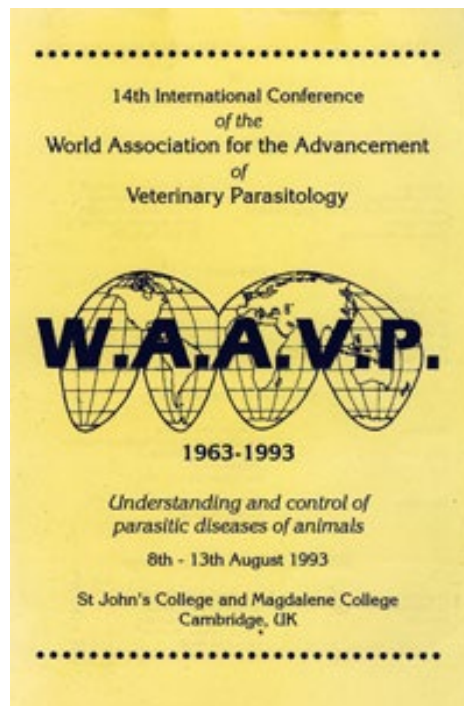


The 14th International Conference of the
**WORLD ASSOCIATION FOR THE
ADVANCEMENT OF VETERINARY
PARASITOLOGY**

“Understanding and Control of Parasitic
Diseases of Animals”

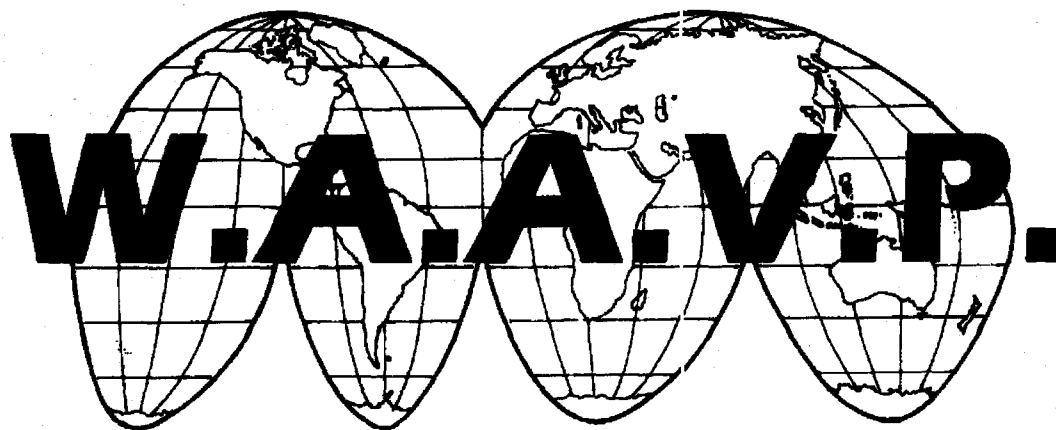
August 8-13, 1993

CAMBRIDGE, UNITED KINGDOM



.....

14th International Conference
of the
World Association for the Advancement
of
Veterinary Parasitology



1963-1993

*Understanding and control of
parasitic diseases of animals*

8th - 13th August 1993

St John's College and Magdalene College
Cambridge, UK

.....

World Association for the Advancement of Veterinary Parasitology

Officers

<i>President:</i>	Dr. O. Slocombe, Canada
<i>1st Vice President:</i>	Dr. Th. Hiepe, Germany
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<i>Dr. P. Nansen</i>	Denmark
<i>Dr. R. K. Prichard</i>	Canada
<i>Dr. J. Tharaldsen</i>	Norway

Organising Committee

<i>Chairman:</i>	Professor Lord Soulsby Department of Clinical Veterinary Medicine University of Cambridge Maddingley Road Cambridge CB3 0ES, UK.
<i>Committee:</i>	Dr. R. Connan Dr. M.A. Gemmell Dr. S. Lloyd

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<i>Chairman:</i>	Professor Lord Soulsby, UK
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<i>Members:</i>	Dr. A.D. Donald, Australia
	Dr. J.L. Duncan, Scotland
	Dr. L. Grisi, Brazil
	Dr. J. Tharaldsen, Norway

3 or 16 / year



Welcome!

Welcome to Cambridge and the 14th Congress of the WAAVP. I trust the scientific programme, the social events and the location will generate the same lasting impressions of this conference as did the previous meetings of the WAAVP.

The WAAVP was established in 1963 in Hannover, Germany, and over the 30 years has grown to an Association with a high reputation for innovative conferences, publications and guidelines for the testing of antiparasitic compounds. Particularly, the global membership has grown greatly on an individual basis as well as affiliated societies and corporate members. The numbers attending each conference continue to grow and this present conference will host nearly 500 delegates.

The city of Cambridge with its University and its Colleges is a unique setting for this 30th Anniversary meeting. Historically, parasitology has had a strong emphasis in the University which is a leading institution for the biological sciences and modern technology.

Those staying in College will particularly savour the pleasure of the Collegiate aspects of a great University; its quiet, beautiful courts, gardens and walks away from the busy streets of the city. But the city, too, is worth exploration and this is easily achieved as both St. John's College and Magdalene College are near the city centre.

We have a busy programme, I believe the longest of any of our conferences so far, but I hope an enjoyable one. Our conferences provide an opportunity for old friendships to be renewed and new ones made; may this be particularly true in Cambridge

LORD SOULSBY

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Schering Plough Animal Health
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The Donkey Sanctuary



CONTENTS

GENERAL INFORMATION	1-2
SUMMARY PROGRAMME	3-4
DETAILED PROGRAMME	5-19
ABSTRACTS OF PLENARY PAPERS.....	22-37
ABSTRACTS OF SUBMITTED PAPERS:	
Session 1	40-49
Session 2	52-61
Session 3	64-73
Session 4	76-85
Session 5	88-96
Session 6	98-103
Session 7	106-115
Session 8	118-127
Session 9	130-139
Session 10	142-151
Session 11	154-162
Session 12	164-173
Session 13	175-179
Session 14	181-184
Session 15	186-190
Session 16	192-196
Session 17	198-202
Session 18	204-207
Session 19	209-211
Session 20	213-217
Session 21	219-223
Session 22	225-228
ABSTRACTS OF HOECHST	
SPECIAL SESSION.....	230-234
ABSTRACTS OF PFIZER	
SPECIAL SESSION.....	236-241
ABSTRACTS OF COST 89 SESSION.....	243-257
POSTER PRESENTATION LIST	258-261
ABSTRACTS OF POSTER SESSION.....	262-342
WORKSHOP PROGRAMME	343-344
ABSTRACTS OF WORKSHOP PAPERS	345-382
MAP OF ST. JOHN'S COLLEGE	Inside back cover



GENERAL INFORMATION

FORMAT

Plenary Sessions will take place during the morning. Submitted papers will be given in sessions on Thursday morning and in the afternoons. Workshops will follow submitted papers. Posters will be displayed at all times.

Full projection facilities are available in all lecture rooms. The Plenary Papers will be recorded on video tape. Tapes will be available for sale (see conference documents).

All papers should be presented in English. Many delegates are from overseas and their native language is not English. Therefore, presenters should speak slowly and distinctly in order to be understood.

Because of the large number of submitted papers, the time limit for presentations is 10 minutes plus 2 minutes for questions. *It is imperative that all speakers keep to their allocated time.*

SPECIAL CONFERENCE ISSUE OF *VETERINARY PARASITOLOGY*

This will contain the text of all the Plenary Papers and summaries (up to 1,500 words) of each workshop. One free copy of the Special Issue will be sent to each delegate. Further copies may be purchased. Manuscripts will have been peer reviewed prior to publication.

MEALS

All meals (except the Conference Banquet) will be served in your college. Meal tickets must be shown at the entrance to the dining hall. Delegates not staying in College, but requiring meals, must purchase tickets at the time of registration. Meals will not be served to delegates without the appropriate meal ticket.

COMMERCIAL EXHIBITION

This is located in a marquee adjacent to the Conference Centre. Tea/coffee will be served in the marquee.



SOCIAL PROGRAMME

A series of social events for all delegates and accompanying persons is offered free of charge on the following days:

Sunday 8th August (evening):	Welcome wine reception
Monday 9th August (evening):	Wine reception following a special session, courtesy of Hoechst Animal Health
Tuesday 10th August (evening):	Wine reception following a special session, courtesy of Pfizer Animal Health Group
Wednesday 11th August (noon):	Presentations by Pfizer Award Winners and wine reception courtesy of Pfizer Animal Health Group

On Wednesday 11th August, the afternoon is free and a series of events will be offered at no charge. These include:

Anglesey Abbey (Historic House) and Garden (limit 50)
National Stud, Newmarket (limit 50)
Duxford Air Museum (limit 50)
Ely Cathedral (limit 50)
Guided Walking Tour of Cambridge (Groups of 20)
Open topped bus tour of Cambridge (limit 50)

All tours will return in sufficient time to permit attendance at the Organ Recital in King's College Chapel at 17.30.

FOR ACCOMPANYING PERSONS ONLY (UNLESS AN EXTRA FEE IS PAID)

MONDAY 9TH AUGUST

A series of walking tours of Cambridge in groups of 10 to 20 persons with a guide:
1. Guided Tour of Cambridge Colleges 2. Guided Tour of Fitzwilliam Museum

TUESDAY 10TH AUGUST

All day bus tour (9.30 to 16.30) to visit the 'Wool Towns' and Constable Country of East Anglia, with visits to old market towns such as Lavenham, Bury St. Edmunds, Long Melford and Newmarket (limited to 50 participants).



SUMMARY PROGRAMME

SUNDAY 8TH AUGUST

- 12.00-18.00 Registration and then daily until noon on 12th August
- 18.00-19.30 Reception, River Court, St. John's College

MONDAY 9TH AUGUST

Palmerston Room

- 8.20-9.30 Opening of Conference
(*Dr. Owen Slocombe, Canada - President, WAAVP*)

Parasitology in the United Kingdom and elsewhere over 30 years of WAAVP
(*Professor Lord Soulsby*)

Presentation of Pfizer Awards for Teaching and Research

PLENARY PAPERS

- 9.30-10.15 *Parasites, Animal Production and Sustainable Development
(*Dr. A.D. Donald*)
- 10.15-10.45 COFFEE
- 10.45-11.30 *Parasite Control in Intensive vs Non-intensive Systems; Ruminants
(*Dr. R.D. Reinecke, Brazil*)
- 11.30-12.15 *Epidemiology and Control of Helminth Infections in Pigs under Intensive and Non-intensive Production Systems
(*Dr. A Roepstorff and Dr. P. Nansen, Denmark*)

***Sponsored by Pfizer Animal Health**

12.15-13.30 LUNCH

13.30-15.30 Submitted Papers - 6 parallel sessions

15.30-16.00 TEA

16.00-18.00 Workshops - 5 parallel sessions

18.00 SPECIAL SESSION
(*Palmerston Room*)

'The Fenbendazole SR Bolus, a New Formulation for the Control of Gastrointestinal and Lung Nematodes in Grazing Cattle'
Sponsored by Hoescht Animal Health (Followed by Wine Reception)

POSTER SESSIONS AVAILABLE ALL DAY

TUESDAY 10TH AUGUST

Palmerston Room

PLENARY PAPERS

- 8.30-9.15 †Epidemiology and Control of Parasites in Nomadic Situations
(*Prof. C. MacPherson, West Indies*)
- 9.15-10.00 †Parasite Control in Transhumance Situations
(*Prof. J. Eckert, Switzerland*)
- †Sponsored by The Donkey Sanctuary
- 10.00-10.30 COFFEE
- 10.30-11.15 ‡Modelling of Parasite Populations - Gastrointestinal Nematodes
(*Drs. B. Grenfell and G. Smith, UK*)
- 11.15-12.00 ‡Modelling of Parasite Populations - Cestodes
(*Dr. M. Roberts, New Zealand*)

†Sponsored by MSD AgVet

12.00-13.30 LUNCH

13.30-15.30 Submitted Papers - 6 parallel sessions

15.30-16.00 TEA

16.00-18.00 Workshops - 6 parallel sessions

18.00 SPECIAL SESSION
(Palmerston Room)

'New Developments in the Control of Parasites of Domestic Animals'
Sponsored by Pfizer Animal Health Group (Followed by Wine Reception)

POSTER SESSIONS AVAILABLE ALL DAY

WEDNESDAY 11TH AUGUST

Palmerston Room

PLENARY PAPERS

8.30-9.15 Genetic Resistance to Parasitic Disease: Particularly of Resistance in Ruminants to Gastrointestinal Nematodes
(Dr. M.J. Stear and Prof. M. Murray, UK)

9.15-10.00 *Vaccine Development
(Dr. M. Lightowlers, Australia)

10.00-10.30 COFFEE

10.30-11.15 †Vector Biology and Control
(Dr. G. Lopez, Colombia)

11.15-12.00 *Vaccines Against Blood Sucking Arthropods
(Dr. J. Opdebeeck, Australia)

12.00-13.00 Special Session: Presentations by Pfizer Awardees for Teaching and Research

*Sponsored by Intervet †Sponsored by Bayer

13.00 LUNCH

POSTER SESSIONS AVAILABLE ALL DAY
AFTERNOON FREE FOR SELECTION OF SOCIAL EVENTS (SEE GENERAL INFORMATION (PAGE 1))

13.00-16.30 Open session on 'Coccidiosis' organised by the COST 89 group

17.30 Organ recital in King's College Chapel

THURSDAY 12TH AUGUST

Palmerston Room

PLENARY PAPERS

8.30-9.15 ‡Chemotherapy and Delivery Systems: Haemoparasites
(Dr. A.F. Peregrine, Kenya)

9.15-10.00 ‡Chemotherapy and Delivery Systems: Helminths
(Prof. Q.A. McKellar, UK)

‡Sponsored by Hoechst Animal Health

10.00-10.30 COFFEE

10.30-11.15 **Anthelmintic Resistance
(Prof. R. Pritchard, Canada)

11.15-12.00 Teaching Veterinary Parasitology
(Prof. A. Verster, South Africa)

**Sponsored by Horserace Betting Levy Board

12.00-14.00 LUNCH

14.00-15.00 Submitted Papers - 4 parallel sessions

15.00-15.30 TEA

15.30-17.30 Workshops - 5 parallel sessions

17.30 General meeting of WAAVP

19.15-19.30 Buses depart for Banquet

FRIDAY 13TH AUGUST

9.00 Possible continuation of Workshops (Delegates to be notified)

12.00 Closing session

12.00 LUNCH

Depart

DETAILED PROGRAMME

SUNDAY 8TH AUGUST

12.00-18.00 Registration and then daily until noon on 12th August

18.00-19.30 Reception, River Court, St John's College

MONDAY 9TH AUGUST

Morning 8.20 Palmerston Room

Chairman: Dr. O. Slocombe

8.20-8.30 Opening of Conference
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8.30-9.15 Parasitology in the United Kingdom and elsewhere over 30
years of WAAVP (*Professor Lord Soulsby*)

9.15-9.30 Presentation of Pfizer Awards for Teaching and Research

PLENARY PAPERS

Chairman: Professor Lord Soulsby

9.30-10.15 *Parasites, Animal Production and Sustainable Development
(*Dr. A.D. Donald*)

10.15-10.45 COFFEE

10.45-11.30 *Parasite Control in Intensive vs Non-intensive Systems,
Ruminants
(*Dr. R.D. Reinecke, Brazil*)

11.30-12.15 *Epidemiology and Control of Helminth Infections in Pigs
under Intensive and Non-intensive Production Systems
(*Dr. A Roepstorff and Dr. P. Nansen, Denmark*)

***Sponsored by Pfizer Animal Health**

12.15-13.15 LUNCH



Afternoon 13.30-15.30

SUBMITTED PAPERS

(*Presenting Author)

Session 1: Parasitology - General Studies

Chairman: Dr. J. Corba

(Durac Room)

Session 2: Arthropod Control I

Chairman: Dr. J. Opdebeeck

(Castlereagh Room)

- 13.30-13.42 Recovery of cestode and dog ascarid eggs from village courtyards of Iran.
A. Eslami, Iran.
- 13.42-13.54 Endoparasitism and management problems of Ethiopian donkeys.
F. Gebreab and N. Yideg, Ethiopia.*
- 13.54-14.06 The occurrence of coccidia in domesticated and free-living small ruminants at the zoo.
M.-A. Hasslinger, T. Ziesche and I. Kiefer, Germany.*
- 14.06-14.18 Prevalence of antibodies against *Babesia divergens* in Austrian cattle by an improved enzyme linked immunosorbent assay.
R. Edelhofer, W. Baumgartner and E. Kutzer, Austria.*
- 14.18-14.30 Carrier state in immune cattle: the implication for the epidemiology and control of East Coast Fever.
D.P. Kariuki and A.S. Young, Kenya.*
- 14.30-14.42 Improvement of chemoprophylactic strategies for the control of African bovine trypanosomiasis: use of isometamidium ELISA.
M.C. Eisler, A.S. Peregrine and P.H. Holmes, U.K., Kenya.*
- 14.42-14.54 Control of cryptosporidiosis: new immuno- and chemotherapies.
R. Fayer, M.C. Jenkins, E. Bostwick and W. Ellis, U.S.A.*
- 14.54-15.06 The role of the Office International des Epizooties (OIE) in Veterinary Parasitology.
R.E. Reichard, France.*
- 15.06-15.18 Choice or chance-teaching of applied Veterinary Parasitology at Pannon University for Agricultural Sciences in Mosonmagyaróvár, Hungary.
B. Egri, Hungary.*
- 15.18-15.30 Arnold of Villanova and the history of Parasitology.
M. Cordero-del-Campillo, Spain*
- 13.30-13.42 Field efficacy of ivermectin against *Oestrus ovis* and ovine helminth parasites.
Ph. Dorchies, J.P. Alzieu, J.M. Yilma and J.L. Crozia, France.*
- 13.42-13.54 Efficacy of moxidectin (injectable and oral drench) against *Oestrus ovis* larvae in naturally infested sheep.
A. Giangaspero, V. Puccini and A. Fasanella, Italy.*
- 13.54-14.06 The efficacy of Cydectin^R 0.5% pour-on against *Hypoderma bovis* in naturally infested cattle: parasitological and serological data.
J.-F. Lonneux, B.R. Mignon and B.J. Losson, Belgium.*
- X 14.06-14.18 The efficacy of topically applied ivermectin for prevention of blowfly strike (myiasis) in sheep in Australia.
J.S. Eagleson, P.G. Scott, L.G. Cramer, D.R. Thompson, J.E. Watts, D. Rugg and R.A. Barrick, U.S.A.*
- X 14.18-14.30 Ectoparasites of livestock in New Zealand. A.C.G. Heath, New Zealand.
- 14.30-14.42 Permethrin spray and wipe-on as useful tools to prevent black fly attacks and simuliotoxicosis in cattle and horses.
G. Liebisch, A. Ziemer and A. Liebisch, Germany.*
- 14.42-14.54 Experience in control of flies and summer mastitis in grazing cattle by use of Bayofly pour on (cyfluthrin).
A. Liebisch and H. Dorn, Germany.*
- 14.54-15.06 The impact of prophylactic mange control in growing swine with Sebacil pour-on on weight gain and economics.
K.J. Krieger and K.-H. Schmitz, Germany.*
- X 15.06-15.18 The efficacy of topically applied ivermectin for the control of the sheep biting louse (*Damalinea ovis*).
L.G. Cramer, J.S. Eagleson, D.R. Thompson, P.G. Scott and R.A. Barrick, U.S.A.*
- X 15.18-15.30 Field efficacy of moxidectin 0.5% pour-on against *Chorioptes bovis*, *Damalinea bovis*, *Linognathus vituli* and *Psoroptes ovis* in naturally infested cattle.
B.J. Losson and J.-F. Lonneux, Belgium.*

Session 3: Anthelmintics - Drug Resistance I**(Palmerston Room)**

Chairman: Prof. R. Prichard

- ⊗ 13.30-13.42 Multiple drug resistance of *Haemonchus contortus* of sheep in Malaysia and the efficacy of moxidectin.
S. Sivaraj*, P. Dorny, J. Vercruysse and V.S. Pandey, Malaysia.
- 6 ⊗ 13.42-13.54 Anthelmintic activity of AB763 in sheep. S.W. Page*, W. Muir, C. Filby, V.J. Theodorides and J.J. Colaianne, U.S.A.
- 13.54-14.06 The use of medicated blocks to control benzimidazole resistant nematodes in Fiji. M.R. Knox, R. Singh, P. Manuelli, L.F. Le Jambre and J.W. Steel*, Australia.
- ⊗ 14.06-14.18 Long term stability of ivermectin resistance following initial diagnosis.
P.F. McKee, T.G. Watson* and B.C. Hosking, New Zealand.
- 4 ⊗ 14.18-14.30 Anthelmintic resistance in southern Latin America: a potential time bomb?
P.J. Waller*, F. Echevarria, C Eddi, S Maciel and A. Nari, Australia, Argentina, Brazil, Paraguay, Uruguay.
- 14.30-14.42 Selection with PYR, LEV, IVM and FBZ on a pyrantel-resistant and an anthelmintic-susceptible isolate of *Oesophagostomum dentatum*.
H. Bjorn*, Denmark.
- ⊗ 14.42-14.54 Nematode resistance to anthelmintics in sheep and goat farms.
C. Hong* and K.R. Hunt, U.K.
- 14.54-15.06 The guinea pig as a model for studying closantel resistance.
P.F. Rolfe* and K. Nicholls, Australia.
- ⊗ 15.06-15.18 The response of naturally infected responder and non-responder male goats to anthelmintic treatments.
F. Jackson*, M. Patterson, E. Jackson, R.L. Coop, Q. McKellar and E.W. Scott, U.K.
- ⊗ 15.18-15.30 Individual variations in goats of febantel efficacy against *Muellerius capillaris*.
C. Chartier*, A. Kulo and J. Cabaret, France.

Session 4: Epidemiology I**(Boys Smith Room)**

Chairman: Prof. P. Nansen

- 13.30-13.42 Seasonal transmission of heartworm (*Dirofilaria immitis*) in dogs exposed to infection in the southern United States.
T.L. McTier*, J.W. McCall, M.T. Dzimiński, J.-P. Raynaud, R.A. Holmes and D.M. Keister, U.S.A., France.
- 13.42-13.54 The epidemiology of *Schistosoma mattheei* in cattle in Zambia.
J. de Bont*, J. Vercruysse, F. Sabbe, V.R. Southgate and D. Rollinson, Zambia.
- 13.54-14.06 Epidemiological study on liver fluke in cattle in France.
J.L. Crosia* and A. Chauvin, France.
- 14.06-14.18 Epidemiology of fascioliasis in Morocco.
K. Khallaayoune*, B.E. Stromberg and A. Dakkak, Morocco.
- 14.18-14.30 Aspects of the biology and epidemiology of *Psoroptes ovis* in Ireland.
D.J. O'Brien*, J.S. Gray and P.F. O'Reilly, Ireland.
- ⊗ 14.30-14.42 Ovine psoroptic otacariasis in Great Britain.
P. Bates, U.K.
- 14.42-14.54 Study of tick populations in the Basque country (Spain).
A.L. Garcia-Perez*, M. Baral, R.A. Juste, D. Fernandez de Luco, J.A. Moreno and V. Dehesa, Spain.
- ⊗ 14.54-15.06 A survey on goat warbles in Iran and evaluation of its treatment with ivermectin. S. Rahbari*, J. Ghasemi and S. Tirgari, Iran.
- 15.06-15.18 Epidemiology and clinical evolution of ovine oestrosis in south west France.
J.P. Alzieu* and Ph. Dorchies, France.
- 15.18-15.30 *Haematobia irritans*: epidemiology and economic impact assay in Argentina.
C. Eddi*, A. Signorini, J. Caracostantogolo, C. Aufranc, J. Peralta, L. Marangunic, A. Noaco and G. Balbiani, Argentina.



Session 5: Biology I

Chairman: Dr. A. S. Peregrine

(Pythagoras School)

Session 6: Genetic Basis of Host Resistance

Chairman: Dr. M. J. Stear

(Music Room)

- 13.30-13.42 Molecular characterisation as an aid to determining the zoonotic potential of *Giardia* and *Cryptosporidium*.
U.M. Morgan*, B.P. Meloni and R.C.A. Thompson, Australia.
- 13.42-13.54 Phylogenetic status of *Neospora caninum* based on 18S rDNA sequence comparison. A. Johnson*, K. Luton and J. Ellis, Australia.
- 13.54-14.06 The phylogeny of *Neospora caninum* and *Toxoplasma gondii* based on ribosomal DNA sequences.
O.J.M. Holmdahl*, J.G. Mattsson, A. Uggla, J.P. Dubey and K.-E. Johansson, Sweden, U.S.A.
- 14.06-14.18 Heterogeneity in *Eimeria acervulina* of British and Bangladeshi origin. M.J. Karim* and A.J. Trees, Bangladesh, U.K.
- 14.18-14.30 *Babesia canis vogeli*: development in and transmission of the Australian strain by *Rhipicephalus sanguineus*.
G.W. Hutchinson*, R. Atkinson and A. Abdulwakil, Australia.
- 14.30-14.42 Natural parasitism of *Boophilus microplus*, biochemical markers, skin surface lipids, and cholesterol in serum on biotypes.
M.M. Cobenas*, P.T. Garcia and M.I. Urrutia, Argentina.
- 14.42-14.54 Odours produced by skin bacteria attract *Wohlfahrtia magnifica*.
G.M. El-Khoga*, K. Marialigeti and R. Farkas, Hungary.
- 14.54-15.06 Target oriented behaviour of *Wohlfahrtia magnifica* and *Lucilia sericata*, agents of myiasis, in Hungary.
M.J.R. Hall*, R. Farkas, F. Kelemen and J. Khoga, London, Hungary.
- ✕ 15.06-15.18 Survey for the presence of nematode-trapping fungi in fresh faeces of grazing livestock in Australia.
M. Larsen*, P.J. Waller and M. Faedo, Denmark, Australia.
- 13.30-13.42 Difference in susceptibility of Suffolk and Louisiana Native sheep to gastrointestinal nematode parasitism.
J.E. Miller*, T.A. Olson and S.R. Barras, U.S.A.
- ✕ 13.42-13.54 Breeding Perendale sheep with resistance or susceptibility to internal parasites following experimental infection.
T.G. Watson*, B.C. Hosking, A.P. Hurford and D.L. Johnson, New Zealand.
- 13.54-14.06 Host responses to helminth infections of sheep selected for enhanced resistance to haemonchosis.
H.S. Gill*, Australia.
- 14.06-14.18 Individual variation in the response to the abomasal nematode *Ostertagia circumcincta*.
M. Doligalska*, E. Sinski and M.J. Stear, Poland.
- ✕ 14.18-14.30 Artificial infections of calves with *Cooperia oncophora* to detect genetic resistance to gastrointestinal nematodes.
A. Kloosterman* and H.W. Ploeger, The Netherlands.
- ✕ 14.30-14.42 Faecal egg count, antibody titre and level of exposure to infection in calves infected with gastrointestinal nematodes.
H.W. Ploeger* and A. Kloosterman, The Netherlands.

15.30-16.00 TEA

16.00-18.00 WORKSHOPS

<i>Workshops</i>	<i>Chairman</i>	<i>Location</i>
Wildlife Situations	Dr. F. Gulland, UK	Boys Smith Room
Myiasis	Dr. C. Benitez-Usher, Argentina	Castlereagh Room
Sustainable Production Systems	Dr. P. Waller, Australia	Pythagoras School
*Parasite and Intermediate Host Population Monitoring	Dr J.B. Malone, USA	Durac Room
Parasitic Zoonoses New Issues	Prof. A. Thompson, Australia	Palmerston Room

*Sponsored by MSD AgVet

18.00 Special Session

(Palmerston Room)

'The Fenbendazole SR Bolus, a new formulation for the control of gastro-intestinal and lung nematodes in grazing cattle'

SPONSORED BY HOESCHT ANIMAL HEALTH

(Followed by Wine Reception)

Pharmaceutical development and quality control of the Panacur SR bolus.

D. Jordan, U.K.*

Pharmacological properties of the Panacur SR bolus, a device for sustained release of fenbendazole in the reticulorumen of cattle.

K. Schmid, Germany.*

The safety profile of fenbendazole and the Panacur SR bolus.

I. Stammberger and D. Mayer, Germany.*

Residues in tissues after administration of the Panacur SR bolus.

E.W. Scott, U.K.*

The Panacur SR bolus in the control of trichostrongyle and lungworm infections in grazing cattle.

