A further two studies investigated efficacy against natural louse infestations with cattle weighing 390-502 kg at treatment. The sixth study examined therapeutic and prophylactic efficacy against *Psoroptes ovis* mites in animals weighing 360-407 kg at treatment. In these six studies the SR Bolus provided high efficacy against endoparasites, sucking lice and *P. ovis* mites. Based on these data, the upper body weight limit for the product can be extended to include cattle weighing up to 400 kg at the time of treatment. Parasite claims previously developed are maintained for animals in the extended body weight range.

Abstract Form

A 2 SAFETY AND EFFICACY OF IVERMECTIN POUR-ON FORMULATION AGAINST STRONGYLOIDES PAPILLOSUS INFECTION IN CATTLE

Nagata T[1], Roncalli R.A[1], Mishiba T[2], Yamada K[2], and Ura S[2].
Animal Science Research, MSD (Japan) Co., Ltd., Inoue Akasaka Bldg. 6-8, Akasaka 1-chome, Minato-ku, Tokyo 107, Japan[1], Kyoto Animal Science R & D Center[2]

The safety and efficacy of ivermectin pour-on formulation were evaluated against *Strongyloides papillosus* in cattle reared in a saw dust litter house in Hyogo, Japan. Eighty cattle (56 castrated males and 24 females) of Holstein breed or Holstein x Japanese Black crossbreed, 100 - 140 days old and weighing 100- 193 kg, were used in the trial. All animals had natural infections of *Strongyloides papillosus* as confirmed by the presence of fecal eggs prior to the study. Replicates of four animals were formed on the basis of similar breed, age and body weight. Within replicates, one animal was randomly allocated to the untreated control group and the other three animals received ivermectin in the pour-on formulation once topically at 500 µg/kg.

Fecal nematode egg counts were effected using the McMaster method on Days 0 (prior to treatment), 14 and 28. No fecal *Strongyloides* eggs were observed in any of the ivermectin-treated animals on Days 14 or 28. All animals in the untreated control group continued to show fecal *Strongyloides* eggs until Day 28. No health problems associated with ivermectin treatment were observed.

These results show that ivermectin, administered in the pour-on formulation at a dose of 500 µg/kg, is highly effective in the control of *Strongyloides papillosus* infections of cattle. This ivermectin formulation will provide a useful and convenient method for preventing sudden death in calves associated with this parasite under Japanese husbandry conditions.

3 - 140-14

A 3 THE PERSISTENT ACTIVITY OF IVERMECTIN AND ABAMECTIN IN CATTLE CHALLENGED DAILY WITH NEMATODES

D.H. Wallace[1], D. Barth[2], J.F.S. Reid[3], J.L. Cox[4], G.F. Ericsson[4].
Merck Research Laboratories, [1]Fulton, MO, USA, [2]Lauterbach, Germany, [3]Brussels, Belgium, [4]Rahway, NJ, USA

To investigate the persistent nematocidal activity of the injectable formulations of ivermectin and abamectin under repeated challenge conditions, two studies were conducted in which the trial animals were administered infective larvae daily. In each study, 21 nematode-free calves were allocated by restricted randomization on weight to one of three treatment groups: unmedicated control, ivermectin or abamectin, injected subcutaneously at 200 µg/kg body weight. Animals were treated at Day 0. Starting at Day 0 and daily thereafter, all animals were artificially infected for 14 or 15 days with third-stage larvae of *Haemonchus placei* (500 larvae/day), *Trichostrongylus axei* (1000/day); and *Cooperia* spp. (1000/day); for 21 or 22 days with *Ostertagia ostertagi* (1000/day) and *Oesophagostomum radiatum* (100/day) and for 28 or 29 days with *Dictyocaulus viviparus* (50/day). Trial animals were humanely sacrificed for nematode quantification using standardized techniques, by replicate on Days 49-51. The data for each parasite from an animal were transformed to ln (count +1). Geometric means were calculated and results for each medication were compared to controls by the modified Friedman's test for combined data. Mean counts for each medicated group were reduced (>99%, $p<0.01$) compared to control counts for *T. axei, H. placei, Cooperia* spp., *O. Ostertagi, Oes. radiatum,* and *viviparus*. These high efficacies within the test periods indicate that both products effectively controlled parasite challenges that simulated field conditions. The practical results of such control are the prevention of clinical parasitism during these periods and the reduced pasture contamination during the same periods plus the prepatent intervals of the respective parasites.

4. 14[15] - 14[30]

A 4 IVERMECTIN PROPHYLAXIS IN CATTLE AND THE DEVELOPMENT OF IMMUNITY

Andrew B. Forbes[1], William T.R. Grimshaw[2] & William G. Ryan[3]
[1]Merck AgVet, Rahway, NJ 08820, [2]MSD AgVet, Hoddesdon, U.K.

Immunity to parasitic nematodes in cattle is a complex phenomenon dependent upon a number of variables including genotype, age and exposure to infection. The objective of all parasite control programs, including those making no use of anthelmintics, is to optimize performance by reducing exposure. By definition, therefore, any program may impact the development of immunity. A quantitative relationship has been suggested between the intensity of control in first season calves and the immunity that is acquired, as measured by worm burdens at the end of the second season or by artificial challenge experiments at the end of the first season. To assess the impact, if any, of first season prophylaxis on subsequent immunity, trials were undertaken with ivermectin, administered topically or by injection in a 3-8-13 week program, or as a sustained (135 day) release bolus to first season calves. Performance was compared to that of salvage - treated controls throughout the first and second grazing seasons. In no trial was there evidence of clinical parasitic gastroenteritis in second season cattle. Performance benefits resulting from first season ivermectin prophylaxis were maintained through the second grazing season. The sporadic occurrence of dictyocaulosis in second season cattle appeared independently of first season prophylaxis and was also observed in salvage-treated control animals. The data indicate that any changes in host immunity to gastrointestinal helminth parasites resulting from first season prophylaxis are subtle and are irrelevant to performance during the second grazing season.

5- 1432 1:5

A 5 EFFICACY OF IVERMECTIN SUSTAINED RELEASE BOLUS FOR CATTLE AGAINST THE TROPICAL WARBLE FLY, *DERMATOBIA HOMINIS*

Carlos
C. Benitez Usher[1], A. Baez Kohn[2], D.O. Farrington[1], R.A. Barrick[1]
[1]Merck Research Laboratories, Rahway, NJ 07065 USA and [2]Juan Max Boettner 1084, e/Juan 23y Narsico R. Colman, Asuncion, Paraguay

Larval stages of the tropical warble fly, *Dermatobia hominis*, infest a wide variety of warm blooded animals. The fly is distributed from the forested areas of Mexico to the south through Central and South America. It causes economic loss due to reduced body weight gain, impaired milk production, death, and hide devaluation. The efficacy of the ivermectin sustained release bolus was tested against *D. hominis* in a clinical trial. The device delivers 12 mg ivermectin per day. Cattle weighing from 273 to 335 kg on the day of treatment were selected from a cattle ranch in Misiones Province, Argentina. Fourteen naturally infested female and male-castrate Zebu cross cattle, 3 1/2 to 4 years old, were used. Seven replicates of two animals each were formed based on total *Dermatobia* body counts on Day 0. They were randomly allocated to either the untreated control group or the treated group. To ensure equal opportunity for exposure to *Dermatobia*, all experimental animals were kept in a paddock known to be heavily infested. Nodular lesions (warbles) caused by *D. hominis* were counted on the whole body on Days 0, 14, 28, 56, 84, 112, 140, 168 and 196. On Day 14 larvae were expressed for identification and determination of efficacy. Counts of lesions on other days were performed to determine residual protective activity. Animals were weighed on the same days, except for Day 14.

Cattle treated with the ivermectin sustained release bolus had significantly ($p<0.05$) fewer *D. hominis* larvae than did the controls from Day 14 to Day 168. Efficacy was 100% on Day 14. No warbles were found on treated animals until Day 196. All control animals were infested by at least one warble at each count through Day 168, and six out of seven controls were infested on Day 196. Treated animals had a weight gain advantage of 26.4 kg over controls during the study.

6- 14[45]-15[15]

A 6 EFFICACY OF IVOMEC® PREMIX AGAINST *STRONGYLOIDES RANSOMI*

D. Barth, S. Rehbein, J.F.S.Reid & R. A. Barrick
Merck Research Laboratories, Rohrdorf, Germany &
Brussels, Belgium and Rahway, New Jersey, USA

The efficacy of an in-feed formulation containing 0.6% ivermectin was tested against *Strongyloides ransomi* in swine. Two trials involving 40 pregnant gilts were carried out to evaluate the efficacy of ivermectin against the somatic larval stages of *S. ransomi* when given at a daily dose of 100 mcg per kg body weight for seven days starting on pregnancy days 66, 78, 92 or 103. The gilts were experimentally infected by three subcutaneous injections of 250,000 infective larvae each with the last infection given 12 - 30 days prior to onset of treatment. Sows were free of pre - existing intestinal stages of *S. ransomi* prior to ivermectin treatment. Fecal nematode egg counts were carried out in sows and piglets. The *Strongyloides* larvae present in sow milk 1, 2 and 7 days post partum were counted. Fourteen days post natum, worm counts were done in four randomly selected piglets from each litter. IVOMEC® premix given to pregnant gilts prevented shedding of larvae in sow milk, egg output in feces and the establishment of *S. ransomi* in piglets. The efficacy of ivermectin against patent infections of *S. ransomi* when given via the feed at 2 ppm for seven days was evaluated in another study with 16 three-month - old piglets. Seven days prior to treatment each piglet was infected subcutaneously with 2,500 infective larvae of *S. ransomi*. Fecal egg counts were carried out on Days -7, 0, 7 and 14, and worm counts carried out on Day 14. Efficacy was 100% in all treated piglets.

A 7 CAT HEARTWORM (Dirofilaria immitis INFECTION IN ITALY: SPREAD AND PROPHYLACTIC TREATMENT.

C. Genchi, G. Venco, B. Di Sacco.
Department of Parasitic Diseases of Domestic Animals, University of Milan and University of Camerino, Italy

Heartworm infection (HWI) was found to range <5% to 22% (1-8 worms/cat) in domestic cats from different areas of Northern Italy. These and further studies carried out recently on pound cats to assess the epidemiology of cat HWI in Italy, suggest that epidemiology of cat HWI is quite impossible to determine but based on a randomized statistical analysis of samples on whole cat population, stratified by age and sex. However, clinical findings show that cat HWI is spreading in Italy. Out of 953 cats with pulmonary diseases examined in the last 2 years, 32 were found HW positive: 14 suddenly died for acute/hyper-acute symptoms, 8 few days later the clinical examination. Because adulticide treatment is very risky in cats, chemoprophylaxis is the most effective and safe option. The safety and efficacy of a chewable ivermectin formulation (24 mcg/Kg per os) given prophylactically once a month for 5 months were evaluated. Seventy domestic cats, negative for circulatinrg microfilariae (Mf) and HW antigen (Ag), were included in the trial. Cats were randomly assigned to an ivermectin-treated group or control group. Cats were checked both for Mf and HWAg 5 months after 1st treatment and 5 and 6 months after the last treatment. Fecal samples were also taken from all cats before 1st, 2nd and 4th treatment and after the 5th. No Mf or HWAg were found in ivermectin-treated. One cat from control group was HWAg positive at both 5th and 6th month after last treatment. In this trial, ivermectin also showed a good efficacy against hookworms and T.cati natural infections. No side effect occurred during the trial.

A 8 EFFICACY AND SAFETY OF TOPICAL IVERMECTIN IN RED DEER

J.L. Cox[1], R. Gogolewski[2], R.K. Fulton[3], D. Barth[4], J.F.S. Reid[5], R.A. Barrick[1]. Merck Research Laboratories, [1]Rahway, NJ, USA, [2]Ingleburn, Australia, [3]Springdale, AR, USA, [4]Lauterbach, Germany, [5]Brussels, Belgium

The clinical efficacy and safety of topical ivermectin in red deer were evaluated under field conditions in three countries. In three studies under conditions of natural infection, deer calves were restrictively randomly allocated to an untreated control group or to a group for treatment on Day 0 with ivermectin at 500 µg/kg body weight applied topically (IVOMEC® Pour-On for Cattle) along the topline. There were at least six deer per group. After selection for a trial, animals were housed to preclude accidental infection with nematodes. From Days 14 to 16 deer were slaughtered for recovery, identification, and counting of nematodes. Adult and fourth-stage larvae of counts of *Dictyocaulus viviparus* (found in 2 trials) were reduced 100% compared to controls; adult *Ostertagia leptospicularis* (2 trials) were reduced >99%; adult *Oesophagostomum* spp. (3 trials) were reduced 100%; adult *Skrjabinagia kolchida* (2 trials) were reduced >99%; and adult *Spiculopteragia asymmetrica* (2 trials) were reduced >99%. In a separate study, 16 deer were randomly assigned to either ivermectin treatment or untreated groups at the time of dosing. Topical ivermectin was administered to four male and four female red deer, about six months old at a dose of 2 mg/kg. Application sites were carefully examined at 7-day intervals after treatment. All treated deer remained healthy throughout the observation period but one control died of suspected yersiniosis. Other than evidence of scurf in the hair along the application site following application of 4X the recommended dose, there were no effects on hair or skin of treated animals. The trials demonstrate that topical ivermectin, at a dose of 500 µg/kg, was safe and highly effective against naturally acquired burdens of pulmonary and gastrointestinal nematodes of red deer.

A 9 THE EFFICACY OF DORAMECTIN AGAINST FIELD NEMATODE INFECTIONS OF CATTLE IN TROPICAL AND TEMPERATE REGIONS IN LATIN AMERICA

R.A. Muniz*, P. Steffan†, J. Divino-Lima‡, O Errecalde*, L.C.B. Goncalves*; *Pfizer Animal Health, New York, USA; †INTA-Balcarce, Buenos Aires, Argentina; ‡University of Minas Gerais, MG, Brazil

Two studies, one in Argentina and one in Brazil, were conducted during equivalent grazing seasons, to evaluate the therapeutic efficacy of doramectin administered S.C. at 200 mcg/kg to cattle harboring naturally acquired infections of gastrointestinal nematodes and lungworms. In each experiment, twenty calves were randomly allocated to a treated or control group based on serial e.p.g. counts. Calves were necropsied 14 to 16 days post-treatment and worm burdens in the lungs, abomasa, small and large intestines were determined. Geometric mean worm burdens were calculated from the log worm counts and used to estimate percentage efficacy. In Argentina, calves originated from a temperate region. Parasite populations found in these animals were: *Cooperia oncophora*, *Dictyocaulus viviparus*, *Haemonchus placei*, *Ostertagia ostertagi* and *Trichostrongylus axei*. In Brazil, calves were from a tropical region. Parasite populations found in these animals were: *Cooperia pectinata*, *C. punctata*, *C. spatulata*, *Dictyocaulus viviparus*, *Haemonchus contortus*, *H. similis*, *Haemonchus spp.*, *Oesophagostomum radiatum*, *Ostertagia ostertagi*, *Trichostrongylus axei*, *T. colubriformis* and *Trichuris discolor*. Doramectin was 100% efficacious against adult stages of all above parasites with the exception of *Trichostrongylus colubriformis*, against which efficacy was 99.4%. Against fourth stage larvae of *Cooperia punctata*, *Dictyocaulus viviparus*, *Haemonchus contortus*, *H. similis*, *Oesophagostomum radiatum*, *Ostertagia ostertagi* and *Trichostrongylus axei*, doramectin efficacy was 99.5% or higher. *C. oncophora* was found only in animals from temperate climates; while other species of *Cooperia*, *H. contortus*, *H. similis*, and *Trichuris discolor*, were found in calves from tropical regions.

A 10 EFFICACY OF DORAMECTIN AGAINST *MECISTOCIRRUS DIGITATUS* AND OTHER ABOMASAL PARASITES OF CATTLE IN VENEZUELA

*L.G. de Moreno, †R.S. Rew, †R.A. Muniz, and †J. Moreno
*Institute of Vet. Investigation, Maracay, Venezuela
†Pfizer Animal Health Group, New York, USA

A study was conducted in Venezuela to evaluate the therapeutic efficacy of doramectin administered S.C. at 200 mcg/kg to cattle harboring naturally - acquired abomasal parasite infections. The presence of parasitism was confirmed by necropsy of 3 animals of the herd before the experiment. Twenty-six male calves were selected and randomly allocated in ranked pairs to a control or treated group based on serial fecal egg counts. Animals were necropsied 14-17 days following treatment and worm burdens in the abomasum were determined. The two most prominent parasites were *Haemonchus similis*, a tropical species of *Haemonchus* found from the southern U.S. to Brazil in the New World and in southern Europe, and *Mecistocirrus digitatus* found in Central America, northern South America and Asia. Efficacy was calculated based on the percentage reduction of the geometric mean number of worm burdens in doramectin-treated animals compared to the untreated controls. Doramectin was 100% efficacious against adult stages of *Mecistocirrus digitatus, Haemonchus similis, H. placei, Cooperia pectinata, C. punctata* and *Trichostrongylus axei*. Efficacy against fourth stage larvae of *M. digitatus, H. similis, C. pectinata* and *C. punctata* was also 100%.

A11 EFFICACY OF DORAMECTIN AGAINST NEMATODE INFECTIONS OF CATTLE IN AUSTRALIA

N. Anderson*, R.S. Rew†, F. Hooke† and M. Pope*
*CSIRO, Parkville, Victoria, Australia
†Pfizer Animal Health, New York, USA

A series of trials were conducted with doramectin, a novel avermectin, to investigate its anthelmintic efficacy properties against natural and artificial nematode challenges in cattle raised under Australian conditions. Three therapeutic efficacy trials and one protective efficacy trial were conducted by comparing calves treated with 200 mcg/kg of doramectin administered subcutaneously in the lateral midline of the neck versus saline treated controls. Results of the therapeutic trials demonstrated that doramectin was 97 - 100% effective following natural or artificial challenge against adult, fourth stage larvae, and inhibited fourth stage larvae of *Ostertagia ostertagi*, *Cooperia oncophora*, and *Trichostrongylus axei*. It was also 100% effective against adult and fourth stage larvae of *T. colubriformis* and *Haemonchus placei*. Results of the protective efficacy trial indicated that doramectin prevented infection of *O. ostertagia* and *T. axei* by >95% for 28 days and for *C. oncophora* by >90% for 21 days when calves were exposed to infective larvae on a weekly basis.

A 12 FIELD TRIALS WITH DORAMECTIN USED IN CATTLE IN THE NETHERLANDS

F.H.M. Borgsteede[1], C.P.H. Gaasenbeek[1], J.N. v.d. Linden[1] and Th. A.A. Rijkenhuizen[2].
[1]DLO-Institute for Animal Science and Health, Lelystad, The Netherlands; [2]Pfizer Animal Health Division, The Netherlands.

Doramectin has been reported to be a very potent anti-parasitic against endo- and ectoparasites of cattle. In 1994, the efficacy of doramectin was tested under field conditions in 34 herds of dairy calves on 29 farms spread across the country. Calves were treated by subcutaneous injection (0.2 mg kg^{-1} bodyweight) at the day of turn out and 8 weeks thereafter. On 5 of the 29 farms, calves were vaccinated against lungworm. Faecal samples were collected 28, 56 and 98 days after treatment, and at housing. EPG-counts and larval cultures were performed according to standard procedures. No clinical signs of parasitic gastro-enteritis or bronchitis were observed during the trials. On all farms, the growth of the calves was good to excellent according to the farmers. After 28 days, calves in all herds had an EPG <25, with 29 larval cultures virtually negative. In all 5 positive larval cultures, *Cooperia oncophora* larvae were found exclusively. After 56 days, calves in 29 herds had an EPG <25, with 3 negative larval cultures. Twelve cultures showed 100% *C. oncophora* and 19 a mixture of *C. oncophora* and *Ostertagia ostertagi*. After 98 days, calves in 30 herds had an EPG <25. Of the larval cultures from the 34 herds, 10 cultures were negative, 5 mixed and 19 exclusively *C. oncophora*. At housing, calves in 18 herds had an EPG <25, 6 had 25, 4 had 50 and 6 had >50. From larval cultures at turn in, three cultures were negative, 24 mixed, 6 exclusively *C. oncophora* and one exclusively *O. ostertagi*. The conclusion from these field experiments is that under conditions which prevail in The Netherlands, treatment with doramectin at turn out and 8 weeks later is an excellent parasite control program.

A 1 3 ACTIVITY OF DORAMECTIN AGAINST INDUCED AND NATURAL INFESTATIONS OF *DERMATOBIA HOMINIS* IN CATTLE

*R.A. Muniz, †O. Soraci, ‡G.E. Moya-Borja, *L.C.B. Goncalves and *O. Errecalde
*Pfizer Animal Health Group, New York; †La Plata University, Argentina; ‡Federal Rural University of Rio de Janiero, Brazil

Two studies were conducted in Latin America to evaluate the efficacy of doramectin administered S.C. at 200 mcg/kg to cattle harboring induced and natural infestations of the tropical warble-fly, *Dermatobia hominis*. In the first study, conducted in Brazil, 12 calves were infested with 25 first instar larvae of *D. hominis*. Twenty-four days later, animals were allocated to a treated or control group based on the number of parasite nodules present. Calves were examined daily for 11 days post-treatment (p.t.) and the number of nodules was mapped and recorded. In the second study, conducted in Argentina, forty 24 month-old cattle, carrying naturally acquired *D. hominis* infestations, were allocated to a treated or control group on the basis of the number of parasitic nodules present 24 hours before treatment. Animals were examined on treatment day and 2, 7, 15 and 30 days p.t. In the study in Brazil, the number of parasitic nodules on doramectin-treated calves was reduced by 74% ($P<0.05$) at 48 hours p.t. Efficacy reached 100% at 6 days p.t. and remained at 100% for the duration of the experiment. In the study in Argentina, no live larvae were found inside nodules after 48 hours p.t. on doramectin-treated animals nor did new nodules develop until the termination of the experiment. When compared with nodule counts in the control group on the same observation day or with counts on the same animal before treatment, efficacy reached 100% at 7 days p.t. and remained at 100% on subsequent observation days ($P<0.05$). In both experiments, dead *D. hominis* larvae were found outside or partially outside the nodules of doramectin-treated animals during the first 48 hours p.t.

A 1 4 FULL SEASON CONTROL OF *DICTYOCAULUS VIVIPARUS* INFECTIONS OF CATTLE IN IRELAND WITH 2 TREATMENTS OF DORAMECTIN: IMMUNE CONSEQUENCES

S.M. Taylor, J. Kenny, H. Edgar
Veterinary Sciences Division, Stormont, Belfast, UK

Two groups of fifteen calves were turned out on to separate paddocks known to be carrying a low overwintered infection of infective larvae of *D. viviparus* and gastrointestinal nematodes. One group was treated at 0 and 8 weeks after turnout with doramectin and the other remained as untreated controls. They were monitored for the entire grazing season, towards the end of which two naive tracer calves were grazed on respective paddocks for two weeks. After housing five of each group were given an infection of *D. viviparus* at the rate of 30 L3/kg and slaughtered 4 weeks later. Between days 28 and 42 of the grazing period the controls had reduced weight gain and intermittent coughing consistent with a subclinical infection of lungworm, although no lungworm larvae were detected from faeces samples. The treated animals were unaffected. After slaughter the tracer calves on both paddocks were found to be lightly infected with lungworm, showing that both areas had been infective during the grazing season. Following experimental infection and slaughter, the calves from the treated group had significantly lower lungworm counts than the untreated controls. As a result of ELISA tests with both larval and adult antigens this was thought to be as a result of disruption and release of parasite antigen from killed worms by the treatment at 8 weeks which acted as immunogens. In the controls the undisrupted infection was presumed to be less immunogenic, and that, coupled with the immunosuppressive effects of the gastrointestinal nematode infections which occurred in that group resulted in less immunity to reinfection. It was concluded that doramectin treatments timed to disrupt low infections resulted in an enhanced immunity to reinfection when compared to similar infections left untreated.

A 15 RESPONSES OF WEANER BULLS DURING ADMINISTRATION OF DORAMECTIN OR IVERMECTIN USING 4- OR 8- WEEK TREATMENT INTERVALS

T.G. Watson*, B.C. Hosking†, F.G. Hooke** and P.F. McKee†
*Pfizer Laboratories Ltd., Auckland, New Zealand; †Ruakura Beef Unit, Hamilton, New Zealand; **Pfizer Pty. Ltd., Sydney, Australia

A replicated study was undertaken on the Ruakura Beef Unit, Hamilton, New Zealand to compare responses of weaner calves injected SC with doramectin (DOR) or ivermectin (IVM) at 4- or 8-week intervals. Twenty calves in each of two replicates per treatment were intensively stocked on paddocks and rotationally grazed for 168 days. Pasture samples were collected for larval densities and animals were weighed every 14 days. Faecal samples were collected every 7 days. *Cooperia* spp. and *Ostertagia* spp. dominated pasture larvae. Larval densities on paddocks grazed by untreated calves were high. Larval densities were very low with 4-week treatment intervals, but were higher with 8-week intervals for both IVM and DOR. With 8-week intervals IVM calves were exposed to more larval contamination than DOR animals. This was consistent for both *Cooperia* spp. and *Ostertagia* spp.. Cumulative faecal egg counts (FEC) were significantly different between all medicated groups and the non-medicated controls ($p<0.0001$). Calves treated at 4-week intervals had significantly lower FEC than those treated at 8-week intervals ($p<0.002$). DOR calves treated at 8-week intervals shed significantly fewer eggs than 8-week IVM. Non-treated animals gained significantly less weight than the treated groups ($p<0.0001$). Though previously 4-week treatment intervals was the recommended program for nematode control for weaner calves under these management conditions, we would now recommend DOR at 8-week intervals which provides a more biocompatible program without compromising productivity.

in NZ to graze at 3–6 wks old & not housed again

A 16 EFFICACY OF DORAMECTIN UNDER FIELD USE CONDITIONS IN NEW ZEALAND: COMPARISON WITH MOXIDECTIN, IVERMECTIN AND OXFENDAZOLE

F. Hooke*. P. Clement†, D. Dell'Osa*, R.M. Porter‡ and D. McCall**
*Pfizer Pty Ltd, Sydney, Australia; †Animal Health Advisory, Auckland, NZ; ‡Tamarunui, NZ; **Te Kuiti, NZ

Two studies were conducted to compare the efficacy of doramectin injectable, moxidectin pour-on, ivermectin pour-on and oxfendazole oral suspension when administered to cattle with naturally acquired nematode infections. Both studies were carried out in central North Island, New Zealand. On day 0, forty cattle were weighed, fecal sampled and allocated to 4 treatment groups on the basis of Day -3 fecal epg. Group 1 received oxfendazole (oxfz) at 4.5 mg/kg (orally); Group 2, doramectin 200 mcg/kg (SC); Group 3, moxidectin (mox) 500 mcg/kg (pour-on); and Group 4, ivermectin (ivm) 500 mcg/kg (pour-on). Individual nematode egg counts and group bulk larval differentiations were done on Days 0, 14, 28, 35, 42, and 56 post-treatment and all cattle were weighed on Day 56. Faecal egg counts (FEC) were reduced by doramectin by 99.1% in the first study and 100% in the second study when sampled 14 days post-treatment. Corresponding FEC reductions for oxfz were 78.3% and 100%, for mox were 80.8% and 85.2%, and for ivm were 86.0% and 80% for first and second studies, respectively. The post-treatment rise in FEC's from Day 28 to 56 in both studies indicated a more rapid reinfection of all groups compared to doramectin. This was especially apparent for oxfz and mox treated cattle, but also ivm treated cattle in one of the two studies. Consistent with the superior efficacy and post-treatment protection, doramectin-treated cattle gained significantly more weight ($p < .05$) over the 56 day period as compared to moxidectin pour-on and ivermectin pour-on in both trials and oxfendazole in one of the two trials.

A 17 MODEL FOR EVALUATING STRATEGIC PROGRAMMES FOR FLEA CONTROL ON CATS

M.A.Fisher, D.E.Jacobs and M.J.Hutchinson,
The Royal Veterinary College, London,
United Kingdom

A model was devised to simulate the control of fleas in the home by use of strategic animal treatments. Four groups of 6 cats in similar, but separate carpeted pens in the same room, were each initially infected with 80 fleas, while 100 eggs, larvae and pupae were put on each carpet. One group was an untreated control. The others were treated every 28th day with either fenthion (30 mg), lufenuron (133 or 266 mg) or with both. A sudden upsurge in flea numbers occurred on control cats after 50 days. At this time, the three control strategies had reduced counts by 91.3, 72.5 and 98.6%, respectively. Thereafter, welfare considerations demanded limitation of the flea burden on control cats. Direct quantitative comparisons were no longer possible, but conditions were shown to remain favourable for flea development. Mean numbers on treated groups after 6 months were 1.2, 11.0 and 0.4, respectively. After this, cats were each infected weekly with 5 fleas (additional to those recruited from the pen environment) to mimic a roaming cat introducing extraneous fleas into the home. This produced no obvious effect on counts, mean values after 3 months treatment being 0.5, 11.0 and 0.2, respectively. Thus, no strategy totally eradicated the flea population but all reduced numbers considerably and all worked equally well whether or not small numbers of new fleas were introduced into the system.

A 18 CLINICAL TRIALS OF LUFENURON (PROGRAM®), FLEA CONTROL AGENT IN JAPAN

A. Arakawa(1), M. Tagawa(2), Y.Hara(2), K. Kagota(3), K. Oda(4), S. Ura(5), T. Toshida(6), & M. Nakano(7)
(1)Osaka Prefectural University, (2)Nippon Veterinary and Animal Science University, (3)Tottori University, (4)Reserch Institute for Animal Science in Biochemistry and Toxicology, (5)Kyoto Animal Health R&D Center, (6)Tosida Animal Clinic, and (7)Ciba Geigy Japan

Lufenuron is a unique flea control agent developed by Ciba Geigy in 1987. It is given orally once a month to dogs and cats, and inhibits chitin synthesis of fleas during their immature stages.

Clinical trials of lufenuron were conducted in a total of 184 dogs and 190 cats naturally infested with fleas at 6 vet institutions during the period of April to October, 1993. In heavy infested dogs 51% and 94% were cleared at 1 and 2 months, respectively. While in cats, 47% and 84% of heavy infestations were cleared in 1 and 2 months, respectively. In both cases, no flea was found after 3 months. Lufenuron was also effective in dogs and cats whether animals were kept outside of houses, kept constantly inside of houses, or temporarily walked out. Results of these clinical trials were similar to those found in artificially infested animals.

No side effects of the product were observed during the study. And, it was confirmed that lufenuron is compatible with other flea control agents.

A 19 FLEA AND TICK CONTOL IN DOGS AND CATS WITH KILTIX A NEW INSECTICIDAL AND ACARICIDAL COLLAR

Dorn, H.; Krieger, K.J.
BAYER AG, D-51368 Leverkusen, Germany

For control and prevention of flea infestations in dogs and cats Propoxur (Carbamate) collars are in use for years. The crystallization of the compound on the surface of the PVC-collars and its distribution in the fur and on the skin of treated animals results in an effective period of up to five month. As Propoxur has minor acaricidal properties collars with Propoxur only do not provide sufficient tick control. KILTIX collars contain a combination of 10% W/V Propoxur and 2.25% W/V Flumethrin, a synthetic Pyrethroid with a pronounced acaricidal and a minor insecticidal profile. The preparation was tested in controlled dog and cat trials with repetitive artificial flea (*Ctenocephalides felis*) and tick infestations (*Rhipicephalus sanguineus, Ixodes ricinus*) and in various field trials. The effective period against fleas and ticks lasted up to 30 weeks. The collars proved to be safe for both, the treated animals and the operators being in contact with the treated animals.

A 20 THE EFFICACY OF A PHYTO-AROMATIC EAR GEL AGAINST AURICULAR MANGE IN RABBITS AND CARNIVORES

B. R. MIGNON and B. J. LOSSON
University of Liège, Faculty of Veterinary Medicine, department of Parasitology and Parasitic diseases, Liège, Belgium.

The efficacy of a phyto-aromatic ear gel (Canidor^{TM}) was evaluated *in vitro* against *Psoroptes cuniculi* and *in vivo* against *Psoroptes cuniculi* and *Otodectes cynotis* in experimentally infested rabbits (n=12) and naturally infested domestic carnivores (n=8) respectively. At 1/1 dilution the gel was 100% active *in vitro* against *Psoroptes cuniculi* and this result was achieved in less than 6 hours of contact. In experimentally infested rabbits the gel was administered daily during 2 periods of 5 consecutive days at 6 day interval. Clinical cure was achieved in all animals but 2 animals which were harbouring a few eggs and mites at the end of the trial. In contrast, all untreated rabbits remained infested until the end of the trial and their clinical condition was deteriorating. In dogs and cats the active formulation was given for 2 periods of 4 consecutive days at 7 day interval. Fifty percent of the animals were parasitologically negative after 4 days. Clinical and parasitological examinations 10 and 30 days after initiating the treatment regimen revealed an 100% efficacy. Taking account that organophosphates and organochlorines which are commonly used in auricular mange are not 100% safe especially in kitten and that ivermectin is not licenced for small animals, this phyto-aromatic ear gel appears as an interesting alternative to classical treatments against otodectic mange in domestic carnivores.