C. Bauer, Germany.*

TUESDAY 10TH AUGUST

Morning 8.30 Palmerston Room

PLENARY PAPERS

Chairman: Prof. J. L. Duncan

8.30-9.15 *Epidemiology and Control of Parasites in Nomadic Situations
(Prof. C. MacPherson, West Indies)

9.15-10.00 *Parasite Control in Transhumance Situations
(Prof. J. Eckert, Switzerland)

10.00-10.30 Coffee

10.30-11.15 †Modelling of Parasite Populations - Gastro-intestinal Nematodes (Dr. B. Grenfell and G. Smith, UK)

11.15-12.00 †Modelling of Parasite Populations - Cestodes (Dr. M. Roberts, New Zealand)

*Sponsored by The Donkey Sanctuary

†Sponsored by MSD AgVet

12.30-13.30 LUNCH

SUBMITTED PAPERS

Session 7: Helminths - Antigens and Immunity

(Palmerston Room)

Chairman: Dr M. W. Lightowers

- 13.30-13.42 The secretory acetylcholinesterases of *Dictyocaulus viviparus*.
J.B. McKeand*, D.P. Knox, M.W. Kennedy and J.L. Duncan, U.K.
- 13.42-13.54 Immunisation of swine with ESP from *Trichuris suis* adult worms induces resistance to a challenge infection.
D.E. Hill*, R.H. Fetterer and J.F. Urban, Jr., U.S.A.
- 13.54-14.06 Evaluation of aspects of the protection afforded to sheep immunised with a gut membrane protein of *Haemonchus contortus*.
W.D. Smith* and S.K. Smith, U.K.
- 14.06-14.18 Cloning, sequencing and expression of H11, a highly protective membrane protein antigen from *Haemonchus contortus*.
E.A. Munn*, M. Graham, T.S. Smith, W.J. Coadwell, S.E. Newton, D.P. Knox, J.J. Oliver, D.S. Smith and F. Smith, U.K.
- 14.18-14.30 The influence of an inhibited L4 *Ostertagia ostertagi* population in calves on the development of immunity.
H. Hilderson*, J. Vercruysse and E. Claerebout, Belgium.
- 14.30-14.42 Development of resistance to *Haemonchus contortus* by Saanen goats.
B.C. Hosking and T.G. Watson*, New Zealand.
- 14.42-14.54 The use of flow cytometry to characterise the responsiveness of peritoneal leukocytes to *Fasciola hepatica*.
K.S. Ovington* and J.A. Smith, Australia.
- 14.54-15.06 Common antigenicity between *Dirofilaria immitis* and the three intestinal nematodes of the dog.
K. Konno* and M. Hayasaki, Japan.
- 15.06-15.18 Third stage larval surface antigens of *Strongylus vulgaris*.
M.S. Philpott*, C. Monahan and T.R. Klei, U.S.A.
- 15.18-15.30 Identification and purification of an *Ostertagia ostertagi* specific antigen from adult worms and its application in an enzyme linked immunosorbent assay.
D.C. de Graaf*, P. Berghen, H. Hilderson, J. Vercruysse and E. Claerebout, Belgium.

Session 8: Helminth Chemotherapy I

(Durac Room)

Chairman: Prof. J. Eckert

- 13.30-13.42 Assessment of the flukicidal action of rafoxanide against immature flukes by the use of antipyrine clearance tests and liver enzyme activity measurements.
H.A. Benchaoui* and Q.A. McKellar, U.K.
- 13.42-13.54 Additive action of two fasciolicides in Criollo sheep.
O.F. Ibarra*, C. Garcia, Y. Vera-Montenegro, J. Escudero and C. Vasquez, Mexico.
- 13.54-14.06 Oral efficacy of two novel pyrroles against mature and immature *Fasciola hepatica* in sheep.
M.E. Doscher*, L.R. Cruthers, R. Slone and P.W. Hayes, U.S.A.
- 14.06-14.18 Efficacy of Flukiver (Janssen) against *Fasciola hepatica* in naturally and experimentally infected sheep.
H.A. Ramisz*, A. Balicka-Laurans and G. Ramisz, Poland.
- 14.18-14.30 Protection of ponies from extended experimental infections of cyathostomes by Strongid-C and evidence for acquired resistance to the late L3-L4 mucosal larvae. T.R. Klei*, M.R. Chapman, D.D. French, M. Monahan and H.W. Taylor, U.S.A.
- 14.30-14.42 Efficacy of moxidectin injectable against lice and endoparasites in cattle.
G.T. Wang* and L. Smith, U.S.A.
- 14.42-14.54 Efficacy of moxidectin and ivermectin against naturally acquired nematode infections in cattle in Brazil.
A.C. Pinheiro and F.A.M. Echevarria*, Brazil.
- 14.54-15.06 Moxidectin: oral and injectable formulations against sheep nematodes in Brazil. F.A.M. Echevarria* and A.C. Pinheiro, Brazil.
- 15.06-15.18 Moxidectin: efficacy against gastrointestinal nematodes of sheep in Argentina. R.R. Ambrustolo, C.S. Eddi, J. Caracostantogolo*, G.M. Bulman, M.E. Morley, A. Noaco, J. Schapiro and P. Cadel, Argentina.
- 15.18-15.30 Moxidectin: an assay of efficacy with injectable and oral formulations against gastrointestinal nematodes and lungworm in sheep in Argentina. P.E. Steffan, G.M. Bulman and R. Ambrustolo, Argentina, U.S.A.

Session 9: Helminth Chemotherapy II

Chairman: Dr. R. J. Reinecke

(Pythagoras School)

- 13.30-13.42 Moxidectin: persistence of activity against cattle nematodes in Argentina. C.S. Eddi, J. Caracostantogolo, L. Marangunich, G.M. Bulman*, M.E. Morley and R.R. Ambrustolo, Argentina, U.S.A.
- 13.42-13.54 Anthelmintic persistency of moxidectin in sheep. K. Bairden*, J.L. Duncan and A.J. Mudd, U.K.
- 13.54-14.06 Chronolysis: practical implementation of the critical and persistent activity of closantel. O. Chiarisoli*, L. Maes, Ph. Dorchie and J.P. Alzieu, France.
- 14.06-14.18 Short-term feed management to increase anthelmintic activity in sheep. D.R. Hennessy* and D.N. Ali, Australia.
- 14.18-14.30 Comparative disposition kinetics of fenbendazole and oxfendazole in sheep: effects of their co-administration with methimazole. C.E. Lanusse*, L.H. Gascon and R.K. Prichard, Argentina.
- 14.30-14.42 On the mode of action of morantel: a single-channel study at nicotinic acetylcholine receptors in muscle membrane from *Ascaris suum*. R.J. Martin* and A.M. Evans, U.K.
- 14.42-14.54 Safety and efficacy of a new formulation of fenbendazole for pigeons. E.M. Abbott, U.K.
- 14.54-15.06 On the chemophrophylactic action of compound 81/470. V.M.L. Srivastava*, J.K. Srivastava, S.P. Singh and J.C. Katiyar, India.
- 15.06-15.18 Design of strategic anthelmintic control programmes in cattle using a mathematical model (PARABAN). W.G. Ryan*, G. Smith and J. Guerrero, U.S.A.
- 15.18-15.30 Chemoprophylaxis and immunity to nematodes in cattle. D.E. Jacobs* and M.A. Fisher, U.K.

Session 10: Epidemiology II

Chairman: Prof. C. N. L. McPherson

(Castlereagh Room)

- 13.30-13.42 Helminth parasitism in farmed red deer. R.M. Connan, U.K.
- 13.42-13.54 Epidemiology of gastrointestinal nematode infections in grazing beef cow-calf herds in Belgium. W. Hollanders*, H. Hilderson, E. Claerebout and J. Vercruysse, Belgium.
- 13.54-14.06 *Nematodirus* epizootiology in summer grazing beef cows and calves: field observations and model estimates. L. Polley* and B. Wagner, Canada.
- 14.06-14.18 Gastrointestinal nematodes in rural goats from the highveld of Zimbabwe. V.S. Pandey*, M. Ndao and V. Kumar, Belgium.
- 14.18-14.30 Epidemiology of helminth infections in traditionally managed Timahdit sheep in the Middle Atlas Mountains, Morocco. A. Dakkak*, K. Khallayoune, A. Ait Taleb, M. Tnibar and M. Sabir, Morocco.
- 14.30-14.42 Seasonal egg output of trichostrongylids in calves under different systems of management in Zimbabwe. D.Z. Moyo*, M.J. Obwolo, F.W.G. Hill, W.M.L. Hendriks and M. Eysker, Zimbabwe.
- 14.42-14.54 Epidemiology and control of helminth infections in pigs under intensive and non-intensive production systems. P. Nansen* and A. Roepstorff, Denmark.
- 14.54-15.06 High endemicity of cysticercosis and echinococcosis in China-Gansu. J. Ci-Peng*, China.
- 15.06-15.18 Echinococcosis in Bulgaria: epidemiology, control, present situation. T. Todorov*, A. Petkov, V. Boeva, N. Nedelchev and P. Zheliaskov, Bulgaria.
- 15.18-15.30 The prevalence of internal parasites in race horses in Japan: a necropsy survey. T. Yoshihara*, M.-A. Oikawa, Y. Katayama and M. Kaneko, Japan.

Session 11: Biology II

Chairman: Dr. B. Grenfell

(Boys Smith Room)

- 13.30-13.42 Dietary mineral supplementation influences plasma pepsinogen concentrations in sheep. *N.F. Suttle*, E. Ortolani, F. Jackson and R.L. Coop, U.K.*
- X 13.42-13.54 Effect of intestinal nematode infection on diet selection of growing sheep. *R.L. Coop*, I. Kyriazakis, D.H. Anderson, S.D.B. Cooper, J.D. Oldham and F. Jackson, U.K.*
- 13.54-14.06 Parasite establishment and pathogenesis of *Haemonchus placei* in immunized calves under different dietary protein. *S.M. Gennari*, A.L. Abdalla, D.M.S.S. Vitti, C.F. Meirelles, R.S. Lopes and M.C.R. Vieira Bressan, Brazil.*
- X 14.06-14.18 The course of experimental cyathostome infections in foals. *C. Bauer* and H.-J. Burger, Germany.*
- 14.18-14.30 Culture of equine strongylidae to the fourth larval stage in a cell free medium. *M.R. Chapman*, G.W. Hutchinson, M.J. Cenac and T.R. Klei, U.S.A.*
- 14.30-14.42 Spontaneous expulsion of worms from pigs massively infected with *Trichuris suis* in a farm using the fermented sawdust litter confinement pens. *N. Taira*, S. Ura and S. Shinkooi, Japan.*
- (X) 14.42-14.54 Goat *Teladorsagia circumcincta* genetic diversity in 14 dairy-goat farms. *J. Cabaret* and N. Gasnier, France.*
- 14.54-15.06 The ever changing status of hydatid disease: an Australian perspective. *R.C.A. Thompson* and D.J. Jenkins, Australia.*
- 15.06-15.18 Inactivation of *Ascaris suum* eggs during storage in lime treated sewage sludge. *L. Eriksen*, P. Andreasen and B. Ilsoe, Denmark.*

Session 12: Diagnosis I

Chairman: Dr. M. G. Roberts

(Music Room)

- 13.30-13.42 A sandwich ELISA technique for the estimation of the antibody response in *Psoroptes ovis* experimentally infested cattle. *J.F. Lonneux*, W. Hollanders and B.J. Losson, Belgium.*
- 13.42-13.54 Application of antigen ELISA in assessment of cure in African trypanosomiasis. *J.M. Ndung'u*, W. Olahu-Makuni, R.M. Ngure and A.R. Nyanyuma, Kenya.*
- 13.54-14.06 IgG and IgM ELISAs for monitoring *Toxoplasma* infection in swine. *P. Lind*, S.A. Henriksen, V. Bille-Hansen, A.L. Schirmer, F. Bager, J. Haugegaard, V. Sorensen, Denmark.*
- 14.06-14.18 Recombinant antigen ELISAs for epidemiological studies on *Toxoplasma gondii* infections in cats. *A.M. Tenter*, M. Rommel and A.M. Johnson, Germany, Australia.*
- 14.18-14.30 Antibody responses against Em18 and Em16 in humans, wild voles and mice infected with *Echinococcus multilocularis*. *A. Ito*, M. Nakao, M. Ito, T. Matsuzaki, M. Kamiya and H. Kutsumi, Japan.*
- 14.30-14.42 Development of an ELISA and western blot for the diagnosis of *Babesia caballi* infections in horses and comparison with immunofluorescence antibody test (IFAT) and complement fixation test (CFT). *B. Peymann*, R. Bose and K.T. Friedhoff, Germany.*
- 14.42-14.54 Diagnosis of taeniasis-cysticercosis by western blot. *O. Hernandez-Montes*, A.A. Hurtado and A. Monroy-Ostria, Mexico.*
- 14.54-15.06 The use of PCR and DNA probe in the epidemiological surveys of natural trypanosome infections. *J.H.P. Nyeko*, L.H. Otieno, O.K. ole Moi Yoi and P.A.O. Majiwa, Uganda.*
- 15.06-15.18 DNA probes for the detection of *Fasciola hepatica* and *Dicrocoelium dentriticum* in intermediate hosts. *J. Kaufmann*, V. Heussler and D. Dobbeleare, Switzerland.*
- (X) 15.18-15.30 Genus specific DNA probes for the identification of strongyle eggs from cattle faeces. *C.M. Christensen*, L.C. Gasbarre and D.S. Zarlenga, U.S.A.*

15.30-16.00 TEA

16.00-18.00 WORKSHOPS

<i>Workshops</i>	<i>Chairman</i>	<i>Location</i>
X Pathophysiology of Gastro-intestinal Parasites	Dr. H. Hertzberg, Switzerland	Palmerston Room
Hydatid- New Approaches	Dr. M.A. Gemmell, UK	Dirac Room
Antiparasitic Testing Guidelines	Dr. V. Theodorides, USA	Pythagoras School
Donkey Parasitology	Dr E. Svendsen, UK	Music Room
Strategies and Economics of Parasite Control in Africa	Prof. K. Pfister, Switzerland	Castlereagh Room
Cyclical and Non-Cyclical Trypanosomes	Dr. L. Touratier, France (and Prof. G. Urquhart, UK)	Boys Smith Room

18.00 Special Session

(Palmerston Room)

'New Developments in the Control of Parasites of Domestic Animals'

SPONSORED BY PFIZER ANIMAL HEALTH GROUP (Followed by Wine Reception)

Discovery and pre-clinical biology of the novel endectocide, doramectin. N.A. Evans*, U.K.

The efficacy of doramectin in the therapy and prevention of nematode infections of cattle. A.J. Weatherley*, N.B. Logan and R.M. Jones, U.K.

The spectrum of activity of doramectin against nematode and arthropod parasites of swine. J.J. Arends*, D.J. Moncol and N.B. Logan, U.S.A.

The efficacy of doramectin against temperate climate arthropods of cattle. J.E. Lloyd, R. Kumar, J.W. Waggoner* and F.E. Phillips, U.S.A.

Efficacy of doramectin against *Boophilus microplus* in cattle. J.C. Gonzales*, R.A. Muniz, A. Farias, L.C.B. Goncalves and R.S. Rew, U.K.

The activity of doramectin against *Cochliomyia hominivorax* in cattle. R.A. Muniz*, G.E. Moya-Borja, C.M.B. Oliveira and L.C.B. Goncalves, U.S.A.

WEDNESDAY 11TH AUGUST

Morning 8.30 Palmerston Room

PLENARY PAPERS

Chairman: Dr. J. Tharaldsen

X 8.30-9.15 Genetic Resistance to Parasitic Disease. Particularly of Resistance in Ruminants to Gastro Intestinal Nematodes (Dr. M.J. Stear and Prof. M. Murray, UK)

X 9.15-10.00 *Vaccine Development (Dr M. Lightowlers, Australia)

*Sponsored by Intervet

10.00-10.30 COFFEE

10.30-11.15 *Vector Biology and Control (Dr G. Lopez, Columbia)
*Sponsored by Bayer

11.15-12.00 *Vaccines Against Blood Sucking Arthropods (Dr. J. Opdebeeck, Australia)
*Sponsored by Intervet

11.30

12.00-13.00 Special Session: Presentations by Pfizer Awardees for Teaching and Research

13.00 LUNCH

Afternoon

Free for selection of social events (see General Information)

13.00-16.30 Open session on 'Coccidiosis' organised by the COST 89 group (Palmerston Room)

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|-------------|--|-------------|---|
| 13.00-13.15 | <i>Eimeria alabamensis</i> coccidiosis in young grazing cattle in Sweden.
<i>C. Svensson*</i> , Sweden. | 15.00-15.15 | Coding of gene fragments encoding antigenic polypeptides of <i>Sarcocystis tenella</i> . <i>C. Mertens*</i> , <i>A.M. Tenter</i> , <i>M. Rommel</i> , <i>J. Ellis</i> and <i>A.M. Johnson</i> , Germany, Australia. |
| 13.15-13.30 | Infection risks in bovine eimeriosis.
<i>H.-J. Burger</i> , Germany. | 15.15-15.30 | TEA |
| 13.30-13.45 | Absorption of vitamin A in <i>Eimeria separata</i> infected rats.
<i>E.M. Whang*</i> and <i>H.-J. Burger</i> , Germany. | 15.30-15.45 | How does in vitro excystation affect membranes of <i>Sarcocystis</i> sporozoite? A TEM study.
<i>K. Horn*</i> , <i>K. Ono</i> , <i>H. Mehlhorn</i> and <i>A.O. Heydorn</i> , Germany. |
| 13.45-14.00 | <i>Isospora suis</i> infections in piglets: oocyst shedding following low level dosing.
<i>S.A. Henriksen*</i> and <i>J.P.B. Christensen</i> , Denmark. | 15.45-16.00 | Assessment of antibodies to recombinant glutathione S-transferase in sera infected with cyst-forming coccidia.
<i>C. Vietmeyer*</i> and <i>A.M. Tenter</i> , Germany. |
| 14.00-14.15 | Characterization and localization of rhoptry antigens in <i>Eimeria</i> . <i>G. Zerzenski*</i> , <i>B. Rick</i> , <i>J.-F. Dubremetz</i> , <i>W. Raether</i> , <i>J. Hofmann</i> and <i>R. Entzeroth</i> , Germany, France. | 16.00-16.15 | <i>Eimeria maxima</i> : ELISA and western blot analyses of protective sera.
<i>N. Smith*</i> , <i>M. Wallach</i> , <i>C. Miller</i> , <i>J. Eckert</i> and <i>M.E. Rose</i> , Switzerland, U.K. |
| 14.15-14.30 | Amplification and characterization of <i>Eimeria bovis</i> DNA fragments. <i>C. Homrighausen-Riester*</i> , <i>B. Barbisch</i> , <i>H.-J. Burger</i> and <i>H. Zahner</i> , Germany. | 16.15-16.30 | Serological response against poultry coccidia, evaluated by ELISA and IFAT. <i>M. Provaznikova*</i> , <i>A. Firmanova</i> and <i>P. Bedrník</i> , Czech Republic. |
| 14.30-14.45 | <i>Sarcocystis muris</i> : cloning of a cDNA encoding a repetitive arginine rich region of a putative microneme antigen.
<i>S. Lechner*</i> , <i>K.-H. Eschenbacher</i> , <i>R. Entzeroth</i> , <i>H. Mehlhorn</i> and <i>W. Ruger</i> , Germany. | 16.30-16.45 | Intraepithelial lymphocytes of the small intestine in lambs infected with <i>Eimeria</i> spp. <i>M. Aleksandersen*</i> , <i>T. Landsverk</i> , <i>B. Gjerd</i> and <i>O. Helle</i> , Norway. |
| 14.45-15.00 | Molecular characterization of a dense granule antigen (32 kDa) from <i>Sarcocystis muris</i> cystozoites.
<i>B. Freyer*</i> , <i>H. Klein</i> , <i>J. Strobel</i> , <i>K.-H. Eschenbacher</i> , <i>R. Entzeroth</i> , <i>H. Mehlhorn</i> and <i>W. Ruger</i> , Germany. | 16.45-17.00 | The effect of chosen immunostimulators on the immunity of chickens vaccinated with coccidia.
<i>M. Mazurkiewicz</i> , <i>A. Gawel</i> , <i>J.A. Madej</i> and <i>J. Kuryszko</i> , Poland. |

THURSDAY 12TH AUGUST

Morning 8.30 (Palmerston Room)

PLENARY PAPERS

Chairman: Dr. V. J. Theodorides

8.30-9.15 *Chemotherapy and Delivery Systems: Haemoparasites
(Dr. A.F. Peregrine, Kenya)

9.15-10.00 *Chemotherapy and Delivery Systems: Helminths
(Prof. Q.A. McKellar, UK)

*Sponsored by Hoechst Animal Health

10.00-10.30 COFFEE

Chairman: Prof. Th. Hlepe

10.30-11.15 Anthelmintic Resistance
(Prof. R. Prichard, Canada)

Sponsored by The Horserace Betting Levy Board

11.15-12.00 Teaching Veterinary Parasitology
(Prof. A. Verster, South Africa)

SUBMITTED PAPERS

Session 13: Protozoa - Antigens and Immunity I

(Palmerston Room)

Chairman: Dr. S. Lloyd

12.00-12.12 Restorative effects of a newly synthesized peptide
(obiopeptide-1) in cyclophosphamide-pretreated mice infected
with opportunistic bacteria. Y. Fujii*, I. Igarashi, Y. Omata,
K. Ono, A. Saito and N. Suzuki, Japan.

12.12-12.24 The effect of immunosuppression caused by cyclophosphamide
to mice chronically infected with *Toxoplasma gondii*. I. Igarashi*,
Y. Fujita, Y. Omata, A. Saito and N. Suzuki, Japan.

12.24-12.36 Immunisation of cats with tissue cysts, bradyzoites and
tachyzoites of the T-263 strain of *Toxoplasma gondii*.
I. Popiel*, A. Freyre, J. Fishback and L. Choromanski, U.S.A.

12.36-12.48 Characterisation of diagnostic antigens for *Babesia caballi*.
R. Bose*, Germany.

12.48-13.00 Analysis of *Babesia equi* antigens by two dimensional
electrophoresis and western blotting.
B. Hentrich*, R. Bose and K.T. Friedhoff, Germany.

Session 14: Anthelmintics - Drug Resistance II

(Durac Room)

Chairman: Prof. A. Verster

12.00-12.12 A field survey on anthelmintic resistance in small strongyles of
the horse.
C.F. Ihler*, Norway.

X 12.12-12.24 Synergistic activity of anthelmintics for the control of
susceptible and resistant strains of *Fasciola hepatica* for the
prevention or management of anthelmintic resistance.
J.C. Boray*, Australia.

X 12.24-12.36 Anthelmintic drug resistance in goats in peninsular Malaysia.
P. Dorny*, J. Vercruysse, E. Claerebout, R. Sani and A. Jalila,
Belgium.

12.36-12.48 Use of anthelmintics by swine farmers in Denmark.
A. Dangolla*, H. Bjorn, P. Nansen and P. Willeberg,
Denmark.

Session 15: Anthelmintics - Control I

Chairman: Prof. Q. A. McKellar

(Castlereagh Room)

- X 12.00-12.12 The effects of different methods of anthelmintic suppression in calves on parasitological parameters. S.M. Taylor*, T.R. Mallon, J. Kenny, H. Edgar and A. Kelly, U.K.
- 12.12-12.24 The strategic control of fascioliasis using triclabendazole - a four year study. S.L. Parr* and J.S. Gray, Ireland.
- 12.24-12.36 The dose and move system for the control of cattle lungworm. M. Eysker*, J.H. Boersema and F.N.J. Kooyman, The Netherlands.
- 12.36-12.48 Strategic control of strongyles in pony foals in Ontario. O. Slocombe* and M. Lake, Canada.
- 12.48-13.00 The benefits of an anthelmintic control programme based on the use of strategic anthelmintic treatments and using the epidemiological data for traditionally managed sheep in the Middle Atlas Mountains, Morocco. A. Dakkak*, K. Hidane and A. Tabarani, Morocco.

Session 16: Pathology I

Chairman: Dr. A. D. Donald

(Pythagoras School)

- 12.00-12.12 Experimental *Ascaris suum* infections in calves. F. Borgsteede*, W.A. de Leeuw, W.P.J. van der Burg and J.B.W.J. Cornelissen, The Netherlands.
- 12.12-12.24 Allergic parasitic abomasitis in Zebu cross cattle in Argentina. M. Mejia*, Argentina.
- 12.24-12.36 Sudden cardiac death of lambs following experimental infection with *Strongyloides papillosus*. Y. Nakamura*, N. Tsuji, N. Taira and H. Hirose, Japan.
- 12.36-12.48 Effect of gastrointestinal nematode infections on serum alkaline phosphatase in sheep. S.M. Thamsborg* and R.J. Jorgensen, Denmark.
- 12.48-13.00 Body composition, water and nitrogen balance in calves infected with *Cooperia punctata*. M.C.R.V. Bressan*, S.M. Gennari, A.L. Abdalla and J.P. Santos Filho, Brazil.

Session 17: Arthropod Control II

Chairman: Dr R. M. Connan

(Boys Smith Room)

- 12.00-12.12 Efficacy of ivermectin against cattle myiasis caused by *Cochliomyia hominivorax*. C. Benitez-Usher*, J. Cruz, L. Carvalho, A. Bridi, J. Eagleson, D. Farrington and R.A. Barrick, Argentina.
- 12.12-12.24 *Dermatobia hominis* control by flumethrin and cyfluthrin pour-on. A. Romano*, J.G. Alberdi and J.R. Keller, Argentina.
- 12.24-12.36 Susceptibility to insecticides of hornfly, *Haematobia irritans*, recently introduced to livestock regions of Brazil. F.B. Scott*, L. Grisi and K. Coumendouros, Brazil.
- 12.36-12.48 Moxidectin: efficacy and dose titration in cattle experimentally infected with *Boophilus microplus* (Can.) in Argentina. J. Caracostantogolo*, C.S. Eddi, G.M. Bulman, M.E. Morley, A. Noaco, R.R. Ambrustolo, L. Marangunich, and J. Schapiro. Argentina, U.S.A.
- 12.48-13.00 Resistance of ectoparasites of cattle to conventional insecticides. L. Grisi*, F.B. Scott, W.M. Passos and K. Coumendouros, Brazil.

Session 18: Diagnosis II

Chairman: Prof. T. R. Klei

(Music Room)

- 12.00-12.12 Applying regression of p.c.v. on e.p.g. for suggesting an effective anthelmintic treatment for ovine helminthosis. G. Asegede*, H.-J. Buerger and J. Steinbach, Ethiopia, Germany.
- X 12.12-12.24 Epidemiological risk factors associated with clinical cyathostomiasis in the horse. S.W.J. Reid*, T.S. Mair, M.H. Hillyer and S. Love, U.K.
- 12.24-12.36 Natural *Ascaris suum* infections in swine measured by coprological and serological methods. A. Roepstorff*, Denmark.
- 12.36-12.48 Evaluation of an ELISA and a histamine release test system for detection of pigs naturally infected with *Ascaris suum*. H.O. Bogh*, L. Eriksen, L.G. Lawson, P. Nansen and P. Lind, Denmark.
- 13.00-14.00 LUNCH

SUBMITTED PAPERS

Session 19: Protozoa - Antigens and Immunity II

(Palmerston Room)

Chairman: Dr. S. Lloyd

- 14.00-14.12 Identification of *Babesia bigemina* infected erythrocyte surface antigens containing epitopes conserved among strains. S. Shompole*, T.F. McLelwain, D.P. Jasmer, S.A. Hines, J. Katende, A.J. Musoke, F.R. Rurangirwa and T.C. McGuire, Kenya.
- 14.12-14.24 Field immunisation of cattle in Ireland against bovine babesiosis with a gerbil-derived live vaccine. J.S. Gray*, B. Kaye, P. Talty and C. McSweeney, Ireland.
- 14.24-14.36 Tropical theileriosis in Morocco - studies on an attenuated cell culture vaccine. S. Williamson*, H. Oubhelli, M. Kachani, R. Melrose and R. Spooner, U.K.

Session 20: Anthelmintics - Control II

(Durac Room)

Chairman: Dr. M. A. Gemmell

- 14.00-14.12 Developmental safety of albendazole in cattle. V.J. Theodorides*, M.C. Carakostas, J.J. Colaianne, J.F. Freeman and S.W. Page, U.S.A.
- 14.12-14.24 Effects of morantel sustained release bolus against gastrointestinal nematodes in field grazing calves in Kiambu district, Kenya. W.K. Munyua*, Kenya.
- 14.24-14.36 Suppression of induced infections of *Dirofilaria immitis* by monthly treatment with ivermectin (6mcg/kg) beginning at four months post infection. J.W. McCall*, T.L. McTier, N. Supakorndej and R.P. Ricketts, U.S.A.
- X 14.36-14.48 World clinical development of melarsomine dihydrochloride for adulticide treatment of canine heartworm. R. Harding*, D.M. Keister and J.M. Postal, France.
- 14.48-15.00 Residual nematocidal effectiveness of ivermectin in cattle. T.A. Yazwinski*, C. Tucker, H. Featherston, Z. Johnson, U.S.A.