A 21 PRAZIQUANTEL TREATMENT OF FREE-RANGING FOXES AGAINST *ECHINOCOCCUS MULTILOCULARIS*

Thomas Roming and Richard Lucius
Div. of Parasitology, University of Hohenheim, Germany

Echinococcus multilocularis the causative agent of human alveolar echinococcosis, is a wildlife parasite whose distribution in Europe was long thought to be largely restricted to a coherent focus in eastern France, southern Germany, northern Switzerland, and Austria. Within recent years however, the parasite was found in foxes from almost every part of Germany. Whether or not this represents a recent extension of the parasite's range is difficult to prove since no reliable large scale investigations have been carried out in northern Germany in former times. The previous absence of the parasite in these areas can only be deducted from the extreme rarity of human cases there. Data from the southern federal states of Baden-Württemberg and Rhineland-Palatinate suggest a considerable increase of *E. multilocularis* prevalences in foxes within the last five to ten years; this coincides with a drastic increase of fox populations possibly due to successful rabies control measures.

Within this context, a pilot control project was recently started in the 'classic' high-prevalence area (approx. 45% of foxes infected) of the southern German federal state Baden-Württemberg. The project is based on results from a previous study conducted in 1990 in Baden-Württemberg, where *E multilocularis* prevalences in free ranging foxes from a small area (156 km^2) could be reduced towards nil within one year by administering praziquantel. In the present study the area was extended to 3500 km^2. Free ranging foxes are treated using praziquantel administered in baits containing 50 mg each; baits and distribution logistics were taken over from rabies control campaigns in the same area. Baits are distributed in densities of 20 / km^2 using small aircraft. Baiting is repeatedly done for two years in intervals of approximately six weeks (prepatent period of the parasite); intervals may be adjusted during the study period depending on the data obtained. Before and during the baiting period, *E multilocularis* prevalences are monitored by post mortems of foxes hunted in the area. Parallel examination of foxes from neighbouring unbaited areas serve as controls. In addition, data are being collected on the prevalence of *E. multilocularis* in various rodent species who are known to serve as intermediate hosts, and the exposure of the human population is measured by serological surveys in selected areas.

The following questions are to be answered by the pilot studies:
-Is control of *E. multilocularis* in a large area feasible by treating free ranging foxes with praziquantel?
-Which efforts (financial and logistical) are necessary?
Depending on the results obtained during the baiting period, consecutive studies will be necessary to determine the time needed for the parasite to re-establish itself in areas with and without possible immigration of infected foxes.

In addition to the project described, a pilot control study is under way in a low prevalence area northwest of Berlin where echinococcosis in foxes exists as a focus surrounded by areas which are largely free of the parasite. In order to compare the results achieved, the methodology of both projects is kept as similar as possible.

B 1 POLYMERASE CHAIN REACTION-BASED MARKER SYSTEM FOR DIFFERENTIATING THEILERIA SERGENTI AND T. BUFFELI

Kawazu SI, Kamio T, Sekizaki T, and Fujisaki K
National Institute of Animal Health, Tsukuba, Ibaraki 305, Japan

Benign Theileria species in cattle, Theileria sergenti and T. buffeli are morphologically indistinguishable. The polymerase chain reaction (PCR) was used to amplify the genes encoding 33/34 kilodalton major piroplasm antigens (p33/34) of T. sergenti and T. buffeli from these parasite-infected cattle blood and T. sergenti-infected tick salivary gland. Following amplification, p33 gene from T. sergenti and p34 gene from T. buffeli were clearly differentiated using the restriction enzymes sites that were not shared between them. The oligonucleotide primers set, designed from the p33/34 genes was specific for these Theileria species, since no amplification was detected with DNA from Babesia ovata, B. bovis, Anaplasma marginale, A. centrale, Eperythrozoon wenyoni, bovine white blood cell and uninfected tick salivary gland. One tenth volume of the template prepared either from 25 μl of blood with 0.5% parasitaemia or individual tick salivary gland with 6 infected acini enabled sufficient amplification to be differentiated by the enzyme digestion. In addition, this system could be used to demonstrate the experimentally induced simultaneous infection of cattle with T. sergenti and T. buffeli. The PCR-based marker system therefore provide a means to differentiate T. sergenti from T. buffeli in the infected cattle blood and in the infected tick salivary gland.

B 2 A STUDY OF THE RELATIONSHIP BETWEEN PARASITE COUNTS, LESIONS AND DAILY WEIGHT GAINS IN PSOROPTES OVIS INFESTED CATTLE

Lonneux J.F., Bossaert K., Leclipteux T., Mignon B., Losson B.

Faculty of Veterinary Medicine, University of Liège, Liège, Belgium.

Psoroptes ovis counts (P.o.) in 12 cm^2 skin scrapings, extend of lesions expressed as percent body surface (Ex) and calculated with a standardized map, daily weight gains (D.W.G.) and anti-Psoroptes cuniculi antibody titres in ELISA (Ab) were recorded during 8 therapeutic field trials. Statistical analysis was performed to study the possible relationship between these different data .
The mean difference between D.W.G. of treated and control animals of the different trials ranged from 0 to 1,180 gr per day. The highest number of P.ovis mites collected from a 12 cm^2 skin scraping was 3,604 and the mean percentages of larvae, nymphs, males and females were 30%, 26%, 18% and 26% respectively. There was no correlation between either P.o. and clinical status or between Ab. and P.o. The correlation coefficient between Ab. and Ex ranged between 0,29 and 0,62 according to the location. Linear regression between mean percentage of Ex and D.W.G. in control animals (n=56)indicated a reduction in D.W.G. of 23 g per % of body surface involved when compared with the mean D.W.G. of the herd.
The D.W.G. of 118 treated animals increased significantly after treatment. However, there was no correlation between the extend of lesions and post-treatment D.W.G.

B 3 FASCIOLOSIS IN CATTLE: CORRESPONDANCES AND DISCREPANCIES BETWEEN SEVERAL METHODS OF DIAGNOSIS

Bossaert K., Leclipteux T., Protz M., Lonneux J.F., Losson B.J.

Faculty of veterinary medicine, University of Liège, Belgium

Different techniques for the diagnosis of Fasciola hepatica infection in cattle were compared: on one hand serology and coproscopy were evaluated in the field (n=383) and on the other hand serology, visual and histopathological liver examinations (n=17) were compared in slaughtered animals. Serological analysis was performed by ELISA test using F.hepatica somatic antigen coated plates.
In the field samples, 11.2 % had an optical density which was in good correlation with a positive coproscopy, 42.5 % had a positive ELISA test with a negative faecal examination, 2.6 % were positive for F.hepatica eggs with a negative serology. Amongst the 17 samples collected from the slaughter house, 10 had a good correlation between serology, histopathology and visual liver examination.
However, a positive serology with compatible microscopical lesions but in the absence of visual modifications was observed in 2 animals. Furthemore, 2 individuals had a positive serology in the absence of macro- and micromodifications and 3 animals had a negative serology together with compatible histopathological changes.
These discrepancies could be explained by either the antigen quality, the use of an anti-bovine Ig G1 labelled monoclonal antibody or some cross reactivity with other helminths.

B 4 FATAL STRONGYLOIDOSIS IN CALVES IN THE SAWDUST LITTER CONFINEMENT PENS IN JAPAN

N.Taira[1], Y.Nakamura[1], H.Kakihira[2] and S.Ura[2]
([1]National Institute of Animal Health, Tsukuba, Ibaraki 305, Japan; [2]Kyoto Animal Science R. & D. Center, Fushimi, Kyoto 612, Japan)

In 1978, it was observed that on two farms in the Kyushu district, the southern part of Japan, calves died suddenly on an unknown disease which was called "Pokkuri-disease". Until 1987, similar cases of this disease were seen on several farms in the Kyushu and Shikoku districts. After this year, our investigations demonstrated that the sudden death of the calves were caused by severe infections with *Strongyloides papillosus*. In 1991, we were able to prove experimentally that calves died if they were exposed in experimental infections to more than 10,000 L_3/kg/BW (Vet. Parasitol., 42, 247-256 (1992)). Therefore, the "Pokkuri-disease" was renamed "Sudden death type of strongyloidosis" in calves. Up to 1994, the disease was also observed in the northern part of Japan. There are now 882 known cases of "sudden death" on 64 farms in 29 of the 46 prefectures in Japan. The economic loss by the disease is estimated to US$6million (¥600million) up to 1994.

B 5 SEROPREVALENCE OF TOXOPLASMOSIS IN FINNISH LYNX (*FELIS LYNX*)

Antti Oksanen, Norwegian College of Veterinary Medicine, Department of Arctic Veterinary Medicine, Tromsø, Norway, and Eero Lindgren, University of Oulu, Department of Zoology, Oulu, Finland

During the winters 1992-93, 93-94 and 94-95, 70 lynx of different ages and both sexes were shot at various locations in Finland, Europe. The carcasses were shipped to the University of Oulu. Blood was collected from the heart or thoracic cavity and deep frozen. Following thawing, the samples were tested for *Toxoplasma gondii* specific antibodies in a commercial Modified Direct Agglutination Test (Toxo-Screen DA, bioMérieux, Charbonnières-les-Bains Cedex, France) in dilutions 1:40 and 1:4000. In case of positive reaction, a quantitative test was performed, either in dilutions 1:60, 1:180, 1:540 and 1:1620, or 1:6000, 1:18000, 1:54000 and 1:162000, depending on the results of the primary (screening) test. From most of the animals, also *Trichinella* sp. larvae were counted from the diaphragm and leg muscles using the digestion method. The age of the animals was determined according to dental cemental annulation, and varied from 1st year juveniles (about 8 to 10 months) up to 14 years. Fifty-one (73 %) of the lynx gave positive agglutination test reaction, indicating specific antibodies to *T. gondii*. The titres varied between 1:40 and 1:18.000. Factors associated with higher proportion of seropositivity were male sex, Odds ratio (OR) 5.89 ($p<0.01$), adult age, OR 4.75 ($p<0.01$), and *Trichinella* infection, OR 8.73 ($p<0.01$). No geographical difference could be demonstrated.

B 6 PORCINE SCHISTOSOMOSIS JAPONICA: THE HOST-PARASITE RELATIONSHIP IN RELATION TO INTENSITY AND CHRONICITY OF INFECTION

A.L.Willingham[1,2] and H.O.Bøgh[1]

[1]Danish Centre for Experimental Parasitology, Royal Veterinary and Agricultural University, Frederiksberg, Denmark and [2]Danish Bilharziasis Laboratory, Charlottenlund, Denmark

The host-parasite relationship of porcine schistosomosis japonica was investigated by experimentally infecting helminth-free Landrace/Yorkshire crossbred pigs once with different cercarial doses of an Anhui, China isolate of *S. japonicum*. Ninety-six pigs were divided into 4 groups (n=24) and injected intramuscularly with either 2000, 500, 100 or 0 cercariae, respectively. One-fourth of the pigs in each group (n=6) were slaughtered at 4, 11, 17 and 24 weeks post-infection (wpi), respectively, and schistosomes recovered. During the study faecal egg counts were conducted biweekly on all pigs from the time of patency and blood samples were taken biweekly from pigs in the 24 week group for clinicopathological assessment. Tissue egg counts were conducted on a 5 g sample from each liver and various tissue samples were taken for pathological examination. Faecal egg counts rose dramatically from the time of patency at 6 wpi, peaking at 8-10 wpi before declining abruptly with few eggs excreted after 18 weeks of infection. Eosinophil counts peaked at 6-8 wpi and then gradually decreased. The magnitude of both faecal egg and eosinophil counts corresponded with the infection dose as did the tissue egg counts. Livers appeared grossly normal in all pigs at 4 wpi, but egg granulomas and intralobular fibrosis were seen at 11 and 17 wpi, the degree of which corresponded to the infection dose. These hepatic lesions appeared nearly resolved at 24 wpi. Almost all of the schistosomes were found in the mesenteric veins of the large intestine with considerable invidual variation in regard to distribution noted in all groups. The results indicate that *S. japonicum* egg production in primary experimental porcine infection subsides rapidly following a brief period of high prolificacy.

B 7 CROSS—TRANSMISSION OF *CRYPTOSPORIDIUM MURIS*

Zhang Xichen, Yang Ju, Yin Jigang
Changchun University of Agriculture and Animal Sciences,
Changchun 130062, P. R. China

Cryptosporidium muris (C. muris) oocysts isolated from cow feces were inoculated into 3 rabbits, 6 suckling mice, 2 dogs, 2 lambs and sheep by oral route at dosage of 6×10^5, 3×10^5, 7×10^5, 10×10^5 and 10×10^5 respectively. The feces of rabbits, suckling mice, dogs, lambs and sheep were collected for examination everyday after inoculation. We had found that a number of oocysts could be detected in rabbits, suckling mice, lambs feces after inoculation on day 7, 7, 4 respectively, but the oocysts could not be detected in the dogs and sheep feces. Moreover, the oocysts isolated from rabbits, mice, lambs inoculated with oocysts isolated from cow are about the same in morphology and biologic properties especially in stained by modified acid—fast, which are quite different from those inoculated; cow C. muris oocysts could be stained by modified acid—fast but oocysts isolated from lambs, rabbits and mice inoculated could not. The C. muris oocysts isolated from lambs inoculated were infectious for mice following oral inoculation. So the significance of new finding of C. muris should be further studied.

GASTROINTESTINAL NEMATODE INFECTION LEVELS ON DAIRY FARMS IN THE NETHERLANDS AFTER THE FIRST GRAZING SEASON.

J. Poot, M. Eysker and T.J.G.M. Lam. Faculty of Veterinary Science, Utrecht University, P.O. Box 80.165, 3508 TD Utrecht, The Netherlands.

A wide variation in nematode infection levels has been demonstrated in dairy cattle herds in the Netherlands (Ploeger, thesis 1989). On most farms infections in young cattle were low and in some herds exposure during the first grazing season appeared to be insufficient to protect against production losses in the second grazing season. Since these surveys the use of highly suppressive early season systems of anthelmintic application has increased in the Netherlands. This implies that underexposure to helminth infections during the first grazing season may be even more a matter of concern. The summer of 1994 was warm and dry, probably enhancing the risk of underexposure. Therefore a seroepidemiological study, using crude worm Ostertagia and Cooperia antigen, was performed on 20 dairy calf herds after the end of the first grazing season. As reference 4 groups with well defined natural nematode infections were used, one with moderate infections, two with light infections and one without infections. Infection levels on the 20 farms were as follows: lower than light infections (13), light infections (2), between light and moderate infections (4), higher than moderate infections (1). High to moderate infection levels were predominantly found in set stocked herds and low infection levels in herds that had been moved regularly. This did not seem to be related with suppressive anthelmintic treatment. Owners of the 6 herds with the lowest infection levels were informed that prophylaxis against parasitic gastroenteritis should be extended to the second grazing season.

B 8

B 9 EPIDEMIOLOGY OF *DIROFILARIA IMMITIS* AND *SPIROCERCA LUPI* IN GUANGDONG PROVINCE

Mingquan Xie, Huixian Wu, Jiangfei Zhang, Fuquan Zhang and Liena Wen
Veterinary Medicine Institute, Guangdong Academy of Agricultural Sciences, P. R. China

D. immitis mainly parasitizes in right-sided heart and lung, and some of it were discovered in liver and trachea with clinic sigh of circular breakdown, difficult respiratory, hemophthisis and falling sickness. *S. lupi* parasitizes the walls of gullet or big artery forming nodes as tumour. Those could cause animal death when they are infested heavily. 85 dogs including 6 small, 30 middle and 49 big animals were investigated for their infection rates and density of both parasites by the method of anatomy in a county named Xingning, which indicated that among 30 middle size dogs, three of them were infected by *D. immitis*, with two parasites for each dog in the hearts, and one was infected by *S. lupi* with 3-5 parasites in the wall of gullet and main artery of the dogs. Among 49 big dogs, 30 infected by D. lupi in hearts with the density 2-27, three in main artery with 2-3 parasites for each dog, one in lung with the density of 4. There were 10 dogs infected by *Spirocerca* in main artery with the density of 2-15, 11 in the wall of gullet. There were not above parasites to be discovered in 6 small dogs. Middle and big dogs were severely infected by the two species parasites in Xingning county of Guangdong Province, China. The main reason is that the intermediate hosts of both kinds of parasites extensively spread in those region. It is important to wipe out intermediate hosts for the control of two diseases.

B10 EPIDEMIOLOGY OF FELINE HEARTWORM INFECTION: LABORATORY STUDIES ON TRANSMISSION AND ON HOST PREFERENCE OF MOSQUITO VECTORS

Abdelmoneim E. Mansour, MS[1], John W. McCall, PhD[1],
Tom L. McTier, PhD[2], Nonglak Supakorndej, MS[2], and Richard Ricketts[2]
[1]Department of Parasitology, College of Veterinary Medicine, University of Georgia, Athens, GA, [2]TRS Labs, Inc. Athens, GA

Two studies were conducted to clarify the vector-host-parasite relationship in the epidemiology of feline heartworm (HW) infection. Nine male and 9 female domestic shorthair cats and a beagle dog were anesthetized and placed in separate mosquito-proof containers. Fifty to 143 *Aedes aegypti* with infective larvae (L_3) of *Dirofilaria immitis* were released into each container for 45 minutes. The average number of L_3 transmitted was estimated by multiplying the average difference in pre- and post-engorgement L_3 counts from dissected mosquitoes by the number that fed on each animal (214 L_3/cat, range 96-240; dog, 120 L_3). One male cat with 36 HW died 194 days postinfection (PI) and one with 14 HW died 211 days PI. The remainder were euthanized 271 days PI. Heartworms were found in 6 of 9 male (mean 14.0 per infected cat; range 2-36) and 5 of 9 female cats (mean 6.0; range 2-12), with 58 HW in the dog. The second study compared the attractiveness of cats and dogs to *A. aegypti*. For each comparison, a male cat and male dog were anesthetized and placed together in a container, into which 50 to 100 mosquitoes were released. Engorging mosquitoes were removed during a 45-minute period, at the end of which all nonengorged mosquitoes were removed. For 10 dog/cat comparisons, 22% of mosquitoes fed on the cats, mainly on the face, ears and paws, and 78% on the dogs, mainly on the face, ears, and body. The data demonstrate that HW infections in cats can be transmitted by mosquitoes and suggest that *A. aegypti* prefers dogs over cats.

B 1 1 UTILITY OF ELISA-BASED ANTIGEN AND ANTIBODY TESTS FOR DETECTION OF HEARTWORM INFECTION IN CATS

John W. McCall, PhD[1], Nonglak Supakorndej, MS[2], William Ryan, BVSc[3] & Mark D. Soll, BVSc[3] [1]Department of Parasitology, College of Veterinary Medicine, University of Georgia, Athens, GA, [2]TRS Labs, Inc. Athens, GA, [3] Merck AgVet, Rahway, NJ 08820

The sensitivity of ELISA antibody (Ab) tests for detecting prepatent, patent, and occult heartworm (HW) infections makes them candidates for diagnosing feline infection, if such tests can be shown to be specific for *Dirofilaria immitis*. A study was undertaken with two objectives. (1) To standardize an Ab test using serum taken from 20 laboratory-bred cats selected for known infection status of (a) no HW, (b) only female HW, (c) only male HW, and (d) male and female HW. Blood was collected prior to and at 2, 4, and 6 to 9 months post infection (PI). (2) To evaluate this test using sera from 215 strays from three separate cat populations -- one from Georgia, in each of 1989 and 1990 and one from South Carolina, in 1990. Cats were bled for Ab and antigen (Ag) tests and necropsied for HW. All of the 20 laboratory cats had negative Ab tests (Ab-) prior to infection (100% specificity), and the test was 80% sensitive (titers ≥ 1:40) in detecting infections at 2 months PI and 100% sensitive at 4 and 6 to 9 months PI. In the populations of strays, the percentage of Ab-positive (Ab+) cats was consistently higher than the percentage infected with adult HW. Of these cats, 28 (13.0%) were Ab+, but HW were found in only 3.7%. Seven of these 8 (87.5%) were Ab+; the Ab- cat had one immature female HW. Only 3 of the 28 Ab+ cats were antigen positive (Ag+) and only 3 of the 8 cats with HW were Ag+. The results suggest that Ab test may be a sensitive tool in the diagnosis of feline heartworm infection and disease.

B 1 2 RESISTIBILITY TO *THEILERIA SERGENTI* INFECTION IN HOLSTEIN AND JAPANESE BLACK CATTLE

Yutaka TERADA, Mitsugi ISHIDA* and Harumichi YAMANAKA
National Institute of Animal Health, Tsukuba, Japan
*National Grassland Research Institute, Nishinasuno, Japan

It has been claimed long time as an experiential knowledge on the field that Japanese Black cattle are more resistant to *Theileria sergenti* infection than Holstein cattle. To examine the difference in those breeds excepting for the external factors, Holstein and Japanese Black calves were infected with *T. sergenti* by infestation with the same number of infected ticks and observed under controlled conditions in artificial environmental chambers. No apparent differences between the two breeds were observed in the feeding numbers and feeding periods of ticks infested. The level and duration of parasitemia were clearly less and shorter in Japanese Black than those in Holstein calves. With progress of parasitemia, packed cell volume(PCV) and erythrocyte number decreased in both breeds. However, the minimum PCV and erythrocyte number recorded in Japanese Black were higher than those in Holstein calves. These results show the advantage of Japanese Black over Holstein calves regarding resistance to the *T. sergenti* infection under the experimentally controlled condition.

B 1 3 GEOGRAPHIC INFORMATION SYSTEMS AND CONTROL OF FASCIOLOSIS IN THE SOUTHERN USA

John B. Malone, Louisiana State University, Baton Rouge, Louisiana, USA

The high environmental sensitivity and focal nature of Fasciola hepatica results in wide variation in infection prevalence in enzootic areas. GIS provides a systematic way to define the preferences and limits of tolerance of parasites and to match these to the spatial and temporal suitability of the environment. Diseases have natural habitats in the same way as a species. GIS risk assessment models were developed for the lower Red River and Chenier Plain ecosystems of Louisiana using the percent of soil types on pastures, slope, stocking rate and linear extent of potential habitat associated with mapped hydrologic features on pastures. In both areas, the rank of egg shedding indices for F. hepatica and Paramphistomum spp. on individual farms were significantly related to soil-hydrology factors that determine the amount of snail habitat on pastures. A regional climate model was used to produce F. hepatica risk maps of Southcentral USA based on the growing degree day concept and Thornthwaite water budget. Used together, we propose that the regional climate model and ecosystem-based GIS models can be used together to predict long term and annual risk of fasciolosis losses in the southcentral USA.

B 14 CORRELATION BETWEEN THE NUMBER OF *Boophilus microplus*, *Babesia* spp. AND *Anaplasma marginale* PARASITAEMIAS IN CROSSBRED-DAIRY CATTLE. IN CHARQUEADA DISTRICT, SOUTHERN BRAZIL.

KASAI*, N.; DELL'PORTO, A.; PENNA, H.F.J.; LOUVANDINI, H. AND GENNARI, S.M.
*Dept. of Veterinary Preventive Medicine and Animal Health, Faculty of Veterinary Medicine and Zootechny-USP-CEP 05340-000-São Paulo-Brazil

From April/94 to March/95, a study related to the populational dynamics of *B.microplus* was made and to assess the existence of correlation with the levels of parasitaemia by *Babesia* spp. and *A.marginale*. Five pairs of animals, each pair represented by a cow and its offspring aged 1 to 3 months were selected from three small farms and monthly 1 or 2 new pairs of animals were added to the group. Blood was taken at each 28 days and accompanied by correspondent tick counts. For the tick counts, the right side of the animal was chosen and the results were duplicated to obtain the valuation of the total number of ticks. The thin blood smears were made each 28 days, by punction of the caudal vein. The quantification of the parasitaemia was made in 2,000 erythrocytes. The results from April to August/94 were tranformed in $\log_{10}(x+1)$ to adjust to the normal distribution, analysed by the SAS 91 system and considered significant the values of $p \leq 0.05$. Among the calves there was observed a positive correlation between number of ticks and *Babesia* spp. parasitaemia in April, May and August. For the cows, no correlation was found. Between the numbers of ticks and *A.marginale* parasitaemia, no correlation was observed, suggesting the possibility of another vector for *Anaplasma* sp. transmission. Monthly comparison of the mean tick numbers showed correlation, meaning that there are fluctuations in the population of ticks. The same occurred when the comparison was made between the farms. It could be related to the tick control system adopted in each property. Cows presented more ticks than calves, this may be explained because the later are separated from the adults.

B 15 FIELD EFFICACY OF POUR-ON AND INJECTABLE FORMULATIONS OF MOXIDECTIN AND IVERMECTIN IN PSOROPTES OVIS INFESTED CATTLE: PARASITOLOGICAL, CLINICAL AND SEROLOGICAL DATA

Lonneux J.F., Losson B.J., Mignon B., Bossaert K., Leclipteux T.

Faculty of Veterinary Medicine, University of Liège, Liège, Belgium.

In a highly infested cattle herd, 33 animals were randomly allocated to five groups on the basis of P.ovis counts performed on day -7. The control group was limited to five animals (Group 1). Groups 2, 3, 4, 5 comprised 7 animals each and were treated at the recomended dose with ivermectin pour-on (I.P.O.), ivermectin injectable (I.I.), moxidectin pour-on (M.P.O.) and moxidectin injectable (M.I.) respectively. Lesions were recorded on a standardized map on days 0 and 28. Living mites were counted in skin scrapings harvested on days 0, 7, 14, 28, 42 and 56 post-treatment (P.T.).The antibody kinetics (Ab.) was studied on serially diluted sera by ELISA according to Lonneux et al. (1993). The Ab. levels were expressed as the dilution giving the positive/negative cut-off. In treated groups, all parasite counts were negative on days 28 and 42 P.T. On day 56, living P.ovis mites were found in one animal of the I.P.O. treated group. The decrease of the Ab. titre in this animal was abnormal. The same observation was made for the two neighbouring animals although they remained parasitologically negative. Similar abnormality was noticed in one animal of the M.P.O. treated group. Mean daily weight gain in theI.P.O. group was slightly lower when compared to the other treated groups. Nevertheless, statistical analysis of the different data did not show any significant difference between the different treatment groups.

B 16 KINETICS OF SPECIFIC ANTIBODY RESPONSE AFTER SUCCESSFUL TREATMENT IN NATURALLY PSOROPTES OVIS INFESTED CATTLE

Lonneux J.F., Bossaert K., Leclipteux T., Mignon B.,Losson B.J.
Faculty of Veterinary Medicine, University of Liège, Liège, Belgium.

After treatment, microscopical examination of skin scrapings frequently fails to detect surviving P.ovis. Methods based on the study of the kinetics of specific antibody response in P.ovis infected animals could be useful for the early detection of a therapeutic failure or natural reinfection.
The decline of anti-P.ovis antibody titres after treatment was compared in 3 groups of cattle naturally infested with P.ovis. Group 1 (n=14), 2 (n=6) and 3 (n=6) were successfully treated with flumethrin pour on (Bayticol T.M.) at 2 mg/kgb.w. at 10 day interval or with a single dose of moxidectin injectable (0.2 mg/kg b.w.) or pour-on (0.5 mg/kg b.w.) respectively. Specific antibody levels were assessed with an ELISA technique using Psoroptes cuniculi as antigenic source. A limiting dilution technique was used. Sera from Group 1 animals were collected on days 0, 7, 14, 28 and 50 post-treatment (P.T.) whereas sera from Group 2 and 3 animals were harvested on days 0, 14, 28, 42 and 56 P.T. All individual antibody titres started to decline between day 7 and day 14 P.T. The exposure to antigen seemed to stop with the end of parasite activity.
A linear decrease was observed. It has been extrapolated that mean geometric titres of Group 1, 2, 3 animals would be seronegative after 153, 141 and 136 days respectively.

B 17 PERSISTENT ACTIVITY OF MOXIDECTIN AGAINST *OSTERTAGIA OSTERTAGI* AND *DICTYOCAULUS VIVIPARUS*.

J Vercruysse, E Claerebout, D Demeulenaere, H Hilderson, P Meeus and E Deroover*. Department of Parasitology, Faculty of Veterinary Medecine, Salisburylaan 133, B9820 Merelbeke, Belgium.*Cyanamid, Rue du Bosquet, 15, B1348 Louvain-la-Neuve, Belgium

Moxidectin is a milbemycin macrocyclic lactone endectocide for the control of both internal and external parasites of domestic animals. A study was conducted to assess the duration of moxidectin anthelmintic persistence against *Ostertagia ostertagi* and *Dictyocaulus viviparus* by measuring post-treatment nematocidal effectiveness of both the topical and injectable formulation. Twenty-one nematode-free calves were randomly allocated to three groups of 7 animals. One group remained untreated (C group), one group was treated with moxidectin 0.5% pour-on at a dose of 0.5 mg moxidectin/kg (P group) and the last group was injected subcutaneously with moxidectin 1% solution at a dose of 0.2 mg/kg (I group). Animals of the C, P and I group were infected daily with 2000 L3 of *O. ostertagia* and 50 L3 of *D. viviparus* from the day after treatment until day 33. Animals were killed for worm counts two days after the last infections in order to define the duration of the prolonged efficacy based on the the efficacy against the different parasitic stages found at necropsy. The percentage efficacy was calculated in terms of the geometric mean worm burden (by parasitic stage) of the treated groups compared with the mean worm burden of the untreated group. The study demonstrated a persistent efficacy, for both moxidectin formulations, of at least 33 days for *O. ostertagia* (>94 %) and at least 29 days for *D. viviparus* (>99), the maximum measurable timepoint in this experiment for the respective parasites. A second study to determine the endpoint of the prolonged efficacy is in progress. The prolonged efficacy of moxidectin could make it possible to reduce the number of treatments required for grazing calves and to consider the use of moxidectin in strategic control programmes.

B 18 EFFICACY AND PERSISTENCY OF MOXIDECTIN 2% EQUINE GEL AGAINST SMALL STRONGYLES (CYATHOSTOMINAE) IN NATURALLY INFECTED HORSES.

C. Genchi, F. Basano Solari, B. Nogara.
Department of Parasitic Diseases of Domestic Animals, University of Milan, Italy.

A field trial to assess efficacy and persistency of moxidectin equine gel against small strongyles in naturally infected horses was carried out. 45 horses were ranked in 3 groups each of 15 animals according to the age and then to mean fecal EPG counts carried out on days -7 and -14. Within each group of 3 animals, 1 was randomly assigned to group receiving moxidectin gel (0.4 mg/Kg), 1 to group receving ivermectin paste (0.2 mg/Kg) and 1 to group receiving pyrantel pamoate paste (60 mg/Kg). Animals were treated on day 0. Efficacy and persistency of treatments were assessed by EPG counts every 14 days from day 14 to day 84, and every 28 days from day 84 to day 196. Throughout the study, when at least 50% of animals of the same treatment group reached a level ≥200 EPG, they were retreated within 14 days after sampling. At pretreatment EPG counts (days -14, -7), all animals were positive for strongyle eggs (mean 519, range 150-2000), identified as Cyathostominae by larval coproculture. On day 196 posttreatment, 5 horses of moxidectin-treated group were positive for strongyle eggs (mean 253, range 150-2100). In the ivermectin-treated group, on day 112, 9 animals had EPG counts ranging 100-1950 and were retreated with ivermectin. On day 196, 9 were still positive (50-1500 EPG). In the pyrantel-treated group, animals were retreated on day 84. On day 196, 13 animals were positive (EPG range 150-4750). No side effects were observed throughout the study. Moxidectin showed high efficacy against small strongyles in horses and a longer period of protection than ivermectin and pyrantel. During the trial a second treatment was not necessary only in moxidectin treated group.

B 19 PREVALENCE OF BENZIMIDAZOLE RESISTANT NEMATODES IN DAIRY GOAT FARMS IN WESTERN FRANCE.

Chartier C., Hubert J., Pors I., Benoit C., Rocheteau D. and Bernard N.

CNEVA/Station Régionale de Pathologie Caprine, 60 rue de Pied de Fond, BP 3081, 79012 NIORT Cedex, France

Fifteen dairy-goat farms, without known problem of strongylosis, were investigated in the western part of France from May to July 1994 to assess the prevalence of benzimidazole (BZD) resistance and the species of nematodes involved. Resistance was determined *in vivo* by a faecal egg count reduction (FECR) test (*Coles et al., 1992*) using fenbendazole at 10 $mg.kg^{-1}$ and *in vitro* by an egg hatch assay (EHA) using thiabendazole (*Beaumont-Schwartz et al., 1987*). FECRs ranged from 0 to 96 %. Resistance (FECR < 95 % and lower 95 % confidence limit < 90 %) to FBZ was present in 14 of the 15 goat farms. Post-treatment larval identifications indicated that *Trichostrongylus* and/or *Teladorsagia* were predominant in 7 farms, *Haemonchus* in 3 farms and a mixture of these nematodes with *Oesophagostomum* in the remaining 4 farms. The values of LD50 (TBZ) in the EHAs ranged from 0.125 to 0.449 $\mu g.ml^{-1}$ indicating BZD resistance in virtually all the farms. The drenching practices in these herds could explain the very high prevalence of BZD resistance found : i) a high frequency (monthly) of drenching during the grazing season ii) an exclusive use of BZD compounds (zero milk withdrawal) and iii) a systematic underdosing due to the pharmacokinetic peculiarities of goats concerning BZD drugs (higher dosages required).