Session 21: Anthelmintics in Productivity

(Castlereagh Room)

Chairman: Dr. J. C. Boray

- 14.00-14.12 Impact of a strategic parasite control programme with ivermectin on weight gain and associated reproductive performance of Angus heifers in Argentina.
C.M. Entrocasso, P.E. Steffan and A. Almada, Argentina.*
- 14.12-14.24 Study of productivity in weaner steers treated with abamectin in the northern humid Pampa of Argentina.
M.E.M. Cobenas, C. Eddi, J. Caracostantogolo, J. Nolasco, S. Gross, J. Guerrero and A. Mascotena, Argentina.*
- 14.24-14.36 Moxidectin: efficacy in a cattle productivity trial under range conditions in the humid Pampas in Argentina.
G.M. Bulman, C.S. Eddi, J. Caracostantogolo, C.G. Ledesma, J. Shapiro, P. Cadel and G. Balbiani, Argentina.*
- 14.36-14.48 Study of productivity in weaner steers treated with abamectin in the province of Cordoba, Argentina.
J. Caracostantogolo, M.E.M. Cobenas, C. Eddi, J. Nolasco, S. Gross, J. Guerrero and A. Mascotena, Argentina.*
- 14.48-15.00 Study of the epidemiology and control of ostertagiasis in the northern humid Pampa of Argentina.
C. Eddi, M.E.M. Cobenas, J. Caracostantogolo, J. Nolasco, S. Gross, J. Guerrero and A. Mascotena, Argentina.*

Session 22: Pathology II

(Pythagoras School)

Chairman: Prof. S. M. Gaafer

- 14.00-14.12 *Babesia microti* and *Trypanosoma musculi* infections in mice concomitantly infected with *Trichinella spiralis*,
S.W. Chiejina and D. Wakelin, Nigeria.*
- 14.12-14.24 Effect of psoroptic mange in housed sheep in Patagonia, Argentina.
F.V. Olaechea, L. Duga and H. Taddeo, Argentina.*
- 14.24-14.36 Clinical picture and serum antibody response in experimental *Sarcoptes scabiei* var. *vulpes* infection in the red fox (*Vulpes vulpes*).
S. Bornstein, P. Thebo and G. Zakrisson, Sweden.*
- 14.36-14.48 Demodicosis in cattle.
H.-F. Matthes, Germany. VIDEO
- 15.00-15.30 TEA

Session 23 Pythagoras Hutton Hemel Hempstead

1530-1730
~~16.00-18.00~~ WORKSHOPS

Workshops

Vaccine Development

Genetics in Parasitology

Anthelmintic Resistance

Vaccination against Coccidiosis

Teaching Veterinary Parasitology

Chairman

Prof. H. Zahner, Germany

Prof. C. Dobson, Australia

Dr H. Bjørn, Denmark

Prof. J. Eckert, Switzerland

Prof. K. Friedhoff, Germany

Location

Pythagoras School

Durac Room

Palmerston Room

Castlereagh Room

Boys Smith Room

17.30 General Meeting of WAAVP

19.15-19.30 Buses depart for Banquet

20.30 Banquet

FRIDAY 13TH AUGUST

Morning 9.00-12.00

9.00 Possible continuation of Workshops (delegates to be notified)

12.00 Closing session (Palmerston Room)

LUNCH

DEPART



ABSTRACTS OF PLENARY PAPERS





PARASITOLOGY IN THE UK AND ELSEWHERE OVER 30 YEARS OF THE WORLD ASSOCIATION FOR THE ADVANCEMENT OF VETERINARY PARASITOLOGY

LORD SOULSBY

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There have been many important developments in Veterinary Parasitology over the three decades since the WAAVP was established in 1963 in Hannover, Germany. They have revolutionised control measures but have identified new problems and served to remind us of the very plastic nature of the phenomenon of parasitism.

Major contributions in the understanding of the biology of parasites include the phenomenon of hypobiosis, the post parturient increases in parasitism and environmental factors in survival of helminths. Pathophysiological responses include the gastrointestinal enteropathies, malabsorption, haemodyscrasias and inhibition of growth and development. Immunological studies have advanced remarkably both in the basic understanding of immune humoral and cellular mechanisms and culminating in genetically engineered vaccines for larval cestodes, gastro-intestinal nematodes and protozoa. Vaccines for blood sucking arthropods, especially ticks, are well under development. However, the responsiveness of young animals still provides a challenge for the would-be vaccinator, though the analyses of the genetic aspects of resistance offer promise for future control programmes.

Chemotherapy, signified by Thiabendazole in 1963 has moved through many generic compounds all with high activity against a spectrum of larval and adult stages of helminth and arthropod parasites. New methods of administration include 'pour-ons', boluses, time-release capsules, etc.

Parasitic resistance to all classes of parasitocidal compounds poses an ever present problem. Though drug resistance is increasingly understood at the molecular level, new approaches to chemotherapeutic control based on a better understanding of parasite biology are required.

Environmental issues increasingly come to the forefront of control programmes and necessitate conscientious study.



PARASITES, ANIMAL PRODUCTION AND SUSTAINABLE DEVELOPMENT

A. D. DONALD

CSIRO Institute of Animal Production and Processing, PO Box 93, North Ryde, NSW 2113, Australia

Ecologically sustainable development is aimed at reducing environmental degradation while enabling economic development with equity between the developed and developing worlds and between generations. Parasite control in livestock can both contribute to, and take advantage of, sustainable agriculture. This will tend towards less-intensive, lower input, diversified crop and animal production with less risk of parasite-induced losses and greater opportunities for integrated control including the exploitation of grazing management. Chemotherapy will continue to play a part but the most serious problem is resistance in the target species. Except for a few isolated issues, currently used parasiticides are relatively minor contaminants of the food supply or the environment. Nevertheless, the compounds of the future will need to be narrow-spectrum, non-persistent and rapidly degraded, with convenience in the hands of the user reduced in importance. Environmentally friendly alternatives to chemotherapy including genetic resistance of hosts, vaccines and biological control, show considerable promise and must be pursued. Sustainable systems pose optimisation problems and more attention must be given to systems research, models and products to aid decisions. If governments are serious about sustainable development, greater support will be needed for longer-term patient, multi-disciplinary research.



PARASITIC CONTROL IN INTENSIVE VS NON-INTENSIVE SYSTEMS - RUMINANTS

R. K. REINECKE

Embrapa Saúde Animal, CxP 74523, Seropédica R.J. 23851-970, Brasil

Control of parasites in sheep grazing intensively (26-36 sheep ha⁻¹) was only possible with frequent use of anthelmintics. All animals had massive mixed infections of *Trichostrongylus*, *Haemonchus* and *Teladorsagia* - fatal for suckling lambs. The worm burdens of ewes dosed with albendazole slow release capsules SRC were compared with those of controls (four standard drenches) and/or another flock receiving weekly therapeutic doses of ABZ. SRC controlled *Trichostrongylus* for 9ld, and *Haemonchus* and *Teladorsagia* for 6ld. Sheep dosed with SRC at 47-6ld intervals for seven months significantly reduced pasture infestation ($P < 0.05$) when compared with controls, but significantly increased infestation ($P < 0.05$) occurred when ABZ was dosed once a week.

In the non-intensive system (12 sheep ha⁻¹) parasite epidemiology in lambs and hoggets showed: development of immunity to *Nematodirus* at 12 weeks; spontaneous cure of *Teladorsagia* in hoggets grazing safe wheat stubble; survival of *Trichostrongylus*. All sheep were drenched with effective broad spectrum anthelmintics in November, May and August and then moved to safe pastures. Lambs and hoggets thrived.

In both systems mature larvae of *Oestrus ovis* formed pupae in winter but imagines failed to develop.



EPIDEMIOLOGY AND CONTROL OF HELMINTH INFECTIONS IN PIGS UNDER INTENSIVE AND NON- INTENSIVE PRODUCTION SYSTEMS

P. NANSEN AND A. ROEPSTORFF

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Swine production systems are characterized by a high diversity with regard to management type and level of intensity, and the number of helminth species as well as infection levels are strongly influenced by the different systems. The present paper, focussing on the situation in Northern Europe with examples from Denmark, describes a decrease in the number of helminth species and in infection levels as result of a shift from non-intensive to highly intensive production systems. Differences in basic biological requirements of the pre-infective larvae, and in transmission characteristics and immunogenicity of the various helminth species explain why some species are more vulnerable to managerial changes than others. Finally, control measures relevant for the different production systems are discussed. Despite the fact that it is well documented that prevention of transmission may be obtained by proper hygiene and management, use of anthelmintics is still the single most important action taken by pig farmers to control worm infections. It is emphasized that anthelmintics should not be used uncritically, but should be integrated with management practice and production system in order to achieve optimal effect and to avoid development of anthelmintic resistance.



EPIDEMIOLOGY AND CONTROL OF PARASITES IN NOMADIC SITUATIONS

C. N. L. MACPHERSON

*School of Veterinary Medicine, Faculty of Medical Sciences, University of the West Indies, St. Augustine,
Trinidad, West Indies*

Nomadism is a specialised mode of life which enables humans and their domestic animals to occupy vast marginal tracts of land especially in Africa, The Middle East and Asia. Nomads are characterised by small, scattered populations which reduces parasite transmission infection pressure. Migrations where people and animals leave environments contaminated with faeces reduces the opportunity for transmission of gastrointestinal parasites, particularly nematodes that require a period of development in the environment before becoming infective. Migrations, however, bring nomads into contact with geographically restricted diseases. Diet appears to both expose and prevent parasitic infection. Rapid transmission of infective stages must occur in the hot, dry environments occupied by most nomads for successful transmission; this may be facilitated by human behaviour. The absence of sanitation, piped water, abattoirs and the close association of people and animals (domestic and wild) increases parasite transmission opportunities. Periodic prolonged droughts and sedenterisation play an important role in the maintenance and proliferation of some parasitic infections. The requirements for appropriate policy, institutions and control of parasites in nomadic communities will be presented.



PARASITE CONTROL IN TRANSHUMANT SITUATIONS

J. ECKERT AND H. HERTZBERG

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Transhumance is defined as "seasonal moving of livestock to regions of different climate" and is an integral part of livestock production in many parts of the world. Its impact to parasite populations of livestock and on parasite control is described, mainly using examples from Europe.

The epidemiology of trichostrongylidosis of cattle, mainly caused by *Ostertagia ostertagi* and *Cooperia oncophora*, is influenced by longer survival of overwintered infective larvae on mountainous pastures as compared to midlands and by grazing management. In order to protect cattle on mountainous pastures during the second half of the grazing season until autumn a late application (June/July) of intraruminal drug-release devices may be necessary. Community pastures used in a transhumant system with mixed grazing of young cattle originating from various farms may enhance transmission of dictyocaulosis. Therefore, specific prophylactic measures are required. In lowland and hill sheep nematode populations may differ in that *Haemonchus contortus* generally plays a minor role in hill sheep in which *Ostertagia circumcincta* and *Nematodirus* spp. predominate. Infections with *Fasciola hepatica* and *Dicrocoelium dendriticum* can be acquired on mountainous pastures by cattle, sheep and other livestock grazing in a transhumant system as intermediate hosts of these parasites may find suitable habitats in these regions. There is evidence that in the prealpine and alpine area both parasites are mainly transmitted in two-season cycles.

Further examples for the impact of transhumance on parasite-host interrelationships include cysticercosis in cattle, echinococcosis, scabies in sheep, tick-borne fever of cattle, and hypodermosis in cattle.



MODELLING OF PARASITE POPULATIONS: NEMATODES

B. T. GRENFELL AND G. SMITH

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Since the 1960s, roughly a dozen models have been produced to describe the epidemiology of directly-transmitted gastrointestinal nematode parasites in agriculturally-important animal host populations. We begin with a brief review of these formulations, in particular contrasting detailed simulations of specific systems with simpler more generic models, which allow the possibility of analytical treatment. We then focus in more detail on the generic models, reviewing how they can be used to derive general epidemiological insights, particularly how the interaction of seasonality, management and host immunity combine to determine observed epidemiological patterns. We then go on to discuss the limitations and future prospects of the analytical modelling approach. There is also considerable current interest in the epidemiology of nematode parasites in wildlife host populations. We therefore conclude with a discussion of current issues in this area and briefly present some new results on the dynamic impact of seasonality on wild host-parasite interactions.



MODELLING OF PARASITIC POPULATIONS-CESTODES

M. G. ROBERTS

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The philosophy of mathematical modelling as it applies to the epidemiology of cestode populations is reviewed. A model provides, via the "threshold theorem", a criterion for deciding in advance if a control programme can succeed in eradicating the parasite. In order to use this criterion it is necessary to have an estimate of the basic reproduction ratio, R_0 , which can only be obtained if reliable epidemiological data are available before the control programme is started. A model has been used to describe the population dynamics of *Echinococcus granulosus*, *Taenia hydatigena* and *T. ovis* in sheep and dogs in New Zealand. For these parasites data from a forty-year longitudinal study, as well as short term field and laboratory studies, were available. A model has also been used to evaluate a proposed control programme directed against *E. multilocularis* in foxes and voles in France. Here the type and extent of control intervention is predetermined by the existing rabies control programme. These two examples, which demonstrate the different techniques required to model cestodes in domestic and wild-animal populations, are reviewed, and the use of a model as the basis for a benefit/cost analysis of control options is discussed. These techniques could, in principal, be used to design control programmes for *T. saginata* or *T. solium* in humans.



GENETIC RESISTANCE TO PARASITIC DISEASE: PARTICULARLY OF RESISTANCE IN RUMINANTS TO GASTROINTESTINAL NEMATODES

M. J. STEAR AND M MURRAY

*Department of Veterinary Medicine, Glasgow University Veterinary School, Bearsden Road,
Glasgow, Scotland*

There is substantial variation among individuals in susceptibility to a wide variety of parasitic diseases and part of this variation in susceptibility is due to genetic factors. The challenge now is to determine the best methods of using the variation to improve our understanding of parasitic infection and to reduce the ravages of parasitic disease. The scientific and commercial applications will depend upon the type of genetic variation. Variation among breeds can be easily exploited by a policy of breed substitution. Variation within a breed can be exploited by selective breeding to improve resistance to infection or to disease. But more work is needed to develop selection indices which are acceptable to livestock breeders. Identifying genes which contribute to the variation in resistance provides a better understanding of the mechanisms of resistance but more work is needed to determine if such genes, alone or in combination, account for a sufficient proportion of the variation in resistance to allow marker assisted selection. A comparison of responses in susceptible and resistant stock provides a powerful tool to distinguish among protective, irrelevant and pathological responses. These themes have been illustrated by three studies of gastrointestinal nematode infections in ruminants.



ANTI-PARASITE VACCINES: THE COMING OF AGE

M. W. LIGHTOWLERS

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A decade of molecular parasitology is beginning to bear fruit, with the appearance of several highly effective, practical vaccines against parasitic diseases. Recombinant antigen vaccines have been developed against cestode, nematode, trematode, protozoan and arthropod parasites. Greatest progress has been made with veterinary vaccines, where the ability to trial numerous vaccine formulations in challenge trials has allowed more rapid identification of host-protective antigens than is possible with many medically important parasites. Several quite different approaches to vaccine development have been successful. The traditional approach using live, attenuated parasites continues to provide effective vaccines against several protozoal and nematode parasites. Recombinant DNA technology, monoclonal antibody technology, protein chemistry and immunochemistry have played critical roles in the outstanding success which has been achieved over the last five years in the development of defined antigen vaccines. Two approaches have been successful in research towards defined antigen vaccines against parasites: 1. The 'natural antigen' approach where immune responses are stimulated to parasite molecules which are normally antigenic, and possibly host-protective, in infected hosts. 2. The 'naive antigen' approach where parasite molecules which are not antigenic, or of very low antigenicity, in infected hosts are used to raise immune responses capable of killing the parasite. This review examines the successful approaches taken towards the development of effective anti-parasite vaccines and the vaccines which have been produced to date. Also discussed are the potential limitations of defined antigen vaccines and approaches to minimising these limitations, prospects for the development of additional anti-parasite vaccines in the near future and new methods for delivery of defined anti-parasite vaccines.



VECTOR BIOLOGY AND CONTROL

G. LOPEZ

Columbia

No abstract has been received at the time of going to press.



VACCINES AGAINST BLOOD SUCKING ARTHROPODS

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Selected personal insights will be provided on the development of vaccines against blood sucking arthropods, with particular emphasis on vaccines against ticks. The emergence of novel or concealed antigens of haematophagous ectoparasites as candidate vaccine antigens will be reviewed and the effect of feeding by the parasite on the expression of protective antigens considered. The distribution of protective antigens through lifecycle stages, the stage of the lifecycle targeted by protective responses and the nature of these responses will be commented on briefly. Concealed antigens of the gut, including the peritrophic membrane, and other internal organs will be commented on for the role they play to induce immunity artificially and some of the work carried out to purify and characterise protective antigens of tick guts will be described. A commentary will be developed on vaccines that combine both 'concealed' and 'exposed' antigens. Some of the problems associated with the infestation and challenge of vaccinated hosts in the field will be identified and the delivery of parasite antigens as vaccines that are both protective and 'user-friendly' will be emphasized as a major problem which remains and that should be solved.



CHEMOTHERAPY AND DELIVERY SYSTEMS: HAEMOPARASITES

A. S. PEREGRINE

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Chemotherapy of haemoparasitic diseases in domestic animals is dependent on a limited number of compounds, many of which are chemically closely related. This review will consider each of the drugs that is currently available for treatment and prophylaxis of trypanosomiasis and the tick-borne diseases theileriosis, babesiosis, anaplasmosis and cowdriosis. In contrast to the situation with the drugs used for control of tick-borne diseases, drug resistance appears to be an increasing problem with the compounds used for trypanosomiasis control. The presentation will therefore review the current methods used to identify and quantify drug resistance in trypanosome populations, reports of resistance to trypanocides, and cross-resistance between trypanocides. The possible reasons for the apparent lack of development of resistance to the compounds used for treatment of tick-borne diseases will also be discussed. In addition to problems with drug resistance, toxicity at the site of injection is a problem that is particularly associated with many of the trypanocides when used on a long-term basis in individual animals. Various alternative preparations of the currently used trypanocides have therefore been developed in an attempt to reduce this toxicity and, in some cases, to extend the prophylactic activity of the compounds. The different preparations will therefore be discussed. Finally, future developments in haemoparasitic chemotherapy will be considered and, for trypanosomiasis, will highlight the importance of integrating chemotherapeutic and chemoprophylactic programmes with control of the insect vector when drug resistance becomes a significant constraint to livestock production.



CHEMOTHERAPY AND DELIVERY SYSTEMS – HELMINTHS

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The milbemycins are the only novel broad spectrum anthelmintic chemicals to reach the market place in the last ten years. Many new systems for delivery strategies for rational use have, however, been introduced. Boluses are now available which are retained by virtue of specific gravity and by variable geometry. They contain benzimidazoles, morantel, ivermectin and levamisole. Their release mechanisms involve preferential corrosion of a retaining metal core, constant diffusion from a laminated ethylene acetate sandwich, and a hydrostatic pump driven by osmotic pressure. Some are biodegradable. Experimental delivery systems have been developed incorporating ear implants and liposomes.

The anthelmintic efficacy of some drugs has been potentiated by the synergistic action of metabolic inhibitors and these combinations hold promise for the future.

Much new information is now available on those factors which affect anthelmintic efficacy such as concurrent administration with food and the presence of the target parasites themselves. This knowledge provides a sound basis for the rational use of anthelmintic drugs.



ANTHELMINTIC RESISTANCE

R. PRICHARD

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Anthelmintic resistance is widespread in nematode parasites of sheep, goats and horses. Resistance is also developing in nematode parasites of cattle and has been detected in pig parasites. Benzimidazole, levamisole/morantel and ivermectin resistances occur in nematodes of sheep and goats and closantel resistance has been found in *Haemonchus contortus*. Anthelmintic resistance is likely to develop wherever anthelmintics are frequently used and be detected if it is investigated.

Worm count or egg count reduction after treatment are useful for the detection of all types of anthelmintic resistances. More economical, faster and more sensitive in vitro assays for the detection of anthelmintic resistance have been developed. Some, such as the egg hatch assay are specific for a particular class of anthelmintic, whilst others such as larval development assays can be used with most anthelmintics. Improvements in our understanding of the biochemistry and molecular genetics of anthelmintic action should lead to the development of more sensitive assays for the detection of anthelmintic resistance in individual nematodes.

Levamisole/morantel resistance appears to be associated with alterations in cholinergic receptors in resistant nematodes. Ivermectin appears to act by binding to a glutamate receptor of a membrane chloride channel. This receptor has been cloned so that further studies of the interaction of ivermectin with this receptor and its possible alteration in ivermectin resistance will be feasible. Benzimidazole resistance in nematodes and fungi appears to be associated with an alteration in B-tubulin genes which reduces or abolishes the high affinity binding of benzimidazoles for tubulin in these organisms. This knowledge can be exploited for DNA probes for benzimidazole resistance/susceptibility in individual organisms.



TEACHING VETERINARY PARASITOLOGY

A. VERSTER

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"I hear and I forget
I see and I remember
I do and I understand"

All teachers in veterinary science should bear the above quotation in mind when drawing up the curriculum of a subject. This is particularly true in veterinary parasitology which is a dynamic subject with exciting new discoveries being made at a startling pace. Seeing and doing certain procedures facilitates the understanding and learning of basic concepts. In veterinary parasitology 'hands on' training and information retrieval should be maximized rather than the mere memorizing of facts. The teacher must also guard against the temptation to teach the students all the detailed information acquired after a lifetime of dedicated study. Such detail camouflages the basic concepts and only serves to confuse the student.

Africa has rich diversity of parasites and, especially in view of the resistance problems that have developed against parasitocides, new measures to control them must be developed. To date attention focussed mainly on the control of parasites in livestock under extensive and intensive conditions. As drought and famine increase in many parts of Africa more attention should be devoted to increasing food production at the subsistence farming level. Such farming need not be limited to domestic livestock but can also include alternative food animals such as the cane rat, *Thryonomys swinderianus*, or the giant rat, *Cricetomys gambianus*.

Undoubtedly the greatest advantage of this Faculty, and particularly of the three disciplines in veterinary parasitology, is the fact that it is cheek by jowl with the Onderstepoort Veterinary Institute. In the seventy years since the establishment of the Faculty of Veterinary Science the symbiotic tradition of co-operation that has developed between these two institutions contributes materially to the thorough grounding the students receive in veterinary parasitology.



**ABSTRACTS OF
SUBMITTED PAPERS**

SESSION 1

Parasitology – General Studies





RECOVERY OF CESTODE AND DOG ASCARIDS EGGS FROM VILLAGE COURTYARDS OF IRAN

A. ESLAMI

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Five hundred soil samples from the court-yards of different villages of Iran were examined to obtain some information on the occurrence of taeniid and dog ascarid eggs in the environment. Ten grams samples of surface soil taken from several sites were mixed with copious amounts of water and sieved through two sieves of 150 Mm and 30 Mm aperture. The final volume was adjusted to 20 ml of which 4 ml was examined for eggs using a clayton-lane centrifuge. Of 500 soil samples 123 (24.6%) and 156 (31.2%) were found to contain 1-88 and 3-42 taeniid and ascarid eggs, respectively. The free movement of sheep dogs inside the court-yard where housewife and school children especially girls spend much of their daily time in sweeping, dish washing, laundrying, meeting friends or playing would appear to be responsible for the high prevalence of *Echinococcus granulosus* in housewives and *E. granulosus* and dog ascarids in school children who become infected at an early age.



ENDOPARASITISM AND MANAGEMENT PROBLEMS OF ETHIOPIAN DONKEYS

F. GEBREAB AND N. YIDEG

Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia

Ethiopia's donkey population is estimated at 3.9 million. Their effective use has been however hampered by problems such as feed deficiency and health hazards. This paper covers a study of babesiosis in donkeys in Bahr-Dar and gastrointestinal helminths in donkeys of Wolisso. To this end blood smears of 348 donkeys and faecal samples of 216 donkeys were examined using standard methods. Furthermore one donkey was examined by postmortem. Babesiosis was detected in 60 (14.4%) donkeys with 51 harbouring *B. equi* and 3 *B. caballi*. Coproscopy of 216 donkeys and ova culture in 12 donkeys with over 5000 epg revealed the presence of the following helminths enumerated in the order of their abundance and frequency: *Trichonema*, *Strongylus vulgaris*, *Strongylus edentatus*, *Strongylus equinus*, *Tridontophorus*, *Oesophagodontus*, *Trichostrongylus axei*, *Gyalocephalus* and *Poteriostomum*. A mean epg. of 400 was determined. In the postmortem examination of the single donkey the additional worms recovered are: *Gasterophilus nasalis*, *Gasterophilus intestinalis*, *Oxyuris equi*, *Parascaris equorum* and *Anoplocephala perfoliata*. It was also confirmed that the donkeys suffer from extreme feed shortages, poor harness leading to the development of traumatic back-sores en-masse. Control of GIT helminths, provision of improved harness and better grazing conditions is a step in the right direction for deriving better pack services.



THE OCCURRENCE OF COCCIDIA IN DOMESTICATED AND FREE-LIVING SMALL RUMINANTS AT THE ZOO

M. -A. HASSLINGER, T. ZIESCHE AND I. KIEFER

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Faecal samples from normal, apparently healthy domestic sheep and goats kept in Munich's Zoological Garden Hellabrunn were examined to determine the numbers and types of *Eimeria* spp. present. In addition the prevalence and the oocyst output was investigated during a 12-month period. Nine *Eimeria* species were encountered in sheep and eight species in goats. The prevalence in both animal groups was 100%, multiple parasitism was the rule. Simultaneously the prophylactic efficacy of a single dose of Toltrazuril (BAYCOX, Bayer AG) against naturally-acquired coccidial infections was evaluated. Toltrazuril given at 20 mg/kg was found to cause a rapid and significant reduction in the oocyst output and the reduced oocyst counts were maintained at low to moderate levels for four to six weeks.

Mountain ruminants (chamois, alpine ibex, wild goat, Rocky Mountain goat, nubian ibex, mouflon, musk oxen) and dama mhorh gazelles of the same area were coproscopically examined concerning their coccidial infestation. For therapy the polyether-antibiotic salinomycin (SACOX or SALOCIN, Hoechst AG) was mixed into forage pellets in a concentration of 30 ppm and administered over a period of two or three weeks. When coccidial levels were high, the best results were achieved by treatment with salinomycin. The parasitic status was influenced by climatic factors. Different husbandry methods, the size and design of the enclosure, the varying intake of food and the antibiotic by which the animals were treated were likewise of importance. Besides, individual immunity influenced by the age of the animals and stress also played an important part in the infectious process.



PREVALENCE OF ANTIBODIES AGAINST *BABESIA DIVERGENS* IN AUSTRIAN CATTLE BY AN IMPROVED ENZYME-LINKED-IMMUNOSORBENT ASSAY

R. EDELHOFER, W. BAUMGARTNER AND E. KUTZER

Institute of Parasitology and General Zoology, VMU, Vienna, Linke Bahng. 11, Vienna, Austria

B *abesia divergens* belongs to a type of parasite that occurs in certain parts of Austria and causes significant economic losses. In order to obtain information on the prevalence of antibodies against *B. divergens* in cattle, 5054 blood samples of eight districts of Austria were tested by an improved enzyme-linked-immunosorbent assay (ELISA). In order to achieve a fairly even distribution of samples in Austria, one serum from every 25th cattle farm out of altogether 140 081 farms in the whole country was taken for testing in the course of the brucellosis - leucosis-IBR/IPV control program of the years 1988/1990.

2309 (45.7%) of the tested animals showed positive titres against *B. divergens*. The highest number of antibody-positive animals could be found in Vorarlberg (80.4%) and Tyrol (59.6%).

The results obtained showed that infections with *B. divergens* occur in different areas of Austria. From 1988 to 1992 in Austria about 55 000 cattle have been immunised by a formalin-killed inactivated vaccine. It may be that the immunisation of cattle in Austria will be a reason for the high percentage (45.7%) of seropositive animals in this study.



CARRIER STATE IN IMMUNE CATTLE: THE IMPLICATIONS FOR THE EPIDEMIOLOGY AND CONTROL OF EAST COAST FEVER

D. P. KARIUKI AND A. S. YOUNG

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East Coast fever, caused by protozoan parasite *Theileria parva*, is considered one of the most important tick-borne diseases in East and Central Africa. The economic losses caused by the disease in 11 countries in the region where over 24 million cattle are at risk from the disease has recently been estimated to be \$168 million annually (Mukhebi *et al* 1992).

Recent demonstration that the majority of cattle in ECF endemic and epidemic areas are carriers of *Theileria parva*, has posed serious questions on the present control strategies of both disease and vector. While frequent acaricide application (once weekly has proved highly effective in the past) this method is becoming prohibitively expensive for most small-holder farmers. Efficient and effective acaricide application results in herds that are totally susceptible creating a state of enzootic instability.

Carrier state maintains the parasite population in a given area, thereby enhancing endemic stability. Frequent application of acaricide to immune cattle which are carriers may control the disease but will not eradicate theileriosis. These studies have shown that immunisation by infection and treatment method will produce carriers beneficial to the maintenance of endemic stability and allow reduction in acaricide application to the benefit of the farmers and the environment.



IMPROVEMENT OF CHEMOPROPHYLACTIC STRATEGIES FOR THE CONTROL OF AFRICAN BOVINE TRYPANOSOMIASIS: USE OF THE ISOMETAMIDIUM ELISA

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Control of African bovine trypanosomiasis relies heavily upon the use of a small number of drugs, of which isometamidium chloride is the only one with clearly established prophylactic activity. There are, however, an increasing number of reports of the development of drug resistant strains of trypanosomes. An ELISA has been developed which has provided the first opportunity to investigate isometamidium concentrations in treated cattle. This study investigated the potential for the ELISA to be used to improve chemoprophylactic regimens in the face of possible drug-resistance.

A competition ELISA for the detection of isometamidium in bovine serum has been developed and validated using serum from (a) untreated European and African cattle, (b) drug-treated African cattle under experimental tsetse challenge with *Trypanosoma congolense* (d) drug-treated African cattle under natural tsetse challenge in the field.

Serum levels following intramuscular drug administration at standard prophylactic doses were similar in both European and African cattle: biphasic drug elimination was observed with a long terminal phase half life (approximately 24 days), and drug levels could be detected for at least three months. The period of prophylaxis against *T. congolense* afforded by isometamidium chloride varied depending on the challenge isolate, from less than one month to six months, and drug levels present at the time break-through infections occurred varied accordingly. Following break-through infection by drug-resistant trypanosomes, drug levels declined more rapidly than in animals which resisted challenge. Sera from drug-treated cattle under field challenge had drug concentrations similar to those in experimental animals.

The isometamidium ELISA has been shown to be useful in determining drug levels in treated cattle, and can provide information regarding the suitability of treatment regimens. Furthermore, it may provide evidence of drug-resistance where infection is demonstrated in animals with levels of drug protective against susceptible isolates.



CONTROL OF CRYPTOSPORIDIOSIS: NEW IMMUNO- AND CHEMOTHERAPIES

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Cryptosporidium parvum, the agent of cryptosporidiosis, is prevalent and widespread. Of over 100 published modalities attempted for prophylaxis or therapy few have reported significant beneficial results. Initial success has been obtained by oral administration of colostral whey from cows hyperimmunized by intramammary infusions of freeze-thawed oocysts. Such whey, with high levels of anti-*C. parvum* Ig, reduced or eliminated *C. parvum* in experimentally infected mice and calves and in human patients. Cloning, and expression of a neutralizable 15 kDa sporozoite surface protein have provided a highly purified, stable antigen that stimulated production of high levels of colostral antibody which is presently being tested for prophylaxis in mice. Anecdotal reports of successful treatment of immunosuppressed patients have led to controlled studies with neonatal BALB/c mice in which we demonstrated prophylaxis with clarithromycin, azithromycin and paromomycin. Paromomycin also exhibited prophylaxis in calves.



THE ROLE OF THE OFFICE INTERNATIONAL DES EPIZOOTIES (OIE) IN VETERINARY PARASITOLOGY

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The three principal aims of the OIE, an organisation comprised of the world's official Veterinary Services, are provision of information on animal health world-wide, international co-ordination of research into and control of certain animal diseases, and the harmonisation of import and export regulations on animal and animal products internationally. Most people working with the major microbial diseases of livestock and poultry know of OIE's work, but veterinary parasitologists should also be aware of the organisation's interest and work in their field.

Specialist Commissions and Working Groups of the OIE are frequently involved with parasitic diseases, their reports sent world-wide. Periodicals such as the monthly *Bulletin*, which reports the evolution of important animal diseases, the quarterly *Scientific and Technical Review*, the annual *World Animal Health*, the *OIE Veterinary Biotechnology* and *Veterinary Drug Registration Newsletters*, cover as applicable veterinary parasitology. Seventeen of the animal diseases reportable internationally through the OIE are parasitic, and officially accepted diagnostic methods for these are described in the *Manual of Standards for Diagnostic Tests and Vaccines*. For their import and export of mammals, birds and bees, in the *International Animal Health Code* there are sanitary and quarantine procedures recommended by Veterinary Services for thirteen parasitic diseases.

Some OIE Reference Laboratories and Collaborating Centres work with parasites. There is an Ad hoc Group on Non Tsetse-Transmitted Animal Trypanosomoses which reports to interested parties. Recently convened was an Ad hoc Group on Wildlife Diseases which, as would be expected, emphasised the importance of parasites.



CHOICE OR CHANCE-TEACHING OF APPLIED VETERINARY PARASITOLOGY AT PANNON UNIVERSITY FOR AGRICULTURAL SCIENCES IN MOSONMAGYARÓVÁR (HUNGARY)

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Its central theme is the possibility and necessity of the education of parasitology with the condition of very few disposable lectures. Since within the *basic subject* - Animal Hygiene there is not much time left, every little chance must be used. Between *optional subjects* - Game-husbandry and Hygiene, and maybe Hygiene of Furbearing Animals, and Horse-breeding and Hygiene gives possibility (choice and/or chance) for the education of Applied Veterinary Parasitology. It's well known, that for the agricultural engineer the explanation of zoo-sanitary prescriptions for trade in animals and animal products, is necessary, as well as for the Public Health Relations. Education about parasitoses in our Subjects, for the most part corresponds with disease of List A and List B of International Zoo-Sanitary-Code (O.I.E.). From List B we detail Echinococcosis, Cysticercosis of cattle and pigs, equine piroplasmidosis, dourine, trichinosis of pigs and wild boar, toxoplasmosis of furbearing animals, sheep mange, acariasis and varroosis of bees. In Hungary by Sanitary Regulations we must detail the dourine, mange and varroosis. However, in some relations we give surplus. Students, who choose in addition to basic subject from optional subjects, get chances to increase the proportion of own parasitological knowledge. Because, future agricultural engineers will have objective parasitological view for the common work with the veterinary surgeon.



ARNALD OF VILLANOVA AND THE HISTORY OF PARASITOLOGY

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In order to determine the contribution of Arnald of Villanova to Parasitology, a study of his authentic and/or attributed works has been undertaken. As main material, several incunabula (XV-XVI century), the first *Opera omnia* (Lyon, 1504) and critical editions have been analysed, and translation from Latin to Spanish prepared, for parts dealing with parasitic diseases. Most of his contributions deal with malaria (tertian and quartan fever), cestoda (*T. solium*, *sensu lato*) and nematoda (ascarids, oxyurids), although some consideration to scabies, lice, fleas and flies is given. Discussion on spontaneous generation, pathogenicity of worms and anthelmintica demonstrates the old roots in the Greek and Latin classics (Hippocrates, Galen, Dioscurides) with the Arabic link (Avicenna, Rhazes, Averroes, Al-Kindi), which passed to Christian Europe through Latin translations, some of them by Vilanova. A critical edition of his *Opera Medica Omnia* is now being undertaken, and it seems as a result of it that the well known *Breviarium practice medicine* may be an addition to the authentic works. A complete study of aspects connected with Parasitology is needed and is currently in preparation.



**ABSTRACTS OF
SUBMITTED PAPERS**

**SESSION 2
Arthropod Control I**



FIELD EFFICACY OF IVERMECTIN AGAINST *OESTRUS* *OVIS* AND OVINE HELMINTH PARASITES

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A field trial was undertaken to assess the efficacy of each of two formulations of ivermectin in prevention and treatment of ovine parasites in a naturally infested flock, grazing on the foothills of the Pyrenees, in south western France. Within the flock, 875 sheep were randomly divided into four groups, and treated twice during the fly season with an interval of 60 days between treatments. Group 1 sheep were treated with albendazole (ABZ) at a dose rate of 3.8 mg/kg to remove trichostrongylid parasites without affecting *Oestrus ovis*; Group 2 received closantel at a dose rate of 10 mg/kg because of the known persistent activity of this compound against *O. ovis*, and lack of efficacy against trichostrongylid parasites, except *Haemonchus contortus*, which is not regarded as being of significance in this region; Groups 3 and 4 received ivermectin at a dose rate of 200 mcg/kg bodyweight by subcutaneous injection (IV) and orally (ID), respectively. In order to assess the prophylactic effect of each treatment, immediately prior to the scheduled second treatment on Day 60, five sheep from each group were chosen at random and necropsied. Similarly, to assess the therapeutic effect, another five sheep from each group were selected on Day 70 and necropsied for parasite counts.

Relative to the closantel group, overall percentage reductions in gastrointestinal parasites for the ABZ, IV and ID groups were 88%, 97.6% and 99%, respectively. Relative to the albendazole group, prophylactic efficacies against *O. ovis* for IV and ID groups were 62.5% and 0%, respectively. For the therapeutic effect, for IV and ID, efficacies were 97.7% and 100% respectively. Closantel was 97.7% effective for prophylactic effect and 100% effective for therapeutic effect.

The trial confirmed the high efficacy of ivermectin as a treatment for gastrointestinal parasites and *O. ovis* in sheep, and indicates that the injectable formulation has some persistent activity against *O. ovis*. Further titration is needed to fully assess the duration of this persistent effect.



EFFICACY OF MOXIDECTIN (INJECTABLE AND ORAL DRENCH) AGAINST *OESTRUS OVIS* LARVAE IN NATURALLY INFESTED SHEEP

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The efficacy of moxidectin 1% injectable solution and moxidectin 0.2% oral drench at a dose rate of 0.2 mg moxidectin/kg b.w. was assessed against natural botfly infestation in sheep. In a commercial flock bearing a natural infection of nasal botfly, two trials were performed. In both trials, the infestation was confirmed at necropsy by parasitological examinations of two sheep. In February 1992 (Trial 1), the flock was shown to have an infestation predominantly with 1st stage larvae of *O.ovis*. In May 1992 (Trial 2), the infestation by 2nd and 3rd stage larvae of *O.ovis* was found to be predominant. In each trial, 15 non lactating sheep of different ages showing clinical signs of oestrosis were chosen. On the basis of clinical signs they were assigned to three treatment groups which were injectable solution, oral drench and untreated control. All sheep were slaughtered 14 days after treatment and examined for the presence of larvae in the nasal and frontal cavities. Results were analysed using geometric means and One-Way analysis of variance. Injectable moxidectin showed an efficacy of 96.00% against 1st stage larvae (Trial 1) and of 100% against 2nd and 3rd stage larvae (Trial 2). The efficacy of the oral formulation was 61.52% against 2nd and 3rd stage larvae but the same formulation was not effective against 1st stage larvae.



THE EFFICACY OF CYDECTIN^R 0.5% POUR-ON AGAINST *HYPODERMA BOVIS* IN NATURALLY INFESTED CATTLE: PARASITOLOGICAL AND SEROLOGICAL DATA

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The field efficacy of CYDECTIN^R 0.5% Pour-on formulation of moxidectin was assessed in heifers naturally infested with *Hypoderma bovis*. After their first grazing season, 38 heifers were selected on results of an ELISA test performed in January. Nine heifers with positive antibody titres were left untreated; the 29 remaining animals (25 serologically positive) were treated with CYDECTIN^R 0.5% Pour-on at a dosage of 0.5 mg per kg body weight. No local or general side effects associated with the treatment were noticed. In May, all the nine controls were warbled; none of the treated animals harboured warbles at any time during the trial. These warble counts were in agreement with antibody kinetics studied on blood samples collected on days 0 (14 January), 6, 27, 48, 90, 119 and 150. In the untreated animals, the antibody titres were rising until May (D119) and then were declining sharply with the emergence of the larvae whereas in the treated animals there was a drop from Day 30 onwards. In conclusion, natural infestations with *H. bovis* first instar larvae can be effectively treated with CYDECTIN^R 0.5% Pour-on at a dosage of 0.5 mg moxidectin per kg body weight.



THE EFFICACY OF TOPICALLY APPLIED IVERMECTIN FOR PREVENTION OF BLOWFLY STRIKE (MYIASIS) IN SHEEP IN AUSTRALIA

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An extensive trial program was conducted to evaluate the efficacy of a topically applied ivermectin-based formulation against blowfly strike (cutaneous myiasis) in sheep. In all trials, the formulation was diluted to the required concentration in water and applied by hand jetting at a pressure of approximately 80 psi or by automatic non-recycling jetting machine. Initial efficacy assessments were conducted in a temperature-controlled blowfly challenge facility. Groups of sheep were housed in the facility at various times after treatment and adult *Lucilia cuprina* were released to simulate exposure of sheep under field conditions. An organophosphate-resistant strain of *L. cuprina* was used in most of the trials. Protection was assessed by inspection of sheep for the presence of myiasis lesions 24 to 48 hours after fly release. A dose level of 0.03 mg/mL was chosen from three dose-selection studies. Efficacy was confirmed in Merino and Corriedale sheep treated by hand jetting and in Merino sheep treated by automatic jetting machine. Rain after hand jetting treatment did not affect efficacy. Established strikes were cured by application of the formulation. A study using larval implants demonstrated that the formulation was also effective against *L. sericata*, *Chrysomya rufifacies* and *Calliphora vicina*. Field trials involving 16,209 sheep were conducted throughout Australia to evaluate the protection of sheep under farm conditions. Each trial included an untreated control group and a hand-jetted group. In 17 trials there was also a group treated by automatic jetting machine. The overall reductions in cumulative number of strikes were 96.5, 95.8 and 93.5% for hand jetting (51 trials) and 88.7, 84.2 and 80.9% for machine jetting (17 trials) for up to 10, 12 and 13/14 weeks after treatment, respectively. The odds of a treated animal being struck 16 weeks after treatment was estimated to be at least 91% less than the odds of a control animal being struck ($p < 0.01$). The data demonstrate that ivermectin jetting fluid is effective in the prevention and treatment of strikes caused by *Lucilia cuprina*, including organophosphate-resistant strains, *L. sericata*, *Chr. rufifacies* and *C. vicina*.



ECTOPARASITES OF LIVESTOCK IN NEW ZEALAND

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Impediments to the growth and well-being of livestock abound under the intensive farming systems used in New Zealand, and ectoparasites are a major concern to the farmer and are a drain on resources.

Lice and blowflies have been present from the earliest days of pastoral agriculture in New Zealand, as was the scab mite, *Psoroptes ovis*, supposedly extinct since the 1880's. The cattle tick, *Haemaphysalis longicornis* was a later addition to New Zealand's arthropod pest fauna which has been extended further by the relatively recent introduction of the Australian sheep blowfly, *Lucilia cuprina*. The sheep ked (*Melophagus ovinus*), mange mites (*Chorioptes bovis*; *Psorergates ovis*; *Psoroptes* spp) and nasal bot fly (*Oestrus ovis*) also occur.

Estimates of the monetary losses due to ectoparasites can be computed, but their long-term value is doubtful. Particularly when seen against a background of fluctuating world prices and occasional reversal of differential values between, say, carcase and by-products. Flystrike alone is estimated to cost >\$30 million annually, so to this can be added the losses due to other ectoparasites, including production losses, quality deterioration, labour and preventive measures.

This paper briefly examines the historical aspects of the ectoparasites of livestock in New Zealand and follows with a view of their current status in terms of prevalence, geographical distribution and economics. Next there is a discussion of the management ('control') methods currently employed, with topics such as insecticide resistance, biological control and molecular biology being considered.



PERMETHRIN SPRAY AND WIPE-ON AS USEFUL TOOLS TO PREVENT BLACK FLY ATTACKS AND SIMULIOTOXICOSIS IN CATTLE AND HORSES

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Comparative field studies were performed in two endemic areas in Germany where black flies (*Odagmia ornata*, *Boophtera erythrocephala*) year by year cause heavy damage and mortality in livestock. Cyfluthrin, cyhalothrin, cypermethrin, deltamethrin and permethrin were applied by spraying as wipe-ons and topically as pour-ons.

In cattle spraying with permethrin (STOMOXIN MO) gave best results compared to all other pyrethroids and types of application. Results could be improved when cattle were sprayed followed by an additional treatment of the less hairy parts with wipe-on. In a critical test in a certain area no case of simuliotoxicosis, neither death nor disease, occurred in the treated cattle. However, 10 animals died after black fly attack in the same area and time when left without treatment, and further cattle had to be treated symptomatically. Permethrin spraying (3 mg/kg BW) prevented 67% of black flies from taking a blood meal for seven days. Horses were protected for five days against black fly attacks after treatment of the whole body surface with permethrin in a wipe-on (Wellcare Emulsion for horses).



EXPERIENCE IN CONTROL OF FLIES AND SUMMER MASTITIS IN GRAZING CATTLE BY USE OF BAYOFLY POUR-ON (CYFLUTHRIN)

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Within the economic impact of parasitism in cattle, parasitic symbovine flies are a primary cause of production losses in all cattle-producing countries. Fauna composition and many other biological data of flies associated with cattle were known from five years lasting studies in Germany. In the period from 1982 until 1992 trials have been performed for control of flies in 6500 dairy cows and 4000 heifers using BAYOFLY Pour-on containing 1% Cyfluthrin. The doses of 10 ml/per animal and 500 kg BW was found to be effective to reduce the nursing fly species (*Musca autumnalis*, *Hydrotaea irritans*) by 90% and the biting fly species (*Haematobia irritans*, *H. stimulans*) by almost 100% compared to untreated animals. The effect was achieved for 30 days, when the highly susceptible and stationary living species *Haematobia irritans* were used as indicator species.

From 1982 until 1992 in selected herds of heifers known for high risk of infection with summer mastitis there was a significant decline in clinical cases (2%) compared to untreated cattle herds (10%).



THE IMPACT OF PROPHYLACTIC MANGE CONTROL IN GROWING SWINE WITH SEBACIL POUR-ON ON WEIGHT GAIN AND ECONOMICS

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Subclinical mange infestations in growing swine may have a considerable impact on feed and weight gain. Therefore, efficacious remedies which are easy to administer to swine in group husbandry are of general interest for a prophylactic mange control in fattening pigs.

Materials and methods: Sebacil Pour-on is a ready to use solution which contains 7.5% Phoxim, an organosphosphorus compound. The recommended dose volume for mange control is 4 ml per 10 kg b.w. and is poured along the dorsal midline of the pigs from the neck to the base of the tail. The investigations of the influence of a single mange treatment of growing swine on the general economics was carried out under field conditions. Ten consecutive groups with an average number of 23 pigs of a German landrace-pietrain-cross were equally divided into treated and untreated control groups. The average body weight at the time of separation and treatment was 64 kg. There were no signs of clinical mange. Pigs were sold for slaughter as soon as they reached an average body weight of 104 kg.

Results: Compared to the control groups the Sebacil groups showed a shorter fattening period (134 versus 147 days = 6%), an improved daily weight gain (577 versus 550 g = 5%) and an almost twofold higher gross profit per animal (DM 29.41 versus DM 15.47 = +90%).

Conclusion: Prophylactic Sebacil Pour-on treatment of fattening pigs protects from economic losses due to the development of a subclinical mange infestation.



THE EFFICACY OF TOPICALLY APPLIED IVERMECTIN FOR THE CONTROL OF THE SHEEP BITING LOUSE (*DAMALINIA OVIS*)

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The efficacy of topically applied ivermectin against the sheep biting louse (*Damallnia ovis*) was evaluated in pen and field trials with long-wooled sheep in Australia. Ivermectin concentrate was diluted to 0.03 mg/mL with water and applied through a pump at a pressure of approximately 80 psi using a jetting handpiece with multiple nozzles that could be combed through the fleece (hand jetting). In the pen studies, the animals were housed in individual outdoor pens exposed to prevailing weather conditions. Efficacy was confirmed in the Merino breed. Further trials examined the effect of rain after treatment, and compared efficacy in Merino and Corriedale sheep. Louse counts were conducted on all trial animals at regular intervals for eight to 15 weeks after treatment. In all pen studies, efficacy was apparent at the first louse count one week after treatment, and geometric mean louse counts in treated animals were reduced by >99% compared with controls at trial termination. Nine field trials were conducted using 7,623 Merino sheep with approximately three months' (two trials), six to seven months' (four trials) or nine months' (three trials) wool growth at the time of treatment. All animals in the trial flocks were treated and were pastured separately from other sheep through to trial termination. Louse counts were obtained periodically from monitor sheep in each trial for 11 to 13 or 15 weeks after treatment. The overall reductions in geometric mean louse counts compared with pretreatment values were 98.9% and 99.8% for 11 to 13 and 15 weeks, respectively. Resistance to synthetic pyrethroids was demonstrated in lice collected from sheep flocks used in four of the field studies and in one pen study. Ivermectin applied to long-wooled sheep by hand jetting controlled biting lice, including pyrethroid-resistant strains.



FIELD EFFICACY OF MOXIDECTIN 0.5% POUR-ON AGAINST *CHORIOPTES BOVIS*, *DAMALINIA BOVIS*, *LIGNONATHUS VITULI* AND *PSOROPTES OVIS* IN NATURALLY INFECTED CATTLE

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This paper presents the results of three field trials carried out to assess the efficacy of a pour-on formulation of moxidectin against a few important ectoparasites of cattle. Two different criteria were used to assess the efficacy of the drug against *C. bovis* and *P. ovis*: a) three skin scrapings were collected from each animal before the start of the trial and weekly or fortnightly thereafter until Day 56. *P. ovis* counts were transformed into *P. ovis* scores; b) the percentage of body area involved (clinical index) was calculated before the start of the trial and on Days 28 and 42 using a standardized map. These data were transformed into clinical scores. Efficacy against *D. bovis* and *L. vituli* was estimated by identifying and counting lice in eight 15 cm long partings at predefined sites. Lice counts were performed on Day 0 and fortnightly thereafter until Day 42. A single application of 0.5% pour-on moxidectin was applied at 0.5 mg/kg body weight (bw) (all trials) or at 0.25 mg/kg bw (*C. bovis* trial) on Day 0 (treated groups) or 28 (control groups). At 0.25 mg/kg bw (*C. bovis* trial) the efficacy was incomplete and two animals out of seven were still harbouring low to high numbers of *C. bovis* mites. In contrast, parasitological and clinical data showed that at 0.5 mg/kg bw this formulation was fully effective against all parasitic species and that parasitological cure was achieved within 14 days. Most of the control animals remained infected until Day 28. These results compare favourably with the efficacy data obtained previously with the injectable formulation of the drug on *C. bovis* and *D. bovis*.



**ABSTRACTS OF
SUBMITTED PAPERS**

SESSION 3

Anthelmintics - Drug Resistance I



MULTIPLE DRUG RESISTANCE OF *HAEMONCHUS* *CONTORTUS* OF SHEEP IN MALYASIA AND THE EFFICACY OF MOXIDECTIN

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The efficacy of benzimidazoles (BZ), levamisole, closantel, ivermectin and moxidectin against gastro intestinal nematodes was studied on a government sheep farm in Selangor, Malaysia. The flock consisting of 1200 sheep had received 13 treatments with ivermectin and 10 treatments with (pro-) BZ over a 22 month period. The efficacy of the anthelmintics was studied by means of a faecal egg count reduction test (FECRT) and controlled tests. An *in vitro* egg hatch assay was also performed for BZ. For the FECRT, groups of 15 sheep were used with the following anthelmintics, namely, oxfendazole (4.5 mg kg^{-1}), fenbendazole (5 mg kg^{-1}), albendazole oxide (5 mg kg^{-1}) and levamisole (7.5 mg kg^{-1}), all of which were given orally, and with ivermectin (0.2 mg kg^{-1}), moxidectin (0.2 mg kg^{-1}) and closantel (2.5 mg kg^{-1}), given by subcutaneous (s/c) injection. The percentage reductions achieved in these FECRT were respectively, 45%, 8%, 0%, 99%, 18%, 100% and 100%. Only L3 larvae of *Haemonchus contortus* were found in the post treatment cultures of the ivermectin and BZ treated groups. The *in vitro* egg hatch assay with thiabendazole gave an LC_{50} of $0.32 \mu\text{g ml}^{-1}$ for samples taken from sheep of the farm compared with $0.02 \mu\text{g ml}^{-1}$ for a BZ susceptible *H. contortus*. Sixteen parasite free young sheep were allowed to graze for four weeks on the farm's pastures before being penned for two weeks, and allocated to four treatment groups of four animals each. They were all slaughtered 10 days post treatment for worm counts. The reductions in *H. contortus* worm counts were, 0% with oxfendazole (at 4.5 mg kg^{-1} orally), 0% with ivermectin (at 0.2 mg kg^{-1} s/c) and 98% with moxidectin (at 0.2 mg kg^{-1} s/c). The results of the FECRT, the egg hatch assay and the slaughter trial provide evidence for the presence of a strain of *H. contortus* on this farm which is simultaneously resistant to ivermectin and BZ. The high efficacy of moxidectin against an ivermectin resistant strain confirms earlier observations in other continents and suggests that side resistance in the macrocyclic lactones group of compounds does not develop as easily as in the BZ group.



ANTHELMINTIC ACTIVITY OF AB763 IN SHEEP

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Widespread and increasing resistance amongst the major pathogenic nematodes of sheep in Australia to most of the currently available anthelmintics is a significant influence adversely affecting the productivity and welfare of sheep. While optimal parasite control should be based on the integration of both management and anthelmintic strategies, a clear need exists for a novel anthelmintic to help reduce resistance selection pressure by existing anthelmintic classes. The comparative anthelmintic activity of AB763 (a combination of albendazole and the new parasiticide AB762), albendazole (ABZ) and AB762 was evaluated in sheep variously infected with benzimidazole resistant (BZ-R) strains of *Haemonchus contortus* (H/c), *Ostertagia circumcincta* (O/c) or *Trichostrongylus colubriformis* (T/c), in three dose titration studies, designed, conducted, and evaluated according to conventional methods (*Veterinary Parasitology* 1982 **10**, 265-284). ABZ administered at 3.8 mg/kg bodyweight (mpk), reduced total worm counts (TWC) of H/c, O/c and T/c by 43.6, 36.7 and 85.4% respectively. AB762 at 10, 20 and 30 mpk reduced TWC of H/c by 95.9, 99.7 and 100%; of O/c by 15.5, 80.0, 93.6% and of T/c by 15.5, 80.0 and 93.6%. Administration of the combination of ABZ (3.8 mpk) with AB762 at 10, 20 or 30 mpk resulted in efficacy against O/c of 63.3, 89.9 and 100%, and against T/c 95.4, 97.5 and 99.4% respectively. ABZ (3.8 mpk) and AB762 (30 mpk) was 100% effective against H/c. These results demonstrate efficacy of greater than 99% against BZ-R strains of H/c, O/c and T/c with the use of AB763 (delivering ABZ at 3.8 and AB762 at 30 mpk), and support the value of this anthelmintic as a component of integrated parasite management.



THE USE OF MEDICATED BLOCKS TO CONTROL BENZIMIDAZOLE RESISTANT NEMATODES IN FIJI

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Internal parasites are a major constraint to commercial small ruminant production in Fiji and intensive use of anthelmintics has led to the development of resistance on some farms. Urea-molasses blocks (UMB) containing fenbendazole (0.75 g/kg) were used to provide continuous low level doses of anthelmintic to animals in two experiments. In Experiment 1, 60 weaner goats were allocated to grazing plots and divided into three groups: unrestricted access to UMB with fenbendazole (FBZ), unrestricted access to UMB without drug, and routine treatment with ivermectin and daily protein supplement (SRS). Faecal egg counts indicated a high level of control in the FBZ group and these goats showed similar bodyweight gain to the SRS group with the UMB group not gaining as much weight. In Experiment 2, 50 pregnant sheep were divided into two groups which grazed separate two ha paddocks and received FBZ or UMB in their night sheds. Faecal egg counts indicated good control in the FBZ block group but no bodyweight advantage was observed for the ewes although their lambs were 2.6 kg heavier at weaning. After weaning the lambs were retained in their treatment groups and the experiment continued for a further six months. At the end of the experiment the FBZ lambs had reached the target saleable weight of 30 kg whereas the UMB lambs were 4.3 kg lighter. It is concluded that FBZ blocks can successfully control benzimidazole resistant strains of nematodes in these production systems.



LONG TERM STABILITY OF IVERMECTIN RESISTANCE FOLLOWING INITIAL DIAGNOSIS

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The first case of ivermectin (IVM) resistance in New Zealand was reported on a goat property in 1989. As with subsequent diagnoses in this country, *Ostertagia* spp. were involved. Following faecal egg count reduction testing (FECRT) a slaughter study confirmed multiple anthelmintic resistance with drug efficacies of 93.5%, 79.6% and 43.8% for IVM, morantel and oxfendazole, respectively. Total spelling of all anthelmintics on the farm has not been a management option. Since isolation, a strict drench rotation has been adopted - levamisole (LEV) 1988-89, IVM 1990 and combination 1991. IVM has been used very strategically as animal health demanded, two occasions in 1988, six in 1990 and twice in each of 1991 and 1992.

Surveillance monitoring of anthelmintic status using lambs in 1992 has indicated that LEV remains highly effective (99.3%) against the resistant parasites and there has been a subtle shift downward for IVM (87.5%). The newer milbemycin/ivermectin, moxidectin (MOX), removed 99.9% of the *Ostertagia* spp. population.

Stability of resistance is an important issue to address when designing and evaluating management options to delay or restrict resistance and minimise the impact on farming profitability. These results suggest that annual rotation and strategic use of IVM have not had a significant effect on the field isolate over the past five years. Currently in the field, there appears to be a significant difference in efficacy between IVM and MOX despite their close structural similarities. It is proposed that MOX will not be used on the site but efficacies will be monitored at regular intervals.



ANTHELMINTIC RESISTANCE IN SOUTHERN LATIN AMERICA: A POTENTIAL TIME BOMB?

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In the subtropical humid zone of South America, which embraces the northern provinces of Buenos Aires, Entre Rios and Corrientes of Argentina, the southern county of Rio Grande du Sul of Brazil and all of Paraguay and Uruguay, approximately 45 million sheep are raised. Here the environment is ideal for parasite transmission throughout the year and up until the present time, farmers appear to have kept nematode parasites in their flocks under control by intensive anthelmintic treatment. Clinical outbreaks of parasitic disease are a regular feature in sheep flocks, even following empirical suppressive (four weekly) drenching practices. An insidious and widespread problem in this region are the practices of drug adulteration and substitution of anthelmintic formulations sold to farmers, despite the greater emphasis now by governments on surveillance and quality control monitoring.

Anthelmintic resistance is not unknown in this region, indeed it was in Rio Grande du Sul that one of the first cases of benzimidazole resistance was reported in 1967. Reports of resistance to all the commonly used anthelmintics, including the avermectins, have been made and limited surveys in Brazil and Uruguay indicate that, at least for the benzimidazoles, resistance is widespread and at a high level.

What is urgently needed in the sheep raising regions of the subtropical humid zone of South America are comprehensive surveys to determine the prevalence and magnitude of anthelmintic resistance. These will provide essential base line data to convince scientists, administrators and farmers alike of the seriousness of the problem and the urgent need to modify internal parasite control practices in sheep flocks before chemotherapy, the only viable control option at this stage, is rendered useless and farmers will be forced to abandon, or drastically alter, their current sheep farming operations.