B 20 ISOLATION AND DROUGHT IN THE DEVELOPMENT OF ANTHELMINTIC RESISTANCE IN NEMATODES

E. PAPADOPOULOS*, C. HIMONAS*, G. COLES**

*ARISTOTELIAN UNIVERSITY, THESSALONIKI, GREECE

**UNIVERSITY OF BRISTOL, BRISTOL, U.K.

A survey was conducted for anthelmintic resistant nematodes in sheep and goats in Greece.
Anaerobically stored faecal samples were returned to the laboratory for testing with the egg hatch and the larval development tests.
Of 204 flocks examined on the mainland only 3 had benzimidazole resistant nematodes. In all cases animals containing resistant nematodes had recently been imported. On the mainland not all animals in one flock are treated and flocks intermingle on common grazing. By contrast 13 out of 90 samples from small islands had benzimidazole resistant *Teladorsagia (Ostertagia) circumcincta*. This represents resistant on 6 out of 15 islands. In one case animals were treated with 66 mg/Kg thiabendazole. At necropsy the efficacy of thiabendazole against *T. circumcincta* was only 44%.
Flocks are smaller on the islands, can be isolated and experience prolonged droughts each year. Although only one or two doses are usually given each year, the whole flock tends to be treated. This suggests that a combination of drought and isolation might be important factors in the selection for anthelmintic resistance.

B 2 1

THE RATE OF RESISTANCE DEVELOPMENT BY H. CONTORTUS TO ENDECTOCIDES

G. T. Wang, H. Berger, K. Simkins and D. Rock;
American Cyanamid Company, Princeton, New Jersey, U.S.A., 08543

Infective larvae (L_3) of Haemonchus contortus, which were originally sensitive to both moxidectin and ivermectin, were used to inoculate sheep to establish patent infections before treatments with subtherapeutic levels of these endectocides. The concentrations of individual compounds were selected on the basis of allowing sufficient egg production for cultivation of L_3 for subsequent passages. After 12 passages, the sensitivity of each of 2 drug passage strains (MO-F12 and IV-F12) and the parent sensitive strain (P12) were evaluated in one nonmedicated control group and 4 dose levels of moxidectin or ivermectin. Based on worm counts at day 14-15 posttreatment, ED95 was calculated for each strain. P12 was determined to have an ED95 of 0.00179 mg/kg b.w. for moxidectin and 0.01125 mg/kg b.w. for ivermectin. Meanwhile, the ED95 for MO-F12 was 0.00949 mg moxidectin/kg b.w. and for IV-F12 it was 0.10874 ivermectin/kg b.w. Relative to P12, the resistant ratio was calculated to be 5.30 for MO-F12 and 9.67 for IV-F12. Against the P12, ivermectin needs a 6.28 times higher dosage than moxidectin. Moxidectin was 11.46 times more potent than ivermectin after 12 passages. The results indicated that moxidectin is more potent than ivermectin and that the rate of resistance development to both endectocides by H. contortus was relatively slow. It is concluded that H. contortus had a relatively slower rate of resistance development to moxidectin than to ivermectin.

B 22 ANTHELMINTIC RESISTANCE IN SHEEP IN THE NETHERLANDS

F.H.M. Borgsteede[1], J.J. Pekelder[2], D.P. Dercksen[2], J. Sol[2], P. Vellema[2], C.P.H. Gaasenbeek[1], J.N. v.d. Linden[1]
[1] DLO-Institute for Animal Science and Health (ID-DLO) Parasitology Section, P.O. Box 65, 8200 AB LELYSTAD, The Netherlands, [2] Animal Health Services in The Netherlands

The prevalence of anthelmintic resistant nematodes in sheep was investigated with a faecal egg count reduction test (FECRT), larval cultures before and after treatment and an egg hatch assay (EHA) on 71 farms in 1994 in the Netherlands. On all farms a questionnaire was filled in with relevant data for the survey, such as anthelmintic usage during the last years, grazing management etc. The farms were visited twice with an interval of 14 days. During the first visit, groups of 15 sheep were formed. All sheep were individually eartagged, weighed before treatment and rectally sampled. One group remained untreated as a control group. Other groups were treated with a benzimidazole (oxfendazole 5 mg kg^{-1} on 70 farms), ivermectin 0.2 mg kg^{-1} as an oral formulation (51 farms), and levamisole 7.5 mg kg^{-1} also as an oral formulation on 36 farms. Procedures were according to the standardised WAAVP methods (Coles *et al.*, Vet. Parasitol., **44** (1992):35-44). Based on the FECRT benzimidazole resistance was present on 56 farms, 2 were suspected, on 9 no resistance was observed, while 3 farms could not be tested due to a too low egg output before treatment. No clear indications for resistance to ivermectin and levamisole were found. Benzimidazole resistance was demonstrated in *Haemonchus contortus, Cooperia curticei, Ostertagia* spp. and/or *Trichostrongylus* spp. No resistance was seen in species from the genus *Nematodirus, Chabertia ovina* and/or *Oesophagostomum* spp. The results of the EHA confirmed the results of the FECRT. On 59 farms the ED50 value was > 0,12 μg ml^{-1} thiabendazole.

B23 ISOLATION OF A FIELD STRAIN OF *HAEMONCHUS CONTORTUS* RESISTANT TO DORAMECTIN, IVERMECTIN AND MOXIDECTIN

C.M. Santos[1], A.A. Bridi[2], L.A. Carvalho[2], J.B. Cruz[2], and C. Benitez-Usher[2]. [1]Marsiaj & Giudice Ltda, 97500-500, Uruguaiana, RS, Brazil and [2]Merck Research Laboratories, Rahway, NJ 07065 USA

There is evidence for side resistance between members of the avermectin/milbemycin (macrocyclic lactone) group, all of which have similar molecular structure and share a common mode of action. A field strain of *Haemonchus contortus* from a commercial sheep farm in Brazil was isolated on the basis of failure to reduce fecal egg counts after treatment with moxidectin. Infective larvae from donors were used to induce infection in a uniform group of 20 worm-free weaner lambs for the conduct of a controlled efficacy study. Twenty-five days after infection, animals were divided into four equivalent groups and allocated to one of the following treatments: untreated control, doramectin, ivermectin and moxidectin; each product was given at the dose of 200 mcg/kg body weight by subcutaneous injection. There were no significant differences ($p>0.25$) between any of the treated groups in the numbers of *H. contortus* recovered at necropsy; efficacy ranged from 0% to 52%. This study confirms the presence of a field strain of *H. contortus* from sheep which shows side resistance between doramectin, ivermectin and moxidectin. Strategies for control of anthelmintic resistance in sheep should reflect this relationship, and the term 'macrocyclic lactone (MCL) resistance' should be used when referring to resistance to this action class.

C 1 DIROFILARIA IMMITIS: ANTIGENIC CROSS-REACTIVITY AMONG NEMATODES

Mineo Hayasaki
School of Veterinary Medicine, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183, Japan.

Purification of specific antigenic molecules in parasites is major purpose on parasitology. For this purpose, many attempts had done to separating or obtaining species specific antigenic molecules. Unfortunately, succesful antigen for protection of parasitic infection were very few and most of it were evaluated under experimental condition.

Even monoclonal antibody(mAb) can react with many antigen franctions separated from parasitic species, despite of recognizing theoretically only one epitope of the antigen. Immunoblotting and immunofluorescent technique using D. immitis-specific mAb showed many antigenic cross-reactions, not only among animal filaria species but also among canine intestinal parasites. From these data, complexicities of antigen components and antigenic cross-reactivity among many kinds of parasite species are revealed, and also a difficulty in obtaining of purified species-specific antigenic molecules is suggested.

C 2 EFFECTS OF VACCINATION WITH A RECOMBINANT SCHISTOSOMA BOVIS-DERIVED GLUTATHIONE S-TRANSFERASE ON EXPERIMENTAL AND NATURAL S. MATTHEEI INFECTIONS IN CATTLE.

J. De Bont, J. Vercruysse, P. Meeus, J.M. Grzych, A. Capron
Department of Parasitology, School of Veterinary Medicine, University of Gent, Belgium.

The potential of a recombinant Schistosoma bovis-derived glutathione S-transferase (rSb28GST) to protect cattle against S. mattheei infection was tested under experimental and natural challenge in Zambia. High specific antibody titres were measured after the second inoculation. Vaccinated calves and non-vaccinated controls were then challenged either with a single dose of 10.000 S. mattheei cercariae percutaneously (Group A) or naturally on a farm infected with S. mattheei (Group B). Calves from groups A and B were perfused 12 weeks and 9 months later, respectively. All vaccinated animals in Group A developed clinical schistosomiasis with high average faecal egg count (742 eggs per gram), worm burden (6515) and tissue egg count (4.2 million) at perfusion. Infections were comparable in vaccinated and non-vaccinated calves indicating that the immunization protocol used did not protect cattle against the massive experimental challenge. Infections in Group B animals were much lighter, as indicated by the mean faecal egg output (12.8 eggs per gram) and worm count(139) in non-vaccinated controls. At perfusion, a significant reduction in the mean worm burden, and average faecal egg and miracidial counts was recorded in the vaccinated group. The average number of tissue eggs per adult female worm was found higher in vaccinated animals than in controls, probably as a result of a crowding effect. However, the mean tissue egg count in vaccinated animals was significantly reduced as compared to controls. It therefore appears that the recombinant rSb28GST can provide significant protection against S. mattheei infection in cattle by affecting worm viability.

C 3 CHANGES OF T-CELL SUBPOPULATION IN THE PERIPHERAL BLOOD OF CHICKENS INFECTED WITH *LEUCOCYTOZOON CAULLERYI*

Takashi Isobe, Shin-ya Shimizu, Naotoshi Tsuji and Kameo Shimura
National Institute of Animal Health, Tsukuba, Ibaraki, JAPAN

Chicken leucocytozoonosis is a hemoprotozoan disease caused by *Leucocytozoon caulleryi*. The cell-mediated immunity is suggested to play an important role for the resistance of chickens to reinfection with *L. caulleryi*. This study identified the effect of *L. caulleryi* infection on specific T-cell subpopulation in the peripheral blood of chickens.

3-week-old specific-pathogen-free chickens were inoculated with 42 sporozoites of *L. caulleryi* intravenously for primary infection. They were reinoculated with 7.5×10^3 sporozoites 35 days after primary inoculation. Packed cell volume (PCV), the number of monocytes and lymphocytes, and the percentage of major T-cell subsets in the peripheral blood were analysed by flow cytometer every week after both infection. Parasites were detected by blood smears.

In primary infection, PCV decreased, and the number of monocytes and lymphocytes increased in the infected chickens 3 weeks after infection (AI). CD8 and T-cell receptor (TCR) $\gamma\delta$ bearing cells extremely increased at 3 weeks AI while no significant differences were found in CD4 and TCR $\alpha\beta$ bearing cells. At 3 weeks AI, the protozoa started to disappear from the peripheral blood. In secondary infection, the major T-cell subsets tended to increase a little but not significant. No parasites were detected in the peripheral blood.

The marked increase of CD8 and TCR $\gamma\delta$ bearing cell in chickens infected with *L. caulleryi* at 3 weeks after primary infection may be indicative of some considerable alternation in cell subsets that are involved in the clearance of the protozoa from the peripheral blood.

C 4 PROTECTIVE IMMUNITY AGAINST CHALLENGE INFECTION WITH *BABESIA MICROTI* IN MICE

Ikuo IGARASHI[1], Reiko SUZUKI[2], Yoshitaka OMATA[2], Atsushi SAITO[2], Yutaka TOYODA[1] and Naoyoshi SUZUKI[1]. [1]The Research Center for Protozoan Molecular Immunology, and [2]Department of Veterinary Physiology, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan.

Mice infected with *Babesia microti* produce transient high parasitemia and thereafter recover naturally from the primary infection. These mice are resistant to challenge infection. In the present study, the role of T cells in the protective immunity against challenge infection was examined by administration of mAb against T-cell subsets and by the cell transfer from immune mice. Mice were challenged with $1x10^7$ parasitized erythrocytes six weeks after the primary infection, and they showed a strong portection against the challenge infection with a very low level of parasitemia (about 0.1%) throughout the experiment. However, $CD4^+$-depleted immune mice showed high parasitemia with a peak of 24.3% on the 18th day and kept moret than 10 % parasitemia until 30 days after challenge infection. Whereas, $CD8^+$-depleted immune mice showed similar infection course with that of unt-reated mice. Transfer of $CD4^+$-enriched spleen cells suppressed parasitemia in recipient mice than $CD8^+$-enriched spleen cells did. Mice transferred with anti-*B.microti* serum did not confer the protection on receipient mice. These results suggest that $CD4^+$T cells play an important role in the protective immunity against challenge infection with *B. microti.*

C 5 THE IMMUNOHISTOCHEMICAL LOCALISATION OF PEPSINOGEN IN THE ABOMASAL MUCOSA OF SHEEP INFECTED WITH HAEMONCHUS CONTORTUS AND IN PARASITE-NAIVE CONTROLS.

Ian Scott, Quintin McKellar, Jane Irvine* and Alma Dick*
Departments of Veterinary Pharmacology and Pathology*, University of Glasgow Veterinary School, Glasgow, United Kingdom.

The mechanism behind the elevation of plasma pepsinogen in ovine haemonchosis has not been extensively researched. We investigated the fate of cell types synthesising pepsinogen in response to parasitic infection in Hampshire Down lambs (a parasite susceptible breed) given trickle infections for ten weeks. Abomasal tissues were obtained at necropsy and stained immunohistochemically for pepsinogen, using a commercially available anti-bovine pepsinogen A antibody. In uninfected animals pepsinogen immunoreactivity was confined to Chief cells and Mucous Neck cells of the fundic mucosa, and was strongest in the former. Tissues from infected animals were significantly thicker ($p<0.001$). Tissue thickness was apparently increased by hyperplasia of cells at the isthmus, i.e. the junction of pit and gland and these cells were positive for pepsinogen. In infected animals chief cell staining was sometimes weaker, but these cells appeared otherwise normal in H&E stained sections. Total tissue pepsinogen was measured biochemically in mucosal homogenates and variation between animals was considerable. There was no significant difference overall between pepsinogen content of tissues of control and infected animals ($p>0.50$) however this was probably due to the conflicting effects of reduced content of chief cells but increased total number of pepsinogen producing cells. The results are consistent with pepsinogen hypersecretion in response to infection, whereby chief cells immediately secrete pepsinogen rather than storing it. Alternatively reduced pepsinogen production by chief cellsmay be offset by increases in the number of cells producing pepsinogen.

C 6 PROTECTIVE EFFECTS OF A STAGE-SPECIFIC, SPECIES CROSS-REACTIVE MONOCLONAL ANTIBODY AGAINST THE MAJOR OOCYST WALL PROTEIN OF EIMERIA TENELLA.

Karim, M. J., Basak, S. C. and Trees, A. J.
Veterinary Parasitology, Liverpool School of Tropical Medicine, Pembroke place, Liverpool L3 5QA, United Kingdom

The oocyst wall of chicken *Eimeria* spp. consists of a 10 nm thick outer lipid layer and a 90 nm thick inner layer of glycoprotein. A monoclonal antibody (Mab) was produced against the major oocyst wall protein of *E. tenella* by inoculation of mice with a gel slice from the 12 kDa region of a SDS-PAGE gel and screening of hybridomas by immunofluorescence against oocyst wall fragments. The IgM isotype Mab also binds to oocyst wall fragments of other chicken *Eimeria* spp., but not to sporozoites of *E. tenella*. In tissue sections stained by immunoperoxidase, the Mab bound to the immature oocyst and to mature macrogametocytes, but not to schizonts, merozoites and microgametocytes. Immunogold labelling revealed that the Mab bound to epitopes on the inner layer of the inner wall of the oocyst. In infected chicks, passively immunized by the intravenous inoculation of the Mab, the total oocyst output was reduced compared with controls. The results suggest the presence of a common antigen both on the oocyst wall and on the macrogametocytes. Although the cross-reactivity of the Mab limits its possible use for identification of oocysts of chicken *Eimeria* spp., it may help isolate gametocyte associated antigens which are targets for a transmission-blocking vaccine.

C 7 DETECTION OF IMMUNE RESPONSE IN CHICKEN INFECTED WITH LOCAL ISOLATE OF ATTENUATION Eimeria tenella THROUGH SELECTION FOR PRECOCIOUSNESS

Umi Cahyaningsih
Faculty of Veterinary Medicine, Bogor Agricultural University
Bogor, Indonesia

The immune response in this research was determine by counting the oocyst productions with Mc Master method and by measuring the antibody titers with ELISA method. To count the oocysts production 45 chickens were used and divided into 3 groups:control, infected with E. tenella precocious and parent line. Dose of infection were $1X10^4$(1^{st} infection) and $1X10^5$(2^{nd}infection). The feces were collected from the 3^{rd} until the 9^{th} day after 1^{st} and 2^{nd} infection (challenge). Four groups each group consist of 15 chickens were used to measure the antibody titers. The serum collection were conducted at 0,7,14 days after the first infection(a.f.i) then the chickens were challenge at 14 days a.f.i. The serum collection were continued at 7 and 14days after second infection(a.s.i.). The results of this research indicated that the oocyst productions of precocious line were higher than parent line at 1^{st}infection, but after 2^{nd}infection precocious line was significantly lower than parent line at 6^{th} and 8^{th} day a.s.i.($P < 0.01$). In the precocious line, the oocyst production after 2^{nd}infection were lower than in the 1^{st}infection and significant difference were found at 5^{th} day($P < 0.05$) and 6^{th} day($P < 0.01$) a.f.i. But in the parent line was lower only until 7 days, however at 8^{th} and 9^{th} day were higher a.s.i Antibody titers to E. tenella were higher in precocious line than in parent line, both after 1^{st} and 2^{nd} infection. Antibody titers of precocious line significantly higher than parent line at 7 days a.f.i.($P < 0.01$) and 14 days a.s.i.($P < 0.05$). The conclusion of this research indicated that the infection with E. tenella attenuated through selection for precociousness showed a higher immune response compare with immune response that caused by E. tenella without attenuated through selection for precociousness (parent line).

C 8

BENEDENIINE (MONOGENEA: CAPSALIDAE) PARASITES OF CULTURED MARINE FISH IN JAPAN

M.G. Bondad-Reantaso[1,2], K. Ogawa[2], and H. Wakabayashi[2]

[1]Fish Health Section, Bureau of Fisheries and Aquatic Resources, 860 Quezon Avenue, Quezon City, Metro Manila 1003, Philippines. [2] Department of Fisheries, Faculty of Agriculture, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113, Japan

Benedeniine parasites affecting cultured marine fish in Japan are limited to two pathogenic and highly host specific species, Benedenia seriolae on yellowtail and amberjacks and B. hoshinai on striped knifejaw. During the last 5 years, new types of benedeniine infection caused by B. epinepheli and Neobenedenia girellae occurred on many species of marine cultured and aquarium fish. The taxonomy, morphological variation in haptoral sclerites, host range, geographic distribution and associated pathology of the two species are presented. In addition, the relation of recent changes or trends in Japanese aquaculture in the occurrence of these parasites are discussed.

IMMUNE RESPONSE OF JAPANESE FLOUNDER (*PARALICHTHYS OLIVACEUS*) AGAINST *NEOBENEDENIA GIRELLAE*, A SKIN PARASITE OF CULTURED MARINE FISH IN JAPAN

M.G. Bondad-Reantaso[1,2], K. Ogawa[2], T. Yoshinaga[3] and H. Wakabayashi[2]

[1]Fish Health Section, Bureau of Fisheries and Aquatic Resources, 860 Quezon Avenue, Quezon City, Metro Manila 1003, Philippines. [2] Department of Fisheries, Faculty of Agriculture, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113, Japan. [3]National Research Institute for Fishery Science, Fuku-ura 2-12-4, Kanazawa-ku Yokohama 236, Japan.

Neobenedenia girellae (Hargis, 1955), an exotic fish parasite, introduced to Japan through infected amberjack (*Seriola dumerili*) fry imported from Hong Kong and Hainan, China, is now an important pathogen of cultured marine fish in Japan. Investigations to determine the immune response of Japanese flounder (*Paralichthys olivaceus*) against the parasite revealed the existence of an acquired protection. This was demosntrated by a reduction in the number and body size of parasites on fish previously exposed to *N. girellae* infection (= primed fish). In this experiment, antibody in the sera of primed fish was not detected by enzyme-linked immunoabsorbent assay (ELISA). In another experiment, antibody in the sera of fish immunized by injection with sonicated adult *N. girellae* antigen was detected by ELISA. However, when challenged with the oncomiracidia of *N. girellae*, the number of parasites established on antigen-injected and PBS-injected control fish were almost similar. Results of the above experiments indicate that serum antibody production was not involved in the protection against *N. girellae*.

C 10 AN EPOZOOTIC OF ANGUILLICOLOSIS IN AQUACULTURED AMERICAN EEL, ANGUILLA ROSTRATA, IN TAIWAN

H.K. OOI, W.S. WANG,*H.Y. CHANG,#C.H. WU, C.C. LIN AND M.T. HSIEH, Dept. of Veterinary Medicine, National Chung Hsing University, Taichung, Taiwan, *Livestock Disease Control Center of Yunlin County, Taiwan, #Taiwan Fisheries Research Institute, Taiwan, R.O.C.

An epizootic due to a parasitic nematode, the swim bladder parasite Anguillicola crassus, was recently observed in aquacultured American eel, Anguilla rostrata, in Taiwan. Besides the swim bladder, adult worms were observed to parasitize in the peritoneal cavity. Larvae were seen also seen in the serosa of the swim bladder. The intestine of the worms were overly dilated and filled red blood cells of the eel. The cause of death of the eel may be attributed to severe anemia and peritonitis. This epizootic of anguillicolosis was seen extensively in many aquaculture farms that reared American and European eel, Anguilla anguilla, in Central Taiwan. Tens of thousands of these eels died in the first few months of 1995 and a huge economic loss was inflicted. Anthelmintic was found to be effective in preventing the death of the affected eels. Until recent years, most of the eels reared in Taiwan were the Japanese eel, Anguilla japonica. No apparent symptom nor fatality has been reported in Japanese eel infected with A. crassus. The recent introduction of American and European eels into Taiwan has given rise to an anguillicolosis epizootic because of the differences in susceptibility of these eels to the nematode. Abberant migration of A. crassus in the European eel has been reported and it is possible that in the American eel such migration is fatal. This is the first report of anguillicolosis epizootic in aquacultured American eels.

C 1 1 SERUM ENZYMES, HEMATOCHEMICAL PROFILE IN TRYPANOSOMIASIS INFECTED CAMEL.

Zia-Ur-Rahman, A.A.Butt, I. U. Haq and A. Ahmad. Department of Physiology and Pharmacology, Univ. of Agriculture, Faisalabad-38040, Pakistan.

Blood was collected from clinically normal and trypanosomiasis infected camels under nomadic condition from several area of punjab province. Health status of these animals was determined by standard trypanosome diagnostic methods. The major change in the serum proteins was the decrease in albumin and increase in τ-globulins. The level of IgM was significantly higher in infected then the healthy camel. A minor fluctuation was observed in the IgG levels in infected camels. Alkaline phosphatase (ALP) was lower while Glutamine oxaloacetic transaminase (GPT) and Sarbitol dehydrogenase (SDH) activity was higher in infected camels. Marked neutropenia with lymphocytosis was observed before treatment. Suramin was 70 percent effective on day 4 and 100 percent on day 9 of treatment.

C 12 EFFECT OF COCCIDIOSIS ON THE HEMATOCHEMICAL PROFILE IN CAMEL.

Zia-Ur-Rahman, A. Ahmed, N. Fatima, I. U. Haq.
Department of Physiology and Pharm., University of Agriculture, Faisalabad- 38040, PAKISTAN.

Coccidiosis is one of the most deadly disease of animals in the tropic. Ten camels infected with coccidiosis were subjected to hematochemical profile and these values were compared with clinically healthy camels. Packed cell volume and erythrocyte values were significantly low as compared to healthy animals and these values returned to normal after Amprolium treatment. Minerals analysis have shown an increase in potassium and decrease in sodium contents in infected camels. Total proteins was decreased due to decrease in gamma globulin in infected animals. Serum glucose, albumin as well as alpha and beta globulin have not shown any significant difference as compared to healthy camel. Amprolium gave the best results in term of oocytes counts reduction, followed by Clopidol and Nitrofurazone.

C 1 3 THE PHYLOGENY OF THE SARCOCYSTIDAE DEDUCED FROM 18S RIBOSOMAL DNA SEQUENCE COMPARISONS

John Ellis and David Morrison
University of Technology Sydney, Gore Hill, NSW, Australia.

The family Sarcocystidae contains a wide variety of parasitic protozoa some of which are important pathogens of livestock and man. The phylogenetic relationships between two of the genera in this family (*Toxoplasma* and *Sarocystis*) have been debated now for a number of years and remains controversial. Recent studies, from comparisons of 18S rDNA sequence data, have suggested that *Sarcocystis* is paraphyletic although a hypothesis supporting monophyly of *Sarcocystis* could not be rejected. In this study it is shown that the phylogenetically informative nucleotide positions within the 18S rDNA are primarily located in the regions that make up the helices in the secondary structure of the 18S rRNA. A phylogenetic analysis of 18S rDNA sequence data aligned by secondary structure contraints, or a subset of the data corresponding to all nucleotides found in the helices, provide unambiguous evidence supporting monophyly of *Sarcocystis*. The wider application of this approach to understanding the phylogenetic relationships among sporozoan parasites in general is discussed.

C 14 IDENTIFICATION OF A COMMON CONOIDAL DETERMINANT AMONG DIFFERENT *EIMERIA* SPECIES WITH A CHICKEN MONOCLONAL ANTIBODY TO *EIMERIA ACERVULINA*

Sasai K., H.S. Lillehoj [1], H. Matsuda [2], Y. Hanioka, T. Fukata, E. Baba and A. Arakawa

Dept. of Vet. Med., Univ. of Osaka Pref., Osaka, Japan, [1]USDA, Parasite Immunobiology Lab., Beltsville, MD. U.S.A., [2]Dept. of Microbiology and Hygiene, Faculty of Applied Biological Science, Hiroshima Univ., Higashi-Hiroshima, Japan

The widespread occurrence of avian coccidiosis has ranked it as one of the most important diseases of poultry. After first infection with an eimerian specie, chickens acquire immunity to re-infection. Many researchers are trying to identify potential vaccine antigens that induce protective host immune response. Recently we have reported that the chicken monoclonal antibody, 6D-12-G10, showed statistically significant inhibition of the sporozoite invasion of CD8 positive T cells *in vitro*. In the immuno-electron microscopic study, the conoid of *Eimeria acervulina* sporozoites was identified as the target of this monoclonal antibody.

In the present study, we examined the reactivities of the monoclonal antibody, 6D-12-G10 to seven different *Eimeria* species, such as *E. acervulina*, *E. brunetti*, *E. maxima*, *E. mitis*, *E. necatrix*, *E. praecox* and *E. tenella* by using an immuno-fluorescence laser microscopic technique. The specific fluorescence was observed in all *Eimeria* sporozoites tested with similar reactive pattern each other. Therefore, these results indicate that conoid antigens recognized by the monoclonal antibody, 6D-12-G10 may play an important role on the invasion of *Eimeria* sporozoites into host cells.

C 15 EFFECTS OF TESTOSTERONE ON THE MUCOSAL DEFENCE AGAINST INTESTINAL HELMINTHS IN INDIAN SOFT-FURRED RATS, *MILLARDIA MELTADA* WITH REFERENCE TO GOBLET AND MAST CELL RESPONSES

Risa Tiuria, Yoichiro Horii, Susumu Makimura and Yukifumi Nawa*
Department of Veterinary Internal Medicine, Faculty of Agriculture, Miyazaki University, Miyazaki 889-21 and *Department of Parasitology, Miyazaki Medical College, Miyazaki 889-16, Japan

Effects of testosterone on the mucosal defence mechanisms against intestinal helminths were examined in *Millardia meltada*. When female *M. meltada* were treated with testosterone at the pharmacological dose, *Nippostrongylus brasiliensis* infection persisted for over 7 weeks with prominent biphasic pattern of faecal egg production, whereas almost complete expulsion was observed by 2 weeks in untreated controls. In spite of biphasic pattern of faecal egg production, worm burden of testosterone-treated animals remained constant up to 3 weeks and then slowly decreaed by 7 weeks. To see whether or not this delayed expulsion in testosterone treated animals was due to altered cellular responses of the intestinal mucosa, goblet and mast cell responses were examined histologically. At 2 weeks post-infection, goblet cell response at the infected site was significantly lower in testosterone-treated animals than that in controls. On the contrary, mast cell hyperplasia were comparable between testosterone-treated and control animals. When *Strongyloides venezuelensis*, of which expulsion is dependent on mucosal mast cells, were infected concurrently with *N. brasiliensis*, testosterone-treated animals could expel *S. venezuelensis* worms by Day 18, but was failed to expel *N. brasiliensis*. Histologically, mast cell hyperplasia was associated with expulsion of *S. venezuelensis*, while goblet cell response was suppressed. From these results, testosterone seems to suppress proliferation/function of goblet cells but not affect on mast cells of *M. meltada*. The present results support our proposal of the selective protective role of goblet cells and mast cells in the mucosal defence against intestinal helminths.

C 16 THE ROLE OF THE MUCOSAL GROWTH FACTORS, TRANSFORMING GROWTH FACTOR-ALPHA AND EPIDERMAL GROWTH FACTOR IN THE PATHOGENESIS OF INFECTION OF SHEEP WITH OSTERTAGIA CIRCUMCINCTA.

Ian Scott, Quintin McKellar, Jane Irvine* and Alma Dick*
Departments of Veterinary Pharmacology and Pathology*, University of Glasgow Veterinary School, Glasgow, United Kingdom.

Transforming Growth Factor-alpha (TGFα) and Epidermal Growth Factor (EGF) are members of the same family of peptide growth factors with important functions in a variety of tissues especially epithelia. Both are potent mitogens and are important in cell differentiation. Both inhibit acid secretion by parietal cells and enhance mucous production in gastric mucosa in-vivo as well as in-vitro. Only TGFα has been shown to be produced in gastric mucosa in significant amounts in species examined so far, but EGF has been demonstrated in salivary secretions and is trophic for the gastric mucosa. Over-expression of TGFα has been implicated in the development of Menetrier's disease of humans, a disease with many histopathological and biochemical similarities to ostertagiasis. In contrast to other species we demonstrated immunohistochemically the coexistence of both growth factors in the fundic mucosa of parasite-naive animals. Immunoreactivity was strongest in Surface Mucous Cells and Parietal cells. Chief cells exhibited only weak apical staining and Mucous Neck cells did not stain at all. Growth factor distributions were also investigated in tissues obtained from sheep grazing predominantly O. circumincta contaminated pasture. In areas of relatively normal mucosa growth factor immunoreactivity was normal. In the hyperplastic nodules metaplastic cells showed strong immunoreactivity for both growth factors and the mature mucous cells lining enlarged pits in these animals were also strongly positive. The results suggest that elevated production of growth factors may be involved in the cellular changes seen in parasite infection, but may simply reflect the change from normal mucosa to one dominated by metaplastic cells.

C 17 GOBLET CELL MUCINS AS THE SELECTIVE BARRIER FOR THE INTESTINAL HELMINTHS

Yoichiro Horii, Naoto Ishikawa* & Yukifumi Nawa* Department of Veterinary Internal Medicine, Faculty of Agriculture, Miyazaki University, Miyazaki 889-21 and *Department of Parasitology, Miyazaki Medical College, Miyazaki 889-16, Japan

The aim of this study was to examine the role of T cells on the alteration of terminal sugars of goblet cell mucins in the small intestinal mucosa of parasitized rats and to clarify the biological significance of the altered mucins in the mucosal defence against intestinal helminths. For this purpose, *Nippostrongylus brasiliensis* adult worms obtained from donor rats at 7 ('normal' worms) or 13 days ('damaged' worms) post-infection were implanted intraduodenally into euthymic and hypothymic (*rnu/rnu*) rats. Expulsion of implanted normal worms and associated goblet cell changes were extremely delayed in hypothymic recipients compared with euthymic recipients. In contrast, intraduodenally implanted damaged worms were expelled by day 5 regardless of the strains. Around the time of expulsion of implanted damaged worms, euthymic recipients showed both goblet cell hyperplasia and alteration of mucins, whereas hypothymic rats showed only the latter. Dexamethasone treatment completely abolished goblet cell changes of both strains of recipients. To clarify the importance of the constitutional changes of goblet cell mucins in mucosal defence, euthymic rats were primed by implantation of damaged worms to induce goblet cell changes, and then 3 or 5 days later they were challenged by implantation with normal worms, recipient rats could completely prevent the establishment of normal worms. When hypothymic rats were primed and challenged in the same manner, a similar but slightly less preventive effect was observed. Such a protective effect of altered mucins seems to be selective because priming of euthymic rats with damaged *N. brasiliensis* did not affect the establishment of *Strongyloides venezuelensis*.

C 18 HYPERGASTRINEMIA AND GASTRIC ACID SECRETION IN RATS INFECTED WITH LARVAL *TAENIA TAENIAEFORMIS*

Y. OKU, T. YAMANOUCHI, K. MATSUDA, H. K. OOI, & M. KAMIYA
Hokkaido University, Sapporo, Japan

Serum gastrin levels and gastric acid secretion in rats experimentally infected with *Taenia taeniaeformis* eggs were examined. It was observed in rats that the greater the number of eggs inoculated, the earlier the hypergastrinemia can be observed. At 11 weeks PI, pathophysiological changes in rats inoculated with 2,000 eggs includes increased in stomach weight (Uninfected: 1.2 g ,Infected : 5.6 g), gastric mucosal hyperplasia, hypersecretion of mucus and elevated gastric luminal pH (Uninfected: pH 3.9, Infected: pH 8.4). Light microscopy revealed that mucosal hyperplasia was restricted to the glandular mucosa. Parietal and Chief cells were rarely seen, and PAS positive mucous cells were the major cell type in the hyperplastic stomach. No difference in gastric acid secretion at the basal acid output level between control and infected groups was observed. However the infected group failed to response to the stimulation with histamine, with the maximum acid output level being 2.8 (μ Eq.H+/15min.) in the infected group, as compared to 12.9 in the Uninfected group.The above results suggested that heavy infection with larval *T. taeniaeformis* can result in suppression of gastric acid secretion, gastric mucous cell hyperplasia, and hypersecretion of mucus. All these events might have led to the resulting elevation of gastric luminal pH and hypergastrinemia.

C 19

IMMUNOHISTOSHEMICAL OBSERVATION ON AVIAN STRONGYLOIDOSIS IN CHICKEN

Sakamoto T[1]* and Aoki M[2]
Dept. of Parasit., Sch. of Vet. Med., Fac. of Agr., Iwate University, Morioka,Japan[1] and Akita University School of Med., Akita, Japan[2]

Four-days-old newborn chickens and 14-days-old baby chickens were percutaneously infected with 1000 infective larvae of *Strongyloides pavonis*. The chickens were dissected with the lapse of time until the 24th day after infection. The tissue materials collected from the intestinal tract were fixed in PLP, Carnoy's fixative or 10% neutral formalin. The caecal tissue pieces fixed in PLP were cut at 4μm in a cryostat. Endogenous peroxidase activity was blocked with 0.3% H_2O_2 in methanol 30min. Anti-chicken IgA goat serum or anti-chicken IgG rabbit serum as the primary antibody were applied to the freezing and paraffin sections. Then, the sections were immunohistochecally stained by ABC method. The other materials were embedded in paraffin. The paraffin sections were stained with HE, HB, PAS, methylgreen pyronin and others. The worms penetrated into the mucosa of caeca 24 hr after infection. At the 4th day, many worms occurred in the lumen and mucosa, and some worms were found in the submucosa and muscular layer. The lymph nodules appeared swollen. PAS-positive capsules were formed around each of the worms in the mucosa. The number of plasma cells in the lamina propria showed a tendency to increase with lapse of time and reached a maximal level between the 17th and 20th days. The inflammatory response in the caeca of the baby chickens was more intensive than that of the newborn chickens. In immunohistochemical examination, most of the plasma cells were IgA positive. Also, the capsules in the mucosa and the surface of the worms in the lumen of caeca were IgA-positive. A part of the plasma cells were IgG-positive. Immunofluorescent antibody technique using antiserum to an antigenic fraction of the worms was performed on the caecal tissue sections. The capsules revealed to be distinct positive.

C 20

DYNAMICS OF ANTIBODY TITER AND FECAL EGG OUTPUT IN CATTLE AND BUFFALO FOLLOWING INFECTION WITH 500 AND 1000 *F. GIGANTICA* METACERCARIAE

Piyanoot Prasittirat, Suree Thammasart, Tasanee Chompoochan, Suwannee Nithiuthai, Noriyuki Taira
National Institute of Animal Health, Bangkok, Thailand

ELISA and beads technique for *Fasciola gigantica* infection detection were used to evaluate antibody titre and egg count per gram (epg) of fluke in cattle and buffalo respectively. Each group of 4 cattle and 4 buffalo were infected with 500 and 1000 metacercaria. The examinations were carried out for 28 weeks with intervals of 1 or 2 weeks. Using ELISA test,the results revealed that the antibody titre against *Fasciola gigantica* infected animals were similar pattern. The earliest antibody detection was at week 2 and high level of antibody titre to 500 metacercaria infected cattle and buffalo was demonstrated between week 12-24. Most of the animals infected with 1000 metacercaria, high antibody titre were found since week 2 after infection. Much later than antibody detection, epg from fecal examination of 500 metacercaria *Fasciola gigantica* infected animals were showed positive between week 16-18 and peak between week 24-26. Whereas the animals infected with 1000 metacercaria were found epg positive at week 10-12 and high egg values were noted at week 16-20 after infection. ELISA, therefore, is a sensitive and useful technique for early detection of *Fasciola gigantica* infection in animals.

C 2 1 DYNAMICS OF ELISA TITER IN CATTLE, BUFFALO AND SHEEP INFECTED WITH 500 F.GIGANTICA METACERCARIAE

Suree Thammasart, Tasanee Chompoochan, Piyanoot Prasittirat, Suwannee Nithiuthai, Noriyuki Taira

National Institute of Animal Health, Bangkok, Thailand

Serological test and stool examination were conducted in 4 cattle, 4 buffalo and 7 sheep experimentally infected with 500 metacercaria of *Fasciola gigantica*. All infected animals were tested for antibodies to *Fasciola gigantica* by ELISA, using a whole body crude extract of adult fluke-derived antigen. These animals were also evaluated egg per gram (epg) in feces for the liver fluke infection by beads technique. The experiments throughout 7 months were performed regularly at 1 or 2 weeks intervals for the detection of antibody titres and epg. Evidence of antibody titre against *Fasciola gigantica* infection in cattle was risen at week 2 (75%), week 4 (100%) and gradually increased with the peak at week 16 then decline thereafter. Similarly, the pattern of antibody titre found in buffalo was risen at week 2 (25%), week 6 (75%) and week 8 (100%) with the peak at week 20. In contrast, the antibody titre detected in infected sheep was risen earliest at week 2 (100%) with obvious irregular peaks until the end of experiment. Correlation with serological pattern, epg of liver fluke in feces in those 3 kind of infected animals were paralleled. The earliest detection of epg in cattle, buffalo and sheep were at week 16, 18 and 12 with the peak (maximum epg) at week 24, 28 and 15-22 (irregular peaks) respectively, Hence ELISA, when used in conjunction with beads technique results, may be a useful indicator for diagnosis of the early stages of fasciolosis in ruminants in endemic areas.

C 22 ANTHELMINTIC EFFECTS OF LEVACID AGAINST SHEEP EXPERIMENTALLY INFECTED WITH *FASCIOLA GIGANTICA*

Tasanee Chompoochan, Piyanoot Prasittirat, Suree Thammasart
Suwannee Nithiuthai, Noriyuki Taira
National Institute of Animal Health, Bangkok, Thailand

Treatment of *Fasciola gigantica* infection with Levacid[R] (Bithional sulphoxide) was investigated in sheep experimentally infected with 500 metacercaria. Ten sheep were devided into 3 groups : group 1(4 sheep) a single dose of Levacid (80 mg/kg) was given orally at week 22 after infection, group 2 (3 sheep) untreated positive control and group 3 (3 sheep) untreated negative control. All animals were determined egg per gram (epg) in feces for the liver fluke infection by beads technique at weekly interval for 28 weeks (epg during week 22 - 23 was examined daily). The eggs disappeared from feces of infected sheep one week after treatment. Levacid was 100% effective in removing adult *Fasciola gigantica* whereas at the postmortem examination all untreated infected sheep had massive infection of mature flukes in bile duct and gall bladder. Changes in liveweight, haematology (Wbc, PCV, MCV, MCHC), serum enzymes (SGOT, SGPT), serum protein and albumin were also performed. No toxic or side effects were observed. The drug showed highly effective against mature *Fasciola gigantica* in sheep.

C23 EFFICACY OF BITHIONOL PASTE AGAINST ANOPLOCEPHALA PERFOLIATA IN NATURALLY INFECTED HORSES

T. Yoshihara, M. Toguchi[1], H. Komazawa[2] and Y. Ohwa[3]
Equine Research Institute, Japan Racing Assoc., Tokyo, [1]Dainippon Pharmaceutical Co., Ltd., Osaka, [2]Hidaka Agr. Mutural Aid Assoc. and [3]Hidaka Horse Breed Assoc., Japan

In Japan, most usage of highly potent anthelmintic drugs has resulted in a change of the prevalence of internal parasites in race horses. An increase in the tapeworm infection rate has been observed recently. This paper presents the results of the antiparasitic efficacy of bithionol paste against Anoplocephala perfoliata in naturally infected horses. The efficacy against tapeworms was confirmed by a single application of 5 or 10 mg/kg body weight (bw) as the bithionol component in two horses. Necropsies were performed 14 days after treatment and examinations performed for the presence of parasites. No tapeworms were found in the alimentary tract of any horses except a control horse used for the necropsy. Two field trials were carried out to assess the same doses in fecal examinations in a total of 81 horses. A control group of six horses was used. Fecal examinations were performed prior to and 14 days after treatment. Bithionol paste showed an efficacy of 86.7 % at 5 mg/kg bw and of 100 % at 10 mg/kg against A. perfoliata. No gross or clinical reactions were observed in the treated horses.

C24

EFFECTIVENESS OF STRATEGIC USE OF CLOSANTEL AND ALBENDAZOLE IN CONTROLLING GASTROINTESTINAL NEMATODES OF SHEEP IN KENYA.

N. MAINGI*, V.M. GICHOHI*, S.M. THAMSBORG**, W.K. MUNYUA*, J.M. GATHUMA* P. NANSEN**

* University of Nairobi, Nairobi, Kenya

** Danish Center for Experimental Parasitology, Copenhangen, Denmark.

The strategic use of anthelmintics in the control of gastrointestinal nematodes of sheep was investigated on a farm in the highlands of Kenya. 30 Corriedale lambs, aged between 9 and 12 months were assigned to three treatment groups of 10 lambs each. The three groups were set stocked on separate paddocks for 12 months. Lambs in group 1 were treated strategically with closantel in combination with albendazole, while those in group 2 were kept "worm-free" by regular treatments with albendazole at 3 weeks intervals. The 3rd group remained untreated. Nematode eggs per gram (EPG) of faeces, weight gain, blood packed cell volume (PCV) , serum albumin and total protein were monitored every 3 weeks. Pasture larvae contamination and worm burdens in tracer lambs were also determined. EPG and pasture contamination were well controlled in the strategically treated group. This resulted in higher weight gains, wool production, PCV, serum albumin and protein compared with the untreated group. These parameters were comparable between the strategically treated and the regulary treated groups. It was concluded that worm control strageties based on the use of closantel could provide effective control of nematodes of sheep in the study area and other parts of Kenya.

C25 THE EFFECTS OF ANTHELMINTICS ON NATURALLY INFECTED SHEEP AND SUGGESTION OF ALTERNATIVES OF TREATMENT PROGRAMMES

Gebrekiros Asegede, Awassa College of Agriculture, Awassa, Ethiopia
H.-J. Bürger and J. Steibach, University of Giessen, Giessen, Germany

A total of 128 lambs (about 6 to 12 months old) were used to investigate the effects of naturally aquired helminthosis by subjecting them to two levels of anthelmintic medications: (1) suppressive treatment (Fenbendazole plus Rafoxanide) at monthly intervals or (2) no treatment at all (controls). Prevalence, worm and e.p.g. counts of gastro intestinal nematodes were 40, 108 and 93%, respectively, higher in the controls than in the treated group at the end of the trial. Treatment resulted also in a higher growth rate (40%), PCV level (9%), Haemoglobin concentration (10%), and higher albumin concentration (10%). The control group, on the other hand, had a 167% higher death rate and 9% higher serum globulin concentration. The experiment indicated that application of anthelmintics at monthly intervals could reduce mortality and improve productivity. Accordingly alternatives of one-time, three- and ten-times antihelmintic treatment programmes are suggested and discussed.

C26 SCREENING OF ANTICOCCIDIAL EFFECTS OF HERB EXTRACTS TO *Eimeria tenella*

Hee-Jeong Youn, Gi-Ok Hong, and Yung-Bai Kang

Department of Veterinary Parasitology, College of Veterinary Medicine, Seoul National University, Suweon, KOREA, *National Veterinary Research Institute, RDA, Anyang, KOREA.

Ionophorous antibiotics have been used in popular for the protection of avian coccidiosis, though Halofuginone which is derived from an extract of the *Dichroa febrifuga*, was developed as an antimalarial and anticoccidial agent. The antibiotics are regarded as the causes of residues in the avian products, therefore the authors have tried to find out more safe herbal materials for the control of avian coccidiosis. Thus, the extracts of 15 kinds of herbs, *Bupleurum chinese* DC, *Sophora flavescens* Aiton, *Artemisia annua* Linne, etc were investigated for the efficacy screening against *E. tenella*.

A reference stock of *E tenella* from the USDA were used in this experiment. All herbs were boiled for 3 hours and divided into groups according to the weight in dilution. Survival rates, lesion scores, body-weight gains, bloody diarrhea, oocysts excretions were investigated at the 1st and the 2nd week after infection.

Bloody diarrhea in the groups of *S flavescens* and *Sinomenium acutum* were milder than those of the other infected groups. Survival rates in the groups of *Ulmus macrocarpa*(100%), *Pulsatilla koreana*, *Torilis japonica*, *Artemisia asiatica* and *S flavescens*(90%) were higher than that of infected control group(70%). Lesion scores in the groups of *U macrocarpa*(1.40+1.14) and *Pulsatilla koreana*(1.60+1.82) were significantly lower than those of infected control group(3.00+1.10).

During the 1st week after infection, the weight gains in the groups of *Quisqualis indica*(232.9+43.5g), *S flavescens*(214.4+46.1g) and *S acutum*(211.3+29.4g) were significantly higher than the infected control group(172.4+17..6).

In a conclusion, analyzing the data of the survival rates, bloody diarrheal symptoms, lesion scores, body weight gains and oocyst excretions, the extract of *S flavescens* was most effective. *P koreana*, *S acutum*, *U macrocarpa* and *Q indica* were effective. The further research on the above herbal materials will be carried out by the authors by means of the chemical analysis of the components.

C27 EFFECT OF NEMATOCIDES ON *ORIENTOSTRONGYLUS EZOENSIS* (NEMATODA; HELIGMONELLIDAE) IN RATS

Fukumoto S.-I.
Veterinary Parasitology, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069, Hokkaido, Japan

Orientostrongylus ezoensis is a small nematode parasitizing in upper small intestine of *Rattus* spp. in Japan (Fukumoto &Ohbayashi,1985). *O.ezoensis* has ideal characteristics for an experimental model of intestinal nematode infection (Fukumoto,1979&1993). We examined efficacy of several nematocides against *O. ezoensis* in experimental rats.

Each male Wistar rat was infected orally with 1000 infective larvae of *O.ezoensis*. Four rats were used in each treated group and in control. On the Day 10 after infection, rats were treated with nematocides at the dose of commercial use . Milbemycin (0.5mg/kg of body weight) , piperazine(200mg), levamisole (7.5mg) and tiabendazole (75mg) were inoculated orally. Ivermecin was injected subcutaneously at a dose of 0.05, 0.1, 0.2 ,0.8, 1.6 and 3.2 mg/kg of body weight , respectively. Animals were autopsied on the day 4 after treatment. The worm reduction rate of treated group against control was caluculated.

Results : Number of recovered worms and reduction rate of each group is shown in Table 1.

Table 1. Efficacy of several nematocides against *Orientostrongylus ezoensis* in rats

nematocides	worms recovered	reduction rate (%)	ivermectin dose (/B.W.)	reduction rate(%)
control	* 549.3±65.3		0.05 mg	13.4
tiabendazol	0	100.0	0.1mg	38.4-52.7
levamisole	11.3± 5.0	98.0	0.2mg	28.0-52.7
milbemycin	181.0±75.7	67.0	0.8mg	48.5-62.3
piperazine	434.0±30.4	20.9	1.6mg	53.9-70.6
	* mean±SD		3.2 mg	75.7

Piperazine , ivermectin and milbemycin were not effective against *O.ezoensis* in rats. *O. ezoensis* used in the present study might have natural resitance against macrocyclic lactons , such as avermectins.

D 1 SELECTIVE PERFUSION OF PIGS INFECTED WITH *SCHISTOSOMA JAPONICUM*. A METHODOLOGICAL STUDY.

H.O.Bøgh, A.L.Willingham and M.V.Johansen*
Danish Centre for Experimental Parasitology, Copenhagen, Denmark.
*Danish Bilharziasis Laboratory, Charlottenlund, Denmark.

The primary goal with the present optimization experiments was to develop a safe, reliable and quick method for collection of adult *Schistosoma japonicum* worms from the mesenteric veins of the pig. In previous experiments, it was found that, in some cases, only 50% of the adult worms were flushed out during perfusion using a non selective perfusion technique. The effect of giving praziquantel to the pigs prior to perfusion was evaluated by applying this known anti-schistosome drug to half of the pigs in each of the two experiments performed. In experiment 1, 8 pigs were infected with 500 *S.japonicum* cercariae each and perfused 16 weeks after infection (wpi). In the second experiment, 12 pigs were infected with 1.000 cercariae and perfused 11 wpi. The mesenteric veins of the intestinal tract were perfused subsequent to the anthelmintic treatment with praziquantel (50 mg/kg body weight *per os*). Basically, the selective perfusion was employed by implanting the tube with the incoming perfusion medium into the descending aorta cranial to the *a. mesenterialis cranialis*. Prior to this, the veins from the kidneys as well as the descending aorta, caudally to the *a. mesenterialis cranialis*) and other places were clamped off. Furthermore, the acetate perfusion buffer was 40°C when used. However, other measures was also taken to make the perfusion of the pigs as optimal as possible. Subsequent to the perfusion of the intestinal tract, the colons, caeca and recta were examined manually and the number of residual worm pairs counted in order to estimate the efficacy of the perfusion. A clear effect of the praziquantel treatment was observed in both experiments. This will greatly diminish the time used for manual dissection of worms from the mesenteric veins of the pigs.

D 2 EXPERIMENTAL SECONDARY PULMONARY ALVEOLAR ECHINOCOCCOSIS IN RATS

A Ito[1], Y. Osawa[1], A. Hashimoto[2], M. Okamoto[3], M. Nakao[4]

[1]Department of Parasitology, Gifu University School of Medicine

[2]Veterinary Hospital, Faculty of Veterinary Medicine, Hokkaido University, Sapporo

[3]Institute of Experimental Animal Sciences, Osaka University Medical School, Suita

[4]Department of Parasitology, Asahikawa Medical College, Asahikawa, Japan

Alveolar echinococcosis (AE), caused by the metacestode of *Echinococcus multilocularis*, is one of the most serious parasitic zoonoses and often misdiagnosed as liver cancer. Usually the primary lesions of metacestodes are established in the liver. However, metastases often occur in almost all other organs. Secondary lesions can often develop in the lung, brain, and other organs. So, pulmonary AE is common in human. Although it has well been established to induce secondary hepatic AE or peritoneal AE in experimental animals, there is no report to establish pulmonary AE in experimental animals. We have used rats and mice for this purpose. Usually the rat is known to be not so suitable intermediate host as mice or jirds. However, almost all rats injected intraperitoneally (i.p.) or intravenously (i.v.) mainly with protoscoleces were susceptible to the secondary AE. All rats injected i.p. harbored fully fertile AE lesions in the peritoneal cavity and survived more than one year at least. All rats injected i.v. from the tail vein or portal vein harbored fertile lesions exclusively in the lung and the liver, respectively. So, the rat is now expected to be highly useful animal model for experimental secondary AE, especially for experimental study of pulmonary AE. Antibody responses in rats with pulmonary or hepatic AE or AE in the peritoneal cavity will be shown for discussion.

D 3 COPROANTIGEN DETECTION FOR DIAGNOSIS OF *ECHINOCOCCUS MULTILOCULARIS* IN FOXES

P. Deplazes, P. Alther, A. Mathis, J. Skaggs, J. Eckert

Institute of Parasitology, University of Zürich, Switzerland

A group of 57 foxes from the Zürich area was parasitologically examined for intestinal *E. multilocularis* infections after necropsy and deep-freezing (one week at -70°C) of the intestines. Prevalence of *E. multilocularis* in this population was found to be 61% (35 animals infected with 4 to approximately 60,000 *E. multilocularis* worms). For the detection of *E. multilocularis* coproantigens an enzyme-linked immunosorbent assay (ELISA) with polyclonal rabbit and chicken egg antibodies against *E. multilocularis* antigens (affinity purified coproantigens and somatic adult worm antigens) was developed. Specificity of the test was 95% (1 positive reaction among faecal samples of 21 foxes without *E. multilocularis* infection but harbouring other cestodes and nematodes). The overall diagnostic sensitivity of the test was 72% but reached 91% in foxes harbouring more than 100 *E. multilocularis* worms and dropped to 43% in animals with less than 100, respectively. Hence, the test enabled detection of the infected foxes harbouring approximately 96% of the calculated total biomass of adult *Echinococcus* present in this population. Therefore, coproantigen detection allows diagnosis of individual intestinal *Echinococcus* infections relevant for the egg contamination of the environment and is a valuable tool to determine the relative prevalence of adult stage *E. multilocularis* in a given endemic area. Other alternative methods to parasitological investigations such as detection of specific antibody with Em2- and other *E. multilocularis* antigens or PCR for identification of *E. multilocularis* eggs in faecal samples will be discussed.

D 4 BIOLOGICAL CONTROL OF NEMATODES OF FREE-RANGED PIGS BY MEANS OF A PREDACIOUS MICROFUNGUS

Nansen P.[1], Larsen M.[1], Roepstorff A.[1], Grønvold J.[1], Wolstrup J.[1] & Henriksen S.A.[1,2]; [1]Danish Centre for Experimental Parasitology, Royal Veterinary and Agricultural University & [2]National Veterinary Laboratory, Copenhagen, Denmark

A series of experiments conducted over the last five years have demonstrated that within the group of nematode-trapping microfungi there are good candidates for biological control against free-living stages of gastro-intestinal parasitic nematodes in ruminants. The aim of this field trial was to test the ability of *Duddingtonia flagrans*, a netforming nematode-trapping fungus, to reduce the worm burden of *Oesophagostomum dentatum* and *Hyostrongylus rubidus* in free-ranged pigs by reducing transmission of infective larvae on pasture. The field trial was performed as a tracer experiment. First, two groups of pigs, experimentally infected with the two parasites at turn out were allowed to graze and infect a pasture, divided in two plots of equal size, for two months. During that period one group (fungus treated group) was given fungal material daily mixed in supplementary fodder while the other group (control group) received a similar amount of fodder but without fungus. The fungal dose was given based upon average live weight of the pigs, with adjustment for the growth during the experiment. After two months two groups of parasite naïve pigs, five in each group, were introduced as tracer pigs for three weeks. After being housed for six weeks all animals were slaughtered and examined for gastrointestinal nematodes. The total average *O. dentatum* population was reduced by approximately 85% in the tracer pigs from the pasture previously grazed by fungus-treated pigs compared to the worm burden in pigs from the control pasture. A similar significant reduction (approximately 80%) was observed for *H. rubidus* in the same pigs. Herbage larval counts of the two parasites were similarly reduced on fungus pasture. This experiment clearly demonstrates that it is possible to significantly reduce the worm burden in pigs when they are given a continuous dose of the nematode-trapping fungus *D. flagrans*. As for other domestic animals this opens up for a possible alternative to chemical control strategies.

D 5 THE EFFECTS OF EXCRETORY/SECRETORY PRODUCTS OF OSTERTAGIA CIRCUMCINCTA ON PEPSINOGEN SECRETION AND SMOOTH MUSCLE CONTRACTION IN ABOMASAL TISSUES DERIVED FROM PREVIOUSLY INFECTED SHEEP AND IN PARASITE-NAIVE ANIMALS

Ian Scott and Quintin McKellar
Department of Veterinary Pharmacology, University of Glasgow Veterinary School, Glasgow, United Kingdom.

The purpose of this study was to assess the effects of excretory/secretory (ES) products of O. circumcincta on pepsinogen secretion and smooth muscle activity using in-vitro methods, and to compare the responses obtained in tissues from sheep with a history of prior exposure to parasites with the responses seen in animals maintained parasite-naive. Adult parasites were collected at necropsy from experimentally infected sheep and cultured in supplemented RPMI-1640 medium. After approximately 24 hours of incubation at 37°C the worm culture fluid containing ES was collected. Tissues were obtained at necropsy by injecting Ringer's solution between the muscle of the abomasum and the mucosa, the two tissues were then separated. Pepsinogen secretion was monitored in intact fundic mucosal sheets bathed in Ringer's in jacketed water baths and smooth muscle activity was assessed by measurement of the tension developed in abomasal longitudinal muscle strips bathed in Krebs-Henseleit solution. All tissues were stimulated with a supramaximal dose of the cholinergic agonist Carbachol to ensure that they were responsive. In tissues from animals previously exposed to parasites, ES produced significant increases ($p<0.05$) in pepsinogen release from mucosal preparations and stimulated contractions in smooth muscle preparations. Maximal contractile responses to ES were less than those to Carbachol. In contrast no responses to ES were seen in tissues from parasite-naive animals. The results were consistent with the hypothesis of immune stimulation of pepsinogen release and smooth muscle contraction in response to antigens present in ES and may be mediated via mast cell degranulation or by neuroimmune mechanisms.

D 6 SOME ECOLOGICAL ASPECTS OF HYALOMMA ANATOLICUM ANATOLICUM IN BANGLADESH

M.M.H. Mondal, M.K. Islam and A.K.M.G. Kibria
Department of Parasitology, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh

Except for the occurrence of Hyalomma anatolicum anatolicum in cattle in the north-western region (i.e. Rajshahi) bordering West Bengal of India, no further details are available.Therefore, an attempt was made to study the ecological basis of this tick in the said region. Of the 240 cattle examined, 155 (64.58%) were found positive to H. a. anatolicum infestation. A total of 1438 ticks were collected (922 male and 516 female). Relatively the young animals (>1 to 2½ years) were more infested than the adults (>2½ to 10 years), but the tick load was found to be higher in adult cattle.A high rate of infestation was recorded during Summer(March to June),71.25%;followed by Monsoon(July to October), 63.75% and Winter (November to February), 58.75%. The tick load on the host body was influenced by the rainfall,temperature and relative humidity. Maximum number of ticks were collected in the month of May having rainfall of 115 mm,temperature 31.45°C and relative humidity 62.50%.Whereas,lowest number of ticks were collected in the month of January with rainfall of 18 mm,temperature 18.40°C and relative humidity 67%.The soil characteristics, types of vegetation, and the meteorological information as have been observed in this study were quite different from the other regions of Bangladesh. The area has been found a bit arid and steppe, which possibly account for the occurrence of H.a.anatolicum in this particular zone of Bangladesh.

D 7 THE HISTORY OF VETERINARY PARASITOLOGY IN JAPAN

Roncalli R. A.

Animal Science Research, MSD (Japan) Co., Ltd., Inoue Akasaka Bldg. 6-8, Akasaka 1-chome, Minato-ku, Tokyo 107, Japan

The development of veterinary parasitology in Japan commenced with the advent of the Meiji Era in 1868. Janson, a German veterinarian, was the first researcher to study systematically the parasites of domestic animals in Japan with the establishment of the Veterinary School at Komaba in Tokyo in 1880. Later, he was assisted in this task by Tokishige, a Japanese veterinarian. Janson discovered *Eurytrema pancreaticum* in sheep in Japan. The first report on the occurrence of *Dirofilaria immitis* in Japan was published in 1872 in the U.K. following receipt of a heart recovered from a hunting dog in Yokohama. Aoyama was the first Japanese veterinarian to describe heartworms in dogs in Japan in 1880. Railliet studied parasites from Japanese domestic animals which were sent to the Paris Universal Exhibition of 1889. Yamaguchi, in the late 30's, published a series of works relevant to helminths of animals in Japan. In 1955 Kume and Itagaki published a classic paper elucidating the life cycle of heartworms in dogs. During the past 50 years notable work has been conducted in several areas of veterinary parasitology including dirofilariasis of dogs and cats, cattle liver fluke, cattle strongyloidosis, poultry cestodes, avian leucocytozoonosis and echinococchosis both in humans and animals.

D 8 *EIMERIA HAGANI* ISOLATED FROM JAVAN JUNGLEFOWL (*GALLUS VARIUS*)

Kameo Shimura, Takashi Isobe, Naotoshi Tsuji (NIAH, Tsukuba, JAPAN), Kaori Koyama, Yoshimasa Watanabe (Saitama LHSC, Sugito and Omiya, JAPAN), and Zhang Shu Min (ASI, Jilin, P.R.CHINA)

A javan junglefowl from Indonesia died of *Escherichia coli* infection during the import inspection period. At necropsy, many coccidial oocysts were found in the caeca of the bird. The oocysts were consisted with single eimerian species, and after serial passages in chickens one line was picked up by single oocyst isolation method.

The oocysts collected on the 7th day after infection were subspherical to broadly ovoid, 15.3 - 22.2 by 14.2 - 19.0 μm with a mean of 19.7±1.12 by 17.4±0.94 μm (Sporulated, N=100) . The oocyst size of the line was almost the same as that of *Eimeria hagani*. A micropyle and oocyst residuum were absent. A polar granule was present. The sporocysts were elongate ovoid, 11.4 - 15.7 by 5.5 - 8.3 μm with a mean of 13.5 ± 0.78 by 7.0± 0.51 μm (N=100), with a Stieda body and residuum.

The parasites were found in the duodenum and upper jejunum. At least 2 types of schizonts (8 by 8 μm and 18 by 18 μm) were present in the duodenum on the 3rd day after infection.

The prepatent period was 113 hours after infection in chicken, and patent period was 3-4 days when chickens were innoculated with 100,000 oocysts. The total discharged oocyst number were 4.8, 5.6, 8.7, and 3.5 million per chicken when infected with 100,000, 10,000, 1,000, and 100 oocysts, respectively.

Infected chickens showed no clinical signs except for slight weight loss. The weight of infected chickens with 100,000 oocysts was 10 % lower than that of non-infected control. No gross lesions were also seen at autopsy on the 8th day after infection.

The line showed no cross-immunity between *Eimeria acervulina*.

As the results of these experimental infections, we identified the line isolated from a javan junglefowl as *E. hagani*.

D 9 SCANNING ELECTRON MICROSCOPY STUDY OF RHINOESTRUS USBEKISTANICUS (GAN 1947) LARVAE AND IMAGO.

CH. GUITTON, PH DORCHIES, ECOLE NATIONALE VETERINAIRE DE TOULOUSE, FRANCE.

Purpose

Rhinoestrus usbekistanicus is developping in the naso-pharyngeal cavities of horse, donkey and Burchell's Zebra. A lot of african donkeys are infected in Morocco and Sénégal and it is very easy to have many samples. Until now, the morphology of this parasite is not wellknown. This study is the first description of the fine structure of the three larval instars and of the imago and allows to show many new details of these stages.

Methods

The three instar larvae have been harvested from the nasal cavities and sinus of naturally infected donkeys of the Veterinary School of Dakar by Pr Pangui (Sénégal). Imago were hatched in his laboratory after pupal period in dry soil. All parasites were preserved in alcohol before treatment.The scanning electronic microscopy was performed according to validated procedures with an Hitachi S 520 system.

Results

The first instar larvae have many strong spines and hooks. They show minor differences with previous description of *Rhinoestrus purpureus*. The second instar larvae have never been observed by any researcher. They are caracterized by prominent antennary lobes and reduced cephalic hooks. The posterior peritremes are small triangular with big holes.Though the third larval instar can be well studied by stereomicroscope, we have utilized be electron since it allows a close study of spinulation of ventral side and also of the posterior peritremes. New fine structures, especially sensorial, have been observed.

Conclusion

This study brings new knowledge on the morphology of larvae and allows comparisons with other Oestrids, so we will have a better understanding of the phylogeny of this family.

D 10 KINETICS OF OESTRUS OVIS (LINNE 1758) LARVAE DEVELOPMENT IN NAIVE LAMBS AFTER NATURAL INFECTION IN FIELD.

PH. DORCHIES, C. DURANTON, J.P. BERGEAUD
ECOLE NATIONALE VETERINAIRE, TOULOUSE, FRANCE.

The bionomics of *O. ovis* larvæ in sheep are greatly dependent upon climatic factors. In many countries, many first instar larvæ need a very long time to develop because of the influence of either the cold weather or the hot and dry climate. The mean time required for the development of the larvæ in immune naive lambs when optimal climatic conditions are present is not well established.

Methods

Sixty naive lambs have been turned out at the beginning of the spring (at the end of april), just before the expected time of emergence of *Oestrus ovis* flies. Six groups of ten lambs were slaugthered on day 42, 50, 70, 84, 98 and 113 after the turn out.The larvæ were picked up in nasal cavities and sinus according to validated procedures.

Results

Infection began few days after the rains in late june and some larvaæ developped from the first to the third stage within less than 20 days. In each group 30 to 80% of lambs were infected. The mean larval burdens were very low : 5,6 to 9,6 per infected lamb. The L1 burdens were lower than the other instars : 0 to 28,7%. The L2 larvæ were at each slaugther more numerous than other stages : 29,8 to 80%, this indicates a quick development of the parasite. The L3 were also numerous : 5,8 to 41,3% of each larval burden.

Conclusion

So far, the indicated larval time by authors is in a range of 25 to 35 days. The present findings indicate that such time can be reduced under the best conditions for evolution : climate and non immune lambs.