SELECTION WITH PYR, LEV, IVM AND FBZ ON A PYRANTEL RESISTANT AND AN ANTHELMINTIC SUSCEPTIBLE ISOLATE OF *OESOPHAGOSTOMUM* *DENTATUM*

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To monitor development of anthelmintic resistance and possible cross-resistance in *Oesophagostomum dentatum* of the pig two selection studies (I and II) were performed. In Study I, an anthelmintic susceptible isolate of *O. dentatum* was selected for 6 to 10 generations into separate lines with PYR, LEV, IVM, FBZ and one line was passaged unselected. Selection pressure was adjusted to give FECR of approximately 80%. In the last selection the following doses were used: PYR: 8xRDR, LEV: 1.5xRDR, IVM: 2xRDR, FBZ: 1/20xRDR; (RDR = recommended dose rate). In Study II a PYR-resistant, but LEV-susceptible isolate of *O. dentatum* was selected with PYR, LEV, FBZ, IVM and a line was passaged unselected. Selection pressure was adjusted to give FECR of approximately 80% in successive generations. In the last generation the following doses were used: PYR: 8xRDR, LEV: 1.5RDR, IVM: 1.5xRDR, FBZ: 1/20xRDR. Infective larvae from all generations of the lines were cryopreserved in saline at -80°C. The selected lines in the two studies were assayed using FECR and a newly developed whole worm muscular contraction assay (MCA) allowing determination of a minimum effective concentration and a agar matrix based egg/larval development assay (LDA).

Results Study I: Based on FECR, MCA and LDA susceptibility was significantly reduced in lines selected with PYR, LEV, IVM, but not FBZ. Reduced PYR and LEV-susceptibility developed in the LEV selected line whereas only reduced susceptibility against PYR was found in the PYR selected line. Reduced susceptibility to IVM was confirmed in the IVM selected line.

Study II: PYR-resistance developed slightly further in the PYR selected line and LEV-R started to appear. In the LEV selected line LEV-R started to appear, whereas PYR-R remained unchanged. Reduced susceptibility to IVM became evident after selection with this drug.

Conclusion: Resistance to LEV developed to similar levels in the two isolates irrespective of their resistance status at the start of selection. Resistance against levamisole and pyrantel seem to develop independently, although the two drugs are thought to share the same (acetylcholine) receptor. Reduced susceptibility to IVM was noticed after selection with this drug in both isolates. It was not possible to select for FBZ resistance in either isolates. This may be explained by a particular pharmacokinetic behaviour of this drug in the large intestine of the pig.



NEMATODE RESISTANCE TO ANTHELMINTICS IN SHEEP AND GOAT FARMS

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Sixty two sheep farms in the north east and 90 in south west England and 72 goat farms in England and Wales participated in a 1992 survey. Faecal samples were tested by a combination of Egg Hatch Assay (EHA), Larval Development Test (LDT), Faecal Egg Count Reduction (FECR) and after coproculture, by 'infected and treated' single lamb trials (SL). 14% sheep farms in north east and 44% in south west, and 65% goat farms showed resistance to benzimidazoles. One sheep (LDT and SL) and one goat farm (LDT, FECR and SL) tested positive for levamisole resistance as well as for benzimidazole. None of the farms tested showed resistance to ivermectin. The current results confirm those of an earlier study of benzimidazole resistance in southern England and in addition, record two cases of dual resistance.



THE GUINEA PIG AS A MODEL FOR STUDYING CLOSANTEL RESISTANCE

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The guinea pig has been used as a model for studying the immunological expulsion of intestinal parasites found in sheep, particularly *Trichostrongylus colubriformis*. This model was examined as a means of studying closantel resistance in *H. contortus*. Young outbred strains of worm free guinea pigs were infected by mouth with 2,000 exsheathed third stage larvae of susceptible or resistant strains of *H. contortus*. Three days after infection (DAI), the pigs were treated, euthanised at six DAI and the larvae recovered and counted. The effective dose to remove 95% of worms (ED95) was estimated by logit transformation of the data and modelling non-linear regression assuming a Poisson distribution of the data. In susceptible isolates, the percentage take varied from 7%-23%; the ED95 varied between 28.9-30.5 mg/kg which is 9-18 times the dose rate required in sheep to achieve the same level of efficacy. The ED95 in resistant isolates varied from 32.6 to 86.3 mg/kg; 1.22-2.97 resistance ratios (RR) (compared to the lowest ED95 to susceptible isolates). The RR values of isolates determined in sheep and the guinea pig were compared. In sheep the RR were up to five times greater than that found in the model with a consistent correlation between strains and between models. In the more resistant isolates the model was not able to differentiate between isolates as well as in the sheep. Good correlations were observed in isolates known to be moderately resistant. The guinea pig model has the advantages of using a less expensive animal that are easily bred, the trials can be performed in seven days and less larvae are required. Disadvantages include toxicity at higher dose rates and the inability to distinguish between more severely resistant isolates.



THE RESPONSE OF NATURALLY INFECTED RESPONDER AND NON-RESPONDER MALE GOATS TO ANTHELMINTIC TREATMENTS

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The aim of the study was to examine the effect of host responsiveness on the transmission of anthelmintic resistant nematodes. Two groups containing naturally infected entire male goats, previously categorised as 'responders' or 'non-responders' on the basis of their response to artificial and natural challenge, were used in the study. The goats had grazed on pastures carrying mixed infections, including anthelmintic resistant strains of *Teladorsagia*, from June 1992 to January 1993. The goats were housed on the first of February (Day 0) and treated with fenbendazole (FBZ, 10 mgs/kg). On Day 28 all animals were treated with ivermectin (IV, 200 µg/kg), on Day 49 they were treated with fenbendazole (10 mgs/kg) and levamisole (LEV, 12 mgs/kg) and the following day (Day 50) with ivermectin (200 µg/kg). Twelve weeks after their first treatment the animals were again treated with fenbendazole (10 mgs/kg). The 'responder' goats had lower faecal egg counts throughout the study, which were significantly different from Day 30, two days after treatment with ivermectin. Almost 90% of the eggs produced in this study came from the 'non-responders'. Efficacies resulting from treatment tended also to be lower in the 'non-responder' goats. Efficacies, calculated using arithmetic mean data 21 days after single and multiple treatments, were 46.8 (FBZ), 36.5 (IV), 0 (FBZ/LEV/IV) and 0 (FBZ) for the 'non-responder' goats compared to 63.5 (FBZ), 73.5 (IV), 0 (FBZ/LEV/IV) and 0 (FBZ) in the 'responders'.



INDIVIDUAL VARIATIONS IN GOATS OF FEBANTEL EFFICACY AGAINST *MUELLERIUS CAPILLARIS*

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Two hundred dairy goats originating from seven farms in Western France were treated individually with febantel (probenzimidazole drug) at the dose rate of 5 mg.kg^{-1} in Spring 1991. Faecal samples were collected on Days 0, 7 and 21 post-treatment and processed for first stage larvae of *Muellerius capillaris* and eggs of digestive tract strongyles. Blood samples were taken on Day 0 to assess pepsinogen concentration. Individual characteristics of the goats were recorded. The procedure was repeated on the same animals in Autumn 1991.

The faecal larval count depressions (FLCD) were similar on Days 7 and 21. Low FLCDs (<20%) were found in 40 (Spring) to 51% (Autumn) of goats and high FLCDs (>80%) ranged from 11 (Autumn) to 23% (Spring) of goats. The FLCD was mainly related to farm and season : Farm 1 in Spring and Farm 6 in Autumn showed a higher proportion of high responder (HR) goats (FLCD > 80%) whereas responses were better in Spring. At the opposite, the characteristics of the goats (breed, physiological status, age, weight, presence or absence of wattles and horns) as well as the initial parasitism (LPG, EPG, pepsinogen concentration) played a limited role on the variability of response to treatment. Goats could not be individually categorised according to their response level as the FLCD values obtained in Spring were not repeatable in Autumn, suggesting that other environmental parameters could be involved.

Further factors related to individual goat physiology and to *M. capillaris* between isolates susceptibility should be investigated.



**ABSTRACTS OF
SUBMITTED PAPERS**

**SESSION 4
Epidemiology I**



SEASONAL TRANSMISSION OF HEARTWORM (*DIROFILARIA IMMITIS*) IN DOGS EXPOSED TO INFECTION IN THE SOUTHERN UNITED STATES

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The seasonality of heartworm (*Dirofilaria immitis*) transmission was studied using tracer beagles to determine the timing, incidence, and intensity of transmission in selected areas of the southeastern USA. Three groups of five tracer (sentinel) beagles were exposed to potential infection during three consecutive four-month periods at each of three sites (Georgia, Florida, and Louisiana); an additional group (control) of five dogs exposed for 12 months was included at each site. Exposure for the control group and the first set of tracers was initiated in April 1988. After exposure, all dogs were held indoors for five months. Transmission occurred at all three sites during the periods of April to August and August to December. No transmission was evident at any of the three sites during the period of December to April of this project year or during the same winter period of the two subsequent years. Most (72%) of the annual transmission occurred from April to August, but some (28%) occurred from August to December. Based on worm measurements, heartworm antigen data, and microfilaremia data, most of the transmission occurred late in the April-to-August period and early in the August-to-December period at the three sites. The seasonal nature of transmission correlated well with an epidemiological model used to predict transmission based on ambient temperature. There was a higher intensity of infection in LA, compared with that in GA and FL. In LA, four of the five control dogs had heartworms (avg. 25.2, range 0-45); all of the dogs in GA (avg. 6.85, range 5-8) and FL (avg. 5.4, range 1-13) had worms.



THE EPIDEMIOLOGY OF *SCHISTOSOMA MATTHEEI* IN CATTLE IN ZAMBIA

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S *chistosoma mattheei* is widespread in cattle in Zambia. Results of *S. mattheei* faecal egg, miracidia and worm counts of calves, heifers and adult cattle in a highly endemic area near Lusaka, Zambia, over an eighteen month period are presented.

Transmission patterns in this focus are also considered. High faecal egg counts were found almost exclusively in calves. They decreased with age to almost zero in adult cows. There is no correlation between the faecal egg counts of *S. mattheei* and the worm burdens. The worm burdens increased with age of the host. The relative numbers of viable eggs and miracidia were highest in calves. Snail infection rates on the farm demonstrated a marked seasonal pattern. Based on these data, the development of immunity by cattle against *Schistosoma* is discussed.



EPIDEMIOLOGICAL STUDY ON LIVER FLUKE IN CATTLE IN FRANCE

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In the period between February 15 to March 15, 1993 to determine the prevalence of infection with *Fasciola hepatica*, blood samples were taken from cattle on 509 farms in 52 French departments. Although selected farms were in potential fluke areas, a criterion for farm selection was no flukicide use within the previous two years. Twenty cattle were selected on each farm. The 20 animals included 10 replacement heifers and 10 cows. Of the samples collected 54.6% were from dairy farms, 39.6% from beef farms and 6.1% from mixed farms. The samples from each farm were pooled into a heifer mix and a cow mix. Both pooled samples from each farm were then examined for the presence of antibodies induced by infection with *Fasciola hepatica*, using a serological method (ELISA).

Results of the ELISA tests indicated that liver fluke was present in all the departments covered by the survey. A serological response to *Fasciola hepatica* was detected on 73.5% of farms, suggestive of contact with the parasite during the second half of the 1992 grazing season. For dairy farms and beef farms, the percentages were 67.7% and 82.0%, respectively.

This survey has demonstrated that liver fluke infection in cattle is widespread throughout France and is prevalent on all types of farms.



EPIDEMIOLOGY OF FASCIOLOSIS IN MOROCCO

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A two year field survey was conducted in Morocco for snails that may be responsible for the transmission of *Fasciola hepatica*. Four *Lymnaea* spp. were identified: *L. truncatula*, *L. peregra*, *L. stagnalis* and *L. palustris*. The former two species were widely distributed throughout the country, the latter species were limited to the North and the Middle-Atlas. Natural infection with *F. hepatica* was detected only in *L. truncatula*. The snail habitats consisted of rivers, small streams, concrete canals, seepages, field ditches, swamps, lakes and springs. The recent extension of modern irrigation networks in agricultural areas has contributed to the creation and expansion of freshwater snail habitats in the country.

Snail activity of *L. truncatula* was monitored monthly in three selected habitats with different types of climate (Rabat, oceanic; Middle-Atlas, mountainous; and Haouz, arid). The prevalence and levels of infection with *F. hepatica* were assessed monthly in sentinel or slaughtered sheep. In the Haouz area, lymnaeid snails were active in their habitat throughout the year, but were more abundant during the hot months of summer (July and August). However, in Rabat and the Middle-Atlas, snail density was high in spring and autumn suggesting the occurrence of two snail generations. Snail populations correlated with fluke transmission as observed in sentinel or slaughtered sheep. In the Middle-Atlas, the fluke burdens in sentinel sheep reached a maximum of 302 in ewes and 345 in lambs in the winter. In the arid zone of Haouz, fluke infection in slaughtered sheep was high in winter and summer, and relatively low in spring and the beginning of autumn. In the same area, snails infected with *F. hepatica* were found in February, June, July and August; their infection rate did not exceed 3%.

These data enabled us to assume that the main periods for transmission of fasciolosis in Morocco were spring and autumn; however, potential infections in other periods of the year were possible depending on the type of climate prevailing in each region.



ASPECTS OF THE BIOLOGY AND EPIDEMIOLOGY OF *PSOROPTES OVIS* IN IRELAND

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A study was carried out involving examination of the infectivity and survival times of the mite off the host during the different seasons, interspecies transmission experiments involving goats and cattle, and a countrywide questionnaire survey of 272 outbreaks of psoroptic mange of sheep over two years.

Strains of *Psoroptes ovis*, from outbreaks of disease in Ireland were found to consistently remain infective off the host for 15 days. These mites failed to cause disease in either cattle or goats.

Questionnaire returns from vets indicated that over 70% of outbreaks involve addition of sheep, other factors included inadequate control measures, sources of purchase and poor fencing.

Only limited control was achieved by various topical applications. Mites, causing outbreaks of disease in Ireland, were effectively eliminated by two injections, ten days apart, of 1% ivermectin at 200 mcg/kg BW and by one injection of 1% moxidectin at 200 mcg/kg BW, however adequacy of dose rate was essential, even slight underdosing resulted in failure.



OVINE PSOROPTIC OTACARIASIS IN GREAT BRITAIN

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Seven cases of psoroptic otacariasis in Great Britain sheep flocks have been investigated by the CVL over the last three years. *Psoroptes (cuniculi?)* mites were isolated from the external auditory canal (EAC), of clinically normal sheep. The isolated mites did not initiate clinical scab on transfer to clean sheep. Further research at the CVL has shown that variations exist as regards virulence within strains of *P. ovis* infesting sheep in Great Britain, and that certain strains will colonise the EAC of heavily infested sheep, and that these in turn have the potential to survive plunge dipping. Further evidence has shown the *P. ovis* and *P. cuniculi* are variants of the same species, and that ovine ear mites are probably derived from body mites retreating into the ear. Psoroptic ear mites could therefore be possible reservoirs of sheep scab.



STUDY OF TICK POPULATIONS IN THE BASQUE COUNTRY (SPAIN)

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A work aimed to know the tick species present in the Basque Country was carried out between March 1992 and March 1993. 69 sampling areas selected on the basis of natural regions, abundance of hosts, specific vegetation, altitude, orientation, use, etc, were sampled at monthly intervals using blanket dragging. Blankets were used to capture active ticks on the vegetation and the procedure adopted was based on dragging at a slow walk for five minutes and then all ticks found were placed in tubes for subsequent identification in the laboratory. A total of 750 samplings were done and the most frequently found genus was *Haemaphysalis* (39% of the samplings), followed by *Ixodes* (33%). *Rhipicephalus* (2%) and *Dermacentor* (3%) were sporadically found. During the study period 10 different species were identified, and all of them represent potential vectors of pathogenic agents of animals and men. *Haemaphysalis punctata* and *Ixodes ricinus* were the species which showed the higher prevalence, and *Haemaphysalis inermis* was identified for the first time in the Basque Country. When data were analysed regarding natural regions clear differences in the presence and abundance of each genus were observed. From the perspective of seasonal activity, the higher active tick population appeared in spring and summer months, and although ticks could be present throughout the year, this time represents a higher infestation risk. *Ixodes* and *Haemaphysalis*, both adults and nymphs, were collected throughout the study period, but larvae in both genera showed a different pattern. Whereas *Haemaphysalis* larvae presented monomodal activity, with a maximum peak in August and total absence in winter, larvae of *Ixodes* showed two peaks in May and July, but even low levels could be found in autumn and winter. Immature stages of *Dermacentor* and *Rhipicephalus* were not found, and adults appeared in autumn-winter months and spring, respectively.



A SURVEY ON GOAT WARBLER IN IRAN AND EVALUATION OF ITS TREATMENT WITH IVERMECTIN

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This paper is dealing with a survey on the incidence of goat warbles in Iran. The prevalence of goat grubs was compared in four regions of fars province. The mean infestation rate was determined to be 93% and related economic loss from 1.6 million infested goats in the sampled area was estimated to be about 400 million rials. In addition, the seasonal variations of grubs in traced animals were determined and a picture of the life cycle of *Przhevalskiana* spp was sketched to some extent.

Our results clearly indicated that treatment with ivermectin is 100% efficient against goat warbles either being used at a dosage rate of 100 mcg/kg body weight by s.c. injection or at half dosage by pour-on administration.



EPIDEMIOLOGY AND CLINICAL EVOLUTION OF OVINE OESTROSIS IN SOUTH WEST FRANCE

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Epidemiological, serological and clinical follow-up of ovine oestrosis has been carried out for a few years in South West of France. Observations allow will more adequate adaptation of treatments in order to prevent infestations and consequently clinical disease.

Slaughter-house surveys indicated that three flying seasons occur in this region: February, May and August. Of course, according to annual weather variations, these events could be modified.

Serological follow-up by weekly ELISA titration indicated the same infection patterns. The more surprising is the winter antibody increase. It may be due to larval evolution and also reinfestations confirmed by increasing L1 populations.

Clinical observations revealed that no clinical signs are present during spring infection. But during summer's reinfestations they are apparent. Clinical score could be graded from serous to muco-purulent discharge. During winter, after a short remission the same nasal signs are seen. In the same time, lung lesions consecutive to progressive pneumonia stimulated by constant antigenic challenge may develop.

These observations allow a plan of two annual treatments with Closantel Drench or Ivermectin injectable. The first is administered during early summer and the second two months later in early autumn. Ovine oestrosis is more important than sheep breeders usually consider.



HAEMATOBIA IRRITANS: EPIDEMIOLOGY AND ECONOMICAL IMPACT ASSAY IN ARGENTINA

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H *aematobia irritans* was observed for the first time in Argentina in November 1991. Due to the lack of basic information on the horn fly in the central breeding area of this country, a trial was designed to study the epidemiology and economic impact in yearling cattle. From June 1992 and through to March 1993, four homogenous Groups of 20 animals each grazing together in a single paddock, received the following treatments: Group I, a benzimidazole drench every 30 days; Group II, Cyfluthrin 'pour-on' every 30 days; Group III, a benzimidazole drench and Cyfluthrin 'pour-on' every 30 days and Group IV, was kept as a non-treated control.

Group I calves, protected only against GI parasites, reached an average weight gain of 137 kg. Group II calves, protected only against flies, gained an average of 97 kg. Group III, protected both against worms and flies, gained an average of 131 kg, and Group IV (non-treated calves), gained 99 kg. Whilst both Groups treated against internal nematodes had a significant increase in weight gain ($P < 0.05$) in relation to the untreated control Group, no significant difference ($P > 0.05$) was observed between this Group IV and Group II, treated only against flies.

H. irritans populations appeared in October (spring), reaching a peak in February/March (late summer) in the control Group IV, when a mean of 140 flies was counted. Using fecal samples taken from the pasture in June 1992 (late autumn), flies born in the laboratory in November (late spring) showed that at least, a five month period of diapause was observed and can be expected, which is important for treatment strategies.



**ABSTRACTS OF
SUBMITTED PAPERS**

SESSION 5

Biology I





MOLECULAR CHARACTERISATION AS AN AID TO DETERMINING THE ZONOTIC POTENTIAL OF *GIARDIA* AND *CRYPTOSPORIDIUM*

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Cryptosporidium and Giardia are both protozoan parasites responsible for diarrhoeal illness in immunocompromised and immunocompetent individuals. Understanding the cross-transmission potential of these organisms is of great importance to the study of the epidemiology of both cryptosporidiosis and giardiasis, particularly with respect to the role of animals as sources of infection to humans. The lack of appropriate morphological markers and problems associated with *in vitro* amplification have made it difficult to define the zoonotic potential of both parasites. The application of molecular characterisation procedures, in particular, PCR-based techniques, has great potential for furthering our understanding of some of these problems. We have applied RAPD (Random Amplified Polymorphic DNA) analysis to both *Giardia* and *Cryptosporidium*. This technique uses single arbitrary primers to produce simple reproducible polymorphisms. As the technique is PCR-based, only minute quantities of material are required. Preliminary data suggests the potential for cross-transmission in both organisms is greater than originally suspected.



PHYLOGENETIC STATUS OF *NEOSPORA CANINUM* BASED ON 18S rDNA SEQUENCE COMPARISON

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N *eospora* - like parasites cause a variety of diseases in dogs, cattle, sheep, goats and horses. Until recently *Neospora* was frequently misdiagnosed as *Toxoplasma gondii* although they share little or no antigenic similarity. Even though the life cycle is not completely known, studies on the morphology of *Neospora caninum* have suggested that it is a cyst-forming coccidian. In order to more accurately define its phylogenetic position, we used the polymerase chain reaction to amplify the small-subunit ribosomal RNA gene and determine its sequence. The complete small subunit ribosomal RNA gene sequence of *N. caninum* was then aligned with those of the apicomplexans *Toxoplasma gondii*, *Sarcocystis gigantea*, *Cryptosporidium parvum*, *Babesia bovis*, *Theileria annulata*, a *Perkinsus* spp. and the free living dinoflagellate *Cryptothecodinium cohnii* as an outgroup.

Phylogenetic analyses by both distance matrix and parsimony methods showed that *Neospora* was unequivocally the sister-group to *Toxoplasma* even to the exclusion of *Sarcocystis*.

This study therefore definitively confirms the classification of *N. caninum* as a cyst-forming coccidian.



THE PHYLOGENY OF *NEOSPORA CANINUM* AND *TOXOPLASMA GONDII* BASED ON RIBOSOMAL RNA SEQUENCES

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During the last decade a protozoan parasite with close morphological resemblance to *Toxoplasma gondii* has been recognised as a cause of clinical illness in animals. The parasite causes paralysis and death in dogs and neonatal mortality and abortion in cattle, sheep, goats and horses. The parasite was characterised and named *Neospora caninum* in 1988. *N. caninum* was arranged in a new genus belonging to the family of *Sarcocystidae* together with the genera *Toxoplasma*, *Sarcocystis*, *Hammondia*, *Besnoitia* and *Frenkelia*. The purpose of this work was to study the phylogenetic relationship between *N. caninum* and *T. gondii*, by utilising sequence information from the small subunit ribosomal RNA (18S rRNA) from the two parasites.

N. caninum and *T. gondii* RH strain were purified from cell cultures and chromosomal DNA was extracted by standard methods. The coding regions for 18S rRNA were amplified by the polymerase chain reaction with a primer pair complementary to universally conserved regions. The forward primer was labelled with biotin. The PCR fragments were collected on magnetic beads coated with streptavidin for solid-phase sequencing.

The 18S rRNA sequences of *N. caninum* and *T. gondii* were aligned with the corresponding sequences of several other protozoan parasites. The alignment revealed a close homology between the two species. An evolutionary tree inferred from the rRNA sequences supported the close relationship between *N. caninum* and *T. gondii*.



HETEROGENEITY IN *EIMERIA ACERVULINA* OF BRITISH AND BANGLADESHI ORIGIN

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Comparative studies using field strains of *Eimeria* spp. of chickens are few. The prospect of having a vaccine against chicken coccidiosis, demands more emphasis on studies on the immunogenic heterogeneity. This paper describes a comparison of seven single oocyst derived field strains of *E. acervulina* isolated from Britain and Bangladesh, by morphometric analysis of oocyst, isoenzyme electrophoresis using thick layer starch gel, biological characteristics and for some pairs by in vivo cross-protection experiments. Regression coefficient of width on length of oocyst, which was observed to be a heritable trait, and analysis of variance showed a degree of intra-specific difference. Four of the seven strains showed a general tendency of parasitizing a more extensive part of the gut than the rest. *E. acervulina* (R) appeared to be more prolific and pathogenic than *E. acervulina* (B), but not from *E. acervulina* (M1). Analysis of six isoenzymes revealed two zymodemes. In vivo cross-protection experiments revealed strain variation on the criterion of oocyst output but not on weight gain. These results, using strains from two widely separated geographical areas, suggest a limited degree of heterogeneity between strains of *E. acervulina*.



BABESIA CANIS VOGELI*: DEVELOPMENT IN AND TRANSMISSION OF THE AUSTRALIAN STRAIN BY *RHIPICEPHALUS SANGUINEUS

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Since the first description of canine babesiosis in Australia in 1966, only limited studies on the disease have been made (Irwin and Hutchinson 1991). It is assumed that *Rhipicephalus sanguineus* is the primary vector as this tick is common in the tropics, and no species of *Dermacentor* have been recorded in Australia. This study aimed to confirm *R. sanguineus* as the vector and to determine the morphology and developmental sequence of *B. c. vogeli* in larvae, nymphs and adult ticks.

Experimental and field infections revealed that adult female ticks acquired parasites via alimentary infection, with subsequent development at low intensities in the haemolymph. Infection rates in eggs (transovarial infection) were as high as 90%. Multiple and binary fission stages were seen during egg incubation with peak infection of >200 parasites per high power field between 6-10 days post-oviposition (PO). Infection persisted vertically into F1 generation adults and F2 egg stages at 6 and 12 hours PO without alimentary reinfection. A variety of morphological forms were seen including 'Strahlenkorper'; haemolymph, egg and intrahaemocyte vermicules; binary and multiple fission bodies; oval gut forms; putative sporozoites, spherical intracellular haemolymph forms and possible cryptozoic intracellular forms in eggs and unfed larvae.

Initial transmission experiments used intact and splenectomised puppies seeded with F1 generation infected larvae, and re-attaching nymphs. This resulted in acute babesiosis with percent parasitised erythrocytes up to 15%, with a variable prepatent period. As no *B. c. vogeli* stages were identified in sections of the larvae it was concluded that nymphs were responsible for transmission. Further studies with unfed F1 ticks on two older intact puppies resulted in asymptomatic low parasitemias, with a prepatent period of six days, despite poor tick attachment. Attempts to achieve infection with F2 generation larvae were unsuccessful.

We conclude that *Rhipicephalus sanguineus* is a competent vector for canine babesiosis in northern Australia, and that persistently infected stages may maintain the organism in the absence of parasitemic carriers. Future studies should examine why, in the face of potentially high tick transmission rates, the disease is of low incidence and severity.

Reference: Irwin, P.J. and Hutchinson, G.W. (1991) *Aust. Vet. J.* **68**, 204-209.



NATURAL PARASITISM OF *B. MICROPLUS*: BIOCHEMICAL MARKERS, SKIN SURFACE LIPIDS, AND CHOLESTEROL IN SERUM ON CATTLE BIOTYPES

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Biochemical markers such as transferrins, hemoglobins and albumins, the fatty acid composition of the triglyceride fraction of the skin surface lipids, and cholesterol in serum were evaluated in different cattle biotypes naturally parasitized with *Boophilus microplus*. Sixty-five female heifers of 12 months old were chosen from a herd in Leales (27° 21' S 65° 18' W) as follows:

10 Nellore (N); 10 Creole (C); 9 C3: 50% C, 38% N and 12% Hereford (H); 10 H7: 34% N and 66% H; 10 B2: 50% N and 50% H (F2); 10 H6: 69% N and 31% H; 6 Cribú (Cr.): 50% C (F2) and 50% N.

Tick burden was evaluated counting the *B. microplus* females 4.5-8.0 mm in length on the left side of each heifer (Guglielmone A.A. *et al*) on five occasions from 12.01.87 until 03.30.88.

In two occasions on 12.01.87 and 03.03.88, blood samples and skin surface lipids from a body portion of 20 cm² were obtained.

Throughout the chromatographic method in three steps (Nicolaidis *et al*) the fatty acids myristic, pentadecanoic, palmitic, palmitoleic, stearic, oleic and linoleic from the triglyceride fraction of the skin surface lipids were evaluated.

Kristjansson - Quinteros and Braend Continuous techniques were employed to determine the phenotypes' albumins, transferrins and hemoglobins respectively.

The cholesterol was determined employing an enzymatic colourimetric method.

The tick burden was lower in N and increased with increasing of C and H genes.

The highest average of cholesterol values was found in Cr. in coincidence with the highest values of estearic ac.

In the case of linoleic ac. the B2 group had the highest value but with an average of cholesterol intermediate between C and N expressing no correlation between them. There have been different genetic frequencies of the biochemical markers related to the characteristics of each breed.

The possible correlation of all the values is discussed.



ODOURS PRODUCED BY SKIN BACTERIA ATTRACT *WOHLFAHRTIA MAGNIFICA*

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In Hungary 35-50% of sheep in a flock can be infested by *Wohlfahrtia magnifica*. Satisfactory prevention measures to stop development of the disease are expected. Possible shifts in skin microbial communities, hereby production of volatile fly attractants by bacteria seem to be in direct connection with stimulation of larviposition. Bacterial communities of healthy adult sheep vulval skin, myiatic lesions, and *W. magnifica* intestines were investigated and compared by numerical taxonomic methods. Dominant gut, and wound colonising bacteria were tested for fly attractance in field experiments by electric net traps. The gut bacterial communities of *W. magnifica* are dominated by coryneforms and pseudomonads. Members of the Enterobacteriaceae family were not detectable in hindgut of 3rd instar larvae. Increase of the population density of *Brevibacterium* sp. in wounds, and fly hindgut communities was observed, as compared to healthy skin. Among different aged cultures of *Brevibacterium* sp., *Bacillus* sp., *Pseudomonas* sp., etc., two days old *Brevibacterium* sp. cultures attracted significantly more flies, especially *W. magnifica*. *Brevibacterium* species produce malodorous volatile components (like thiols) and *B. linens* could be repeatedly isolated from lesions in poultry and man. Our preliminary results corroborate the supposition, that *Brevibacterium* sp. can be responsible for the *Wohlfahrtia magnifica* wound infestation.



TARGET ORIENTED BEHAVIOUR OF *WOHLFAHRTIA* *MAGNIFICA* AND *LUCILIA SERICATA*, AGENTS OF MYIASIS, IN HUNGARY

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The target-oriented behaviour of adults of the myiasis species *Wohlfahrtia magnifica* and *Lucilia sericata* was studied in the field: such targets have potential both for population monitoring and control. All studies were conducted near to Sárbogárd, Hungary, where incidence of *W. magnifica* myiasis in sheep (N>800) varied over a 14-day period from 23-39%: 52 of 112 infested sheep (46.4%) had multiple infections, from >1 larviposition.

Experiments with electrified, cloth targets (baited with a potent screwworm attractant, Swormlure-4) demonstrated that: 1) a black target was most attractive for both *W. magnifica* and *L. sericata*, with other colours in the following order of effectiveness, blue>white>yellow; 2) larger targets (1.0x0.5 m) caught approximately twice as many flies of both species as smaller targets (0.5x0.5 m); 3) *W. magnifica* did not respond to Swormlure-4 in the strong manner that *L. sericata* did. The sex ratios of *W. magnifica* caught on targets (67.2% males) and hand-netted from fence posts (68.8% males) were similar and biased towards males, while that of *L. sericata* on targets was strongly biased towards females (15.6% males), indicating a fundamental difference in the response of these two myiasis species towards the baited targets: *W. magnifica* responded towards them as 'waiting stations' for mate seeking, *L. sericata* responded towards them as oviposition sites.



SURVEY FOR THE PRESENCE OF NEMATODE-TRAPPING FUNGI IN FRESH FAECES OF GRAZING LIVESTOCK IN AUSTRALIA

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Recent research has demonstrated the potential of nematode-trapping microfungi as an alternative or supplement to anthelmintic treatment in the control of nematode parasites of ruminants. The aim of this survey was to screen faecal samples of livestock, mainly sheep, in Australia for nematode-trapping fungi. The isolates obtained were further tested to confirm their ability to survive passage through the gastro-intestinal tract of sheep. During a 12 month period 1742 fresh faecal samples were examined, whereby a few grams of faeces were spread onto water agar plates, to which approximately 2,000-4,000 infective larvae were added as bait. The plates were inspected once a week for three weeks and nematode-trapping fungi present were isolated. To confirm the ability of the isolates to survive passage through sheep a single daily dose of approximately 2.4×10^6 fungal units was given to *Trichostrongylus colubriformis* infected animals and the subsequent reduction in number of infective larvae in faecal cultures was recorded. During the year, 48 fungal isolates were recorded, from sheep (36), cattle (6), horse (4) and goat (2). Of these 16 were identified as *Duddingtonia flagrans*, whilst the remainder were *Arthrobotrys* spp. Out of 11 *Arthrobotrys* spp. and nine *D. flagrans* isolates tested by oral dosing of sheep only *D. flagrans* reduced the number of infective larvae by 90% or higher. This survey showed that nematode-trapping fungi are acquired naturally and survive passage through livestock. Isolates of the species *D. flagrans* appear as very promising candidates for future experiments with fungi as biological control agents against trichostrongyles in livestock.



**ABSTRACTS OF
SUBMITTED PAPERS**

SESSION 6

Genetic Basis of Host Resistance



DIFFERENCE IN SUSCEPTIBILITY OF SUFFOLK AND LOUISIANA NATIVE SHEEP TO GASTROINTESTINAL NEMATODE PARASITISM

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The epidemiology of nematodiasis (specifically haemonchosis) in Suffolk and Louisiana Native sheep was investigated at Louisiana State University over a five year period. During the last 2.5 years (April 1989 through September 1991) of that investigation the two breeds were maintained together on the same pasture system to ensure similar exposure for both breeds. Fecal egg counts (FEC) and blood packed cell volume (PCV) were determined at two week intervals. Anthelmintic treatment was administered to individual animals when PCV dropped below 15 and/or increasing FEC with clinical signs. Periodically from 1988 through 1990 two age matched lambs of each breed were used as tracer lambs to evaluate relative differences in nematode burdens acquired under equal exposure. Overall mean FEC and PCV were consistently and significantly higher and lower, respectively, for Suffolk mature ewes (882 EPG and 23.3, $n=15/\text{yr}$) than for Louisiana Native mature ewes (80 EPG and 27.0, $n=15/\text{yr}$). Overall mean FEC and PCV were consistently and significantly higher and lower, respectively, for Suffolk ewe lambs one to nine months of age (2,083 EPG and 20.9, $n=5/\text{yr}$) than for Louisiana Native ewe lambs one to nine months of age (763 EPG and 30.2, $n=5/\text{yr}$). The number of anthelmintic treatments administered to Suffolk and Louisiana Native mature ewes and ewe lambs was 53 and 50, and 0 and 6, respectively. Overall mean FEC and nematode count for Suffolk ($n=47$) and Louisiana Native ($n=46$) tracer lambs were 2,693 EPG and 15,011, and 713 EPG and 2,045, respectively. The results of this study indicate that Louisiana Native sheep are more resistant to nematodiasis than Suffolk sheep.



BREEDING PERENDALE SHEEP WITH RESISTANCE OR SUSCEPTIBILITY TO INTERNAL PARASITES FOLLOWING EXPERIMENTAL INFECTION

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Selective mating of Perendale sheep to generate lines with increased or reduced susceptibility to *Haemonchus contortus* and *Trichostrongylus colubriformis* following experimental challenge commenced in 1986. Five years later changes were assessed by monitoring faecal nematode egg count (FEC) of selected breeding ewes and their progeny. In the absence of drenching, mature flock ewes were monitored four times during the periparturient period. Ewe and ram lamb progeny were screened on five occasions between weaning (November 1990) and May 1991. Lambs were given anthelmintic at sample dates. Progeny were run as single sex mobs subsequent to weaning. All stock were grazed across pasture contaminated previously with mixed parasite populations.

Mature ewes selected as lambs with Low FEC shed lower numbers of eggs than High FEC line ewes two weeks pre- and three, six and nine weeks post-parturition. At the periparturient peak, approximately three weeks after lambing, Low FEC line ewes were shedding over three times fewer nematode eggs than their High FEC line counterparts.

Lambs from the Low FEC line had significantly lower FECs on four or five sample dates ($P < 0.01$). At the final sample date (April/May), there was a 10-fold difference in mean FEC. Although ram lambs had significantly higher egg counts than ewes differences between lines were consistent with the selection criteria. Bulk faecal cultures demonstrated that *Haemonchus* and *Trichostrongylus* were the dominant genera.



HOST RESPONSES TO HELMINTH INFECTIONS OF SHEEP SELECTED FOR ENHANCED RESISTANCE TO HAEMONCHOSIS

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The aim of the study was to examine the parasitological and immunological responses to infections with *Trichostrongylus colubriformis* and *Ostertagia circumcincta* of sheep with genetic resistance to *Haemonchus contortus*. Groups of genetically resistant and random-bred lambs were infected with 20,000 *H. contortus*, *T. colubriformis* or *O. circumcincta* larvae and their faecal egg counts, worm burdens, packed cell volume, peripheral eosinophilia, anti-parasite antibody and mucosal mast cell responses monitored. *Haemonchus*-resistant lambs exhibited higher resistance to *Trichostrongylus* and *Ostertagia* than random-bred sheep. Resistance to challenge infection was accompanied by mucosal mastocytosis and higher levels of antibodies against antigens of larval parasites. Resistant lambs consistently showed lower eosinophil counts than random-bred lambs following infection with all the three parasites and there was no correlation between peripheral eosinophilia and resistance to challenge infection. Together, these results suggest that selection for resistance to *Haemonchus* also results in enhanced resistance to other gastrointestinal nematodes.



INDIVIDUAL VARIATION IN THE RESPONSE TO THE ABOMASAL NEMATODE *OSTERTAGIA CIRCUMCINCTA*

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Twenty four, six month old, female Scottish Blackface sheep with the same history of exposure to nematode infection were given a single subclinical dose of 50,000 infective larvae of *O. circumcincta*, monitored for eight weeks, then slaughtered. There were strong correlations between egg count, plasma pepsinogen, peripheral eosinophilia and worm burden. There was also a strong negative association between worm burden and growth rate. There was substantial variation among sheep in the numbers of mast cells, globule leucocytes, IgA positive plasma cells and eosinophils in the abomasum. There were strong correlations between cell numbers in the fundus and in the pylorus, although in general concentrations were higher in the pylorus. There were also correlated responses among the different cell types. There was substantial variation among sheep in the IgA or IgG1 responses in the plasma or the abomasal mucus to a somatic extract of 3rd stage larvae and to a somatic extract or excretory secretory antigen preparation from 4th stage larvae. There was a significant association between high levels of mucus IgA specific for 4th stage larvae and reduced adult worm length. There were also significant associations of increased number of IgA positive plasma cells and globule leucocytes with reduced adult worm burdens. The most resistant sheep had the highest globule leucocyte concentrations. The most susceptible sheep had the lowest IgA concentrations.



ARTIFICIAL INFECTIONS OF CALVES WITH *COOPERIA ONCOPHORA* TO DETECT GENETIC RESISTANCE TO GASTROINTESTINAL NEMATODES

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Analysis of the data from more than 470 calves, most of them belonging to paternal halfsib groups, showed that infection with a single dose of 100,000 *Cooperia oncophora* L3 larvae given to three months old, male, Friesian calves offers a good model for detection of genetical differences.

Within this model faecal egg counts (FEC) between Day 28 and Day 42 are found to display the largest between sire variation. At this stage of infection FEC also correlates best with the antibody response (AB).

It is suggested that using this infection model and examining both parameters (FEC and AB) it should be possible to select young sires for resistance against G.I. nematodes.



FAECAL EGG COUNT, ANTIBODY TITRE AND LEVEL OF EXPOSURE TO INFECTION IN CALVES INFECTED WITH GASTROINTESTINAL NEMATODES

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In two trials in which several levels and patterns of natural infection with *Cooperia oncophora* and *Ostertagia ostertagi* were artificially stimulated, the relationships between faecal egg count (FEC), IgG antibody titre and level of infection were investigated. In total, 94 female Friesian calves were used. These calves were at the start of the trials on average 4.5 months old.

Between groups of calves, both faecal egg count and antibody titre were positively correlated with the level of exposure. Within each group of calves, i.e. within each level of exposure, FEC and antibody titre were significantly negatively correlated. The latter finding indicated a genetic component in the host response to infection which is influencing both FEC and antibody titre. This negative correlation between FEC and antibody titre was over a wide range independent of the level of exposure to infection.

Although no prior selection was made to obtain balanced paternal half-sib groups in these trials, some sires could be identified having more than one offspring. Analysis of the sire effect revealed some sires possibly inheriting susceptibility and others possibly inheriting resistance to gastrointestinal nematode infection.

It is suggested that herd mean antibody titres and herd mean FEC can estimate the level of exposure to infection. However, within herds antibody titre and FEC are correlated negatively with each other independent from the level of exposure, indicating genetic differences among calves in their responsiveness to infection.



**ABSTRACTS OF
SUBMITTED PAPERS**

**SESSION 7
Helminths - Antigens
and Immunity**





THE SECRETORY ACETYLCHOLINESTERASES OF *Dictyocaulus viviparus*

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Natural infection with the cattle lungworm, *Dictyocaulus viviparus*, induces a strong acquired immunity. Although a vaccine based on irradiated larvae has been used successfully for thirty years, little is known regarding the mechanisms of protective immunity in this infection. Studies were designed to investigate the potential significance of excretory/secretory (ES) products released by adult parasites in the development of immunity in parasitic bronchitis. *D. viviparus* ES materials, which induced up to 85% protection against challenge in guinea pigs, were found to contain a heterogeneous mixture of polypeptides. Acetylcholinesterase (AChE) activity was detected in the adult ES and immunoglobulin, purified from the serum of infected and vaccinated calves, bound and/or inhibited these enzymes. This was indicative of their *in vivo* release and suggested that parasite AChE might have a role in the immunobiology of this infection. One possibility is that AChE may be involved in evasion of the host immune response by increasing the breakdown of acetylcholine which is known to stimulate several immune effector mechanisms. Secretory AChE's were purified by affinity chromatography to permit their more detailed characterisation and to facilitate an evaluation of the host responses to these enzymes. The information gained from these studies has provided interesting insights into interactions which may be occurring at the *D. viviparus*: host interface.



IMMUNIZATION OF SWINE WITH ESP FROM *TRICHURIS SUIS* ADULT WORMS INDUCES RESISTANCE TO A CHALLENGE INFECTION

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Trichuris suis is a nematode infection of swine which is highly pathogenic, causing anemia, weight loss, anorexia, mucohemorrhagic diarrhea, and death in heavy infections. Acquired resistance to trichuriasis is inferred from the fact that infective eggs are ubiquitous and long lived, but infections are mainly observed in growing pigs. There are few reports on immunity to *T. suis* in swine. The purpose of this study was to determine the effects of immunization of pigs with *T. suis* adult excretory/secretory products (ESP) on the development of resistance to *T. suis* infection. Procedures were developed for the in vitro cultivation of adult *T. suis* that result in the production of culture derived ESP. Pigs were immunized against a combined experimental inoculation and natural exposure. Four groups of 8 pigs each were injected at day 0 and 7 of the experiment as follows: 1) control (uninjected); 2) 2 mg ESP in FCA i.m., then 1 mg ESP in IFA i.p. (high dose Freund's); 3) 2 mg in alum i.p., then 1 mg ESP in alum i.p. (high dose alum); 4) 0.6 mg ESP in alum i.p., then 0.3 mg ESP in alum i.p. (low dose alum). All pigs were challenged with 2000 eggs/kg body weight and then placed on a dirt lot contaminated with *T. suis* eggs for 52 days. Immunized pigs had increased serum IgG, IgA and IgM antibodies to ESP. Control, unimmunized pigs had 2205 ± 465 *T. suis* adults recovered at necropsy, while immunized pigs had adult recoveries reduced by 31% in the high dose Freund's group, 86% in the high dose alum group, and 94% in the low dose alum group. In addition, immunization with ESP completely eliminated the pathology (weight loss, diarrhea, dehydration) normally associated with trichuriasis in swine. Fractionation of the crude ESP into high, medium and low molecular weight fractions and immunization of pigs with these fractions in alum as above resulted in identification of the medium fraction as that which contained the protective antigen. This fraction contained a highly active zinc metalloprotease which has been characterized.



EVALUATION OF ASPECTS OF THE PROTECTION AFFORDED TO SHEEP IMMUNISED WITH A GUT MEMBRANE PROTEIN OF *HAEMONCHUS CONTORTUS*

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Experiments were undertaken to evaluate the type of protection conferred against *Haemonchus contortus* by immunising sheep with H11, a 'hidden' integral gut membrane protein isolated from this parasite.

A serial kill experiment showed that worms began to be lost from immunised lambs between 7 and 14 days after challenge with a single dose of larvae, and adult females were more susceptible than male worms. Seven day old juveniles survived immunisation even though their intestinal cells became coated with sheep antibody. Immunisation was equally effective against both benzimidazole resistant and susceptible strains of *Haemonchus*. When immunised lambs were subjected to a trickle infection, they were largely protected against anaemia and egg output observed in the challenge controls. Moreover they grew fast and as efficiently as uninfected lambs fed on the same high protein diet and acquired a natural immunity during the course of the trickle infection.

It was concluded that if protective antigens like H11 can be reproduced by recombinant DNA techniques, the prospects for a vaccine containing gut membrane proteins are promising for blood feeding nematodes like *Haemonchus*. The finding that the artificial immunity generated by vaccination is likely to be replaced by natural immunity before vaccine immunity wanes, suggests that frequent boosting may not be necessary.



CLONING, SEQUENCING AND EXPRESSION OF H11, A HIGHLY PROTECTIVE MEMBRANE PROTEIN ANTIGEN FROM *HAEMONCHUS CONTORTUS*

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Lambs vaccinated with the 110kDa microvillar integral membrane protein H11 isolated from the parasite's intestine, are highly protected against challenge infections with *Haemonchus*. Levels of protection correlate with specific antibody levels. cDNA expression libraries derived from UK and Australian worms were immunoscreened with antibodies to H11 from vaccinated sheep. Clones of three small positive inserts were obtained. They were used to select longer, overlapping, inserts by hybridisation. All the clones hybridised to mRNA of 3.5kb, a size compatible with coding for a 110kDa glycoprotein. Partial and full-length PCR products have been generated and sequenced. N-terminal amino acid sequences obtained from proteolytic and cNBr fragments of H11 match the predicted amino acid sequence. The enzymatic function of H11 deduced from sequence identity has been confirmed by assay and specific inhibitor studies. The activity is localised in the intestinal microvilli, co-purifies with H11 and is inhibited by serum antibodies from vaccinated animals. The full-length PCR product has been expressed in active form in the baculovirus-Sf9 insect cell system.



THE INFLUENCE OF AN INHIBITED L₄ *OSTERTAGIA* *OSTERTAGI* POPULATION IN CALVES ON THE DEVELOPMENT OF IMMUNITY

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The aim of the present study was to determine if the presence of an inhibited L₄ *O. ostertagi* population during stabling (winter), influenced acquired immunity in heifers the second grazing season.

Twenty calves were divided into five groups. Four groups (A-D) were grazed on heavy infected pastures for five months, the fifth group was kept as uninfected control group (Group E).

At housing (October) calves of Group A were necropsied for wormburden determination and the calves of Group C were treated with ivermectin to eliminate the present worm population. At the end of the housing period Group B was also necropsied for worm counts. The remaining three groups were treated with oxfendazole and turned out on the same pasture as the previous year for a challenge period of four weeks, after which all animals were necropsied. At monthly intervals faecal egg counts, pepsinogen, and *Ostertagia* antibodies were determined.

At housing a geometric mean wormburden of 80,000 *O. ostertagi* was recovered from Group A calves with 85% at the EL₄ stage. During the stabling period only a reduction of 26% of the total *O. ostertagi* wormburden was observed, with still 78% at the EL₄ stage. However, the number of developing stages in Group B was significantly higher than in the Group A calves.

The *O. ostertagi* wormcounts of the three remaining groups together with the pepsinogen values, egg counts and *O. ostertagi* antibody levels are discussed in relation to acquired immunity.



DEVELOPMENT OF RESISTANCE TO *HAEMONCHUS* *CONTORTUS* BY SAANEN GOATS

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General opinion is that unlike sheep, goats do not develop an effective resistance to infection by nematode parasites. An experiment was carried out to determine if Saanen kids can acquire resistance to *Haemonchus contortus*. Twenty-five buck kids reared without exposure to nematodes were randomised into five similar groups on the basis of source, age and liveweight. Each kid was given 350 infective larvae per kg liveweight 0, 4 and 15 weeks into the study. Infections in Group ND were not abbreviated with anthelmintic. Those in the DD group were abbreviated with levamisole (LEV) prior to each challenge (three and 14 weeks). Kids in the PD group were treated only at three weeks and those in the SD group only at 14 weeks. Animals in Group C were infected and slaughtered four weeks later to determine the infectivity of the parasite culture whereas all other animals were killed 10 weeks after the last challenge. Mean numbers of worms recovered did not differ between the groups. Establishment rate for the primary infection (Group C) was 24.0%. Based on the size of the challenge after anthelmintic abbreviation recovery rates were 7.0%, 20.0%, 17.1% and 15.2% for ND, DD, PD, SD, respectively. The tendency was for lower male:female ratios when infections were not abbreviated 0.42 versus 1.38, 0.90 and 1.06. Results from this experimental study suggest that existing worm burdens may be required to regulate establishment of newly ingested larvae. Populations appear to be self-regulating. This can be one explanation for the continued susceptibility of goats and the dependency farmers have on anthelmintics to control nematode parasite burdens.



THE USE OF FLOW CYTOMETRY TO CHARACTERISE THE RESPONSIVENESS OF PERITONEAL LEUKOCYTES TO *FASCIOLA HEPATICA*

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Passive immunity to *Fasciola hepatica* can be induced in rats by giving them sera from infected sheep which are themselves not immune. Therefore, it appears that in sheep it is the cellular effector mechanisms rather than the humoral immune mechanisms which are absent or dysfunctional. We have been developing a method using flow cytometry to characterise the oxidative responsiveness of individual peritoneal cells from rats and sheep. In a flow cytometer peritoneal cells have been separated on the basis of forward and right angle light scatter. Oxidative responsiveness of individual cells was then determined using dihydrorhodamine which is a dye that becomes trapped in the cytoplasm of leukocytes and becomes fluorescent upon oxidation by reactive oxygen intermediates. Granulocyte and monocyte populations from infected rats and sheep were heterogeneous in their response to fluke antigens. Additionally, in rats following infection there is a greater percentage of peritoneal granulocytes which are highly fluorescent in response to fluke antigens. We have still to determine whether this also occurs in infected sheep. Flow cytometry will enable us to characterise the immune responsiveness of individual leukocytes in an attempt to identify differentiation in the cellular effector mechanisms of sheep and rats.



COMMON ANTIGENICITY BETWEEN *DIROFILARIA IMMITIS* AND THE THREE INTESTINAL NEMATODES OF THE DOG

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Antigenicity of *Dirofilaria immitis* was compared with that of *Toxocara canis*, *Ancylostoma caninum*, and *Trichuris vulpis* by SDS-PAGE and immunoblotting. Somatic proteins of adult worms were separated into 44, 48, 38, and 30 bands by Coomassie blue staining, respectively. Among them, 11 bands in *D. immitis*, 19 bands in *T. canis*, 6 bands in *A. caninum* and two bands in *T. vulpis* were specific to each species. The total number of common bands was 25. Among them, 18 bands were common to two species, five bands were common to three species, and two bands were common to all species, in terms of molecular weight.

The antigenic bands of these extracts were then analysed by immunoblotting, using mouse sera immunised with the extract of *D. immitis*. The immunoblotting showed 26 bands in *D. immitis*, 20 bands in *T. canis*, 21 bands in *A. caninum*, and 25 bands in *T. vulpis*. Among them, molecular weights of nine bands were common to two species, three bands were common to three species. However, no band was common to all species.

This study reveals that *D. immitis* possess a partial common antigenicity to *T. canis*, *A. caninum*, and *T. vulpis*, although it is uncertain whether this cross-reactivity may be dependent on a single antigenic epitope or several epitopes. Such a cross-reactivity is also demonstrated by means of immunoblotting and fluorescent antibody technique with monoclonal antibodies produced from *D. immitis* extract. From these results, it is conceivable that these four nematode species may be originated from some unique nematode species and may still possess partially a common antigenicity throughout their biological evolution and adaptation.



THIRD STAGE LARVAL SURFACE ANTIGENS OF *STRONGYLUS VULGARIS*

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Vaccination of ponies with irradiated L₃ but not extracts of adults or larvae induces protective resistance to challenged infections. Antibodies in serum of protected but not sensitised and susceptible individuals react with the surface of *S. vulgaris* L₃ but not *S. edentatus* L₃. Antibodies from both groups react with L₄ of these parasites. To further define antigens on the surface of *S. vulgaris* L₃ larvae, a panel of monoclonal antibodies was prepared which bind to molecules exposed on the surface of the worm. All the monoclonals are specific for *S. vulgaris* L₃ and did not bind to *S. vulgaris* L₄ or to L₃ or L₄ of *S. edentatus*. The molecules recognised by the antibodies fall into two groups: those present immediately after exsheathment of the larvae which are lost within two days in culture and molecules present on the L₃ larvae which are expressed until the molt to the fourth stage larvae. Some antigens could be removed from the surface of the worm by treatment with the detergent CTAB, while others could not. Observations indicate that a dynamic change in L₃ cuticular antigen expression occurs and that antibody recognition of these antigens may be important in the protective immune response observed.



IDENTIFICATION AND PURIFICATION OF AN *OSTERTAGIA OSTERTAGI* SPECIFIC ANTIGEN FROM ADULT WORMS AND ITS APPLICATION IN AN ENZYME LINKED IMMUNOSORBENT ASSAY

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Total extracts from adult *O. ostertagi* were studied by Westernblot analysis to identify species specific antigens. Two antigens with apparent molecular weights of 19.7 (OA19.7) and 20.7 kDa (OA20.7) were recognised only by serum from *O. ostertagi* infected calves. The purification of OA19.7 was achieved by different subsequent chromatographic separations, i.e. gelfiltration, ion-exchange and reversed phase chromatography. Heterologous sera from *Cooperia*, *Dictyocaulus*, *Nematodirus* and *Fasciola* monoinfected calves did not cross-react with the purified OA19.7. The use of this antigen in an enzyme linked immunosorbent assay resulted in an increased specificity of the antibody detection test, when compared to total worm extracts. However, strong individual differences in antibody response were observed between animals which received the same *Ostertagia* infection dose. The anti-OA19.7 response started six to eight weeks after initial infection, and reached its highest level at week 15.



**ABSTRACTS OF
SUBMITTED PAPERS**

**SESSION 8
Helminth Chemotherapy I**





ASSESSMENT OF THE FLUKICIDAL ACTION OF RAFOXANIDE AGAINST IMMATURE FLUKES BY THE USE OF ANTIPYRINE CLEARANCE TEST AND LIVER ENZYME ACTIVITY MEASUREMENTS

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The effect of rafoxanide against 4-week old *Fasciola hepatica* was tested in sheep by measurement of antipyrine plasma clearance, glutamate dehydrogenase (GLDH) and gamma-glutamyl transferase (γ GT) activities bi-weekly up to 14 weeks post-infection. Three animal groups were used. In two groups, sheep were infected with 200 metacercariae each, while in the third group sheep were left uninfected. Four weeks after infection, one of the two infected groups and the uninfected group received rafoxanide at a recommended dose rate. GLDH activity fell dramatically two weeks after treatment but increased again at 12 weeks post-infection in the infected treated sheep. Antipyrine clearance decreased between eight and 14 weeks post-infection in untreated sheep. In the infected-treated sheep plasma clearance of antipyrine remained unchanged until 10 weeks after treatment when it decreased from the pre-infection value of 5.09 ml.kg/min to 3.90 ml.kg/min. Rafoxanide did not affect antipyrine disposition in the uninfected sheep. Infections were confirmed by post-mortem examination of the livers. It was concluded that rafoxanide acts at an early stage of *Fasciola hepatica* infections as evidenced by the unchanged clearance of antipyrine and the decline of GLDH activity during the pre-patent period of the infection. However, changes in these parameters which occurred later in the course of infection indicate that rafoxanide may not kill immature flukes but temporarily inhibit their development.



ADDITIVE ACTION OF TWO FASCIOLICIDES IN CRIOLLO SHEEP

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With the aim to determining the synergistic effect of combinations of fasciolicides, 55 criollo sheep were infected with 20 metacercariae of *Fasciola hepatica* given at weekly intervals for 13 weeks. At week 14, the sheep were divided into 11 groups of five animals each and the corresponding treatments given, with one remaining as controls. The flukicides used were Diamphenetide, Rafoxanide and Albendazole, all of which were administered at the manufacturer's recommended dose and half this dose, both in combined form and as single treatments. Two weeks after treatment all animals were slaughtered and the number of immature and adult flukes present in the liver counted to evaluate the percentage of *Fasciola* reduction relative to the control group. The best combination was Diamphenetide and Rafoxanide given simultaneously at half the recommended dose, 50.0 and 3.7 mg/kg respectively, removing 98.1% immature and adult flukes.



ORAL EFFICACY OF TWO NOVEL PYRROLES AGAINST MATURE AND IMMATURE *FASCIOLA HEPATICA* IN SHEEP

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Two 2,4,5-tribromo-3-carbonitrile pyrroles that showed antiluke activity *in vitro* and in rats were evaluated against induced mature and immature *Fasciola hepatica* infections in sheep. Following infection with 200 *F. hepatica* metacercariae, sheep were divided into 10 treatment groups of eight sheep each. Five groups were treated at two weeks and five at 12 weeks post-infection. Clorsulon at seven mg kg⁻¹ was the positive control. All sheep were necropsied at 14 weeks post-infection and remaining flukes counted. Efficacy results are tabulated below.

Drug	Oral Dose mg kg ⁻¹	Remaining Flukes (Median)		Percent Efficacy	
		2 Wk	12Wk	2 Wk	12 Wk
AC301814	1.7		2*		93
	3.3	17		39	
	5.0		0*		100
	10.0 ^a	27		4	
AC322706	3.3	17	0*	39	100
	10.0	6	0*	68	100
Clorsulon	7.0	29.5	0*	0	100

* Significantly different from controls ($P < 0.05$)

^a Four of eight sheep died following dosing.

The 18 untreated control sheep had a median fluke burden of 28. The compounds represent a new class of flukicides with activity against *F. hepatica*.