D 1 1 SUSCEPTIBILITY OF IMMUNODEFICIENCY (SCID AND NUDE) MICE TO HIGH AND LOW VIRULENT STRAINS OF *TRYPANOSOMA BRUCEI GAMBIENSE*

D. NARUMI[1], N. INOUE[1], A. SAITO[1], N. SUZUKI[2] and H. HIRUMI[2].
Department of Veterinary Physiology[1] and The Research Center for Protozoan Molecular Immunology[2], Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan

African trypanosomiases are caused by salivarian trypanosomes which are of variable virulence for host animals. Among those, *Trypanosoma brucei gambiense*, the pathogen of chronic human trypanosomiasis, is in general of low virulence for laboratory rodents (e.g. mice). Mechanisms that regulate the pathogenicity and differentiation of salivarian trypanosomes, the susceptibility of host animals to the parasites and the virulence (relative capacity) of the parasites to cause the disease have not yet been fully understood. In this study the suitability of an experimental model to elucidate the mechanisms was examined by using a combination of (1) two strains of *T. b. gambiense* which are high (IL2343) and low (IL3253) virulence for mice and (2) three strains of mice: (i) severe combined immunodeficiency C.B-17*scid* (SCID), congenitally absent of functional B- and T- cell- mediated immunity, (ii) athymic BALB/*c-nu* (nude), and (iii) immunocompetent BALB/c mice. Each mouse was inoculated (i.p.) with 5x10, $5x10^2$ or $5x10^3$ bloodstream forms (BSFs). The parameters chosen were prepatent periods (PP), levels of parasitaemia (LP, number of BSFs in tail blood), numbers of parasitaemic waves (PW), duration of survival (DS), packed cell volumes (PCV) and pleomorphism of the parasites at the peak parasitaemia. Firstly, it was confirmed that IL2343 strain is of high virulence for immunocompitent BALB/c mice (PP: 4 days, PW:2.5, high LP:$1.4x10^9$ / ml, relatively short DS:18 days and low PCV:28%) (2 mice) expressing pleomorphism and that IL3253 is of low virulence (PP: 6.5 days, PW:1.5, LP:$2x10^3$ - $2.1x10^6$ / ml, DS: >100 days, and PCV:>45%) (12 mice). Secondly an examination of the growth pattern of IL3253 strain in SCID mice revealed that the parasites displayed high virulence (PP:6.2, PW:1, LP:1.6-$2.3x10^9$ / ml, DS:17.6 days, all mice dead by day 20, PCV: 32.2%) (5 mice) expressing monomorphism at the peak parasitaemia. Thirdly IL3253 displayed an intermediate virulence for nude mice (PP: 5.5 days, PW: 5.2, LP: $8.0x10^6$ - $2.2x10^7$ / ml, DS:>37 days at the time when this abstract was prepared, PCV:>45%) (6 mice) expressing moderate pleomorphism. The results obtained indicated that the model system reported here would be highly suitable for the study of the virulence and pathogenicity of salivarian trypanosomes.

D 12 *TRYPANOSOMA BRUCEI GAMBIENSE*: IN VITRO CULTIVATION OF BLOODSTREAM TRYPOMASTIGOTES OF A LOW VIRULENT STRAIN (IL 3253)

N. Inoue[1], K. Hirumi[2], D. Narumi[1], A. Saito[1], N. Suzuki[2] and H. Hirumi[2]
Department of Veterinary Physiology[1], Research Center for Protozoan Molecular Immunology[2], Obihiro Univ., Obihiro, JAPAN

Trypanosoma brucei is divided into three subspecies: *T.b. brucei*, *T.b. gambiense* and *T.b. rhodesiense*. Although, they are all infective to mice, *T.b. gambiense* is a pathogen of variable virulence and of low virulence for the rodents (Hoare, 1970). Recently we reported(Narumi, et al.) that a *T.b. gambiense* strain of low virulence for immunocompetent mice was highly virunable to severe combined immunodeficient (SCID) mice. To elucidate mechanisms that regulate the pathogenicity and virulence of salivarian *Trypanosomes*, we have also established an axenic culture system that supports the growth of bloodstream forms (BSFs) of such a "low virulent" *T.b. gambiense*. Firstly BSFs of IL2343 and IL3253 were cultured in the axenic culture system described earlier (Hirumi, et al., 1994) to replace the basal IMDM medium (Flow Lab., Irvine, Scotland), which was used in the original system but has become commercially unavailable, with a currently available medium. Four such basal media, namely Iscove's modified DMEM Hybri-Max® (S-IMDM/H-M) (Sigma, St Louis, MD), IMDM (Sigma), IMDM (Gibco, Grand Island, NY) and S-Clone SF-B (S-C/SF-B) (Sanko Junyaku, Tokyo) supplemented with 20%(v/v) fetal bovine serum (FBS), were tested for their ability to support the growth of BSFs. Although all the media supported the growth of "high virulent" IL2343 BSFs, non of them supported "low virulent" IL3253 BSFs. Secondly the growth of IL3253 BSFs was examined in the feeder cell layer system (Hirumi, et al., 1977) using RPMI-1640 supplemented with 20% FBS, adult bovine serum, adult bovine plasma, horse serum (HoS), horse plasma, human serum (HuS) or human plasma (HuP) in the presence of embryonic fibroblast-like cells of *Microtus montanus* (MEF) (Brun, et al., 1981). The best growth was obtained in the medium supplemented with HuP, moderate growth in the media supplemented with FBS, HoS or HuS, and no growth in the media with others. Finally S-IMDM/H-M and S-C/SF-B media supplemented with 20% HuP were examined for their ability to support the growth of IL3253 BSFs. The shortest population doubling time of the parasites was shorter in S-IMDM/H-M (8.7 h) than that in S-C/SF-B(13.0 h) and the maximum population density was 1.7×10^6 and 6.1×10^5, respectively. The result demonstrated that S-IMDM/H-M with 20% HuP was the most suitable system for culturing the "low virulent" *T.b. gambiense* BSFs and would be a useful tool for studying not only the virulence and pathogenicity but also growth-promoting factors, drug-sensitivity test, mode of trypanocidal activity, gene-selection/cloning and cell division cycle which have been difficult earlier.

D 13 THE CULTIVATION OF *EIMERIA NECATRIX IN VITRO*

Hongliao Xie, Mingquan Xie, Huixian Wu, Xinyu Peng, Wenkang Wei and Liena Wen
Veterinary Medicine Institute, Guangdong Academy of Agricultural Sciences, P. R. China

The life cycle of *E. necatrix* was studied *in vitro*. Primary chicken kidney cells were prepared by standard method of cell culture. The cells of three-day culture were inoculated with *E. necatrix* sporozoites excysted with 0.25% trypsin and 0.75% taurodeoxycholic acid in the concentration of 830,000 sporozoites per milliliter medium. Different stages of life cycle of parasite was observed under inverted microscope with the results that the first generation schizonts was discovered at 48 hours postinoculation with the size of 25-35 x 15-28μ, average 29.58 x 21.21μ. There were 150 merozoites of size 5.5-10 x 1.5-2.2μ, average 7.63 x 2.00μ in the first generation schizonts. The second generation schizonts, size 31-46 x 23-32μ, average 37 x 27.4μ, were observed in cells which contained about 110 merozoites of size 9-13 x 2-2.4μ, average 11.29 x 2.23μ at 72 hours postinoculation. The third generation parasite was smaller than the first and second generation ones with the merozoites of size 3.1-4.5 x 1.2-1.5μ, average 3.75 x 1.31μ at 144 hours. Oocysts were not seen *in vitro* and it should be inspected continuously.

D 14 NEMATODE CONTROL USED BY DAIRY FARMERS IN SOUTHEASTERN BRAZIL.

Terezinha P. Charles & John Furlong
EMBRAPA-Centro Nacional de Pesquisa de Gado de Leite, Rodovia MG 133, km 42, Coronel Pacheco MG 36155-000, Brazil

A survey on nematode control used by dairy farmers in Southeastern Brazil, which is a region responsible for 49.5% of the national milk production, was conducted through farmer's interviews. To select the producers to be interviewed, the region was divided in 15 non-contiguous clusters according to the level of milk production. A systematic sample was then selected in each of the clusters. The interview questionnaire consisted of one-way, multiple-choice and open-ended questions. Data collected were represented by numbers, manually entered on a data base (Epi Info, version 5,01) and analyzed. Among the 71 producers interviewed, 98.5% deworm their herd based on general appearance or weight loss of the animals (50.0%) or according to the season (46.8%). Most farmers seek advise from public or private institutions about deworming their animals (60.8%). Generally, anthelmintics are applied from one to eight times a year (mean of 3 times/year) to all categories. Of the anthelmintics used in the last deworming, levamisole was used exclusively by 16.9% of the farmers, benzimidazole by 18.5% and ivermectin by 9.2%, while 55.4% used more them one anthelmintic base to deworm their animals. To decide the dose, farmers consult the label (92.6%) and most of them apply the same dose to all animals based on the estimation of the mean body weight of all animals (55.9%). Improvements on the general appearance of the animal and weight gains are observed by most farmers after deworming (89.4%). Most farmers (92.4%) intend to continue using the same control measures in the following year. However, if better methods were available they would be willing to change. Veterinarians of public and private institutions play an important role on farmer's decision to deworm their animals. Programs aimed at technology transfer should include continuous updates on the subject, specially for veterinarians.

D 15 A RANDOM AMPLIFIED POLYMORPHIC DNA MARKER ASSOCIATED WITH MURINE VIRULENCE OF *TOXOPLASMA GONDII*

Zhi-Gang Guo and Alan M Johnson

Molecular Parasitology Unit, Department of Cell and Molecular Biology, University of Technology, Sydney, Australia.

Genomic DNA from eight *Toxoplasma gondii* virulent strains (RHa, RHu, ENT, PT, GT1, CT1, S48, and ts4) and seven avirulent strains (Me49, PLK, CEP, Tg51, TPR, Tg68, and Tg132) were amplified by Random Amplified Polymorphic DNA (RAPD) PCR using a single 10-mer arbitrary primer (B12) (5'-CCTTGACGCA-3'). This primer was found to be able to generate a virulence-specific DNA fragment which we called B12-243. The RAPD PCR products of all eight virulent strains tested had the B12-243 fragment, but B12-243 could not be visually detected in those of the seven avirulent strains analyzed. Southern blotting analysis confirmed that the B12-243 fragment hybridized exclusively to the RAPD PCR products of the eight virulent strains that were amplified from genomic DNAs by using primer B12 but not to those of the avirulent strains. However, B12-243 hybridized to genomic DNA from both virulent and avirulent *T. gondii* strains digested with restriction enzymes *Msp I* and *Pst I*, and produced identical hybridization band patterns. This result suggested that the B12-243 RAPD PCR amplified product is not caused by a deletion in the genome between virulent and avirulent strains. B12-243 was cloned into the plasmid pUC18 and sequenced. We are currently cloning DNA fragments from genomic libraries of the RH strain and the TPR strain in order to sequence the primer binding sites for B12 in each strain. The differences in the specific sequences between virulent and avirulent *T. gondii* strains will allow us to determine why primer B12 amplifies B12-243 from virulent strains but not from avirulent strains.

D 16 NEW MOLECULAR EVIDENCE FOR ZOONOTIC INFECTIONS WITH MICROSPORIDIA (ENCEPHALITOZOON CUNICULI)

A. Mathis[1], Ch. Müller[1], H. Kuster[2], J. Akerstedt[3], R. Weber[2], P. Deplazes[1].
[1]Institute of Parasitology and [2]University Hospital, University of Zürich, Switzerland; [3] Department of Virology and Serodiagnostics, Central Veterinary Laboratory, Oslo, Norway.

Microsporidia are among the most prevalent groups of intracellular protozoan parasites. The species *Encephalitozoon cuniculi* is parasitic in different mammals including rodents, rabbits, carnivores, and primates. Recently, microsporidia have emerged as important opportunistic protozoa in AIDS patients. Three new species were discovered (*Enterocytozoon bieneusi, Encephalitozoon hellem* and *Septata intestinalis)* which, thus far, have been detected only in humans. Spores of *E. hellem* and *S. intestinalis* cannot be distinguished morphologically from those of *E. cuniculi* of animal origin and are referred to as *Encephalitozoon*-like. Therefore, earlier reports of human infections with *E. cuniculi* remain uncertain because the diagnosis was done when *E. hellem* and *S. intestinalis* were not yet known. We have isolated *Encephalitozoon*-like spores from 7 HIV-infected patients and 9 rabbits from Switzerland as well as from 3 farmed blue foxes from Norway. The spores were cultivated in MRC 5 cells and characterized by Western blot analysis and by restriction enzyme analysis of the amplified SSU rRNA gene. All 9 isolates from rabbits and all 3 from foxes showed identical banding patterns in both methods as an *E. cuniculi* reference isolate. Three of our isolates from humans had similar patterns as the *E. hellem* reference isolate, one isolate was identified as *S. intestinalis*, and another three isolates were indistinguishable from the *E. cuniculi* reference isolate. The SSU rRNA sequence of one *E. cuniculi* isolate from human was determined and found to have a 99.5% similarity with the sequence of an isolate from rabbit. These findings strongly suggest that *E. cuniculi* is a zoonotic parasite.

D 17 HORSE STRONGYLES - PROSPECTS FOR NEMATODE-TRAPPING FUNGI AS BIOLOGICAL CONTROL AGENTS

Larsen M.[1], Nansen P.[1], Henriksen S.A.[1,3], Grøndal C.[2], Thamsborg S.M.[1], Grønvold J.[1] & Wolstrup J.[1]; [1]Danish Centre for Experimental Parasitology, [2]Royal Veterinary and Agricultural University & [3]National Veterinary Laboratory, Copenhagen, Denmark

As for sheep nematodes, anthelmintic resistance has also become a problem within the small horse strongyles (cyathostomes) in the horse. Thus, there is a great need for considering alternatives to traditional drug usage. Methods of pasture management and pasture hygiene can reduce the level of pasture infection. Biological control of free-living stages by nematode-destroying fungi could serve as a valuable adjunct or alternative to the mentioned types of control. Since the first description of the predacious fungus *Arthrobotrys oligospora* more than 100 years ago a relatively limited number of experiments has been carried out to evaluate the potentials of these fungi as biological control agents against parasitic nematodes in livestock. While research has clearly demonstrated that nematode-trapping fungi are able to significantly reduce the number of free-living larvae of parasitic nematodes in cattle and sheep, only few experiments have been performed to test the effect of these fungi against horse strongyle larvae. Recent studies show that nematode-trapping fungi belonging to the genera *Arthrobotrys* and *Duddingtonia* may kill preinfective larvae in faeces of horses, cattle & sheep. When spores of these fungi are mixed into faecal cultures of cyathostome infected horses the number of developing infective larvae may be significantly reduced. Dosing horses perorally with defined doses of *D. flagrans* spores (10^5-10^7 units/kg bodyweight) significantly reduce the number of developing larvae in the excreted faeces. In 1994 we performed a field experiment to test the effect of daily dosings of strongyle infected horses with *D. flagrans* spores. Besides measuring herbage infectivity during the grazing season, acquisition of infections in tracer foals were assessed by the number of eggs excreted per gram of faeces (epg) and the number of large and small strongyles present at post mortem examination. There was a highly significant reduction in herbage infectivity on the pasture grazed by fungus treated horses, which was also reflected in reduced epg and burdens of strongyles in the tracer foals at post mortem. Prospects for implementing biological control is discussed.

D 18

THE EFFECT OF NEMATOPHAGOUS FUNGI FED TO CATTLE, SHEEP AND HORSES ON THE DEVELOPMENT OF INFECTIVE LARVAE.

J. Bird, R.P. Herd. Dept. of Vet. Prev. Med., The Ohio State University, Columbus, OH, USA 43210-1092

Six-month old heifers, adult ewes, and adult mares were fed spores from 2 species of nematophagous fungi, Arthrobotrys oligospora and A. flagrans. For each host group of 5 fungi-fed animals, feces were collected, pooled and mixed with nematode ova harvested by a filter technique from feces of naturally-infected donor animals. Feces from naturally-infected donor animals were the controls. For each species of fungus, 8 experimental and 8 control fecal pats of uniform weight were formed for each animal group and randomly deposited 2 m from each other on a clean pasture. The number and genera of infective larvae (L3s) found on herbage in a 20 cm diameter semi-circle around each pat were determined 4 weeks and 8 weeks after deposition. The dominant species of parasites present were Ostertagia and Cooperia (cattle), Haemonchus (sheep), and cyathostomes species (horse). For A. oligospora, reductions in the number of L3s were 47.3% and 37.3% in the cattle study, zero in the sheep study, and 38.1% and zero in the horse study, at 4 and 8 weeks, respectively. For A. flagrans, reductions in the number of L3s were 93.2% and 72.4% in the cattle study, 36.0% and 60.1% in the sheep study, and 55.1% and 17.0% in the horse study, at 4 and 8 weeks, respectively. Overall, A. flagrans exhibited superior ability to A. oligospora in reducing pasture infectivity.

D 19

J. EUZEBY
Parasitologie
Ecole Vétérinaire de LYON
69280 MARCY L'ETOILE - FRANCE

Specificity of host-parasite relationship. What about the development in Man of parasites of animal origin ?

As an introduction to the selected topic, the author, after conjuring up the problem of parasite specificity, describes the various biological behaviour of parasites of animal origin having got into Man. So doig, he quotes two main types of parasitic zoonoses (1) holozoonoses, in which the parasites are able to pass from animals to Man and back ; (2) hemi-zoonoses, in which parasites cannot go back from Man to animals. The latter are due : (a) either to the inability for the parasite to reach, in man, the stage which would enable it to follow on its life cycle ; this is a biological phenomenon : Man is a dead-lock for the parasite ; (b) or to the necessity for a parasite having reached a suitable stage in Man, to go back -to animal through predatio of Man by the animal ; this is an ethological phenomenon : Man is a cul-de-sac for the parasite.

Parasitic diseases

Host-parasit relationship

Zoonoses.

D 20 RODENT ATERNATIVE DEFINITIVE HOST MODEL FOR *ECHINOCOCCUS MULTILOCULARIS* IMMUNODIAGNOSIS AND PROPHYLAXIS

M. KAMIYA, Y. OKU, J. INOHARA, N. NONAKA , R. OTUBO, Y. KONDOH & H.-K. OOI Dept. of Parasitology, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060, Japan

The use of predonizolone-treated golden hamster and Mongolian gerbil as alternative definitive host model for *Echinococcus multilocularis* has been well documented. However, *E. multilocularis* adult stage tapeworm had also been recovered from the small intestine of experimentally infected golden hamster on day 25 postinfection (PI) without predonizolone treatment. To examine the range of rodents that can serve as alternative definitive host for *E. multilocularis*, wild rodents of North America belonging to Heteromydae(*Perognathus* sp. and *Dipodomys* spp.) and Hesperomynae (*Peromyscus* sp., *Onychomys* sp., *Reithrodontomys* sp., *Neotoma* sp. and *Sigmodon* sp.) were orally infected with protoscoleces followed by attempts to recover the tapeworm from their digestive taracts. Between 20-80% of the tapeworm were recovered from *Perognathus* sp. and *Dipodomys* sp. during the initial stage of the experiment, that is, between day 2-5 PI. However, worms could only be recovered only from *Perognathus* sp. thereafter, until day 25 PI, with a recovery rate of 0.9-5.2%. No tapeworm could be recovered from the Hesperomynae group of rodents. Thus, with further modifications, the golden hamster and *Perognathus* sp. may be developed into research models for the study of coproantigen detection in immunodiagnosis of the infection in the definitive host and also the elucidation of the phenomenon of worm expulsion from the degestive tract. Rationale and limitations on the use of Cricetid and Heteromid rodents as alternative difinitive host model for *E. multilocularis* will also discussed.

SCIENTIFIC PROGRAM

POSTER SESSION

Wednesday August 30 ~ Friday September 1, 1995 **Room E**

IN VITRO EFFECTS OF PF1022A ON *ANGIOSTRONGYLUS CANTONENSIS, ASCARIS SUUM* AND ISOLATED FROG RECTUS PREPARATIONS

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ABSTRACTS OF POSTER SESSION

E 1 *IN VITRO* EFFECTS OF PF1022A ON *ANGIOSTRONGYLUS CANTONENSIS*, *ASCARIS SUUM* AND ISOLATED FROG RECTUS PREPARATIONS

Mamoru Terada, Wency Chen, Motohito Sano, Juei-Tang Cheng*
Department of Parasitology, Hamamatsu University School of Medicine, Hamamatsu, Japan
* Department of Pharmacology, College of Medicine, National Cheng Kung University, Tainan City, Taiwan

PF1022A is an anthelmintic newly developing in Japan. In this study we examined neuropharmacologically mode of action of PF1022A. 1. Effects of PF1022A on the motility of the whole worm preparation of *Angiostrongylus cantonensis* by means of isotonic transducer method: PF1022A inhibited the motility at such a low concentration as 10^{-13} g/ml (1.05×10^{-13} M), and paralyzed the worm at 10^{-12} - 10^{-6} g/ml. The paralysis by the drug at 10^{-12} g/ml was partially antagonized by gabergic antagonists like picrotoxin and bicuculline, and completely reversed when N-methylcytisine (N-MC, a stimulator of the release of ACh) was added with gabergic antagonists. On the other hand, in the preparations paralyzed by PF1022A (10^{-10} g/ml), the spasmogenic effects of N-MC and eserine were kept inhibited even with gabergic antagonists, while those of pyrantel (a nicotinic receptor stimulant) were not inhibited. 2. Effects of PF1022A on GABA receptor binding in *Ascaris* muscle membrane fraction: PF1022A (10^{-10}-10^{-5} M) inhibited dose-dependently the binding of [methyl-^{3}H]-bicuculline. On the other hand, PF1022A at higher concentrations such as 10^{-5} M also inhibited the binding of [butyl-^{3}H]-baclofen. These results suggest that PF1022A selectively binds the $GABA_A$ receptors and paralyzes nematode worms. 3. Effects of PF1022A on the guanidine (2.5×10^{-3} M)-induced twitch responses in isolated frog rectus: The twitch responses were inhibited by PF1022A (10^{-6} g/ml), while contraction by pyrantel was not inhibited in the paralyzed preparation. As all gabergic anthelmintics except piperazine are known to stimulate the release of ACh from the nerve endings at their higher concentrations, PF1022A is unique in affecting nematodes synergistically by stimulating the gabergic mechanism and inhibiting the cholinergic mechanism.

E 2 *IN VIVO* EFFECTS OF PF1022A ON *ANGIOSTRONGYLUS CANTONENSIS* IN RATS

Shigeo Kachi, Mamoru Terada, Akira Ishih, Motohito Sano, Hisakuni Hashimoto[1], Mitsuyo Matsumoto[2], Tomoko Shomura[2] Department of Parasitology, Department of Pharmacy[1], Hamamatsu University School of Medicine, Hamamatsu, Japan, Department of Formulation, Pharmaceutical Research Center, Meiji Seika Kaisha, Yokohama, Japan[2]

PF1022A is known to be effective against many intestinal nematodes *in vitro* and *in vivo*. Regarding tissue-dwelling nematode, Terada *et al.* (1993) has reported that PF1022A was completely effective against larval *Angiostrongylus costaricensis* in mice by 5 successive doses at 10 mg/kg (oral, p.o.) and 0.625 mg/kg (intraperitoneal, i.p.). In addition, effects were markedly influenced by the formulation of the drug and the formulation named as the oral cream was the most effective among 4 formulations examined. In this study we examined effects of PF1022A in the oral cream against *A. cantonensis* in rats, our another model. When we use this model, we can examine effects of drugs on (a) adult worms in the pulmonary arteries, (b) larval and young adult stages staying in the CNS and (c) larvae migrating into the CNS by selecting timing of treatment. 1. Effects of PF1022A on adult worms: Five successive oral doses at 10 mg/kg killed completely female worms but not male worms. About 80 % of male adults were also killed when the successive treatment was repeated 4 times. Five successive i.p. doses at 0.5 mg/kg killed about 60 % females and 40 % males. 2. Effects of PF1022A on larvae and young adult worms staying in the CNS of rats: Five successive doses at 20 mg/kg, p.o. and 2.5 mg/kg i.p. were little effective. 3. Effects of PF1022A on larvae migrating into the CNS: About 80 % females and 60 % males were killed by 5 successive oral doses of PF1022A at 10 mg/kg. By 5 successive i.p. doses at 0.5 mg/kg, similar relation in worm recovery between females and males was observed, but less effective. These results suggest that PF1022A is effective against *A. cantonensis* in the sites other than the CNS of rats probably because it does not pass through the blood-brain barrier. As the drug is extremely less toxic, PF1022A will become a promising anthelmintic available for tissue-dwelling as well as intestinal nematodes.

E 3 THE EFFICACY OF LUFENURON AGAINST THE DOG LOUSE (*Linognathus setosus*) - A CLINICAL TRIAL

V. Rantanen, S. Saari and S. Nikander
College of Veterinary Medicine, Laboratory of Parasitology, Helsinki
Finland

The benzoylphenyl urea, lufenuron is an insect development inhibitor developed by Ciba-Geigy that has been proven to be useful against cat fleas (*Ctenocephalides felis*). The purpose of this study was to find out the usefulness of orally administered lufenuron against the dog lice (*Linognathus setosus*), that is still an important ectoparasite of dogs in Finland, being more common than *C. felis*. The material consisted of 68 dogs of various breed, sex and age with suspected naturally occuring infestation by *L. setosus*. The infestation was confirmed on 11 dogs by demonstrating and counting live lice, on predilection sites (head, chest, neck and back). Lufenuron was administered orally according to the manufacturer's protocol. Each dog got totally 3 tablets (1 tablet monthly = effective dose for 90 days). The patients were examined every 30 days (30, 60, 90 and 120 days) and the predilection sites were examined for lice and eggs. The five dogs that had been most heavily infested still had living lice 30 days after the first treatment although the number of the lice was remarkably lower. The dogs that had only few lice seemed to be free of them at the time of the first control. After 60 days all dogs but one were considered as lice-free and after 90 days also that dog was cured. In this clinical trial lufenuron seemed to have development inhibiting capacity against dog lice. Lufenuron has no effect on the adult fleas and seemed to be uneffective against adult dog lice as well. This is a disadvantage of lufenuron, when it is used in treatment of canine linognathosis. The fairly long total elimination time of adult lice may cause inconvinience (pruritus etc.) for the dog. Our suggestion is to treat the dog with lice with topical insectiside followed by lufenuron course to avoid the reinfestations, and inhibit development of hatching immature lice. Lufenuron is also valuable as a preventive treatment above all in kennel conditions.

E 4 EFFECTS OF SALINOMYCIN ON THE NUMBER OF *LACTOBACILLUS SPP.*, *CLOSTRIDIUM PERFRINGENS* AND METABOLITES LEVELS IN CECAL CONTENT OF BROILER CHICKEN

KYUYA SUDA, KENICHIRO TAKEI, SHI EIE CHENG, YASUO KOBAYASHI*, MASAAKI WAKITA* AND SADAO HOSHINO*

Ag-Vet Div., Kaken Pharmaceutical Co., Ltd., Fujieda-shi 426, Japan

*; Faculty of Bioresources, Mie University, Tsu-shi 514, Japan

Two experiments were conducted to evaluate the effects of salinomycin (SL) on the number of bacteria *Lactobacillus spp.*, *Clostridium perfringens* and metabolites levels in cecal content of broiler chicken. Exp.1: Four two-week-old commercial female chickens were orally administered 5mg of SL suspending in 1ml of water twice a day and other five received just water as Control. On the following day, their ceca were aseptically removed and the cecal contents were diluted in an anaerobic buffer solution every 10-fold to 10^{-9}. Exp.2: Forty two-week-old female chickens were assigned into two groups and given un-medicated (Control) or SL 50ppm medicated commercial feed (SL group) during the experiment. Five each were killed 1, 2, 3 and 5weeks later and their dilutions of cecal contents were obtained by the same way as in Exp.1. In both experiments, the dilutions were inoculated in 2 agar media (TS and DHL) for aerobes and 5 (EG, BL, BS, LBS and NN) for anaerobes and incubated at 39°C for 2 or 3 days. The number of colony in each media was counted. After centrifugating the 10^{-1} dilution, the supernatant was stored in feezer for the later assay of VFA and lactate.

The numbers of *Lactobacillus spp.* and *C. perfringens* in SL group were less than Control in Exp.1. SL incresed the minor VFA resulting in higher total VFA level than Control. L(+)-lactate level in SL group was also higher than Control. Same results were obtained in Exp.2, especially significant differences ($p<0.05$) were observed on the number of *Lactobacillus spp.* in first week, *C. perfringens* in second week and L(+)-lactate level throughout the experiment. These results suggest that SL may affect the intestinal metabolites by shifting micro-flora and its anticoccidial activity.

E 5 IN VITRO SELECTION OF *FASCIOLA HEPATICA* FOR RESISTANCE TO CLOSANTEL

Klockiewicz M., Wedrychowicz H.

Department of Parasitology, Warsaw Agricultural University, Warsaw, Poland

Resistance of helminth parasites to anthelmintics is an increasing problem in world economy. Suspected resistance of *Fasciola hepatica* to salicylanilides has been recently confirmed in the fields. However, the mechanisms of the resistance have not been recognised yet. In the present experiments we selected *in vitro* populations of the liver fluke showing increased resistance to salicylanilide derivative - Closantel by incubation of liver fluke eggs in media containing increasing concentration of the drug. Three populations with increasing levels of resistance were acquired and somatic protein patterns were compared in normal and drug resistant flukes. Preliminary results indicate that production of approximately 12 polypeptides is reduced in drug resistant flukes and 3 new polypeptides with MW 29,5 ; 25.4 and 13.3 kDa (PI 6.4; 5.4; 5.6 respectively) are expressed. DNAs complementary to mRNA isolated from adult worms showing normal or increased resistance to closantel are presently being analysed by RAPD-PCR technique. Six arbitrarily selected 10--mers as well as 10 *F. hepatica* specific primers are used in order to find out whether the increased resistance to closantel is associated with a selection of a particular DNA sequence.

E 6 Parasiticidial effects of bovine Lactoferricin to *Toxoplasma gondii* parasite

T. TANAKA[1], Y. OMATA[1], A. SAITO[1], K. SHIMAZAKI[3], I. IGARASHI[2] and N. SUZUKI[2]
[1]Dept. of Vet. Physiol., [2]Res. Ctr. for Protoz. Mol. Immunol., Obihiro Univ. of Agr. & Vet. Med., Obihiro [3]Dept. of Dairy Sci., Fac. of Agri., Hokkaido Univ. Sapporo, Japan.

Bovine lactoferricin (B-LFcin), an active peptides, which is isolated and sequenced from pepsin digestion of lactoferrin, has inhibitory effects to the growth of Gram-negative and Gram-positive microorganisms due to direct damage to outer membrane of them. This peptides is contained asymmetric clustering of basic amino acid residues such as lysin and arginin. In the present study, the effects of B-LFcin to infectivity and viability of *Toxoplasma gondii* (*T.gondii*) parasite was examined in vitro and in vivo assay.

When high virulent strain of *T.gondii* (RH strain) were incubated with 1000 μ g/ml of B-LFcin at 37 ℃ for 30 minutes, 90 % of parasites had lost trypanblue dye exclusion activity and decreased significantly penetration ability of mouse embryonal cells.

When mice were inoculated with either non-treated or B-LFcin treated parasites, four out of five mice inoculated with treated parasites survived until end of experiment without any clinical sign. While, all mice inoculated with non-treated parasites were died within 9 days post inoculation. Likewise, mice inoculated with treated cysts of *T.gondii* (S273 strain) had lower numbers of cysts in the brain (300/mouse), in comparison with that of mice inoculated with non-treated ones (1500/mouse).These results indicate parasiticidial effects of B-LFcin to *T.gondii*.

E 7 Resistance to Toxoplasma gondii Infection in Cats Inoculated with ^{60}Co-Irradiated Parasites

Y. OMATA[1], M. KANDA[3], A. SAITO[1], I. IGARASHI[2], and N. SUZUKI[2]
[1]Dept. of Vet. Physiol., [2]Res. Ctr. for Protoz. Mol. Immunol., Obihiro Univ. of Agr. & Vet. Med., Obihiro [3]Aburahi Lab., Shionogi & Co., LtD., Kohka-cho. Shiga, Japan

The effects of ^{60}Co-irradiation on the viability and immunogenicity of Toxoplasma gondii (T. gondii) was examined in comparison with different strain. ^{60}Co-irradiated parsites remained invasion activity in mouse embryonal cells and sustained the antigenicity in mouse peritoneal macrophages, although the parasites have lost the development activity in the host cells. Preinoculation of mice with ^{60}Co-irradiated parasites of either RH or Beverley strain appeared to induce resistance to exposure of high virulent strain (RH strain). On the other hand, preinoculation of kittens with ^{60}Co-irradiated parasites of Beverley strain had tendency to prevent oocyst shedding post orally exposure of T. gondii cysts, Whereas, kittens preinoculated with ^{60}Co-irradiated parasites of RH strain had shedding oocysts post the exposure, as well as non-inoculated ones. These results suggest that the development of protective immunity in feline toxoplasmosis requires stimulation of strain and /or stage-specific parasite antigen(s). Interaction between parasitized cells and immune recognition system would be also concerned with the induction of protective immunity.

E 8 SOME CHARACTERISTICS OF IMMUNE RESPONSE OF CALVES INFECTED WITH IRRADIATED AND NON-IRRADIATED L3 OF *HAEMONCHUS PLACEI*

M.C.R.Vieira-Bressan, S.M.Gennari,R.Nurmberger Jr., M.L.Z.Dagli
Depto.de Parasitologia,Instituto de Ciências Biomédicas, USP, Av. Prof. Lineu Prestes,1374-São Paulo-CEP 05508-900-BRASIL.

Twelve Holstein male calves aged 4 months were separated in 3 groups: G1 and G2, immunized with two doses of 50,000 irradiated L3 (60Co-400Gy) and non-irradiated L3, respectively, with 5 weeks interval between doses, and unimmunized G3(control). Four weeks later, G1, G2 and G3 received a challenge infection with 100,000 L3. After five weeks, all calves were necropsied for worm counts, and abomasal tissue samples were harvested for immunohistochemistry with monoclonal antibodies anti-bovine IgA. Blood and faecal samples were collected at weekly intervals. Calves immunized with irradiated larvae (G2) and control group became positive for egg counts at week 4 after the challenge, whereas calves receiving non-irradiated larvae showed egg counts from week 4 after the 1st dose, increasing until the sacrifice. The differences between worm burden means was not significant . PCV values of calves G2 decreased significantly from week 4 to 11. After that the other two groups showed an abrupt PCV values decrease until the end of the experiment. Antibody response was measured by ELISA using crude *H.placei* adult antigen (10 ug/ml). The serum dilution (1:50) was used in duplicate. A peroxidase-conjugated rabbit anti-bovine IgG(whole molecule) at dilution 1:1,000 was used as primary antibody. The substrate was o-phenylenediamine and the optical density was measured at 492 nm. Two weeks after the 1st dose, a similar gradual increase of *H.placei* IgG antibodies was detected in G1 and G2, which were significantly higher than G3 until week 11. However, the values of G2 showed a tendency to be superior of those from G1.The absorbance values from the controls showed a background activity until the 2nd week after the challenge, when occurred a sudden significant increase of these values. The IgA positive cells were counted in 40X power fields of abomasal mucosa sections. The results showed that the G1 animals presented IgA positive cells amounts similar to the G3, and G2 animals presented less IgA positive cells than the other two groups.

E 9

CHARACTERIZATION OF EPITOPES ON AN 18kDa SURFACE PROTEIN OF *B. EQUI*

Ali, S[1]., Sugimoto, C.[1], Kanemaru, T.[2], Kamada, T.[2], Onuma, M.[1]

[1]Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060,Japan

[2]Equine Research Institute, Japan Racing Association,Tochigi 329-4, Japan

Equine babesiosis is one of the major problems in the horse trade especially due to the danger of disease transmission during the movement of horses from country to country. There is a need to improve the existing diagnostic assays. In our previous study (Ali *et al.* 1993), proteins of *B. equi* piroplasms were characterized. Immunodominant proteins identified in immunoblotting with serum of an experimentally infected horse were p18, p28 and p30, all of which were membrane-bound proteins. It is necessary to determine the utility of these proteins in various serological tests for equine babesiosis. In this study, monoclonal antibodies (MoAbs) were produced against *B. equi* piroplasms. Three MoAbs reacting with an 18 kDa surface membrane protein (p18) of *B. equi* in immunoblot analysis, were used to characterize the epitopes on p18. All the three MoAbs recognized the same epitope on p18 as indicated by competitive ELISA. Negative results in two-site ELISA suggests absence of repetitive epitopes on p18. Triton X-114 phase partitioning confirmed that 18 kDa antigen is an integral membrane protein of *B. equi* piroplasms. As these MoAbs identified a single protein and showed no crossreaction with *B. caballi* or equine erythrocyte proteins, these can be a candidate to be used in the differential diagnosis of mixed equine piroplasma infections.

E 10 CLONING AND CHARACTERISATION OF *UNCINARIA STENOCEPHALA* RECOMBINANT ANTIGEN

H. Wedrychowicz & A. Orzel
Department of Parasitology, Warsaw Agricultural University, Warsaw, Poland

Uncinaria stenocephala infections are wide spread in dogs of Northern Hemisphere and known to be associated with pathology, including anaemia, eosinophilia , dilatation and varicosity of the capillaries of intestinal villi. To our knowledge, there have been no reports so far on functional antigens of the nematode.

In the present study antigens inducing serum antibody responses of naturally and experimentally infected dogs were investigated using serological and molecular biology techniques.

ELISA tests showed that both naturally and experimentally infected dogs produce antibodies against surface and somatic antigens of infective larvae and adult nematodes. Western blot analysis revealed that sera from infected dogs specifically recognise at least three antigens on the surface of infective larvae of the nematode (MW 245; 52.5 and 44 kDa). A cDNA expression library was constructed from *U. stenocephala* infective larvae mRNA in the UNI-ZAP vector. A clone encoding a 44 kDa *U. stenocephala* molecule was isolated on the basis of specific recognition by sera from dogs experimentally infected with the nematode. Protein sequence data from this clone is being examined to obtain information on the possible biological function of this protein.

E11 ANTIBODY RESPONSE AND PROTECTION AGAINST *STRONGYLOIDES PAPILLOSUS* IN RABBITS

Y. Nakamura, C. Ooba[1] and N. Taira
National Institute of Animal Health, Tsukuba, Ibaraki 305, Japan ([1]Hokkaido Prefecture)

The relation between serum IgG response and protective immunity against *Strongyloides papillosus* was investigated in rabbits receiving infective larvae (L_3) by skin exposure. Specific antibodies were produced against 30–200 kDa and 21–160 kDa proteins of L_3 somatic and adult excretory/secretory (ES) antigens, respectively, by 4 weeks post-infection (pi). The responses to L_3 somatic antigens were enhanced in rabbits with secondary infection. No antibody responses were detected against any proteins of L_3 ES and adult somatic antigens. In rabbits immunized with either L_3 somatic or adult ES antigens by intradermal inoculations, antibodies were produced against 30–200 kDa or 67–210 kDa proteins of each homologous antigens, respectively. No responses were detected against any proteins of each heterologous antigens. Percentage reductions of maximum egg counts after challenge infection were 99.8, 65.7 and 9.3% in rabbits receiving secondary infection, immunization with L_3 somatic and adult ES antigens, respectively, as compared to rabbits with primary infection. Percentage reduction of migratory larvae recovered from the lungs 5 days pi was 72 % in rabbits immunized with L_3 somatic antigens, while no larvae in the lungs of rabbits with secondary infection. These results suggested that immune responses, including effective antibody production, are induced by L_3 somatic antigens to play a role in the protection in the early stages of reinfection.

E 1 2 γδ T CELLS PLAY AN IMPORTANT ROLE IN HSP65 EXPRESSION AND IN ACQUIRING PROTECTIVE IMMUNITY IN MICE INFECTED WITH *TOXOPLASMA GONDII*

Hideyuki Nagasawa, Hajime Hisaeda, Tohru Sakai, Hiroyuki Ishikawa, Youichi Maekawa, and Kunisuke Himeno
Department of Parasitology and Immunology, School of Medicine, The University of Tokushima, Tokushima, JAPAN

Heat shock proteins (HSPs) are evolutionarily highly conserved polypeptides. They are immunodominant antigens in a wide variety of bacteria and parasites. Recently, the role of HSPs in infection and immunity is receiving much attention. We previously reported that the expression of 65-kD HSP (HSP65) within host macrophages closely correlates with protection against infection with *Toxoplasma gondii* in mice. Here, we propose that γδ T cells play a crucial role in the induction of HSP65 and also in the protective immune response to *T. gondii* infection. Intraperitoneal inoculation with this protozoan (Beverley strain bradyzoites) resulted in HSP65 being expressed on and in host peritoneal macrophages and an increase of γδ T cells in the peritoneal cavity and spleen. When mice were depleted of γδ T cells by the administration of a mAb, HSP65 expression was markedly decreased. By contrast, the expression of this protein was rather enhanced and γδ T cells prominently expanded in mice depleted of αβ T cells. The protection in mice treated with the mAb paralleled the magnitude of HSP65 expression. Mice depleted of γδ T cells died most frequently in the early stages of infection, whereas most of those depleted of αβ T cells survived the early stages of lethal infection with *T. gondii*. However, the latter group of mice did not definitely control the *T. gondii* infection in its late stages. These findings indicated that γδ T cells contribute to express HSP65 in macrophages and to induce the protective immunity at early stage of *T. gondii* infection.

E 1 3 " CROSS-REACTIONS BETWEEN *Toxocara canis AND Ascaris suum* IN THE IMMUNODIAGNOSIS OF VISCERAL LARVA MIGRANS"

NUNES, C.M.**; TUNDISI, R.N.; HEINEMANN, M.B.; RICHTZENHAIN, L.J.; OGASSAWARA, S.
Faculdade de Med. Veterinária e Zootecnia - U.S.P. - São Paulo - BRASIL.

Visceral Larva Migrans (VLM) is a clinical syndrome of man caused by tissue migration of larval stages of *Toxocara canis,* the common roundworm of dogs. Diagnosis of this disease depends mainly on immunological tests because neither eggs nor larvae are eliminated by the host (GLICKMAN; SCHANTZ,1981). After the introduction of the enzyme-linked immunosorbent assay (ELISA) using the larval secretory-excretory (ES) antigen of *T. canis* (de SAVIGNY *et al.*, 1979), the diagnosis specificity was greatly improved although cross-reactivity with other helminths are still being reported (LYNCH *et al.*,1988). In Brazil, diagnosis is routinely made after absorption of serum samples with *Ascaris suum* antigens, a nematode antigenicaly related with *Ascaris lumbricoides* which is a common intestinal nematode of children.

In order to identify *T. canis* antigens that will increase the specificity of diagnostic tests we analysed ES antigen by SDS-PAGE and Western blotting techniques (TOWBIN *et al.*, 1979). When we used serum samples from patients suspected of VLM numerous bands were seen. Among these there is at least one band with molecular weight around 55-60 kDa that seems to be responsible for the cross-reactivity between *T. canis* e *A. suum* once it desapear when previous absorption of serum samples with *A. suum* antigens is performed.

E 1 4 CANINE BABESIOSIS: CLINICAL ASPECT, HEMOGRAM AND BONE MARROW CYTOLOGY.

ASSIS, C.T., KOHAYAGAWA, A., LARANJEIRA, S., BOMFIM, S.R.K.M.
Veterinary Medicine Faculty - Veterinary Clinical Department - Universidade Estadual Paulista - PO.BOX 560 - Botucatu - SP - Brazil - 18.618-000

Eleven mixed breed dogs, weighting approximately 10 kilograms each were used in the trials. These animals were serologically negative for babesiosis and were maintained tick-free in individual cages. One dog was splenectomized to enhance babesia growth while the remainig were alloted into Group I (six animals) and Group II (four animals). All the parameters were determined for each animal prior to the inoculation with Babesia canis. Animals from Group I were inoculated with 4.8×10^9 B. canis infected erythrocytes and four blood samples were collected at seven days intervals. The animals were then splenectomized. Twelve blood samples were collected afterwards, at weekly intervals, and the animals reinoculated on day 115th with a different B. canis strain. After this, one blood sample was collected. Animals from Group II were splenectomized, and 45 days after the surgery they were inoculated with 5.0×10^9 B. canis infected erythrocytes and monitored for 63 days. The follow up of both Groups consisted of: daily rectal temperature and parasitemia measurements, hemograms, total protein (TP), fibrinogen and bone marrow cytology. All the animals from Group I showed a slight decrease in erythrocytic values before and after splenectomy. Four out of six had no clinical changes while the other two developed babesiosis. In the Group II animals, the disease was characterized by temperature increases, initial leukopenia an anemia. Both Groups showed responsive bone marrow and unchanged TP levels. Temporary increase of fibrinogen concentrations were also observed, but only in Group I animals.

E 1 5 THE DYNAMIC OF IgG RESPONSE IN CALVES UNDER DIFFERENT DIETARY PROTEIN AND IMMUNISED WITH *Haemonchus placei.*

S. M. Gennari, S. M. Nishi, L. J. Richtzenhain, M. C. R. Vieira Bressan, D. M. S. S. Vitti
Faculdade de Medicina Veterinária e Zootecnia - USP, São Paulo, Brazil.

The experiment was conducted to examine the influence of dietary protein and immunisation on IgG production in calves infected with *Haemonchus placei.* Four groups of 4 to 6 month old calves (n=4) were given a low protein diet (LP) containing 213g/head/day crude protein (CP) or high protein diet (HP) containing 469g/head/day CP. Five weeks later, calves in one of the two groups of each dietary treatment were given 50,000 *H.placei* L3. Twenty five days later, infection in these groups was terminated by dosing with oxfendazole. This immunisation process was repeated 4 days later. Four days after termination of the second immunisation all calves were challenged with 100,000 L3. Five weeks later they were slaughtered for worm counts. Serum samples were collected at weekly interval during all the trial and an ELISA, using adult *H.placei* extract as antigen (10ug/ml) was used to evaluate the IgG response. A chequerboard titration was done previously and the optimal dilution for the serum and conjugate (peroxidase-conjugate rabbit anti-bovine IgG) were 1:50 and 1:1000 respectively. The o-phenylenediamine dihydrochloride was employed as chromogen and the optical density (OD) measured at 492 nm. A linear model for analysis of variance was used and changes within groups over time were analyzed by Tukey test. From the pre immunisation period until the challenge the OD values showed similar pattern. The HP-Immunised group started to show antibodies rise after the 2nd immunisation, however only after the challenge this rise was significant ($p<0.05$). The results showed a positive correlation between IgG and protein level in the diet in both immunised and control groups ($p<0.05$). However, the immunisation had no effect on the IgG values ($p>0.05$).

E 1 6 USE OF A SOLUBLE *Haemonchus placei* ADULT ANTIGEN IN AN ELISA FOR IMMUNODIAGNOSIS OF BOVINE HAEMONCHOSIS.

S.M.Gennari, S.M.Nishi, L.J.Richtzenhain, M.C.R.Vieira Bressan
Depto. de Medicina Veterinária Preventiva e Saúde Animal, Faculdade de Medicina Veterinária e Zootecnia -USP- São Paulo - BRAZIL.

Enzyme-linked immunosorbent assays (ELISA) were used to measure the anti-*Haemonchus placei* antibody response (IgG) in serum of calves. Serum samples were collected from 20 calves aged 4 to 6 months before and 8 weeks after experimental infection with 100,000 *H.placei* L3. Individual negative and positive sera were used to determine the optimal antigen and conjugate concentration by chequerboard titration. The conjugate used was a peroxidase-conjugate rabbit anti-bovine IgG (whole molecule) and the microtitration plates were from NUNC. The plates were blocked with 10% horse serum and *o*-phenylenediamine dihydrochloride was employed as chromogen. The optical density (OD) was measured at 492 nm. For standardisation of the different readings, the OD of all samples were converted into indices (S/P). Adult worms of *H.placei* were homogenised with anti-proteases and centrifuged at 10,000g for 5 min. and supernatants used as antigen for ELISA. Protein yield was estimated by the BCA Protein kit. The significance of the differences between the two periods was calculated using analysis of variance. The optimal antigen concentration was 10 ug/ml. A conjugate dilution of 1:1000 (v/v) gave the optimal results. The best discrimintion between positive and negative sera was seen at a serum dilution of 1:50. All 20 negative sera showed a background activity and the OD increased significantly ($p<0.01$) 8 weeks after infection. The cut-off point for the discrimination between positive and negative findings was 0.53 S/P (mean OD for the negatives + 2 SD). The sensitivity and specificity values were respectively: 95% and 90%.

E 1 7 IgG LEVELS IN CALVES DURING TWO CONSECUTIVE INFECTIONS WITH *Haemonchus placei* MEASURED BY ELISA.

S. M. Gennari, S. M. Nishi, L. J. Richtzenhain, L. R. Meireles, M. C. R. Vieira Bressan
Faculdade de Medicina Veterinária e Zootecnia - USP, São Paulo, Brazil.

The dynamic of the humoral response (IgG) in calves during two experimental *Haemonchus placei* infection was investigated. Two groups of five 5 to 6 month old calves were infected with 100,000 infective larvae. Group A received the total larvae amount at once; Group B received the same amount in five inocula of 20,000 L3 each at two days interval between doses and Group C functioned as controls. When the first infection, measured by faecal egg counts, finished, the calves received oxfendazole and they were reinfected using the same scheme of infection. At the end of the second patent period, all calves were necropsied for worm counts. Blood samples were collected at weekly intervals. ELISA was used to evaluate the IgG response during the infections. An adult *H. placei* soluble extract was used as antigenic material (10 ug/ml). One serum dilution was used (1:50) in duplicate. A peroxidase-conjugate rabbit anti-bovine IgG (whole molecule) was used as a second antibody (1:1000). The substrate was o-phenylenediamine and the optical density (OD) was measured at 492 nm. Analysis of variance was used and changes within groups over time were analysed by Tukey test. *H.placei* IgG antibodies started to rise 4 weeks after the first infection ($p<0.05$) and peaked for the first time at week 6 after infection. Another increase was noted after the second infection with a peak between weeks 8 and 10. The infected groups showed similar pattern and since the 4th week the OD values were significantly superior than the values from the controls, that only presented a background activity.

E 18 A MAJOR GENE DETERMINING THE RESISTANCE OF SHEEP AGAINST *FASCIOLA GIGANTICA*.

Roberts JA[1], Widjayanti S[1], Hetzel DJS[2], Partoutomo S[1]
Balai Penilitian Veteriner[1] & CSIRO Australia[2],
Bogor, Jawa Barat,
Indonesia.

Indonesian Thin Tail (ITT) sheep have resistance against *F gigantica* when infections are compared with those in Merino sheep and St Croix sheep. The inheritance of the resistance has been investigated by counting the yields of mature parasites in groups of ITT sheep, St Croix sheep and F2 and F3 crossbred sheep. Approximately half of the crossbred sheep were as resistant as the parental ITT sheep, one quarter were relatively resistant and one quarter were as susceptible as the parental St Croix sheep. It was concluded that the resistance is controlled by a major gene with incomplete dominance. There is potential for substantial benefit from utilising ITT sheep for breed substitution and crossbreeding in regions of the humid tropics where fasciolosis is a constraint on meat production from small ruminants. Identification of the *F gigantica* resistance gene could lead to benefits arising from gene transfer, or detection of the gene in other breeds of sheep, and subsequent development of resistant strains of those breeds.
The fasciolosis project at Balitvet is sponsored by the Australian Centre for International Agricultural Research.

E 19 CELLULAR RESPONSE TO INFECTION WITH CANINE HOOKWORM *UNCINARIA STENOCEPHALA*

Wedrychowicz H.,[1] Piusinski W.,[2]Krawiec M.[2], Gorski P.[1]
[1]Department of Parasitology & [2]Department of Pathology Warsaw Agricultural University, Warsaw, Poland

The host-parasite relationship of the post-infection tissue resident and migratory stages of hookworms are areas which have received very little attention to-date, particularly in relation to host immune responses to *U. stenocephala.* Although, this nematode matures only in carnivores, the L3 larvae can penetrate the skin of many mammal species and are able to survive there for several weeks. Like other canine hookworms, *U. stenocephala* is a cause of the so-called "creeping eruption" in humans.

In the present study eosinophil and mast cell responses in the skin, thoracic lymph nodes , spleen and blood were evaluated using a differential staining methods.

The level of eosinophils in the blood of mice infected with one dose of larvae was 2 and 3.4 times higher than in controls on 14 and 28 days post infection (dpi) respectively. In the blood of mice subjected to 2 infections eosinophils increased 2.5 to 2.1 times (14 and 28 dpi respectively) as compare to non-infected controls. A clear eosinophil infiltration was observed in the skin at the site of infection. Interestingly, the number of these cells increased significantly ($p<0.01$) and dramatically at the site of the first infection (abdomen) within 24 hours of exposure to the second dose of larvae which was administered at a different site (back). An infiltration of mast cells to the site of larval penetration was also observed however, the number of mast cells was much lower than eosinophils and statistically significant differences between control and infected mice were only observed in the group exposed to two infections.

The results suggest that eosinophils may play a major role in trapping and damaging the larvae during their migration in the skin.

E 2 0 *ECHINOCOCCUS GRANULOSUS* IN FINLAND

J. Lundén, S. Saari and S. Nikander
College of Veterinary Medicine, Laboratory of Parasitology, Helsinki
Finland

This retrospective study is based on the records from the College of Veterinary Medicine, the National Veterinary and Food Research Institute and the Finnish Medical Board.The intention was to documentate all the diagnosed cases of hydatidosis, in animals and man, in Finland. The hydatidosis is very rare, seen almost exclusive in reindeer. Since 1968 when the law of reindeer meat inspection came into force, reliable records have been available. During the past 37 years 59 cases of hydatidosis have been diagnosed in the lungs of the reindeer in the northern part of Finland (about 100 000 reideer are slaughtered and inspected annually). In 1986 a case of bovine lung hydatidosis was registered in the southern part of our country. This is interesting, because it is the only bovine case ever recorded in Finland, as far as we know. Four cases (1978, 1979, 1992 and 1993) of equine hydatidosis has been recorded in the Department of Pathology of the College of Veterinary Medicine. Three horses had typical liver cysts, one had cysts in the lungs. Three horses had unilocular cysts, while one had a multilocular cyst. All these horses were imported. There is no reliable figures on hydatidosis in sheeps and pigs because there has been a confusion between hydatid cysts and cysticercus in the statistics from the slaughterhouses. In the late 1940's a cyst was detected in the lung of a reindeer owner in the obligatory x-ray examination for tuberculosis. The cyst was surgically removed and the final diagnosis was hydatidosis. In 1963 an analogous case was diagnosed in another reindeer owner. Since then sporadic cases has been detected in immigrants. To our knowledge, echinococcosis has never been diagnosed in *Canidae* in Finland.

E 2 1 IgG1 AND IgG2 ANTIBODY RESPONSES IN SYMPTOMATIC AND ASYMPTOMATIC DOGS NATURALLY INFECTED WITH *LEISHMANIA INFANTUM*

P. Deplazes[1], N.C. Smith[2], P. Arnold[3], A. Mathis[1], I. Tanner[1] and J. Eckert[1]. [1]Institute of Parasitology and [3]Department of Internal Veterinary Medicine, University of Zürich, Switzerland, [2]Queensland Institute of Medical Research, Queensland, Australia

Sera from dogs naturally infected with *Leishmania infantum* were analysed for the IgG subclass specificity of their antibody responses by ELISA. Specific IgG1 was detected only in dogs with progressive disease (symptomatic dogs before chemotherapy and non responders to chemotherapy). IgG1 levels decreased markedly within 3 months after successful clinical chemotherapy with Glucantime®. In all these 5 cases, however, persistence of the infection was proven by diagnostic in vitro cultivation and PCR of lymph node aspirates. Specific IgG2 was detected in dogs with symptomatic and asymptomatic infections. Furthermore, blood mononuclear cells of asymptomatic dogs responded to *L. infantum* antigen in the lymphocyte proliferation assay whereas cells of symptomatic dogs failed to respond. The differential responses of IgG1 and IgG2 serum antibodies in asymptomatic and symptomatic dogs may indicate a dichotomous immune response to infection with *L. infantum*. To confirm this on a broader scale, sera from dogs naturally exposed to *Toxoplasma gondii* (asymptomatic infections) were analysed as well as sera from dogs exposed to the nematodes *Dirofilaria immitis* and *Toxocara canis*. Only specific antibodies to *T. gondii* antigen of the IgG2 subclass were detected in sera of 17 dogs. Both IgG1 and IgG2 antibodies to *D. immitis* and *T. canis* antigens were present in the sera of naturally infected dogs but IgG1 appeared to be the predominant subclass. The association of IgG1 with disease caused by *L. infantum* may be extremely useful in veterinary practice as an adjunct to monitoring and predicting the severity of disease and the success of chemotherapy.

E 2 2 A METHODOLOGICAL STUDY ON TISSUE EGG COUNTS IN PIGS INFECTED WITH *SCHISTOSOMA JAPONICUM*

H.O.Bøgh, A.L.Willingham, E.H.Barnes,
M.V.Johansen*, N.Ø.Christensen* and P.Nansen
Danish Centre for Experimental Parasitology, Copenhagen, Denmark
Danish Bilharziasis Laboratory, Charlottenlund, Denmark

The present study examined the possibility of using a tissue subsample, taken directly from the host tissue, to estimate the total number of S.japonicum eggs in a specific organ. Sixteen male Landrace/Yorkshire crossbred pigs were infected with 500 S.japonicum cercariae and killed 12 weeks post infection. Five gram samples were taken from specific areas of the liver, colon, caecum and rectum and the tissue egg counts (TECs) were determined by standard digestion of tissue in 3% KOH for 18 hours. The rest of each organ was also processed. Five 1ml samples were counted for each 5g sample the mean of which was used to determine eggs per gram. For the liver, counts from 5g samples taken from the left medial and left lateral lobes were not significantly different from counts for the whole liver, but counts from 5g samples from the right lateral, right medial and central lobes did differ significantly from whole liver counts. These results suggest that the processing time of liver tissue from large scale pig experiments can be greatly decreased by using a 5g sample from certain lobes of the pig livers to estimate the TEC of the whole organ. Variable results were obtained for 5g tissue samples taken at 6 specific points in the colon compared to the whole colon digests. This latter result corresponded well to observed pathological findings. The observations made using a single 5g sample from either the caecum or rectum in order to estimate the whole organ count were inconclusive because of the high variability in the counts. A strong correlation was observed between the worm burdens and faecal egg counts.

E 23 DETECTION OF CRYPTOSPORIDIUM OOCYSTS BY MONOCLONAL ANTIBODIES IN SEWAGE SLUDGE, SURFACE AND PUBLIC WATER IN THE CZECH REPUBLIC

Lukešová,D.-Novák,P.

University of Veterinary and Pharmaceutical Sciences Brno, The Czech Republic

A fluorescence assay with monoclonal antibodies-MAbs developed for the detection of Cryptosporidium oocyst (Monofluo® kit,Pasteur Diagnostic, France) in samples of sewage sludge,surface-water and unfiltred public water was compared with the Ziehl-Neelsen modified acid-fast stain in 150 samples collected between 1989 -1994 in the Czech Republic. Cryptosporidium oocysts were indentified in samples of sewage sludge by direct methods in 6,9-55,2% and by MAbs in 33,3-80,0%. The small oocysts were detected in surface-water of the river Svratka in 4,4-6,7% exclusively by MAbs. In unfiltred public water oocysts were tested directly in 5,9%, by MAbs in 10,5-17,7%. In a total of 743 fecal smears of calves Cryptosporidium oocysts were detected in 46,3% using direct methods. Other pathogens (E.coli 83%,Giardia 8,4%,Salmonella 6,0% and Eimeria 2,4%) were identified. Cryptosporidium antibodies were diagnosed in 75,8% by IFAT in serum of 297 calves (positive titres 10-640). The most positive samples (54,2%) were detected in dilution 1:10. The sensitivity,specificity and simplicity of the fluorescence assay with MAbs is suitable for this type of samples and enables a definitive diagnosis of Cryptosporidium oocysts to be established.

E24 A RAPD-PCR DERIVED MARKER CAN DIFFERENTIATE BETWEEN PATHOGENIC AND NON-PATHOGENIC *SARCOCYSTIS* SPECIES OF SHEEP

A. Joachim*, A. M. Tenter*, A. C. Jeffries†, A. M. Johnson†

* Tierärztliche Hochschule, Hannover, Germany
† University of Technology Sydney, Sydney, Australia

Random Amplified Polymorphic DNA (RAPD) PCR was applied to differentiate among four cyst-forming coccidia of sheep, *Sarcocystis tenella*, *S. gigantea*, *S. arieticanis* and *Toxoplasma gondii*. Genomic DNA of the four parasite species was amplified using RAPD-PCR and the DNA fragments were separated on agarose gels. A RAPD-PCR band derived from *S. tenella*, which only occurred in the *S. tenella* and S. arieticanis band patterns, was isolated from the gel and subcloned into pUC18. The insert was completely sequenced and found to be 1278 nucleotides long. This sequence is cryptic in nature as it showed no significant sequence peculiarities or similarity with any other known sequences either at the nucleotide or derived amino acid levels. The recombinant was radiolabelled and used as a probe in Southern hybridisation. This probe, termed STF10, hybridised to *Mbo* I restriction enzyme digested genomic DNA of *S. tenella* and *S. arieticanis*, but not to DNA of *S. gigantea*, *T. gondii*, mouse or sheep. It is likely that STF10 will become a valuable diagnostic tool for *Sarcocystis* infections in sheep to differentiate between pathogenic species of this genus and *S. gigantea* or *T. gondii*.

E 2 5 DIAGNOSIS OF *ECHINOCOCCUS* INFECTED DEFINITIVE HOSTS BY DETECTION OF COPROANTIGENS

H. SAKAI[1], N. NONAKA[1], K. YAGI[2], R. MALGOR[3], I. BASMADJIAN[3], M. IIDA[1], Y. OKU[1] & M. KAMIYA[1]

1. Hokkaido University, Sapporo, Japan
2. Hokkaido Institute of Public Health, Sapporo, Japan
3. Instituto de Higiene, Montevideo, Uruguay

A sandwich ELISA, using polyclonal antibody against excretory/secretory antigens and a monoclonal antibody (MoAb) against the somatic antigen of adult *E. multilocularis*, was used for diagnostic detection of *Echinococcus* coproantigens in definitive hosts; dogs and foxes. The antibodies used for ELISA recognized heat-resistant antigens, thus all fecal samples could be heated to render it safe for handling before the test is performed. Biochemical analysis of the coproantigen showed that the carbohydrate moiety may be an integral part of epitope recognized.

By using sandwich ELISA, we were able to detect both *E. multilocularis* and *E. granulosus* coproantigens during the initial phase of the infection in dogs experimentally infected with these cestodes. Similar results were obtained using foxes experimantally infected with *E. multilocularis*.

The specificity of the assay was evaluated using faecal samples of animals infected with other cestodes, i. e. *Taenia hydatigena*, *T. crassiceps* and *T. taeniaeformis*. No cross reaction was observed in feces containing *T. crassiceps* and *T. taeniaeformis* coproantigens. However, a slight cross reaction was observed in samples with *T. hydatigena* coproantigen during the patent phase of the infection.

This assay was also applied for the detection of *E. multilocularis* coproantigen in feces of red foxes (n=435) in Hokkaido, Japan, and of *E. granulosus* coproantigen in feces of dogs (n=67) in Uruguay. All foxes were necropsied and the posterior one sixth of the small intestine was evaluated for worm burden. Dogs were treated with arecoline hydrobromide and the excreted parasites were identified. The diagnostic sensitivity evaluated from these results was 88% in foxes and 40% in dogs.

E 2 6 A STUDY ON THE INFLUENCE OF PSOROPTES OVIS INFESTATION ON THE IMMUNE RESPONSE IN CATTLE

Lonneux J.-F., Bossaert K., Leclipteux T., Mignon B., Nguyen T.Q., Losson B.J.
University of Liège, Faculty of Veterinary Medicine, Department of Parasitology and Parasitic diseases, Liège, Belgium.

Immunity in 24 calves were investigated in two trials. Six control animals were compared to 18 animals infected with 600 Psoroptes ovis (P.o.). A challenge with 100 P.o. was given on day 28 in trial 2. After development of lesions i.e. on day 35 in trial 1 and on day 56 in trial 2, all animals were immunized with keyhole limpet hemocyanin (K.L.H.) in incomplete Freund adjuvant and with an attenuated vaccine against infectious bovine rhinotracheitis (I.B.R.). Six animals were given the recommanded dose of ivermectine 21 days after immunization and all animals received a final boost with vaccine and K.L.H. on day 98. Blood samples were collected fornightly. Antibodies against P.o., K.L.H. and I.B.R. were followed using specific ELISA. The non-specific mitogens and P.o. antigen induced responsiveness of peripheral blood lymphocytes were studied in a lymphocyte transformation assay (L.T.A.). Lesions and mites counts were recorded monthly. Twelve calves developed an active infection. There were no differences in the development of anti-K.L.H. and anti-I.B.R. antibodies between infested and control animals. Anti-P.o. antibodies appeared early in some infested animals and this was correlated with marked cell-mediated immune response as shown by L.T.A. There was no significant variation in lymphocyte responsiveness to mitogens regardless of treatments. Immunosuppresssion seems not to be a feature in psoroptic mange.

E 2 7 DIFFERENCE IN MITOCHONDRIAL DNA SEQUENCE WITHIN AND BETWEEN *FASCIOLA* SPECIES

Itagaki T.[1], Tsutsumi K.[1], Ito K.[1], Sakamoto T.[1] and Tsutsumi Y.[2]
[1]Faculty of Agriculture, Iwate University, 3-18-8 Ueda, Morioka 020, Japan, and [2]Faculty of Medicine, Kyorin University, 6-20-2 Shinkawa, Mitaka 181, Japan.

Fasciola sp. in Japan has not been identified as *F. hepatica* nor *F. gigantica* because of its intermediate morphological and ecological characters. The present study was carried out to compare genetic variations in parts of cytochrome c oxidase subunit I (COI) and NADH dehydrogenase subunit I (NDI) genes of mitochondrial DNA among *F. hepatica*, *F. gigantica* and Japanese *Fasciola* sp. Polymerase chain reaction - single strand conformation polymorphism (PCR-SSCP), which is a method to detect nucleotide differences in DNA amplified by PCR as mobility shifts caused by conformational changes in a non-denaturing polyacrilamide gel, was used to detect inter- and intra-specific variation of *Fasciola* species and nucleotide sequences were determined from DNA amplified by PCR. By PCR-SSCP analysis, PCR products in region of COI and NDI showed similar mobility shifts between *F. hepatica* and Japanese *Fasciola* sp. Level of intra-specific variation is low in *F. hepatica* and Japanese *Fasciola* sp. and high in *F. gigantica*. By nucleotide sequence analysis, nucleotide divergence in a fragment (187bp) of NDI was low among *Fasciola* species. Nucleotide sequence of Japanese *Fasciola* sp. was identical to that of *F. gigantica*.