EFFICACY OF FLUKIVER (JANSSEN) AGAINST *FASCIOLA HEPATICA* IN NATURALLY AND EXPERIMENTALLY INFECTED SHEEP

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The studies were carried out on 150 ewes naturally and on 14 lambs (17-21 kg) experimentally infected with 200 metacercariae. Flukiver (5% solution) was administered subcutaneously as a single 5 mg/kg dose to the infected animals. The ewes were treated before pasturage and were divided into three groups: I control - nontreated during pasture season; II group - treated eight weeks after beginning of the pasture season and III group - treated twice, eight and 15 weeks after beginning of the pasturage. The lambs were divided into two groups: I - control (infected non-treated) and II - experimental (infected, treated). The lambs were treated six weeks after infection. The extent as well as the intensity of *F. hepatica* infection was established by using the sedimentation method. The efficacy of Flukiver on gastro-intestinal nematode infection was estimated the basis of coprological examination (Willis-Schlaaf method) and on larvae culture. Flukiver was 98% effective on adult *F. hepatica* infection of ewes and the infection rate of gastro-intestinal nematodes was reduced in 65% of ewes. After treatment of seven experimentally infected lambs only 8% only two were single flukes (4,5,) found in the liver. Only 8% of ewes in group III were infected after the pasture season. Flukiver is a valuable drug, very useful for strategic control of ovine fasciolosis. This drug is highly effective against adult and immature *F. hepatica* stages as well against some important haematophagous nematodes.



PROTECTION OF PONIES FROM EXTENDED EXPERIMENTAL INFECTIONS OF CYATHOSTOMES BY STRONGID-C AND EVIDENCE FOR ACQUIRED RESISTANCE TO THE LATE L₃-L₄ MUCOSAL LARVAE

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The efficacy of daily pyrantel-tartrate (Stongid-C) at the recommended dose in reducing total cyathostome numbers and associated pathology was tested using 16 ponies previously exposed to parasites. Ponies were divided into three groups. A fourth group of four, age matched ponies reared under parasite free conditions was also used. Two weeks prior to initiation of experimental infections all ponies were treated with ivermectin at 0.2 mg/kg body weight, and daily for five days with oxibendazole at 20 mg/kg body weight. Necropsies were performed on three ponies on day 0. The remaining ponies in this group were not experimentally infected and were necropsied at the end of the experiment. All other ponies received 10⁴ small strongyle L₃/day for six weeks. One previously infected group received daily Strongid-C during this period. Strongid-C was > 98% efficacious against cyathostome larvae. Marked changes in the large intestinal mucosae were not noted. However, weights of large intestine biopsies suggest an increase in tissue mass associated with increased parasite burdens. Comparisons of parasite numbers in nontreated parasite-free ponies with untreated, previously infected ponies suggest an acquired resistance (82% reduction) against late L₃-L₄ larvae occurred in the previously infected ponies. Supported in part by Pfizer, Inc.



EFFICACY OF MOXIDECTIN INJECTABLE AGAINST LICE AND ENDOPARASITES IN CATTLE

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In order to confirm efficacy of moxidectin (M) against lice and nematodes, one group of 20 cattle was injected SC with 0.2 mg M/kg b.w. and another group of 20 received blank vehicle. Animals were examined weekly for 8 weeks for lice, and fecal samples were taken for egg counts and larval cultures. Against extremely heavy infestations of sucking lice, moxidectin was 100% effective in eliminating adult and nymph stages of *Haematopinus eurysternus* and *Linognathus vituli* throughout the 56-day posttreatment period, but it was ineffective against the biting louse, *Damalinia bovis*.

Moxidectin was 100% effective in reducing nematode egg counts to 0 during Days 7 and 14 posttreatment. A single subcutaneous treatment remained >96% and >88% effective against four genera of gastrointestinal nematodes (*Haemonchus*, *Ostertagia*, *Cooperia*, and *Nematodirus*) at 28 and 56 days posttreatment, respectively. Based on the life cycle, the presence of relatively low numbers of nematode eggs in the treated animals at these time periods was due to reinfection from the premises.

Over the 56-day experimental period, the treated animals gained an average of 0.679 kg per head per day compared to 0.314 kg for the controls. No swelling or irritation was observed at the injection site on any of the calves.



EFFICACY OF MOXIDECTIN AND IVERMECTIN AGAINST NATURALLY ACQUIRED NEMATODE INFECTIONS IN CATTLE IN BRAZIL

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Twenty nine Devon calves, aged seven to eight months, naturally infected with both lung and gastrointestinal nematodes were allocated according to their epg to three experimental groups and treated as follows: moxidectin (n=10), ivermectin (n=9) and untreated control (n=10). All treated groups received 0.2 mg/kg subcutaneously of 1% formulations. Ten days later all the animals were slaughtered for worm counting. A 10% sample of abomasum and small intestine contents was counted whereas all nematodes present in the large intestine and lungs were counted. Worm counts were log transformed before being submitted to statistical analysis. Moxidectin and ivermectin treatments were 100% effective against *Haemonchus* sp., *Trichostrongylus axei*, *T. colubriformis*, *Oesophagostomum radiatum* and the lung worm *Dictyocaulus viviparus*. Against *Cooperia* (*C. punctata* and *C. onchophora*) the reduction was 95% for the moxidectin treatment and 98.4% for the ivermectin ($P>0.05$).



MOXIDECTIN: ORAL AND INJECTABLE FORMULATIONS AGAINST SHEEP NEMATODES IN BRAZIL

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The efficacy of two moxidectin formulations: a 1% injectable solution and a 0.2% oral drench were evaluated for the control of nematodes in naturally infected sheep. A 0.08% ivermectin oral drench was also included in the trial as a positive control. Seventy Corriedale wethers aged six to seven months and naturally infected with gastrointestinal nematodes were allocated according to their epg counts to seven experimental groups of 10 animals and treated according to individual body weights as follows: moxidectin oral at 0.1 mg/kg, 0.2 mg/kg and 0.4 mg/kg; moxidectin injectable at 0.2 mg/kg and 0.4 mg/kg; ivermectin oral at 0.2 mg/kg and untreated control. On Days 14 and 15 after treatment all animals were slaughtered (five animals/group/day) to estimate gastrointestinal worm burdens. Moxidectin and ivermectin were 100% effective against *Haemonchus contortus*, *Ostertagia* spp., *Trichostrongylus axei*, *T. colubriformis*, *Cooperia* spp., *Oesophagostomum venulosum* and *Trichuris ovis*. On *Nematodirus spathiger* all treatments were also 100% effective with the exception of injectable moxidectin at 0.2 mg/kg where the efficacy was 99.7%.



MOXIDECTIN: EFFICACY AGAINST GASTROINTESTINAL NEMATODES OF SHEEP IN ARGENTINA

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A total of 24 male and female cross-breed Corriedale hoggets, naturally infected with gastrointestinal nematodes, was divided into four groups as follows:

Group 1 was treated with Moxidectin 1% at 0.4 mg/kg SC; Group 2 with Moxidectin 1% at 0.2 mg/kg SC; Group 3 with Moxidectin 0.2% (2.0 g/L) at 0.4 mg/kg orally, whilst Group 4 was left as the untreated control.

Percentage reduction of parasites in Group 1, 2 and 3 was 100% for *Ostertagia circumcincta*, *O. trifurcata*; *Haemonchus contortus*; *Trichostrongylus axei* and *T. colubriformis*; *Trichuris ovis*; *Oesophagostomum venulosum* and *O. columbianum*. Percentage reduction of *Nematodirus spathiger* and *N. abnormalis* was 100%, 92% and 99%, and for *Cooperia curticei*, 100%, 92% and 100%, in Groups 1, 2 and 3, respectively. Differences among treated groups was not significant ($P > 0.05$). However at necropsy, the total parasite burden in all the treated groups was significantly lower ($P < 0.05$) than the worm burden in the untreated control.

A significant ($P < 0.05$) improvement in the mucosal surface of the abomasum measured by pH and mean scores for mucosal lesions was registered after treatment in Groups 1 and 3 when compared with the untreated control Group 4.

Moxidectin 1% at the dose rate of 0.2 and 0.4 mg/kg subcutaneously, or Moxidectin 0.2% at 0.4 mg/kg in an oral formulation (2.0 g/L), was proven highly effective against natural infections with the most important nematodes of sheep in Argentina.



MOXIDECTIN: AN ASSAY OF EFFICACY WITH INJECTABLE AND ORAL FORMULATIONS AGAINST GASTROINTESTINAL NEMATODES AND LUNGWORM IN SHEEP, IN ARGENTINA

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A total of 56 young Lincoln breed sheep with a natural nematode infection on a farm in the central east of the Humid Pampas, was treated in January 1990 (summer) with an oral benzimidazole and kept untreated in a single paddock with a high level of GI larval infection until May (autumn), when they were taken to the INTA Research Centre in Balcarce. Kept on concrete floor pens and fed only lucerne pellets, they were allocated by EPG counts to seven homogenous Groups of eight animals each: Group 1 was left as the non-treated control; Groups 2 through 4 inclusive, received Moxidectin 0.2% (2.0 g/l) oral, at 0.1, 0.2 and 0.4 mg/kg, respectively; Groups 5 and 6 received Moxidectin at 0.2 and 0.4 mg/kg, respectively, and Group 7 ivermectin SC, at 200 mcg/kg. EPG counts were made on Days +2, +5 and +10 posttreatment (PT) and necropsies on Days +13 to +15 for total worm counts and identification. Results showed EPG counts were reduced 99.9 to 100% by Day +5; worm burden reduction was between 99.6 and 100% in all formulations. There were no apparent differences between Moxidectin oral or injectable formulations or the dose applied, thus determining the high degree of efficacy of this new macrocyclic endectocide with a single dose against gastrointestinal nematodes and lungworms in sheep. In non-treated controls, the following parasites were identified: in abomasum, *Haemonchus contortus*, *H. similis*, *Ostertagia circumcincta*, *O. trifurcata*, *O. lyrata* and *Trichostrongylus axei*; in the small intestine *T. colubriformis*, *Cooperia punctata*, *C. curticei*, *Nematodirus fillicolis* and *N. spathiger*; in the large intestine, *Oesophagostomum venulosum*, *Trichuris ovis* and *Chabertia ovis*, and in the lungs, *Dictyocaulus filaria*. The low EPG counts on Days +2 and +5 PT did not yield positive larval cultures, suggesting the existence of a larvicidal effect with the drug excreted in the faeces. Compared with ivermectin, Moxidectin was not surpassed in efficacy.



**ABSTRACTS OF
SUBMITTED PAPERS**

**SESSION 9
Helminth Chemotherapy II**



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A single injection of Moxidectin 28, 21, 14 and 7 days prior to infection gave the following levels (%) of protection: *O. ostertagi* (adults) 75, 93, 98, 100; *Haemonchus* adults 86, 100, 99, 100; *O. radiatum* (adults) 90, 100, 100, 100; *O. radiatum* (L₄) 98, 70, 91, 100 *T. colubriformis* (adults) 79, 76, 76, 98. Protection (PA) against infection due to *T. axei* and *Cooperia* spp. (total count) at seven days post-treatment was 98 and 84% respectively (*C. oncophora* 84; *C. punctata* 93; *C. mcmasteri* 99; *Cooperia* spp L₄, 98), and was at or below 60% at the other time points. In comparison to controls, treatment of Group 5 on Day +25 pi produced a 100% reduction of all parasites. Due to the therapeutic efficacy and this remarkable PA, Moxidectin 1% should be considered a first choice in strategic control programmes against gastrointestinal nematodes in cattle.



ANTHELMINTIC PERSISTENCY OF MOXIDECTIN IN SHEEP

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One hundred and forty five Suffolk-cross lambs were divided into groups of five animals per group. Treatments were Moxidectin injectable or oral drench, compared with Ivermectin injectable or oral drench, a positive group treated with Moxidectin injection 23 days after infection and negative controls. The first group of lambs was treated at 42 days prior to infection with known quantities of *Haemonchus contortus*, *Ostertagia circumcincta*, *Cooperia curticei* and *Trichostrongylus colubriformis*.

Thereafter one group was treated at weekly intervals. Evidence of persistency effect was determined by nematode counts performed at necropsy 23-25 days after infection and compared with positive controls.

Results: The table shows the number of days of persistency where a >90% level of efficacy was obtained compared with the negative control.

Species	Injection		Oral	
	Moxidectin	Ivermectin	Moxi	Iverm
<i>Cooperia curticei</i>	14	14	0	7
<i>T. colubriformis</i>	7	7	14	0
<i>Ostertagia circumcincta</i>	42	7	35	0
<i>Haemonchus contortus</i>	35	7	35	7

Conclusion: With the exception of *Cooperia*, the limiting species, the data show that Moxidectin persistency is high in young lambs. This would be of value in the control of ovine PGE, especially at the time of lambing.



CHRONOLYSIS: PRACTICAL IMPLEMENTATION OF THE CRITICAL AND PERSISTANT ACTIVITY OF CLOSANTEL

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Closantel is a drug with characteristic pharmacological properties. After systemic absorption, closantel becomes strongly bound to plasma albumin (>99%) and the peak plasma concentrations explain its immediate cidal activity against hematophagous parasites. The limited metabolism and the long plasmatic half-life (two to three w.) form the basis for the intrinsic persistent activity period. The practical advantages of these effects were explored in sheep, artificially or naturally infected with *H. contortus*, *O. ovis* and *F. hepatica*. The sheep were treated using a 5% closantel oral formulation at a dose of 10 mg/kg *per os*, which resulted in peak plasma concentration of about 50 µg/ml. Previous dose-titration studies have established the Minimal Effective Plasma Concentration for each of the parasites. Laboratory and field studies demonstrated that the period of residual activity was determined by the time at which the actual drug plasma concentrations dropped below the MEPC. For *H. contortus*, the MEPC is 7 µg/ml and the ensuing persistent activity period lasts about eight w. This supports a prophylactic use of closantel to control haemonchosis. The MEPC against *O. ovis* is 15 µg/ml with a persistent activity period of about six w. and validates its metaphylactic use to control oestrosis. Against *F. hepatica*, the persistent plasma levels cause severe 'stunting' of young immatures, whereby egg excretion is inhibited for at least 10 w. after a single dosing. The combination of the strong immediate cidal effect and the long-lasting activity, allows us to rationalise the number of dosings for an optimal epidemiological control of the three sheep parasites. The principle of 'chronolysis' sustains a flexible approach of strategic dosing in different management systems within the economic constraints of current sheep farming.



SHORT-TERM FEED MANAGEMENT TO INCREASE ANTHELMINTIC ACTIVITY IN SHEEP

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Anthelmintic activity is principally determined by the duration which the target parasite is exposed to sufficiently 'toxic' metabolite concentrations. This duration is largely defined by a compound's intrinsic metabolism but is also influenced by the residence time of the orally administered drug within the rumen, slow digesta passage contributing to the rumen drug 'reservoir' action. As digesta flow rate is directly related to the level of feed intake, the kinetic disposition of orally administered oxfendazole (OFZ), albendazole (ABZ) and ivermectin was compared in sheep fitted with rumen and abomasal cannulae and fed 400 and 800 g lucerne wheaten chaff daily. Cr-EDTA and Ruphenanthroline markers were used to measure the flow rate of fluid and particulate digesta, respectively. Metabolite disposition at these sites in the digestive tract and bloodstream was determined by HPLC and radiotracer analysis.

Rumen digesta residence time was longer and the digesta flow rate through the abomasum was slower in sheep on low compared to high feed intake. This prolonged the duration for metabolite absorption and recycling which resulted in a greater and more protracted anthelmintic availability. Digesta flow rate responds quickly to changes in feed intake, and within 24 h of halving the 800 g daily ration the abomasal digesta flow rate had reduced by approximately 30%. Administration of OFZ or ABZ at this time to parasitised sheep significantly increased the efficacy of these compounds against benzimidazole-resistant nematodes. Short-term feed reduction preceding drug administration can therefore be of significant benefit in increasing anthelmintic activity.



COMPARATIVE DISPOSITION KINETICS OF FENBENDAZOLE AND OXFENDAZOLE IN SHEEP: EFFECTS OF THEIR CO-ADMINISTRATION WITH METHIMAZOLE

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Understanding of the pharmacokinetics of benzimidazole molecules in the host may be used to predict and enhance their anthelmintic efficacy. The goals of this work were a) to compare the plasma disposition kinetics of fenbendazole (FBZ) and oxfendazole (OFZ) orally administered to sheep, and b) to characterize the effects of methimazole (MTZ) on the disposition of FBZ/OFZ metabolites. Twelve (12) parasite-free Finn Dorset sheep (45-50 kg) were divided into two groups. Animals received either FBZ (Group A) or OFZ (Group B) (oral, 5 mg/kg) (phase I). After a four-week washout period, animals in both groups (phase II) were treated with either FBZ (Group A) or OFZ (Group B) co-administered with MTZ (oral, 3 mg/kg). The plasma profiles of the three HPLC-recovered analytes (FBZ, OFZ and FBZ sulphone (FBZSO₂)) followed similar disposition patterns after the administration of both anthelmintics; OFZ being the main component detected in plasma between two and 144 h post-treatments. Low concentrations of FBZ, the molecule with the highest affinity for nematode β -tubulin, were found in plasma between four (FBZ treatment) or eight (OFZ treatment) and 72 h post-treatment. The peak concentration (C_{max}) of FBZ was significantly higher ($P < 0.05$) and reached earlier when animals were treated with FBZ than when they received OFZ. The co-administration of FBZ with MTZ resulted in higher AUC (30%) and C_{max} (50%) ($P < 0.05$), reached at an earlier T_{max}, for FBZ parent drug, than those obtained with the administration of FBZ alone. FBZ body clearance was lower (30%) in the presence of MTZ. Consistently, significant higher AUC and C_{max} (< 0.05) values for FBZ were obtained when animals received the OFZ+MTZ treatment compared with those obtained after the administration of OFZ alone. As earlier shown for albendazole metabolites, MTZ-induced changes to the kinetics of FBZ/OFZ metabolites may lead to improved anthelmintic efficacy.



ON THE MODE OF ACTION OF MORANTEL: A
SINGLE-CHANNEL STUDY AT NICOTINIC
ACETYLCHOLINE RECEPTORS IN MUSCLE MEMBRANE
FROM *ASCARIS SUUM*

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Morantel is an anthelmintic which produces spastic paralysis of nematodes by an agonist action at nicotinic acetylcholine receptors (nAChR) found in somatic muscle membranes. We have now investigated the mode of action of morantel, in further detail, by using the patch-clamp technique to examine effects of morantel on nAChR channel currents produced in membrane vesicles prepared from somatic muscle of *Ascaris suum*. Morantel was added to the patch-pipette solution in concentrations between 0.6-600 μM . At low concentrations (0.6-6 μM) morantel acted as an agonist and opened channels which had a main-state conductance of around 40 pS and brief open-times with a mean value of 0.8 ms. These channels showed inward rectification and carried more current in the inward direction than in the outward direction.

At high concentrations (10-600 μM) morantel also entered the open channel pore but could not pass through since it is a large cation; it produced open channel-block. Two blocked states were observed at negative membrane potentials; a brief block (4-20 ms), and a long block (up to 7s). In addition the concentration-block relationship had a Hill coefficient of 1.6, suggesting the involvement of at least two blocking molecules. The block produced by morantel increased as the membrane potential was made more negative (voltage-sensitive block) and as the concentration of morantel was increased. We have produced a simple sequential block model to explain these observations in which morantel acts as an agonist to open the nAChR channel and in addition two molecules of morantel enter the opened channel pore to produce open-channel block. The therapeutic significance of these observations will be discussed.



THE SAFETY AND EFFICACY OF A NEW FORMULATION OF FENBENDAZOLE FOR PIGEONS

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Pigeons are commonly infected with the roundworms *Ascaridia columbae* and *Capillaria spp.* Heavy infections can reduce growth rate and affect performance. Initial studies indicated that fenbendazole given orally at 7.5 mg/kg was highly effective against *Ascaridia spp.* but that a dose rate of 20 mg/kg was required to achieve similar efficacy against *Capillaria spp.* Fenbendazole medicated feed over three to four days was also highly effective against both nematode species. However, in-feed medication does not guarantee that each pigeon receives a therapeutic dose. Furthermore potential mixing problems could cause toxicity. Therefore, a capsule formulation of fenbendazole for individual administration orally, was developed and is now commercially available in the UK. Initial dose-seeking studies in naturally infected birds indicated that a single treatment with 8 mg fenbendazole/bird ($\approx$ 20 mg fenbendazole/kg body weight) was highly effective in reducing faecal egg output. The efficacy of this dose rate was confirmed in a controlled test where *Ascaridia spp.* and *Capillaria spp.* worm burdens were reduced by 100% and 91% respectively. Controlled comparative efficacy trials confirmed that the capsule formulation of fenbendazole was more effective and better tolerated than either levamisole (orally/in water) or piperazine (in feed). The capsule was easy to administer and well tolerated. Further tolerability studies in young (two months old) and adult birds confirmed the wide safety margin of this formulation of fenbendazole in pigeons (x9).



ON THE CHEMOPROPHYLACTIC ACTION OF COMPOUND 81/470

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Compound 81/470 which is methyl [5] [4-(2-pyridinyl)-1-piperazinyl] carbonyl]-1H-benzimidazol-2-yl] carbamate, exhibits fairly good activity against a variety of helminth parasites. The drug is quite safe and is under comprehensive investigation for developing it as a broad spectrum anthelmintic for clinical and veterinary use. Interestingly, this compound at a single 100 mg/kg i.m. dose shows strong prophylactic action against *Ancylostoma ceylanicum* in hamsters. With an aim to understand the basis for such an action, metabolic disposition of tritiated 81/470 as well as effect of the compound on antioxidant system of the parasite and the host jejunum were examined. The results indicated that the i.m. dose formed a depot at the site of administration, from where it was released at a very slow rate. Consequently, the radioactivity in the serum could easily be detected upto at least seven weeks whereas the oral dose was undetectable after only one week. 81/470 *per se* did not affect activities of superoxide dismutase and catalase of either infective larvae or adult worms of *A. ceylanicum*. The compound, nevertheless, greatly enhanced the generation of O_2 and H_2O_2 by the jejunum. The combination of depot action and enhancement of reactive oxygen intermediates production by the host intestines appears to account for chemoprophylactic effect of the compound.



DESIGN OF STRATEGIC ANTHELMINTIC CONTROL PROGRAMS IN CATTLE USING A MATHEMATICAL MODEL (PARABAN)

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Trickle and single infection experiments provide a basis for understanding host/parasite interaction of trichostrongylid parasites. Published data on population biology permit the development of a model for the processes that regulate and control parasite abundance. Input of local climatic data, as well as locally identified patterns of inhibition of *Ostertagia*, allow tailoring of the model to project epidemiology on a regional basis. Incorporation of published data on anthelmintic efficacy allows comparison of different treatment strategies, according to management objectives identified by the user. Validation studies completed in Europe and South America suggest that the model has a sound base for use throughout the world as a guide in development of parasite control strategies. Informed use of such a model will provide an educational basis to facilitate responsible and cost effective use of anthelmintics.



CHEMOPROPHYLAXIS AND IMMUNITY TO NEMATODES IN CATTLE

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Control of nematode infections in grazing calves by early season anthelmintic suppression can be so effective that, in theory, there could be insufficient antigenic exposure to induce adequate immunity. To investigate this possibility, five matched groups of nine autumn-born calves were grazed on similar, contaminated paddocks: controls; two groups given oxfendazole pulse release boluses, one of which was also vaccinated against lungworm; a morantel sustained release bolus group; and a group treated with ivermectin at 3, 8 and 13 weeks after turnout. These strategies were found to influence patterns of infection during the first grazing season to different degrees enabling groups to be ranked according to exposure to pulmonary or gastrointestinal nematodes. After autumn housing, three calves from each group and two parasite-naïve controls were challenged with *D. viviparus*, *O. ostertagi* and *C. oncophora*. Worm counts revealed clear differences in parasite establishment correlating in each case with the appropriate ranking order. In the second grazing season, remaining animals were grazed together with parasite-naïve sentinels. Respiratory signs occurred in all groups but sentinels were worst affected.



**ABSTRACTS OF
SUBMITTED PAPERS**

SESSION 18

Diagnosis II





APPLYING REGRESSION OF P.C.V. ON E.P.G. FOR SUGGESTING AN EFFECTIVE ANTHELMINTIC TREATMENT FOR OVINE HELMINTHOSIS

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To determine the degree of helminth pathogenesis and predict an intervention with anthelmintics, 64 six to twelve month old sheep were grazed for one year on an area predominantly infected with *Haemonchus contortus*. Every month eggs per gram of faeces (e.p.g.) by McMaster and packed cell volume (p.c.v.) by Microhaematocrit Centrifuge methods were used to estimate the worm burden and resulting helminthic pathology in the sheep. Simple and quadratic linear regressions of the p.c.v. on the e.p.g. were then analysed.

The quadratic regression was found to be more informative. It showed a gradual fall in the p.c.v. until it reached 25% with a corresponding e.p.g. of 1800. Thus it is concluded that anthelmintic treatment until an e.p.g. of 1800 and p.c.v. of 25% could possibly save the sheep. Treatment after this stage has been reached becomes ineffective to reverse the condition and the sheep continue suffering from progressive debility.



EPIDEMIOLOGICAL RISK FACTORS ASSOCIATED WITH CLINICAL CYATHOSTOMIASIS IN THE HORSE

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Multiple logistic regression was used to assess epidemiological risk factors associated with the diagnosis of cyathostomiasis in 87 cases of chronic diarrhoea in the horse. Bivariate analysis identified age, season and the length of time since last deworming as important parameters whereas access to grazing and recurrence of symptoms were only weakly associated with a diagnosis of cyathostomiasis. Multivariate analysis of these parameters using logistic regression was performed. The final model included age, season and time since last deworming. The model had a specificity of 86.0%, sensitivity of 66.7%, overall correct classification of 79.3%, a positive predictive value of 71.4% and a negative predictive value of 83.1%. The results of this study indicated that only the specified variables may be useful in the differentiation of clinical cyathostomiasis from other causes of chronic diarrhoea.

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NATURAL *ASCARIS SUUM* INFECTIONS IN SWINE MEASURED BY COPROLOGICAL AND SEROLOGICAL METHODS

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The purpose of this study was to examine the antibody response against *Ascaris suum* and to compare the results with faecal egg counts. In each of 20 medium to large size sow herds in Denmark faeces and blood were collected from 15 weaners (appr. 25 kg b.w.), 15 large fatteners (appr. 90 kg b.w.) and 10-15 lactating sows together with blood from two piglets of each litter. The samples were analyzed by a modified McMaster technique and an indirect ELISA, using *A. suum* L₂/L₃-excretory-secretory antigens and anti-swine-IgG. The results showed an increasing percentage of coprologically positive samples with age (weaners: 4%, fatteners: 20%), followed by a decrease in the sows (9%). The serological results on the other hand showed much higher numbers of positive cases, which clearly increased with age (weaners: 28%; fatteners: 63%; sows: 80%). The *A. suum* specific antibodies were to a large extent transferred to the suckling piglets, which sometimes had even higher antibody levels than their mothers. However, the 25 kg weaners were often totally seronegative, which reflected that the maternal antibodies had been catabolized and that the piglets in these herds didn't become infected in the farrowing pens. In some of the latter herds the fatteners continued to be helminth-free during the whole fattening period. In some other herds the pigs acquired infection at an early stage as illustrated by coprologically and serologically positive weaners, indicating that a heavy transmission had taken place in the farrowing pens. In individual pigs there was no significant correlation between egg counts and antibody level. It is concluded that serodiagnosis is superior to coprological examination in studies on prevalence rates and transmission patterns of *A. suum* in swine herds.



EVALUATION OF AN ELISA AND A HISTAMINE RELEASE TEST SYSTEM FOR THE DETECTION OF PIGS NATURALLY INFECTED WITH *ASCARIS SUUM*

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An indirect ELISA and a histamine release test system were evaluated for the detection of pigs naturally infected with *Ascaris suum*. Histamine, released by antigen provocation of blood leukocytes was selectively bound to glass microfibres and detected fluorometrically, following coupling to o-phthaldialdehyde. Two antigens were used in the two tests: Adult body fluid (ABF) and L2/L3 excretory/secretory antigens, obtained by in vitro cultivation of hatched infective larvae. The number of worms in the small intestines of the pigs and the number of eggs in the faeces were determined and the liver milk spots counted, together with differential blood leukocyte counts. A total of 150 pigs, weighing approximately 90 kg, from 23 farms were tested. Seventy one (47%) of the pigs had either adult worms, faecal eggs or liver milkspots. Twenty out of 23 farms (87%) delivered *A. suum* infected pigs to the slaughter-house. Liver milk spots were detected in 23.4% of the pigs. When the presence of three or more liver milkspots was considered evidence of an *A. suum* infection, the ELISA using L2/L3-ES as the antigen gave a test sensitivity of 97% and a specificity of 89%. Significant associations were achieved between the presence of milkspots and the results obtained in the tests using both antigen types in ELISA and using L2/L3 in the histamine release assay. No significant association was found between milkspots and the histamine release test using ABF as antigens, between number of milkspots and presence of intestinal *A. suum* worms and between the immunological test systems and the hematological data. The present material provided a model for calculating the probability of a pig having three or more liver milk spots originating from a natural *A. suum* infection. This model could be of value in epidemiological surveys as well as in combination with surveillance of other infections in pigs, which is regularly performed by blood sampling. Furthermore, it can be used as a tool to classify herds as *A. suum* free.



**ABSTRACTS OF
SUBMITTED PAPERS**

**SESSION 10
Epidemiology II**



HELMINTH PARASITISM IN FARMED RED DEER

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The predominant helminth parasites of farmed red deer are the ostertagids and the lungworm, *Dictyocaulus eckerti*. In hinds there appears to be a loss of resistance to both parasites associated with calving which is reflected by a rise in the faecal egg and L counts. These take place during the summer and they ensure the presence of infective larvae on pasture for the transmission to the calves.

Hypobiotic larvae of both parasites are present in adult animals from September to May and in both they form the nucleus of the early summer populations of adult worms. Of the anthelmintics available neither fenbendazole at 15 mg/kg nor ivermectin at 400 µg/Kg is fully effective against these larvae. The consequences for resistance will be discussed.