E 2 8 HELMINTH PARASITES EMPHASISING ON *FASCIOLA GIGANTICA* INFECTION IN DAIRY CATTLE IN THAILAND

Tasanee Chompoochan, Manvika Pholpark,
Itipol Chaichanapunpol, Usa Chethanond
National Institute of Animal Health, Bangkok Thailand

This study reports the number and species of helminth parasites in dairy cattle determined by fecal examination were conducted from 4 parts of Thailand (ie. middle, southern, northern and north-eastern) during the period of October 1993 to September 1994.Seven genera of helminths were found. Evidence of such helminth parasites were 25.72% (1,375 out of 5,345), 57.41% (813 out of 1,416), 34.98% (551 out of 1,461) and 11.59% (337 out of 2,907) with the emphasise of *Fasciola gigantica* infection at the rate of0.34, 5.0, 4.4 and 2.7% from middle, southern, northern and north-eastern of Thailand respectively. The importance of these findings in order to improve the yield of dairy cattle and products, the relation of epidemiology of parasitic infections and economic losses should be considerated.

E29 EXTRACTION OF ENCYSTED METACERCARIAE OF PHAGICOLA FROM THE TISSUES OF MULLETS MUGIL BY HOMOGENIZATION AND PEPTIC DIGESTION TECHNIQUES.

Castro,J.M.de & Ogassawara,S.
Dept.Medicina Veterinária Preventiva,Faculdade de Medicina Veterinária,Universidade de São Paulo,São Paulo-Brazil 05340-000

Phagicola, a possible zoonotic agent acquired by ingestion of raw mullet's flesh,has encysted metacercariae in the tissues. Homogenization and digestion techniques were used to extract metacercariae from the host tissues. From each of 50 fishes bought at a market,two 5g samples from a pool of visceral tissue (heart,spleen,liver,kidney) and two 10 g samples of muscle tissue were collected. Tissue homogenization: each minced sample plus water (1:5) was homogenized at 3000 rpm 15 s.The cup with homogenate was completed with water and sieved into a conical glass. Supernatant was discarded 5 min later, the sediment suspended and completed with water.This process was repeated 2-3 times.Sediment was examined under stereomicroscope.Tissue digestion:each minced sample plus digestive fluid (1:5) plus glass beads was incubated at 37°C 90 min.Thereafter,the digested suspension was completed with water and sieved into a conical glass. Supernatant was discarded 5 min later. From here the procedure was as described above.These techniques permitted recovering motile metacercariae with unbroken cystic membranes and free of host tissues. The average number of recovered cysts by both techniques was not statistically significant(Student t test) but the homogenization technique was faster and more economical.

E30 RESPONSE TO TRICHOSTRONGYLE INFECTION IN DAIRY GOATS AND CONSEQUENCES ON MILK PRODUCTION: COMPARISON BETWEEN HIGH- AND LOW- PRODUCING GOATS.

C. Chartier, H. Hoste, H. Coutineau, I Pors, M.-P. Mallereau, C. Benoit, and C. Koch

CNEVA/Station Régionale de Pathologie Caprine, 60 rue de Pied de Fond, BP 3081, 79012 NIORT Cedex, France

The objectives of the study was to examine the influence of the level of milk production (high or low producers) in dairy goats on their ability to develop a resistance to nematode parasites. Two groups of 50 goats were initially submitted (Group I: « Immunised ») or not (Group NI: « Non Immunised ») to an immunising protocol with 3 repeated infections by a mixture of *Haemonchus contortus* and *Trichostrongylus colubriformis* followed by a fenbendazole drenching 50 days after each infection. At the end of this period, the goats remained free of worms for 1 month before kidding. One month after the beginning of lactation, 25 goats from both groups were challenged with a mixture of *H. contortus* and *T. colubriformis* and were sampled at fortnight interval. Parasitological, pathophysiological and milk production parameters were examined for 3 months. Within this I and NI groups, the effects of parasites and the consequences of immunisation were compared between the 25 % goats with the lowest level of milk production (subgroups LPI and LPNI ; 2-2.5 $l.day^{-1}$) and the 25 % goats with the highest level of milk production (subgroups HPI and HPNI ; 4-4.5 $l.day^{-1}$). During the immunising protocol, no difference was seen between HP and LP goats. In the second part of the study, HP and LP animals showed similar pathophysiological and parasitological measurements when non immunised. In contrast, major differences were observed in the response to immunisation depending on the level of milk production (subgroups HPI and LPI). In the LP goats, a previous contact with parasites lead to a decrease in fecal egg count with minor variations in the pathophysiological and milk production measurements. In the HP goats, no difference in FEC was found between HPI and HPNI subgroup, whereas immunisation was associated with major disorders on pathophysiological parameters (pepsinogen, phosphate, blood eosinophils) and with a more severe decrease in milk production. These results confirm that differences in the level of milk production in dairy goats are associated with differences in their response to parasitism : the response in LP animals was restricted to a decrease in FEC whereas the response in HP animals had severe and negative effects for the host.

E 3 1 RESPONSE TO TRICHOSTRONGYLE INFECTION IN DAIRY GOATS AND CONSEQUENCES ON MILK PRODUCTION.

H. Hoste, C. Chartier, H. Coutineau, I. Pors, M.-P. Mallereau, C. Benoit and C. Koch
INRA, Station de Pathologie Aviaire et de Parasitologie, 37380 NOUZILLY, France

This study aimed at examining the ability of goats to develop a response to nematode infection. In the first part of the experiment, 100 dairy goats were divided into two groups. One remained uninfected (Group NI: « Non Immunised ») ; the other one (Group I: « Immunised ») was infected three times, at 50 days interval, with a mixture of *Haemonchus contortus* and *Trichostrongylus colubriformis* infective larvae (L3). In this last group, parasitological and pathophysiological parameters were examined at the end of each infection (fenbendazole : 10 $mg.kg^{-1}$). At the end of this immunising protocol, the animals were drenched and remained free of parasites for 1 month before kidding. The second part of the study began 1 month after kidding. Twenty five lactating goats from each group were then challenged with 5 000 *H. contortus* L3 and 20 000 *T. colubriformis* L3. Parasitological, pathophysiological and milk production parameters were measured in both groups at fortnight interval for 3 months. In the first part of the study, only slight variations in fecal egg count were observed after each infection. In a similar way, the inorganic phosphate concentrations remained similarly depressed after each infection. In contrast, consequences of the infection gradually decreased concerning blood parameters related to the abomasal parasitism (pepsinogen, PCV) and a progressive increase in blood eosinophilia was observed. In the second part of the study, no difference was found in FEC between groups I and NI. However, the pathophysiological consequences of infection were more severe in group I (pepsinogen and inorganic phosphate concentrations). In addition, the milk production was also more severely depressed in the « immunised » goats compared to the « non immunised » ones. These results indicate that the repeated inoculations were not able to produce a resistance to a further parasitic challenge in goats unlike sheep in similar conditions. However, results from the immunising protocol suggest the ability to develop a premunition to *H. contortus* but not to *T. colubriformis*. In addition, the response of the animals although inefficient on worm population seems to contribute to enhance the pathophysiological consequences for the host.

E 3 2 TRICHODINID CILIATES FOUND FROM THE GILLS OF TWO CULTURED FISHES, TIGER PUFFER (*Takifugu rubripes*) AND YELLOWTAIL (*Seriola quinqueradiata*)

Soichi Imai, Satoshi Matsumoto, Kazuyoshi Kotani, Kishio Hatai* and Yutaka Fukuda**
Department of Veterinary Parasitology, *Department of Fish Disease, Nippon Veterinary and Animal Science University, Tokyo, Japan and ** Oita Prefectural Fisheries Experimental Station, Oita, Japan

Ciliate species of the genus *Trichodina* were surveyed on two major cultured fishes in Japan, tiger puffer and yellowtail. The materials examined were the gills of juveniles of tiger puffer cultured in Shizuoka Pref. and of yellowtail cultured in Oita Pref. A part of the samples was smeared for Klein's silver impregnation and Giemsa's stain, and the other was treated for scanning electron microscopy. As a result of survey one species of *Trichodina* from tiger puffer and two from the yellowtail were recognized. Though one trichodinid species from the yellowtail was identified as *T. jadranica* Heider which has been described not only from various marine but from freshwater fishes, the other two trichodinids seemed to be new to science because of their differences of morphological characters and measurements to those of already described species. *Trichodina* sp. from the tiger puffer was 50-60 μm in body diameter with 30-39 μm adhesive disc having dark center area and 29-35 denticles. Blade of the denticle was rod-like being one of the characteristics of this species. The species from the yellowtail was 33-47 μm in body diameter with 29 - 39 μm adhesive disc having dark center part and 23-25 denticles. The denticle had short and sharp thorn with round blade. Presence of new trichodinid species from respective fishes examined might show relatively high host specificity of the ciliates of this genus. In SEM study, injury of the surface of gill by attachment of adhesive disc of trichodinid ciliates was suggested in tiger puffer.

E 3 3 SCANNING ELECTRON MICROSCOPY ON THE LARVAE, PUPAE AND ADULTS OF *Hydrotaea irritans* (Fallen, 1823)

Yung-Bai KANG

Foreign Animal Disease Research Division,
National Veterinary Research Institute, Anyang, Republic of Korea

Myiasis is the term describing an infestation of living vertebtrate host tissue by the larvae of flies belonging to the Order Diptera, and has been classified into two types, obligate if the larvae can only exist on living tissues and facultative where larvae that normally feed on dead tissue.

A scanning electron microscope (Hitachi S-570) was applied on the larvae collected from the traumatic injuries caused by the larval feeding folled by the opportunistic laying of the *Hydrotaea irritans* flies on the hide of bovine animal.

Some significant features were noticed in the 3rd stage larvae having a pair of anterior spiracles with 9 papillae and a pair of posterior spiracles with 3 sets of respiratory slits and the peritreme.

The ultrastructures of the pupae in metamorphosis process and the adult flies showing the fine surface structures of the wing veneations and of the pupae and adult flies were also taken.

In conclusion, the identification of the maggot species implicated in myiasis in the bovine rawhides in the Republic of Korea has confirmed that the fly species involved is *Hydrotaea irritans* with the morphological charateristics showing in the scanning electron microphotographs and in the legends of photographs included.

E 3 4 TICK FAUNA (IXODIDAE) IN CATTLE RAISING AREA AND WILDLIFE SANCTUARY IN THAILAND

Nopporn Sarataphan, Darunee Tuntasuvan, Suthisak Boonchit and Yasuhiro Ito

National Institute of Animal Health, Bangkok, Thailand

A preliminary survey was carried out for the purpose of elucidating the ixodid tick fauna in the cattle raising area in comparison with highland wildlife sanctuary. The survey was conducted in 17 provinces including two sanctuaries in Thailand during the period of 1993 to 1995 by picking up ticks from cattle and buffaloes or dragging a flannel cloth on vegetation. *Boophilus microplus* was a dominant species in all provinces of the cattle raising area, whereas fewer species *Rhipicephalus haemaphysaloides* and *Amblyomma testudinarium* were found from cattle and buffaloes. A few adults of *Haemaphysalis* (*Kaiseriana*) *wellingtoni* were obtained togather with uncertain species of *Haemaphysalis* larvae and nymphs by dragging at dairy farms. On the other hand, *H.(K.) lagrangei, H.(K.) shimoga* and *H.(K.) semermis* were collected from Sambar deer (*Cervus unicolor*) and vegetation in the sanctuaries and from the former 2 species were abundant. These results showed that *B. microplus* was the dominant species and followed *R. haemaphysaloides* in the cattle raising areas. *H.(K.) wellingtoni* and *A. testudinarium* distributed in a lesser extent of the area. The main habitat of *H.(K.) lagrangei, H.(K.) shimoga* and *H.(K.) semermis* was the sanctuary.

E 3 5 AN IMPROVED ELISA TECHNIQUE FOR THE DIAGNOSIS OF PSOROPTES OVIS INFESTATIONS IN CATTLE

Lonneux J.F., Mignon B., Bossaert K., Leclipteux T.,Losson B.J.

Faculty of Veterinary Medicine, University of Liège, Liège Belgium.

A sandwich enzyme-linked immunosorbent assay (ELISA) was developped using polyclonal antibodies from Psoroptes cuniculi infested rabbits and a crude P.cuniculi antigen.
Sera from 133 P.ovis infested and 137 uninfested cattle were serially diluted from 1:100 to 1:102,400 and analyzed. Curves obtained by plotting log dilution versus optical densities were used and a working dilution of 1:400 was selected. Positive/negative cut-off values were calculated using the mean optical density percentage (6%) of all negative sera plus 3 standard deviations. Sensitivity and specificity according to the resulting cut-off (25%) were 98.5 % and 97.8 % respectively. Expressed as the dilution giving the cut-off value, results in ELISA demonstrated a wide range of antibody titres (from 1:19 to 1:2,240,000) with a geometric mean value of 1: 35,480 probably related to the well described hyperglobulinaemia in P.ovis infested cattle.
This ELISA test might be useful for the assessment of the presence of sub-clinical P.ovis infestations and could be a tool for the control of P.ovis disappearance after treatment in individual animals or cattle herds.

E 3 6 DEVELOPMENT OF AN ELISA-BASED TEST FOR THE DETECTION OF *FASCIOLA HEPATICA* IN STOOLS AND SERUM SAMPLES FROM CATTLE.

Leclipteux Th., Bossaert K.,Protz M.,Lonneux J.F., Losson B.
University of Liège, B-4000 Liège; Belgium

Currently, Fasciolosis infections is still a real problem throughout several EEC countries, because of its economical impact or potential health hazard. Diagnosis of faciolosis in domestic ruminants and man is often based on the detection of so-called specific antibodies and coprological examinations. The results obtained with these different available tests present often marked discrepancies. Furthermore numerous studies have clearly shown that this type of techniques are not sensitive and specific enough. This work will define new detection tool mainly ELISA-based in term of antigen detection in serum samples and/or in stools.
Monoclonal antibodies have been generated in order to monitor and quantify the *F. hepatica* specific antigens during *Fasciola hepatica* infection in cattle. Mice have been exposed to an active infection followed by one boost consisting of Excretory/Secretory products produced by adult larvae. Different isotypes have been selected, i.e. IgG(1 and 3), IgA and IgM. Previous results showed that specific monoclonal antibodies are able to distinguish between E/S and somatic antigens. Infected and non infected animals have been checked with encouraging results in term of diagnosis. Moreover previous western blot studies have shown marked differences in the kinetics of appearance of several antigens. These monoclonals will allow us to follow this kinetics more accurately.

E 3 7 SET-UP OF A DIAGNOSTIC KIT FOR DETECTION OF WARBLE FLY INFECTION IN POOLED SERUM SAMPLES.

Leclipteux[1] Th., Protz[1] M., Losson[1] B., Boulard[2] C. and Rimmele[3] D.
1: University of Liège, B-4000 Liège; Belgium
2: INRA, 37380 Tours; France
3: Vetoquinol Diagnostic, 70204 Lure; France

Control of warble fly became of importance in certain region of France since 1988. French eradication programme started in 1989. The test has been developped for pooled blood samples. Therefore, the classical serodiagnostic test has to be improved by using a semi-purified antigen produced from the first instar larvae stage. This antigen contains a hypodermine C higly concentrated fraction and was prepared from *Hypoderma lineatum*. Antigen concentration, dilution buffer and pH were the main coating parameters checked. 99 % of specificity and sensitivity were determined on 200 sera, with a cut-off fixed at 33,7 % of the positive reference. Anti-Bovine IgG polyserum or monoclonal antibody were also evaluated as conjugate in order to reach optimal specificity. Saturation solutions as well as different quality plates and drying process were evaluated in term of background and long term stability. Results showed that low quality plates, giving the best results regarding background were unfortunately those giving the worst stability at 37 °C. Therefore, Nunc® maxisorp plates have been choosen regarding adsorption capacity and stability criteria. Concentration of 5 µg/ml in PBS was optimal on these plates. A donkey anti-bovine IgG was choosen as conjugate. Plates were dried packaged and stored at 4 °C.

E38 MORPHOLOGICAL, SEROLOGICAL AND ANTIGENIC CHARACTERISTICS, AND PROTEIN PROFILE OF NEWLY ISOLATED JAPANESE BOVINE *BABESIA* PARASITE WITH PARTICULAR REFERENCE TO THOSE OF *B. OVATA*

Masato OHTA, Naotoshi TSUJI, Shin-ichiro KAWAZU, Yutaka TERADA, Tsugihiko KAMIO and Kozo FUJISAKI
National Institute of Animal Health, Tsukuba, Japan

Babesia ovata has been the only species of *Babesia* that has been identified as the causative agent of bovine babesiosis in Japan, except for the Okinawa islands. An intraerythrocytic large protozoan, tentatively designated *B.* sp.1, was recently isolated from cattle in Hokkaido Prefecture, Japan. This parasite closely resembled *B. ovata* in shape of piroplasms, but was distinguishable by other morphological, immunological, and biochemical characters. The paired pyriform piroplasm of *B.* sp.1 was larger than that of *B. ovata*. The results from serological and antigenic examination by enzyme-linked immunosorbent assay and Western blot analysis showed that there were cross- but distinguishable-reaction between *B.* sp.1 and *B. ovata*. Protein profiles of both *Babesia* parasites piroplasms analyzed by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) were apparently different from each other. Several major proteins revealed by 2D-PAGE and the immunodominant proteins resolved by Western blot analysis (40 kDa for *B.* sp.1 and 29 kDa for *B. ovata*) were unique to each parasite. These results indicate the possibility that *B.* sp.1 is a species different from *B. ovata*.

E 3 9 *Hydrotaea irritans* (Fallen, 1823): THE CAUSE OF TRAUMATIC INJURIES IN THE BOVINE HIDES

Yung-Bai KANG* and Guk-Hyun Jang**
National Veterinary Research Institute, Anyang* and Kangwon Provincial Veterinary Service Laboratory, Kangreung** Republic of Korea

An epidemiological and morphological study was performed in order to reveal the cause of traumatic injuries in the bovine rawhides. The morphological characteristics of the lesions were spot-like, various round in shape and liquidized traumatic gangrene. The perforation and disfigurement reduce the market value of the hides. Flies are attracted to the manure around the anus and in the vicinity of the hip on the hides. Numerous maggots milk-white in colour, brownish pupae and tiny black flies were collected from the surface of the salted hides.

For the observation of morphological characteristics and identification of the parasites, the specimens were applied to a binocular stereoscopic microscope.

Hydrotaea irritans is a non-biting muscid, 3-5mm in length in adult stage, resembling the housefly *Musca domestica* but rather smaller. It was characteristic that the 3rd larvae have a pair of anterior spiracles with 9 papillae and a pair of posterior spiracles with 3 sets of respiratory slits and the peritreme. The fly is mostly active in summer, June to August, and the prevalence of the maggot injuries is seasonal as the female flies opportunistically lay on the rawhide during the summer.

In conclusion, the cause of the traumatic injuries in the bovine rawhides as related to the maggots of *Hydrotaea irritans* belonging to the Family Muscidae, Suborder Cyclorrhapha, Order Diptera.

E40 EXPERIMENTAL INFECTION WITH *Strongyloides papillosus* IN YOUNG CALVES AND LARVAE RECOVERY FROM THE TISSUES.

Marcos Moreira Braga, Alvaro Luiz Marinho Castro, Denise Botelho de Oliveira e Adivaldo Henrique Fonseca. Universidade Federal Rural do Rio de Janeiro. Brazil.

A hyperinfection by *Strongyloides papillosus* (SPL) may be due to deficiences on sanitary or zootecnic management. Four crossbred calves of "Holandez X Gir", 120 days old were used for this experimental study. These animals received percutaneously 10.000 000 of SPL per 100 kg body weight. After the prepatent period, the animals started to develop clinical abnormalities such as itching at the inoculation site, hyporexia, decreased urinary flow, ocular and nasal discharges, besides diarrhea or constipation. All animals were very debilitated just before death, and each of them had eliminated millions of eggs in their fezes. The natural death had occurred between 15 to 25 days after infection. At necropsy, it was observed lung congestion, and hydropericarditis. The bladder was full, and there was erosive enteritis of the duodenum and jejunum. The bile showed a thick and flocky appearance. It was collected 10 grams samples of each of the following organs: brain, tongue, heart, lung, diaphragm, liver and spleen. All the samples were analysed by the Baermam technic, and it was possible to recover larvaes from the diaphragm, lungs, livers, and tongue. No such findings neither clinical signs were observed in the control group.

E41 COMPARISON OF DOG ORIGINATED *Pneumocystis carinii* WITH HUMAN AND RAT ORIGINATED ORGANISMS

Antti Sukura[1], Seppo Saari[1], Anna-Kaisa Järvinen[1], Mats Olsson[2]. [1]College of Veterinary Medicine, Helsinki, Finland, and [2]Swedish Institute for Infectious Disease Control, Stockholm, Sweden.

Pneumocystis carinii causes pneumonia in immunocompromised hosts. It has been earlier considered as a potential zoonosis, but recent studies have shown host species specific genetic and immunological variations. We studied in more detail *P. carinii* organisms from one clinical canine *P. carinii* pneumonia case with transmission electron microscopy, immunoelectron microscopy, immunohistochemistry and polymerase chain reaction. All employed commercially available human origin *P. carinii*-specific monoclonal antibodies (Detect If Pneumocystis carinii Diagnostic Kit®, Shield Diagnostics Ltd, Dundee, UK (SMo); Pneumo-Cel I.F. Test®, Cellabs PTY Ltd, Brookvale, NSW, Australia and Dako-Pneumocystis®; Dakopatts A/S, Copenhagen, Denmark (DM0)) showed a positive reaction in immunohistochemistry carried out on either lungimprints, cytological specimens or paraffin embedded lung sections. All but DMo reacted also well with rat origin organisms. Ultrastructurally *P. carinii* organisms were undistinguishable from human and rat *P. carinii*. Immunoelectron microscopical localization of SMo only on the electron lucent middle layer of cyst pellicle, but not in trophozoites was in correspondence to that reported on rat and human originated organisms, which might indicate that the epitope of this monoclonal antibody could be a group specific one. The PCR amplification protocol, based on the thymidylate synthase sequence of rat origin *P. carinii* showed positive reaction, however it needed lowered annealing temperatures than for human or rat originated organisms which might indicate that the DNA sequence of the dog origin *P. carinii* could be different compared to rat or human origin *P. carinii*.

E 4 2 INCREASE OF *Pneumocystis carinii* PREVALENCE IN CANINE DISTEMPER INFECTED DOGS

Antti Sukura[1], Juha Laakkonen[1], Eeva Rudbäck[2]. [1] Department of Anatomy, College of Veterinary Medicine, [2] Pathology Unit, National Veterinary and Food Research Institute, Helsinki, Finland

Pneumocystis carinii is an extracellular opportunistic pathogen with an uncertain taxonomic status. It causes pneumonia on immunocompromised hosts and is best known as a pathogen of AIDS patients and immunosuppressed transplant and cancer patients in human medicine. In Finland, after a long silent period of canine distemper (CD) infection in dogs, clinical cases have occurred in the beginning of 1990's and thereafter. We studied retrospectively lung specimens of 35 CD-cases diagnosed 1990 - 92 in the Pathology Unit, National Veterinary and Food Research Institute (NVFI). *P. carinii* organisms were identified with Gomori's methenamine silver stained paraffin embedded lung sections.The mean age of cases was 8.7 months (0.5 - 72). Fifty controls were collected from NVFI (18) and from the Department of Pathology, College of Veterinary Medicine (32). Criteria for the controls were: young dog without diagnosis of CD or any other viral infections. The diagnosis of controls were mainly trauma or dysontogenesis, mean age of controls was 8.1 months (2 - 30). *P. carinii* organisms were found from 5 cases (5/35, 14%) but not any in 50 controls (0/50; $p<0.01$). The frequence of histologically confirmed pneumonia in *P. carinii* positive CD infected dogs (2/5) did not statistically differ from the frequence of pneumonia in other CD infected cases (7/30; $p=0.59$). In the literature CD is considered to be immunosuppressive, but with retrospective data is not possible to deduce if the higher prevalence of *P. carinii* was due to immunosuppressive effect of CD infection or if the CD was due to some underlying immunodeficiency which also allowed manifestation of *P. carinii*.

E 4 3 EPIDEMIOLOGY OF GASTROINTESTINAL PARASITISM IN POITOU DONKEYS IN WESTERN FRANCE.

R. Simon, C. Chartier, I. Pors, M.-P. Mallereau

CNEVA/Station Régionale de Pathologie Caprine, 60 rue de Pied de Fond, BP 3081, 79012 NIORT Cedex, France

The epidemiology of gastrointestinal parasitism was investigated in a herd of 30 animals from December 1991 to December 1992, the grazing season beginning in March and ending in December. At 45 days intervals, faecal egg counts and standard haematological parameters were assessed individually and faecal cultures were realized on an age group basis. In addition, 3 anthelmintics (febantel, pyrantel, ivermectin) were used to check the susceptibility of the small strongyles. The helminth fauna consisted mainly in *Cyathostomum* spp (80 % of egg output), *Trichostrongylus axei* and *Strongylus vulgaris*. Other important nematodes included *Dictyocaulus arnfieldi* and *Parascaris equorum*. Two peaks in gastrointestinal strongyles eggs output occurred in March (1900 EPG) and September (1600 EPG) with more than 80 % of the animals showing EPG>500. A significant correlation was seen in paired December faecal egg counts, despite the March drenching, indicating that individual donkeys may exhibit predisposition to helminth infection. In young animals prevalence reached 100 % by 5 months of age for gastrointestinal and respiratory strongyles and for *P. equorum* infections. Beyong 18 months of age digestive strongyles infection was similar for egg outputs and coprocultures whatever the age. Significant correlations were assessed between values of EPG and haematological parameters : negative for PCV and RBC counts, positive for eosinophils and neutrophils. All of the 3 anthelmintics tested gave a faecal egg count reduction > 90%.

E 4 4 ATTEMPTS TO IDENTIFY A SMALL PIROPLASM FROM LIONS IN THE KRUGER NATIONAL PARK

LM López-Rebollar*, BL Penzhorn**, DT de Waal*, BD Lewis** and DGA Meltzer**

* Protozoology Division, Onderstepoort Veterinary Institute, South Africa

** Dept of Veterinary Tropical Diseases, University of Pretoria, South Africa

A small piroplasm was detected in blood smears prepared from lions in the Kruger National Park. The parasite was provisionally identified as *Babesia felis*, but serum from these lions tested negative on *B. felis* antigen in the indirect fluorescent antibody test (IFAT). Blood from a lion was sub-inoculated into a domestic cat in an attempt to identify this parasite. When parasites first appeared in blood smears, blood was collected and antigen slides prepared for the IFAT. One lion was infected with *B. felis* (from a cat) and two leopards with blood stabilate containing the unidentified small piroplasm. The 3 animals were immobilized at monthly intervals and blood collected for serum and blood smear preparation. All serum samples were tested against *B. felis*, the unidentified small piroplasm and *Cytauxzoon felis* antigen. The serological test results indicate the small piroplasm to be distinct. No fluorescence was observed with serum from lions on either *B. felis* or *C. felis* antigen and the unidentified piroplasm probably represents an undescribed *Babesia* species

E45 EXPRESSION OF HEAT SHOCK PROTEINS AND HEAT SHOCK COGNATE 70 GENE DURING TRANSFORMATION FROM FREE-LIVING INFECTIVE LARVAE TO THE PARASITIC STAGE IN *STRONGYLOIDES VENEZULENSIS*

Tsuji N., Ohta M., Kawazu S., Sekizaki T., and Fujisaki K.
National Institute of Animal Health, Tsukuba, Ibaraki, Japan

Strongyloides venezuelensis possess two developmental stages, both free-living and parasitic stages. Infection with this parasite to the host is due to the penetration of the skin by infective larvae. The transformation from infective larvae to parasitic stage is a critical phase in the life cycle of *S. venezuelensis*. The biochemical pathways that regulate this transitional period upon different environment are unknown. In the present study, protein synthesis during transformation from infective larvae to the parasitic stage were examined.

Comparison of protein profile labelled with [^{35}S]-methionine of infective larvae cultured at 25 ℃ and 37 ℃ with a marked morphological transformation revealed an increase of 70 kDa protein and new appearance of two complexes between 16 kDa and 22 kDa proteins at 37℃. The 70 kDa protein cross-reacted with monoclonal antibody against heat shock protein 70 (HSP70) of human, was constitutively expressed. These proteins synthesized during the transformation of the infective larvae to the parasitic stage may be crucial biochemical events that regulate infectivity of the parasite for the host.

In addition, a partial-length of heat shock protein 70 (HSP70) gene was cloned from cDNA library constructed in λ ZAP II vector from mRNA of infective larvae. A gene pSH70-1 had high homology with mammalian HSP70 genes. One of three deduced amino acid sequence showed high homology to heat shock cognate 70 (HSC70) of other organisms. Northern blot analysis using the pSH70-1 as probe revealed that the expression of 3.2 kb mRNA, which was constitutively expressed was increased in the transformation from infective larvae to parasitic stage. These results suggest that the *S. venezuelensis* HSC70 like gene is associated with the transformation from infective larvae to the parasitic stage in *S. venezuelensis*.

E 4 6 HELMINTHOSES OF DOGS AND CATS IN MOSCOW

Yastreb V.B., Belousov M.N., Bessonov A.S.
The All-Russian K.I.Skryabin Institute of Helminthology, Moscow, Russia

In 1981-1993 in Moscow 422 stray dogs and 567 stray cats were post-mortem examined for helminthoses. Samples of muscles from 39 dogs and 395 cats were examined by the method of compressor trichinelloscopy, faecal samples from 798 adult dogs, 129 puppies and 85 cats were examined by the method of flotation. During post mortem examination of dogs their infection with Toxocara canis (31,99%), Toxascaris leonina (1,89%), Uncinaria stenocephala (0,47%), Dipylidium caninum (7,82%) and Taenia hydatigena (1,18%) and at post mortem examination of cats infection with T.mystax (33,16%), T.leonina (1,06%), D.caninum (48,68%), Hydatigera taeniaeformis (4,6%), Ollulanus tricuspis (5,84%), Taenia pisiformis (0,18%) and Dyphyllobothrium latum (0,18%) were found out. Trichinella larvae in muscles of examined dogs and cats were not found. Coproscopic methods helped to detect in adult dogs and cats eggs of the same helminths that were found during post mortem examination. The very high rate of infection - 93,8% - with T.canis was revealed in 129 puppies at the age of 1-4 months. It is promoted by great biotic potential of Toxocara (one female can produce tens of thousands eggs per a day), possibility of intrauterine and lactogenic infection of puppies, somatic migration of Toxocara larvae and their keeping viability for a long time in muscles and internal organs of adult dogs. The number of dogs in Moscow (registered and stray) is not less than 200000. Every day 54 tons of faeces are remained in the street, at lawns, public gardens and open-air kindergartens. In 1992 single cases of toxocarosis were registered for the first time in inhabitants of Moscow.

E47 IN VITRO CULTIVATION OF ANGIOSTRONGYLUS COSTARICENSIS IN A CHEMICALLY DEFINED MEDIUM

Hidekazu Hata
Department of Parasitology, Chiba University School of Medicine, Inohana, Chiba City
Japan

The third stage larvae of A. costaricensis were successfully cultured to young adults in Waymouth's chemically defined medium MB 752/1, which contained 18 amino acids, 11 vitamins, glutathione, hypoxanthine, and glucose in a balanced salt solution. Nutritional requirements were examined by deletion of single components from Waymouth's medium. Ten amino acids, namely L-arginine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-threonine, L-tryptophan, and L-valine, were shown to be essential for the parasite's development. Among the 11 vitamins, only choline chloride was essential for the development. The deletion of pyridoxine from the medium adversely affected parasite development. Glucose was also required by the worms, but glutathione and hypoxanthine were not required for their development. When the 10 essential L-amino acids were replaced individually by D-isomers of the same amino acids, none supported larval development in the manner of the L-amino acids. A. costaricensis eggs were also successfully cultured to the first stage larvae in chemically defined Ham's F-12 medium. Ten days after cultivation in this medium, 34% of the eggs developed to the first stage larvae. When these first stage larvae were infected to the first intermediate host snails, Biomphalaria glabrata, they developed into the third stage larvae.

E48

STUDIES ON THE LIFE CYCLE OF *BABESIA GIBSONI*

S. Higuchi, H. Kuroda, M. Sekine, H. Hoshi, S. Kawamura and Y. Yasuda

Department of Internal Medicine, School of Veterinary and Animal Sciences
Kitasato University, Towada, Aomori 034, Japan

There are many reports on the development of *Babesia* species in the vector ticks.However, no detailed observations on the developmental stages of *Babesia gibsoni* in the ticks have been made yet. *Babesia* species are known to undergo morphological change during the growth in the vector ticks. The present was carried out to study the growth of *B. gibsoni* in the vector ticks, *Haemaphysalis longicornis, Rhipicephalus sanguineus.*

In the tick *H. longicornis*, *B. gibsoni* gose through gametogony(ring form,sperical form,fission form, bizarre form, elongate form, microgamete and zygote) in the gut. Kinetes of *B. gibsoni* are formed in the haemolymph of adults ticks. After that, kinetes were found in reproductive organs oftick,such as the ovarian cavity, epithelial cells, cell cord and ovum in ovary.

In the tick *R. sanguineus*, the developmental stages of *B. gibsoni* in the gut shows a close similarity to that *B. gibsoni* in gut of *H. longicornis.* *B. gibsoni* shows the sporogony (sporont and sporozoites) in the salivary glands.

E49 ERYTHROCYTE OXIDATION IN DOGS ARTIFICIALLY INFECTED WITH *Babesia gibsoni*

Morita, T., Saeki, H., Imai, S. and Ishii, T.

Department of Veterinary Parasitology, Nippon Veterinary and Animal Science University, Tokyo, Japan

We have reported that anti-erythrocyte antibody in the dogs infected with *B. gibsoni* (aEAb) bound stronger against artificially aged or oxidized erythrocytes *in vitro* than against intact ones, suggesting that lifespan of erythrocytes in the infected dogs might be shorten by aEAb. But the role of aEAb on anemia caused by *B. gibsoni* infection has not been known enough yet. To clarify the role of aEAb on the anemia *in vivo*, methemoglobin rate (metHb%) was comparatively obseved in intact and splenectomized dogs infected with the organisms artificially. In intact dogs, metHb% and aEAb titer increased, suggesting that erythrocytes oxidized by the infection are attacked by aEAb stronger than intact erythrocytes. On the other hand, Coombs' test resulted in all negative in the present experiments. However, based on the previous reports that some human patients of the autoimmune hemolytic anemia (AIHA) showed Coombs' negative, aEAb should not be denied as a cause of the anemia by the results of Coombs' test. In contrast, in splenectomized dogs, metHb% did not increase in spite of showing higher peak of average PE rate (50.2%) compared with that in intact dogs (13.1%) (and Coombs' test showed the same results as those in intact dogs). These results suggest that there would be some correlation between the presence of spleen and the erythrocyte oxidation. We thought that there are at least some relations between aEAb and the anemia in *B. gibsoni* infected dogs.

E50 FIRST CASE OF *Hepatozoon canis* INFECTION OF WILD CARNIVORES IN BRAZIL

ALENCAR, N.X., KOHAYAGAWA, A., SANTARÉM, V.A.
Departamento de Clínica Veterinária da Faculdade de Medicina Veterinária e Zootecnia - UNESP, 18618-000 -Botucatu-Brazil

A young crab-eating fox (*Cerdocyon thous*), tramped in a highway in the region of Botucatu, São Paulo, Brazil, was attended in February, 1995, by the area of Small Animal Surgery of FMVZ, where was diagnosed with radiographics exams aid, bilateral fracture of olecranon, being later corrected by osteosynthesis. It was solicited hematologic examination of the animal, that presented red blood cell counts 4.79 x 10^6 /µl, haemoglobin 11.0 g/dl, packed cell volume 32%, total plasma protein 7.6 g/dl, fibrinogen 800 mg/dl, white blood cell counts 25,800/µl, and in the Leishman-stained blood film it was observed neutrophilia, eosinophilia and monocitosis, with moderate anisocytosis and polychromasia, presentation of 03 metarubricytes in 100 leukocytes, and gametocytes of *Hepatozoon canis* in neutrophils, presenting 9.1 ± 0.54 x 5.3 ± 0.46 µm in measurement of parasites made with an ocular micrometer. During the post-surgery treatment, which was based on antibioticotherapy, the dog presented diarrhea and other complications that culminated in euthanasia seven days after surgery. Anatomicopathologic findings consisted of liver degeneration, petechiae patches in lungs, haemorragic cystitis and hyperplasia of white pulp spleen. The laboratorial and necropsy exams associated to the clinical features, indicate based on the scientific literature, the diagnostic of the first case of hepatozoonosis in wild carnivores in Brazil.

E51 EFFECT OF EIMERIA TENELLA INFECTION ON THE PRODUCTION OF SALMONELLA ENTERITIDIS-CONTAMINATED EGGS AND SUSCEPTIBILITY OF LAYING HENS TO S. ENTERITIDIS

Z. Qin, A. Arakawa, E. Baba, T. Fukata and K. Sasai
Department of Veterinary Science, College of Agriculture, University of Osaka Prefecture, Sakai, Osaka 593, Japan

There were two groups of laying hens: one group infected with S. enteritidis, and the other group infected with S. enteritidis and E. tenella. Chickens in Experiments 1, 2 and 3 were infected with 10^4 CFU, 10^6 CFU and 10^8 CFU of S. enteritidis per day for 2 consecutive days beginning 3 days after infected with $2x10^5$ oocysts of E. tenella respectively. Half of fresh eggs in Experiments 1 and 2 were examined for S. enteritidis in egg shells and contents; the other half for S. enteritidis in the egg contents after stored at 37 C for 1 week. In Experiment 3, eggs laid by individual hens within 1 week were pooled for S. enteritidis culture. Hens were killed for bacteriological examination 2 weeks in Experiments 1 and 2, and 3 weeks in Experiment 3, respectively, after S. enteritidis infection. E. tenella infection resulted in a significant increase ($P<.05$) of S. enteritidis-contamination of eggshells, while E. tenella infection did not cause significant increase of S. enteritidis-contamination of egg contents. Surface-sterilized eggshells showed significantly higher S. enteritidis cells than the pooled egg content samples too. There was a significant difference ($P<0.01$) in both cecal S. enteritidis positive rate and cecal S. enteritidis counts between chickens infected with coccidia and hens without exposure to coccidia. Similarly, coccidial infection resulted in a significant increase of S. enteritidis in the coacal samples.

SCIENTIFIC PROGRAM

WORKSHOP

Workshop

Biological control of internal parasites of livestock

15:30 ~ 17:30 Wednesday August 30, 1995

Room A

Chairperson: **Dr. P.J. Waller**, *Australia*
Dr. M. Larsen, *Denmark*

Workshop

Haemosporidia

15:30 ~ 17:30 Wednesday August 30, 1995

Room B

Chairpersons: **Dr. M. Onuma**, *Japan*
Dr. I. Kakoma, *U.S.A.*

15:35 **COMPARATIVE DNA SEQUENCE ANALYSIS FOR A MAJOR PIROPLASM SURFACE PROTEIN OF BENIGN BOVINE *THEILERIA* SPP. DISTRIBUTED IN EAST ASIA**

Tsuji, M., Kobota, S., Ishihara, C. and Onuma, M., *Japan*

W 1

16:00 **VERTICAL TRANSMISSION OF *THEILERIA SERGENTI* IN COWS VERIFIED BY POLYMERASE CHAIN REACTION**

Baek, B-K., Kim, J.H., Lee, H.I., Han, S.S., Kubota, S., Onuma, M. and Kakoma, I., *Korea*

W 2

16:25 **EPIDEMIOLOGY AND CONTROL OF *THEILERIA ANNULATA* INFECTION**

Pipano, E., *Israel*

W 3

16:50 **IMMUNOLOGICAL CONTROL OF *THEILERIA SERGENTI* INFECTION: VACCINE TRIAL WITH A RECOMBINANT PIROPLASM PROTEIN AND SYNTHETIC PEPTIDE**

Sugimoto, C., Kubota, S., Sako, Y., Matsuba, T. and Onuma, M., *Japan*

W 4

17:15 **DISCUSSION**

Ectoparasiticide resistance 15:30 ~ 17:30 Wednesday August 30, 1995
Room C

Chairperson: **Dr. L. Grisi**, *Brazil*

Workshop

Antiparasitic testing guidelines 15:30 ~ 17:30 Wednesday August 30, 1995
Room D

Chairperson: **Dr. V.J. Theodorides**, *U.S.A.*

Beechinor, J.G., *EC*

ANTIPARASITIC TESTING GUIDELINES

Holdsworth, P.A. , *Australia*

W 5

US/FDA ANTIPARASITIC TESTING GUIDELINES

Letonja, T., Berson, M., Dobson, J., Messenheimer, J. and Olsen, J., *U.S.A.*

W 6

CLINICAL TRIALS FOR ANTIPARASITIC TESTING FOR ANIMALS IN JAPAN

Taira, N. and Ishikawa, M. , *Japan*

W 7

Grisi, L., *Brazil*

Tropical livestock industry and technology transfer

15:30 ~ 17:30 Thursday August 31, 1995

Room A

Chairperson: **Dr. R. Prichard**, *Canada*

15:30 **OPENING REMARKS**

Prichard, R., *Canada*

15:35 **TECHNOLOGY TRANSFER IN DEVELOPING COUNTRIES: THE CASE OF DAIRY CATTLE CONTROL IN SOUTHEAST BRAZIL**

Charles, T., *Brazil*

16:00 **EFFECTING DEVELOPMENT AND ADOPTION OF BREEDING TECHNOLOGIES FOR PRODUCING SHEEP RESISTANT TO NEMATODE PARASITES**

Watson, T., *New Zealand*

16:25 **THE ADOPTION OF WORM CONTROL PROGRAMS FOR SHEEP IN AUSTRALIA**

Donald, A., *Australia*

16:50 **INDUSTRY VIEW OF TECHNOLOGY TRANSFER**

Rew, R., *U.S.A.*

17:15 **CONCLUDING DISCUSSION**

Zoonoses - Meat transmission 15:30 ~ 17:30 Thursday August 31, 1995

Room B

Chairperson: **Dr. J. Eckert**, *Switzerland*

ZOONOSES: MEAT - TRANSMISSION

Eckert, J., *Switzerland*

W 8

Workshop

Antiprotozoal resistance 15:30 ~ 17:30 Thursday August 31, 1995

Room C

Chairperson: **Dr. K. Hoji**, *Japan*

EXPERIMENTAL DRUG RESISTANCE OF CHICKEN COCCIDIA

Saitoh, Y., Itagaki, H., *Japan*

W 9

RESISTIVITY AGAINST ANTICOCCISTATS ON AVIAN COCCIDIUM IN JAPAN

Ohara, E., Itahana, H., Kuwano, A. and Furuki, M., *Japan*

W 10

Equine parasitology

15:30 ~ 17:30 Thursday August 31, 1995
Room D

Chairperson: **Dr. T.R. Klei**, *U.S.A.*

CURRENT STATUS OF EQUINE PARASITE PROBLEMS IN JAPAN

Yoshihara, T., *Japan*

BIOLOGY OF CYATHOSTOMES

Eysker, M., *The Netherlands*

DRUG RESISTANT CYATHOSTOMES

Slocombe, O., *Canada*

CONTROL OF INTERNAL PARASITES

Duncan, J.L., *United Kingdom*

Zoonoses - Environmental transmission

15:30 ~ 17:30 Saturday September 2, 1995

Room A

Chairperson: **Dr. M. Kamiya** *Japan*

Workshop

Analytical approaches to detect drug in animal products

15:30 ~ 17:30 Saturday September 2, 1995

Room B

Chairperson: **Dr. F. Kondoh**, *Japan*

THE DEVELOPMENT OF ANALYTICAL METHODS FOR SULFONAMIDES IN MEAT

Horie, M., *Japan*

W 11

CHEMICAL ANALYSIS OF TETRACYCLINE ANTIBIOTICS

Oka, H., *Japan*

W 12

OFFICIAL ANALYTICAL METHOD FOR RESIDUAL ANTIPARASITIC DRUGS IN ANIMAL PRODUCTS

Murayama, M. and Saito, Y., *Japan*

W 13

CURRENT OVERVIEW OF FEED ADDITIVES AND VETERINARY DRUGS AND THEIR RESIDUAL ANALYSIS IN JAPAN

Nakazawa, H., *Japan*

W 14

Anthelmintic resistance 15:30 ~ 17:30 Saturday September 2, 1995

Room C

Chairpersons: **Dr. F. Borgsteede**, *The Netherlands*
Dr. M. Roos, *The Netherlands*

15:30 **INTRODUCTION**

Borgsteede, F., *The Netherlands*

15:40 **MOLECULAR ANALYSIS OF BENZIMIDAZOLE RESISTANCE**

Roos, M., *The Netherlands*

15:55 **THE UTILITY OF SIMPLE MODELS FOR ANTHELMINTIC RESISTANCE**

Smith, G.

16:10 **IMPLICATIONS OF RESISTANCE ALLELE FREQUENCY FOR TREATMENT STRATEGIES**

Prichard, R., *Canada*

16:25 **DISCUSSION**

ABSTRACTS OF WORKSHOP

W 1 COMPARATIVE DNA SEQUENCE ANALYSIS FOR A MAJOR PIROPLASM SURFACE PROTEIN OF BENIGN BOVINE *THEILERIA* SPP. DISTRIBUTED IN EAST ASIA

Masayoshi Tsuji[1], Shuichi Kobota[2], Chiaki Ishihara[1] and Misao Onuma[2]
[1] Department of Veterinary Medicine, Rakuno-gakuen University, Bunkyodai, Ebetsu 069, Japan; [2] Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060, Japan.

Many veterinary scientists in east Asia have been aware of the presence of relatively benign *Theileria* parasites which are widespread among cattle. Although the parasites are presumed to belong to *Theileria sergenti/buffeli/ orientalis* group, systematic studies across countries have not been conducted. We have collected DNA samples of *Theileria* parasites from various countries in east Asia, and studied the sequences of the DNA encoding ORF of 32/33 kDa major piroplasm surface protein. This protein shows diversity among isolates, as three similar but distinct DNA sequences were reported for *T. sergenti* Chitose stock (C-type), *T. sergenti* Ikeda stock (I-type), and *T. buffeli* Warwick stock (B-type). For this study, blood samples were collected from various regions in Korea, Taiwan, China and Japan. Genomic DNAs were prepared from the blood samples, followed by PCR amplification of the DNA encoding 32/33 kDa protein. The PCR products were cloned in a plasmid vector. More than 20 DNA clones were picked up for a DNA sample obtained from individual cattle, and the insert DNAs were cut with various restriction enzyme to examine digestion pattern. The DNA clones were found to be classified into four groups having distinct restriction patterns. Three of them were identical to the restriction patterns expected for C-, I- and B-type sequences. Sequencing of representative DNA clones in each group revealed that many parasites collected in Japan, Korea and China have C- and I-type sequences. The parasites in Taiwan had C- and B-type sequences. Furthermore, we found three new sequences (two in Korea, one in China), which show 75-91% homology to the three reported sequences described above. DNA clones derived from a single cattle often showed two to three distinct types of sequences, indicating that the cattle were infected with mixed population of *Theileria* parasites. The results indicate that in addition to the parasites reported as *T. sergenti/buffeli/orientalis*, there may be several new benign *Theileria* parasites distributed in east Asia, and also that many cattle are infected with a mixed, geographical variable, population of *Theileria* parasites.

W 2 VERTICAL TRANSMISSION OF *THEILERIA SERGENTI* IN COWS VERIFIED BY POLYMERASE CHAIN REACTION

Byeong Kirl Baek[1], Jin Ho Kim[1], Ho Il Lee[1], Sang Seop Han[4], Shuichi Kuboda[2], Misao Onuma[2], Ibulaimu Kakoma[3]

1. College of Veterinary Medicine, Chonbuk National University, Chonju, Korea. 2. Faculty of Veterinary Medicine, Hokaido University, Sapporo, Japan. 3. College of Veterinary, University of Illinois Urbana, Illinois USA 4. KRISCT, Taejeon, Korea.

Bovine theileriosis due to *Theileria sergenti* is economically very important. Knowledge on the transmission of the disease including the vertical mode will influence control strategy. Six pregnant cows naturally infected with *Theileria sergenti* were used to test for the possible vertical transmission of *T. sergenti*. Blood samples were collected from 6 newborn calves and their dams. *Theileria sergenti* infection was confirmed by both microscopic and single polymerase chain reaction(PCR). Two different primer sets were sequentially used to demonstrate *T. sergenti* in formalin-fixed specimens. All six dams and five of six newborn calves were *T. sergenti* positive and only one calf was negative in both test. In the positive PCR, a predicted amplification product of 875bp was detected. The specific amplification band 684bp by nested PCR was also found in DNA samples from spleens of three aborted fetuses. These data are discussed in relation to the unequivocal confirmation of the transplacental transmission of *T. sergenti* infection in cattle. The PCR data correlated with parasitologic data but PCR had the advantage that it could detect *T. sergenti* in formalin-fixed fetal tissue that were unsuitable for parasitologic investigation.

W 3

EPIDEMIOLOGY AND CONTROL OF *THEILERIA ANNULATA* INFECTION

E. Pipano: The Kimron Veterinary Institute,
Bet Dagan 50250, Israel

Theileria annulata (*T.a.*) is transmitted stage to stage by ticks of the genus *Hyalomma*. The infection occurs around the Mediterranean basin and in much of Asia. In some areas *T.a.* overlaps less pathogenic species of *Theileria*. Eradication of the vector on pastures is not achievable as a practical alternative. Acaricide treatment of cattle has a limited success mainly because of the short period of time required for transmission after the tick attaches. Chemotherapy is used when other control methods have failed or have not been applied. Various vaccination regimes using live schizonts or sporozoites have been tried. The developmental stages of *T.a.* are antigenically different and schizonts confer only partial protection against infection with sporozoites. Virulent schizonts engender stronger immunity than low virulence schizonts. Immunological strain differences have been demonstrated by cross-protectiontests with field isolates. Long-term cultivation of *T.a.* schizonts yields non-virulent parasites. Immunization of cattle with attenuated schizonts does not prevent infection by ticks but inhibits parasite multiplication and severe clinical symptoms. Tick challenge of vaccinated cattle results in appearance of erythrocytic merozoites, therefore vaccination cannot eradicate theileriosis. More than 2 decades experience with culture-derived,attenuated vaccine has shown this to be a practical, safe and efficient procedure for preventing losses caused by *T.a.* infection in cattle.

W 4 IMMUNOLOGICAL CONTROL OF *THEILERIA SERGENTI* INFECTION: VACCINE TRIAL WITH A RECOMBINANT PIROPLASM PROTEIN AND SYNTHETIC PEPTIDE.

C. Sugimoto, S. Kubota, Y. Sako, T. Matsuba and M. Onuma
Faculty of Veterinary Medicine, Hokkaido University
Sapporo, Japan.

Bovine piroplasmosis caused by *Theileria sergenti* is a major cause of economical loss in grazing cattle in Japan. We have revealed that the parasite stocks and isolates consisted of genetically and antigenically mixed populations. Parasite populations bearing two to three different allelic forms of p32/34, a piroplasm major surface protein are generally recognized within a single isolate or stock. Differences in immunogenicity among these allelic products against calves were also demonstrated. We immunized calves with a recombinant p32/34 expressed by a baculovirus system or peptides which contain B-cell epitopes and are exposed on p32/34 molecule, and challenged with a sporozoite stabilate containing two populations with two allelic forms of p32/34. The immunizations did not protect the animals against the infection, but immunization with synthetic peptides delayed appearance of piroplasms in peripheral bloods and lowered the level of parasitemia. Interestingly, parasites with a p32 allelic form corresponding to one used as the immunogen were suppressed. These results suggested that host humoral immunity might select certain parasite populations. Parasite population analysis of *T. sergenti* is indispensable for epidemiological studies on benign theileriosis in Asian countries and development of effective control methods.

W 5 ANTIPARASITIC TESTING GUIDELINES

P.A.Holdsworth
National Registration Authority for Agricultural and Veterinary Chemicals,
P.O. Box E240, Queen Victoria Terrace, ACT, 2600, Australia

The registration of veterinary parasitological products in Australia is undertaken by the National Registration Authority for Agricultural and Veterinary Chemicals (NRA). One component of this process is the evaluation of submitted efficacy and safety data for new end use products or modification to the use patterns of existing registered products.

Applicants of new products are expected to show, through the submission of comprehensive data, results of laboratory and field scale experiments/trials which prove that the product, when used according to the directions, is effective and safe for the intended animal species. As a general rule, efficacy and safety studies should be conducted using the formulation to be marketed.

Efficacy data from overseas countries may be used to support an application. However due to Australia's diversified animal production industries and a history of rapid development of chemical resistance in parasites, confirmatory efficacy trials are required to show that results under typical Australian conditions are at least equal to those obtained elsewhere. Specific efficacy parameters are set for particular parasite groups with respect to what product label claim will be registered [e.g. for ruminant anthelmintic therapeutic and prophylactic claims, >95% and >99% efficacy, respectively must be demonstrated]. Field trials should be carried out at geographical locations which are important for that particular animal industry and preferably where the parasite occur under optimum rather than under marginal conditions.

Specific efficacy guidelines have been developed for various categories of veterinary parasitological products e.g. ectoparasiticides, anthelmintics and anticoccidials. Some of these guidelines (ovine miticides, lousicides and blowfly specifies; cattle acaricides and cattle buffalo fly treatments) have recently (1994-95) been updated. Others (ruminant anthelmintics; poultry anticoccidials; anthelmintics for dogs, cats, swine and horses) are in the process of being modified throughout the 1995-96 year. The latter group is based on specific WAAVP Antiparasitic Testing Guidelines.

The NRA is investigating the avenues that exist for utilising overseas assessment reports in the evaluation process to avoid duplication of effort and reduce assessment time. Risk assessment in this area needs to be carefully considered and researched to maintain quality of assessment.

W 6 US/FDA ANTIPARASITIC TESTING GUIDELINES

T. Letonja, M. Berson, J. Dobson, J. Messenheimer, and J. Olsen, Center for Veterinary Medicine, US Food and Drug Administration

The Center for Veterinary Medicine has created guidelines to help in the preparation of new animal drug applications supporting the approval of efficacy claims for antiparasitic compounds. Currently, there are six documents that contain recommendations for the testing of anticoccidials in chickens and turkeys, for anthelmintic compounds in cattle, swine, equine, cats and dogs, and minor species (game birds, sheep and goats, rabbits, and food fish species).

Three phases are recommended for evaluating the efficacy of the products: dose titration, dose confirmation, and field trials. The Center encourages sponsors to submit protocols and discuss them before conducting the studies. Effectiveness of anthelmintic compounds is measured as % efficacy, and in the case of anticoccidial drugs, correction of intestinal lesions. Experimental or natural infections are acceptable in the trials. Recent parasite isolates should be used in the experimental infection studies.

The current guidelines are being revised to incorporate general recommendations for broad spectrum compounds that are both ecto and endoparasiticides, and address persistent efficacy claims. In addition, we intend to create a poultry anthelmintic guideline.

The three largest pharmaceutical markets, the United States, the European Union and Japan are involved in active discussions in several International Conferences on Harmonization. The objective of the discussions is to harmonize requirements for registration of pharmaceuticals among the three parties. The pharmaceutical industry is interested in generating a single worldwide database or master file, including high quality information that will reduce research and development time and costs, and consider ethical treatment of animals. At the same time, for the regulatory bodies, the efforts of harmonization will impact on the regulatory requirements, evaluation of the submitted data, and criteria for acceptance or rejection of the data package.

In the first stage, guidelines will be exchanged for tripartite consultation. English will be used as the common language.

effective = >90% arithm. mean.

W 7

CLINICAL TRIALS FOR ANTIPARASITIC TESTING FOR ANIMALS IN JAPAN

N.Taira and M.Ishikawa
(Nat. Inst. of Anim. Health, Tsukuba, Ibaraki 305, Japan)

In Japan, antiparasitic drugs for animals are under the jurisdiction of the Ministry of Agriculture, Forestry and Fishery. The total procedure of the development has been summarized in the 14th WAAVP (Cambridge, 1993; Abst. on P361). At this time, the outline of clinical trials for antiparasitic drugs for animals in Japan is summarized.

1. General items
   1) Studies should be performed in at least 2 institutions.
   2) Sophisticated and objective assessments are required.
2. Number of animals
   1) Cattle, horse, sheep, goat, swine, dog and cat : At least 60 animals.
   2) Poultry and/or other animals : At least 200 animals.
3. Administration route and dosage
   1) Clinical application route, and anticipated dose and its higher and lower levels must be performed.
4. Other items
   1) The control group is required.
   2) Diagnostic criteria for diseases should be described.
   3) Criteria for efficacy assessment are required.
   4) Data concerning the pre-treatment condition of animals and disease contamination are necessary.
   5) Trials should be performed under a common protocol.

W 8 Introduction to the Workshop: Zoonoses: meat- transmission

J. Eckert

Institute of Parasitology, University of Zürich and WHO Collaborating Centre for Parasitic Zoonoses

Meat-borne parasitic infections continue to represent a potential risk factor for the health of the population in various parts of the world and should therefore be a concern of governments and health authorties. In recent years consumers increasingly demand meat and meat products free of pathogens and chemical residues. Therefore, parasitologists are asked for more efficient methods of diagnosis, control and prevention of certain meat-borne zoonoses. The present status of knowledge will be discussed in the workshop.

Toxoplasmosis is in part a meat-borne infection because of the persistence of cysts of *Toxoplasma gondii* in raw or undercooked meat. Special risk exists for women and their foetuses if primary infection occures during pregnancy. In addition, reactivation of latent toxoplasmosis with cerebral and generalised manifestations is a growing problem in immunocompromised patients (AIDS patients etc.). There is little public awareness that the infection is normally not diagnosed in slaughter animals so that raw or undercooked meat may serve as an uncontrolled source of infection. Effective modern methods for diagnosing the infection in living animals (immunodiagnosis, PCR) are essentially available but they are not applied to large-scale use in practice. Efficient control strategies are not available.

Microsporidiosis. Human microsporidiosis is a growing problem in immunodeficient patients but little is known on sources of infection and ways of transmission. A few reports suggest that microsporidia species from fish may be infective to humans.

Taeniosis. Transmission of cysticerci (metacestodes) of *Taenia saginata* or *Taenia solium* by consumption of raw or undercooked meat of cattle and swine, respectively, may lead to intestinal taeniosis in humans and, in the case of *T. solium*, indirectly to cerebral, ocular or muscular cysticercosis. In the intermediate hosts (cattle, swine) infection with cysticerci may be a cause of serious economic losses. (The same applies for *Taenia ovis* cysticercosis in sheep). Diagnostic methods for the diagnosis of intestinal taeniosis of humans could by improved by the development of ELISAs for copro-antigen detection. A reliable *in vivo* diagnosis of cysticercosis in living animals is presently not possible, but new prospects exist for vaccination of cattle against cysticercosis based on knowledge gained from the development of a vaccine against *Taenia ovis* cysticercosis in sheep.

Trichinellosis. Trichinellosis is still an unsolved zoonotic problem with new actuality in some countries, mainly by transmission of *Trichinella spiralis* with horse meat. Great advances have been made using biochemical and molecular techniques in the identification of *Trichinella* species and strains, and serodiagnostic methods using highly specific antigens in the ELISA have given very promising results in mass screening studies of animal populations.
These zoonoses and others shoud be discussed in the workshop.

W 9 EXPERIMENTAL DRUG RESISTANCE OF CHICKEN COCCIDIA

Yasuhide SAITOH and Hiroshi ITAGAKI
Department of Parasitology, School of Veterinary Medicine, Azabu University.
SAGAMIHARA City, JAPAN

The line of *Eimeria acervulina* derived from a single oocyst to 9 aniticoccidials was examined for sensitivity. The line was multiple drug resistant. This line also developed resistance against robenidine and sulfadimethoxine(SD) through serial passages in chickens that were medicated with the drug at suboptimal doses respectively.

The experimentally induced SD resistant line of *E.acervulina* became sensitive again to SD at 2000ppm after 15 serial passages in chicken fed with normal rations. Serial passages necessary to become sensitive were reduced when the sensitive oocysts were added to the resistant at a rate of 10% in the starting material for passages. However, sensitivity to SD was not changed after 50 serial passages in chickens fed with normal rations when the SD resistant sub-line derived from a single oocyst as starting material was used. Twelve clones each derived from a single oocyst of the SD resistant line were examined for the sensitivity to SD. One of the 12 clones was completely inhibited in endogenous growth by the medication with SD at 2000ppm.

These results suggest that in the case of SD, sensitive *E.acervulina* organisms proliferate more dominantly than the resistant ones when both the organisms are passaged in the same chickens fed with normal rations.

W 1 0 Resistivity against anticoccistats on avian coccidium in Japan

Eiji Ohara, Hidenobu Itahana, Akira Kuwano, Makiko Furuki

DAIICHI PHARMACEUTICAL CO.,LTD.

Avian coccidiosis is a disease induced by parasitic protozoa in the digestive tract of poultry. The mass-rearing trend of poultry in the mass management is one of the most favorable conditions for this infectious disease, and it is a cause of mass infection. Once infected by coccidiums, it damage is significant, and it causes mortal sacrifice and seriously influences the rearing rate, body weight gain, feed convension, egg production rate, and other factors. Oocyst, the infectant of this disease, is very strong in resistance, and any disinfectant to destroy it is not known yet. As its countermeasure, therefore, method have been attempted, such as prevention and treatment by drugs, but there arises problem of resistance to these drugs.

This investigation was carried out for Avian coccidiosis drug-sensitivities to compare the two period since 1979 to 1984 and 1991 to 1995. The results indicated that resistance to clopidol, polyether antibiotics and sulfa drugs were decrease in the period since 1991 to 1995 than since 1979 to 1984. But about amprolium , an improvement of drug sensitivity were recognized. And for a long time, there was high sensitivity about nicarbazin, convinations of sulfa drugs and folic acid antagonists. There was once to fourth drug-resistant, and many combinations were exist about amprolium and clopidol in the period since 1979 to 1984 and about only polyether antibiotics or polyether antibiotics and sulfa drugs combination in the period since 1991 to 1995.

W 1 1

THE DEVELOPMENT OF ANALYTICAL METHODS FOR SULFONAMIDES IN MEAT

M. HORIE

Saitama Prefecture Institute of Public Health, Kami-Okubo 639-1, Urawa City, Saitama 338, Japan

Sulfonamides such as sulfadimidine (SDD), sulfadimethoxine (SDMX), sulfamonomethoxine (SMMX) and sulfaquinoxaline (SQ) have been widely used for the prevention and treatment of infectious diseases and coccidiosis in animals. So, food hygiene concerns have arisen regarding the presence of these drugs' residues in livestock products. Such residues may have direct toxic effects on consumers (e.g., carcinogenicity of some drugs) or may indirectly cause problems through the induction of resistant strains of bacteria.

In Japan, according to the Food Sanitation Law, no livestock products should contain antibiotics and synthetic antibacterials. On the other hand, in the United States, as in many other countries, tolerance limits for sulfonamides in livestock products have been set. In most cases, the tolerance for sulfonamides in livestock products is 0.1 ppm. Therefore, simple and reliable analytical methods are required to monitor these drug residues in edible tissues of livestock animals. Many analytical methods have been developed for determination of the drugs. The extract from a tissue sample contains many diverse compounds in addition to the possible traces of the target sulfonamides. To exclude these physically- or chemically-interfering substances, a variety of techniques may be employed. In this workshop, I will describe an overview of chemical analysis methods for sulfonamides.

W 1-2

CHEMICAL ANALYSIS OF TETRACYCLINE ANTIBIOTICS

H. OKA

Aichi Prefecture Institute of Public Health, Nagare 7-6, Tsuji-machi, Kita-ku, Nagoya 462, Japan

Tetracycline antibiotics (TCs), which represent oxytetracycline (OTC), tetracycline (TC), chlortetracycline (CTC) and doxycycline (DC), are commonly applied to food-producing animals including honeybees as veterinary drugs and feed additives because of broad spectrum activity and cost effectiveness. Their widespread utilization has is required. As with most antibiotics, microbiological assays have commonly been used for the measurement of TCs in food, but they are time consuming, cannot identify or differentiate certain TCs and their precision appears to be variable. Therefore, the need for precise chemical analysis methods has been clear.

Taking physicochemical properties of TCs into consideration, many chemical analysis methods for the determination of residues of TCs in food have been reported over the last 20 years or more. However, most of them require complicated procedures, because of the propensity of TCs to form chelate complexes with metal ions and to bind with proteins and silanol groups in the stationary phase. These properties inhibited the development of a simple chemical method for the analysis of TCs. Recently, it became possible to control these undesirable properties through the use of ethylenediaminetetraacetic acid and oxalic acid at each analytical step (extraction, clean up, and separation). Their use has remarkably improved the available analytical methods for TCs in food.

In this paper, the author describes recent developments in the chemical analysis of TCs, including the control of their undesirable properties, and make recommendations on the application of chemical methods for the determination and confirmation of TCs in food to routine regulatory use.

W 1 3

OFFICIAL ANALYTICAL METHOD FOR RESIDUAL ANTIPARASITIC DRUGS IN ANIMAL PRODUCTS

M. MURAYAMA AND Y. SAITO

Division of Food, National Institute of Health Science, Kamiyoga 1-18-1, Setagaya-ku, Tokyo 158, Japan

More than hundred kinds of veterinary drugs are used to remedy livestock. The number of veterinary drugs has been increasing every year. Almost all of the drug has a possibility of affection on human health, and some drugs are very harmful.

Our institute has major responsibilities for testing and research work on the safety of foods. We have been investigated on the determination method for residual veterinary drugs in animal products. So we introduce Japanese official analytical method for residual veterinary drugs, especially for antiparasitic and anticoccidial drugs. Objective drugs are listed as follow, closantel, flubendazole, ivermectin, morantel, amprolium, clopidol, decoquinate, ethopabate, nicarbazin, pyrimethamine and robenidine.

W 1 4

CURRENT OVERVIEW OF FEED ADDITIVES AND VETERINARY DRUGS AND THEIR RESIDUAL ANALYSIS IN JAPAN

H. NAKAZAWA

Department of Analytical Chemistry, Faculty of Pharmaceutical Science, Hoshi University, Ebara 2, Shinagawa-ku, Tokyo 142, Japan

The livestock and marine culture industry in Japan has been enlarged and become intensive. In order to decrease economically the cost of production, and to improve the quality of product and raise the productivity, various compounds as feed additives and veterinary drugs are used as an effective means. The residue of drug in foods of animal origin has increasingly become of interest to the entire industry as growing consumer health concerns. Based on the legal restrictions of drug residue in products, it was required to analyze synthetic antibacterials and antibiotics in food with a simple and reliable methods.

The current overview of feed additives and veterinary drugs including anabolic agents used in Japan and their residual analysis with the related regulatory laws are reported.

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