EPIDEMIOLOGY OF GASTROINTESTINAL NEMATODE INFECTIONS IN GRAZING BEEF COW-CALF HERDS IN BELGIUM

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The epidemiology of gastrointestinal nematode infections in beef cows grazing with their spring-born calves was investigated in Belgium.

Two groups of cow-calf pairs (A and B) were monitored during the 1990 grazing season and two groups of cow-calf pairs (C and D) during the 1992 season. In both studies faecal egg counts, faecal cultures and herbage larval counts were monitored at monthly intervals. In addition two parasite-naïve tracer calves were grazed for 2-week periods on each pasture at the start and end of the calf grazing period.

In the 1990 study both pastures were initially contaminated with mainly *Ostertagia* as indicated by tracer worm counts. At turnout cow egg output was distinctly higher in Group A as compared to Group B. During the grazing season cow and calf egg counts were low. At the end of the grazing season pasture A tracers harboured the highest *Ostertagia* worm burdens, indicating the role of cow egg output at the start of the grazing season as source of pasture contamination. In the 1992 study both *Ostertagia* and *Cooperia* were initially present on the pastures. Cow egg output was low both at turnout and throughout the study in Groups C and D. In calves from both groups high faecal egg counts were observed, *Cooperia* being responsible for most of the egg shedding. Moderate *Ostertagia* and high *Cooperia* burdens were present in the tracers from both pastures at the end of the study. These results suggest that in the 1992 study calves were the major source of pasture contamination.

It appears that the epidemiological pattern of gastrointestinal nematode infections in grazing cow-calf pairs is strongly influenced by farm management practices, herd type (winter- or spring-born calves), the overwintered larval population on pasture and climatological conditions. These factors should be considered when designing anthelmintic control programs for grazing cow-calf pairs.



NEMATODIRUS EPIZOOTIOLOGY IN SUMMER GRAZING BEEF COWS AND CALVES: FIELD OBSERVATIONS AND MODEL ESTIMATES

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This study's purpose was to use a field data-based mathematical model, relating temperature and egg development rate for *Nematodirus helvetianus* (*Nh*), to dissect the epizootiology of this parasite in grazing beef cows (from which *Nh* eggs are very rarely recovered) and calves (from which such eggs are very commonly recovered). Hatched *Nh* infective larvae (IL) were first observed in overwintered faecal masses from fall-grazed pastures on 7 June 1991 and 28 May 1992. Model-estimated dates were 17 June 1991 and 24 June 1992 (using observed daily mean air temperatures - ODMAT) and 28 May 1991 and 27 May 1992 (using calculated daily mean grass temperatures - CDMGT). Grazing calves first developed patent *Nh* infections in mid-July (1991 and 1992). Model estimates suggest that only eggs deposited on pasture by the calves between then and 3 August (using 30 year ODMAT) or 30 August (using 30 year CDMGT) would develop to hatched IL before the end of the grazing season. Peak *Nh* egg production by the calves occurred during the first two weeks of September (1991 and 1992); field observations and model estimates suggest that these eggs would contribute to *Nh* infection in the next year's grazing calves. These results together support the view that in the Canadian prairies cows may play little part in *Nh* epizootiology, which depends instead on pasture-overwintered free-living stages for transmission from one year's calves to the next.



GASTRO-INTESTINAL NEMATODES IN RURAL GOATS FROM THE HIGHVELD OF ZIMBABWE

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The seasonal variations in the gastro-intestinal nematodes were studied in goats grazing on communal pastures in the highveld area near Harare, Zimbabwe. The climate of this region is characterised by a warm rainy season from mid November to April and a dry season from May to October/November. On six occasions during a one-year period, one to two year old goats were acquired from the traditional farmers, housed for three weeks under worm-free conditions and necropsied. All the animals (4 October, 4 November, 6 January, 6 June, 6 August) were found infected with nematodes. *Haemonchus contortus* was the most prevalent (97%) and dominant species followed by *Trichostrongylus axei* (91%), *T. colubriformis* (84%), *Oesophagostomum columbianum* (91%), *Trichostrongylus* spp. (21%), *Strongylodes papillosus* (9%) and *Bunostomum* spp. (3%). The total nematode burden of individuals varied between 13 and 4791 and mean of each sampling period from 230 to 2222. It was lowest at the end of the dry season, increased gradually through the rainy season, to reach a peak at the end of rains in April. The fourth stage larvae of *H. contortus* accounted for 0-7% of the total *H. contortus* population at all samplings except in August when they constituted 46% of the burden. In a separate study monthly faecal examination of goats from the same area showed peak nematode egg counts in January with low counts during dry period. It is concluded that there is a direct relationship with rainfall and gastro-intestinal nematode burden and the hypobiosis of *H. contortus* is not of major importance.



EPIDEMIOLOGY OF HELMINTH INFECTIONS IN TRADITIONALLY MANAGED TIMAHDIT SHEEP IN THE MIDDLE ATLAS MOUNTAINS (MOROCCO)

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The epidemiology of helminth infections in sheep was studied in the Middle Atlas over a continuing three year period by the monthly determination of (i) pasture herbage populations of infective larvae of gastrointestinal nematode parasites (GI), (ii) snail intermediate host of *Fasciola hepatica* (*Lymnea truncatula*) populations, and (iii) helminth populations in the digestive tract, the lungs and the liver of sentinel sheep. The density of infective larvae of GI was high from October to June and null from July to August. *L. truncatula* populations followed an annual cyclical pattern, the massive numbers occurring in the spring and somewhat irregular numbers in the autumn. In the digestive tract the period of high worm burdens occurred from September to March, but two peaks were observed; from September to November and from January to March. Either young sheep (less than one year old) or ewes were infected with *Moniezia* spp. during the whole year. However, the worm population was high in young animals particularly from January to July, while the weight of these parasites was high from June to January, with a peak during June and July. The lungs are infected with *D. filaria* from October to April, but two peaks were observed; October to December and April. Infection with small lungworm did not show any variation during the year, but the worm burdens are higher in ewes than in young animals. The average rate of infection with *E. granulosus* hydatid cysts was 10% and 90% respectively in young sheep and in ewes. High fluke burdens were recorded in the autumn and winter. Burdens reached maximum levels during the winter and then declined to low numbers by late spring and summer. The rate of *E. granulosus* hydatid cyst infection ranged from 27.77% in the lambs to 93.33% in the ewes, while that of *Cysticercus tenuicollis* ranged from 27.77% in the ewes to 48.11% in the lambs.



SEASONAL EGG OUTPUT OF TRICHOSTRONGYLIDS IN CALVES UNDER DIFFERENT SYSTEMS OF MANAGEMENT IN ZIMBABWE

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Trichostrongylid faecal egg output was studied over a 12 month period in calves in the highveld of Zimbabwe. Two traditional African communal lands, two standard commercial beef farms, and two beef farms irrigated with treated effluent were included in this study. Faeces of calves were collected monthly per rectum and parasite egg counts carried out according to the McMaster technique. Faecal cultures were done to differentiate the trichostrongylids. Mean trichostrongylid egg output during the dry and wet seasons ranged from 195-745 e.p.g. in communal lands, 100-391 e.p.g. in standard commercial beef farms, and 77-501 e.p.g. in effluent irrigated beef farms. During the dry season *Cooperia* sp. were dominant followed by *Haemonchus* sp. and *Trichostrongylus* sp. respectively. Transition from *Cooperia* dominance to *Haemonchus* dominance occurred during the rainy season in all management systems.



EPIDEMIOLOGY AND CONTROL OF HELMINTH INFECTIONS IN PIGS UNDER INTENSIVE AND NON- INTENSIVE PRODUCTION SYSTEMS

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Swine production systems are characterized by a high diversity with regard to management type and level of intensity, and the number of helminth species as well as infection levels are strongly influenced by the different systems. The present paper, focussing on the situation in Northern Europe with examples from Denmark, describes a decrease in the number of helminth species and in infection levels as result of a shift from non-intensive to highly intensive production systems. Differences in basic biological requirements of the pre-infective larvae, and in transmission characteristics and immunogenicity of the various helminth species explain, why some species are more vulnerable to managerial changes than others. Finally, control measures relevant for the different production systems are discussed. Despite the fact it is well documented that prevention of transmission may be obtained by proper hygiene and management, use of anthelmintics is still the single most important action taken by pig farmers to control worm infections. It is emphasized that anthelmintics should not be used uncritically, but should be integrated with management practice and production system in order to achieve optimal effect and to avoid development of anthelmintic resistance.



HIGH ENDEMICITY OF CYSTICERCOSIS AND ECHINOCOCCOSIS IN CHINA-GANSU

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Two parasitic zoonoses are endemic in Gansu Province. From 1957 to 1962, the detection rate of pig cysticercosis was low, ranging between 0.1%-1%. But the rate increased obviously in 1979, especially in Zhang-Xei and Lin-Zia prefectures reaching 5.7% and 8.3% respectively. In 1979, the infection rage of 1261358 pigs was 1.21% with obvious differences in some areas, the lowest only 0.02% and the highest 13.9%. After a comprehensive control strategy begun in 1979, the prevalence rate of human cysticercosis decreased from an original 599 per 100000 to 20 per 100000 in 1991 and the detection rate of pig cysticercosis also reduced from 1.76% to 0.37%. The ratio between governmental investment and economic benefit was 1:20.18. The endemic areas may be divided into single E.g., single E.m. and mixed infection. The infection rates of intermediate or final hosts of E.g. were high, especially sheep (85.2%), yak (60.34%) and dog (19.2%) in Ganan prefecture. According to incomplete statistics of Ganan slaughterhouse from 1985 to 1989, the total amount of sheep and yak condemned offal (liver, lung, heart) cumulatively weighed 13.1 tons. From Lanzhou's three major hospitals, 1674 patients of cystic echinococcosis were collected in the period of 30 years (1960-1990). In Zhang County, final host of E.m. was dog (10%), but liver alveococcus was only detected in 1 *Mus musculus* (housemouse). Two surveys from this county reported that Casoni's test positive rate, ultrasonic positive rate and the morbidity of the patients were 19.2% (73/380), 5% (65/1312) and 2.4% (9/380) respectively. In Hydatid Research Laboratory of Lanzhou Medical College, 70 cases of liver AE were seen during 1970-1991.



ECHINOCOCCOSIS IN BULGARIA: EPIDEMIOLOGY, CONTROL, PRESENT SITUATION

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The purpose of our study is to record on some epidemiological peculiarities of hydatid disease, to describe the control measures carried out and to evaluate the present situation in Bulgaria. The study included two periods, the first ranging over 13 years (1950-1962) and the second 20 years (1971-1990). During the first period of study the average infestation rate for sheep was 67%, for cattle 41% and for pigs 3%. The infestation rate among dogs was found to be 14% but varied from one region to another. A total of 6469 surgically confirmed cases of hydatid disease were recorded, the annual infestation rate being 6.5 per 100,000 population. The organization of echinococcosis control campaign was initiated in 1960. Priority was given to dogs and their control, including registration and reduction of the number of stray dogs, periodical medical treatment. Most essential components of the control measures were the improvement of the health education and slaughterhouse control. The control measures carried out during the second period have led to a considerable improvement of the situation of echinococcosis among animals. The percentage of infected sheep fell to 19%, cattle to 17% and pigs to 1.2%. The number of dogs, especially of stray dogs, has been markedly reduced and the infestation rate fell to 4%. The morbidity among people gradually decreased. During the second period the number of new surgical cases was 4176 and the annual incidence rate amounted 2.4 per 100,000. The dynamics of the incidence rate in animals and morbidity in humans after 1981 showed a fluctuation and tended to increase during the last five years (1986-1990). The infestation rate for sheep increased to 30%, for cattle to 19% and for pigs to 1.5%. The annual incidence rate for humans reached 3.2 per 100,000. The unfavourable patterns seen with echinococcosis prevalence were an indication for reinforcement of hydatid parasite transmission which reflected some changes of the economic conditions in the country.



THE PREVALENCE OF INTERNAL PARASITES IN RACE HORSES IN JAPAN: A NECROPSY SURVEY

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The introduction of highly potent antiparasitic drugs such as ivermectin has resulted in a change in the prevalence and nature of parasitic burdens in race horses in Japan. A gradual decrease in the prevalence on some nematodes from a previously high rate has been observed. This study examined the current internal parasite prevalence and the gross and histopathological lesions associated with these parasites. The parasite burdens of 178 horses submitted for routine necropsy between January 1989 and December 1992 were identified and counted with special emphasis being placed on examination of the small intestine and caecum for the presence of tapeworms and associated gross/histopathology. The prevalence of gastrointestinal parasites was as follows; large strongyles, 6.2%, ascarid - *Parascaris equorum*, 14.6%; tapeworms, *Anoplocephala perfoliata*, 52.2%; *A. magna* was not found; bots - *Gasterophilus intestinalis*, 7.3%; *Setaria equina* (a filariid) was recovered from the abdominal cavity in 2.8% of horses. *A. perfoliata* caused ulcerative and/or degenerative lesions at the region of the ileo-cecum valve and the cecal. There is a need for the development of a suitable anthelmintic for equine tapeworms.



**ABSTRACTS OF
SUBMITTED PAPERS**

SESSION 11

Biology II





DIETARY MINERAL SUPPLEMENTATION INFLUENCES PLASMA PEPSINOGEN CONCENTRATIONS IN SHEEP

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Plasma pepsinogen (PP) concentrations are inconsistent indicators of worm burden for reasons not fully understood. In studying the effects of mineral supplements on the pathogenicity of abomasal parasites, marked influences of diet on PP became apparent. In Experiment 1, three groups of 10 worm-free lambs were given a diet based on whole barley, naturally low in Mo (0.3), Cu (5.0) and S (1500 mg/kg DM) supplemented with either Mo (5.0 as Na_2MoO_4), S (3000 as CaSO_4) or Mo + S: six from each dietary group received a trickle infection of 500 L3 *H. contortus* larvae, 5d/week, from Day 17 to 52 of a 62d study. Mean PP values, measured weekly from the onset of infection, were 0.45, 0.27 and 0.34 ± 0.05 pooled s.e. d.m. ($P < 0.01$) in groups Mo, Mo + S and S respectively, the effect of diet being almost equal to and largely independent of that of infection (0.45 v 0.21 ± 0.04 u/l) which was within the normal range. In Experiment 2, four groups of lambs were given whole barley diets high (H, 0.65%) or low (L, 0.15%) in Mg with (I) or without (O) a trickle infection of *O. circumcincta* ie a 2 x 2 factorial design. Mean PP concentrations between Days 21 and 56 of infection were HI, 1.82; LI, 1.11; HO, 0.25; LO; 0.34 ± 0.13 u/l. Thus Mg raised PP by 64% in 1 but not 0 lambs (interaction $P < 0.01$): Mg supplementation also delayed the return to normal PP values when infection ceased. Worm burden was not influenced by diet in either study. Mineral intake can thus affect baseline PP values (Experiment 1) and the response in PP to a given worm burden (Experiment 2).



EFFECT OF INTESTINAL NEMATODE INFECTION ON DIET SELECTION OF GROWING SHEEP

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The aim of the experiment was to test whether young growing sheep are able to select a diet, when offered a choice of feeds, that enables them to meet the increased protein requirements associated with parasitic infection. Six Texel x Scottish Blackface lambs (growing from 28-48 kg), infected daily with 2500 L3 of *T. colubriformis* and 6 worm-free controls were offered a free-choice between two feeds of different crude protein content (Low, 90; High, 214 g/kg⁻¹ fresh food) but similar energy (10.4MJ kg⁻¹). In order to assess the effects of the diets alone further groups of six infected and six uninfected lambs were offered either the Low or High CP feeds or a mixture of the two (164 CP kg⁻¹) ad libitum. There was no significant interaction between parasitic infection and CP content of the food. There was no significant effect of infection on feed intake, diet selection or growth rate during the first four weeks of the trial. From weeks four to the end of the trial the proportion of high CP feed selected by the infected lambs increased compared to control (622 g vs 475 g feed H kg⁻¹ total feed intake), although their overall food intake was reduced. Infection also caused a marked decrease in serum P and a slight decline in serum albumin concentration which were more pronounced in the mixed or low CP groups. In conclusion parasitised lambs are able to maintain their CP intake by modifying their selection of diet.



PARASITE ESTABLISHMENT AND PATHOGENESIS OF *HAEMONCHUS PLACEI* IN IMMUNISED CALVES UNDER DIFFERENT DIETARY PROTEIN

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The experiment was conducted to examine the influence of dietary protein and immunisation on parasite establishment and pathogenesis of *Haemonchus placei* in calves. Four groups of five to six month old worm free calves were used. Groups A and C were introduced to normal protein diet (213.3g crude protein (CP) kg^{-1} dry matter (DM)) and B and D to high protein diet (469.3g CP kg^{-1} DM) at five months old. Five weeks later, Groups A and B were given 50,000 *H. placei* infective larvae. At Day 25, infection in these groups was removed with oxfendazole. Four days later the same animals received a second infection with an equal dose of L3 and again it was terminated with oxfendazole, 25 days later. After four days, all groups were challenged with 100,000 larvae. Blood and faecal samples were collected for analysis weekly and bodyweights fortnightly. Five weeks after challenge, all calves were slaughtered for abomasal worm counts. The results demonstrated that the immunisation associated with normal and low dietary protein have no effect on worm establishment, although the immunised and normal dietary protein group showed a significantly lower worm burden. However, pathogenesis was milder in the immunised calves and for all studied parameters animals from Group B consistently showed milder alterations. During the immunisation period, the groups that received low protein diet, showed the lowest body weight changes and, after challenge, only the non immunised control groups had a significant body weight loss.



THE COURSE OF EXPERIMENTAL CYATHOSTOME INFECTIONS IN FOALS

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The course of experimental cyathostome infections was investigated in two trials with a total of 15 strongyle-naïve pony foals during the prepatent and early patent period. Each animal was infected with 130,000 third stage larvae of a benzimidazole-resistant population, that had been cold conditioned for one to eight months. The prepatent period was 43 to 52 days. At one and two weeks of patency, the mean egg output was 105 and 65 epg (trial one) and 335 and 850 epg (trial two), respectively. At post mortem examinations 15 (trial one) or 10 weeks (trial two) after infection, the geometric mean total cyathostome burden (*Cyathostomum*, *Cylicocyclus*, *Cylicostephanus*) was significantly lower in trial one (11,475) than in trial two (45,335). The mean relative proportion of tissue dwelling stages was also lower in trial one (4%) than in trial two (20%). Clinical signs and deviations from the so-called normal values were not recorded with any of the haematological and biochemical parameters measured during nine weeks p.i. However, mean serum protein concentrations rose significantly, and mean serum magnesium levels decreased continuously in both trials during nine weeks p.i. There was also a decreasing trend in the mean haematocrit and haemoglobin levels in the higher infected foals (trial two) versus the lower infected foals (trial one) between weeks three and six p.i. These alterations may be attributed to cyathostome infections.



CULTURE OF EQUINE STRONGYLIDAE TO THE FOURTH LARVAL STAGE IN A CELL FREE MEDIUM

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An efficient and reliable method for the culture of equine strongyles from the third to the fourth larval stage in medium of 50% fetal calf serum and 50% NCTC with additions of L-glutamine, NaHCO_3 , yeast extract, bactopectone and dextrose and a gas phase consisting of 10% CO_2 , 5% O_2 , and 85% N_2 . *Strongylus vulgaris*, *S. edentatus*, *S. equinus*, *Tridontophorus brevicauda*, *T. serratus*, *T. tenulcollis*, *Oesophagodontus robustus*, *Cyllococyclus insigne*, and mixed cyathostome spp. have been cultured to the L_4 stage. *O. robustus* has been cultured to the L_5 stage. Depending on species, 44% to 95% of strongyliinae L_3 s inoculated into this system molt to L_4 . Cyathostome larvae develop to the late L_3 stage, but ecdysis is markedly reduced (<1%). Viability of all spp. is high (>60 to 70% larvae surviving) for at least four weeks (cyathostomes) and as long as six months (*Strongylus edentatus*). The addition of equine hemin to cultures of *S. vulgaris* and *O. robustus* L_4 enhances development and prolongs viability of these larvae, but has little or no effect on cultures of *S. edentatus*, *S. equinus*, and cyathostomes.



SPONTANEOUS EXPULSION OF WORMS FROM PIGS MASSIVELY INFECTED WITH *TRICHURIS SUIS* IN A FARM USING THE FERMENTED SAWDUST LITTER CONFINEMENT PENS

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Rearing and management for pigs in confinement pens on fermented sawdust litter is a common practice in Japan. Such litter generally remains in use for more than six months with large accumulations of faeces and urine 'fermented' in the litter. Additionally, pigs eat the litter, and may acquire massive infections with *Trichuris suis*.

In 1986 Farm-H in Kagoshima, about 100 out of 400 fattening pigs died from *T. suis* infections. The sawdust litter from this site contained a large number of *T. suis* eggs; the mean egg count in 1 gram of litter was 221, and 3.3% of the eggs were embryonated. On 1st October, seven pigs with severe symptoms were transported to the Institute for examinations. The next day, four of these pigs (Nos 1, 3, 4, 7) were treated with dichlorvos (15 mg/kg bodyweight), and the other three (Nos 2, 5, 6) pigs were not treated. Pig No 2 died at two days and Pig No 1 died at 10 days after treatment and 43,600 and 124,800 *T. suis* were detected in their large intestines. All pigs expelled many immature *T. suis* into the faeces for up to four weeks after their transportation to the Institute. The maximal number of worms expelled in 25 g faeces in six pigs were 230 (No 1), 365 (No 3), 238 (No 4), 108 (No 7), 34 (No 5) and 315 (No 6), respectively. The mean body length of the worm was 18.4 (13.6-22.7) mm, the body width anterior and posterior were 11.5 (9.6-13.9) μ m and 18.3 (13.2-25.4) μ m, respectively. One pig, retained for long term observation, had expelled *T. suis* the faeces for 50 days. The efficacy of dichlorvos against the infection was not clarified.



GOAT *TELADORSAGIA CIRCUMCINCTA* GENETIC DIVERSITY IN 14 DAIRY-GOAT FARMS

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Allozyme variation was studied in 23 natural populations of *Teladorsagia circumcincta* from 13 farms in Touraine and from one farm in Poitou-Charentes (center region of France). These populations were investigated using starch-gel electrophoresis. Five enzymes (polymorphic and interpretable) were assessed. Biosys-1 program was used to calculate eventual heterozygote deficiencies and Rogers distances. Clustering among populations was visualised using the unweighed pair-group method with arithmetic averaging (UPGMA) based on Rogers' genetic distance. Genetic distances were also assessed by means of principal component analysis (PCA) based on the genotype frequencies.

Genotypic frequencies were compared to the expected Hardy-Weinberg proportions. An important deficiency in heterozygotes was recorded in all enzymes and all worm populations. It may be the result of the fact that farms do not exchange infected goats and thus gene flow is often nil. We distinguished three groups of farms:

- 1) Group 1:
 - presence of a third allele at locus MDH-1. That is a potential characteristic of goat derived strain, whereas sheep derived ones do not harbour this allele.
 - important deficiency in heterozygotes at locus MDH-1
- 2) Group 2:
 - reduced presence of third allele at locus MDH-1
 - slightly less important deficiency in heterozygotes
- 3) Group 3:
 - slow migrating LDH, rapid migrating GPI and slow migrating MPI were more frequent than in the other groups.

Genetic diversity between farms was thus evidenced and future work should be to relate this genetic diversity to farm-breeding conditions.

MDH
LDH
PGM
GPI
MPI

} enzymes
stud



THE EVER CHANGING STATUS OF HYDATID DISEASE: AN AUSTRALIAN PERSPECTIVE

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In Australia, hydatid disease caused by *Echinococcus granulosus* can no longer be considered to be a rural problem, confined principally to sheep raising areas. There is increasing evidence of urban foci of transmission across mainland Australia, which are associated with an upsurge in recreational hunting activities and the incursion of foxes into residential areas. The red fox is now known to be a suitable host for the common sheep strain of *E. granulosus* in Australia and surveys which have revealed infection rates of up to 47% must be extended throughout Australia. Human involvement in the perpetuation of such cycles is of paramount importance and must be taken into account when implementing control strategies which should be focused and targeted to particular groups.

Although a wild animal cycle of transmission involving dingoes and macropod marsupials has been recognised in Australia for over 50 years, recent surveys have revealed such cycles, and the range of hosts involved, to be much more extensive than previously was thought to be the case. There is interaction between these cycles and those involving domestic hosts, and in Western Australia there is evidence that human activity is responsible for a spill-over of infection from domestic animals to wild and sylvatic hosts!

Also of concern in Australia, is the increase in levels of hydatid infection in cattle. This has been seen across Australia and of particular significance have been the high levels, of up to 60% in some lines, seen in cattle from the Kimberley region of Western Australia. This is an area previously considered to be free of hydatid infection and the high levels now seen in cattle appear to reflect spill-over from a previously unrecognised wild and/or feral animal cycle. *Echinococcus* infection in cattle in Australia represents a potentially serious economic problem due to the increase in live cattle exports to countries in Asia.



INACTIVATION OF *ASCARIS SUUM* EGGS DURING STORAGE IN LIME TREATED SEWAGE SLUDGE

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The aim of the present laboratory experiment has been to examine the survival and development of *Ascaris suum* eggs in sewage sludge mixed with 10% of quick lime (CaO). Freshly collected *A. suum* eggs were mixed with sewage sludge during lime treatment at a concentration of 8.000 *A. suum* eggs per ml. The development of the eggs was followed every second week for 10 weeks and again after five months together with control eggs in 1% formalin.

The results showed that lime treatment of sewage sludge resulted in a pH > 12 and no development of *A. suum* eggs occurred during the observation period, whereas larvated eggs developed within four weeks in the control eggs. When the *Ascaris* eggs were isolated from the sewage sludge and suspended at neutral pH, the proportion that developed to larvated eggs decreased with time. Thus, the inactivation of *A. suum* eggs was reversible and incubation at neutral pH imitates spreading on agricultural land, where the eggs could be a potential infective hazard. In contrast, prolonged storage of sludge with pH > 12 completely destroy the ability of *A. suum* eggs to embryonate and after five months no intact eggs could be identified in the sludge. In conclusion, lime treatment and storage of sewage sludge at pH > 12 for at least three months is now accepted by the Danish veterinary authorities as a method that allows sludge to be used on agricultural land without hygienic restrictions.



**ABSTRACTS OF
SUBMITTED PAPERS**

SESSION 12

Diagnosis I





A SANDWICH ELISA TECHNIQUE FOR THE ESTIMATION OF THE ANTIBODY RESPONSE IN *PSOROPTES OVIS* EXPERIMENTALLY INFESTED CATTLE

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A sandwich ELISA technique using *Psoroptes cuniculi* crude antigen and serum from *P. cuniculi* infested rabbits was used to estimate the antibody response in cattle experimentally infested with *Psoroptes ovis*. Eleven 1-year-old parasite-naïve cattle, eight Belgian White Blue (BWB) and three Friesian-cross animals, were used: two BWB animals served as uninfested controls, all others were infested with a single dose of 600 *P. ovis* mites. Presence of mites and skin lesions and *P. ovis* antibody titres were monitored at regular intervals from 17 days prior to infestation until trial end at 99 days post infestation (p.i.). Three BWB animals were treated with ivermectin (200 mcg/kg SC) at Day 64 p.i..

Three of the infested BWB animals and all Friesian-cross animals developed clinical mange with presence of persistent skin lesions. A distinct antibody titre increase was observed in all these animals. In all other animals titres remained at pre-infestation levels. No decrease in antibody titres was observed from the day of ivermectin treatment through the end of the trial although regression of skin lesions and disappearance of live mites demonstrated this treatment to be effective.

It appears that the sandwich ELISA technique used in this trial may be useful for detection of developing active mange infestations in a cattle herd but not for early evaluation of the efficacy of an acaricide treatment.



APPLICATION OF ANTIGEN ELISA IN ASSESSMENT OF CURE IN AFRICAN TRYPANOSOMIASIS

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In an attempt to determine the diagnostic and prognostic value of the antigen-detection enzyme-linked immunosorbent assay (AgELISA) in sleeping sickness patients, vervet monkeys (*Cercopithecus aethiops*) were infected with *Trypanosoma rhodesiense*, then treated with various trypanocidal drugs. Following infection, serum antigens remained below detectable levels for a few days after onset of parasitaemia. Subsequently, the levels increased until treatment was carried out, after which they dropped depending on the drug used. When melarsoprol or suramin were used in acute disease, the blood became antigen-negative within 12 weeks. In animals treated with melarsoprol in late-stage disease antigenaemia persisted for more than 20 weeks. Two weeks after infection, antigens were detected in the cerebrospinal fluid (CSF). With treatment during the acute stage, an initial increase in CSF antigens occurred and persisted for up to 20 weeks. In two animals that relapsed, serum antigen levels started increasing several weeks before trypanosomes could be detected in the blood. The fall in antigen levels after curative treatment, and then increase several weeks before relapses indicates that the AgELISA technique has great potential as a seroepidemiological tool for sleeping sickness surveillance and control.



IGG AND IGM ELISA'S FOR MONITORING *TOXOPLASMA* INFECTION IN SWINE

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With the aim of developing routine serological tests for monitoring the *Toxoplasma* infection status of Danish swine herds, four ELISA's based on tachyzoite lysate were set up: 1) an indirect ELISA for IgG-antibody, 2) a blocking ELISA for antibody to the membrane antigen, P₃₀, 3) an indirect ELISA for IgM and 4) a reverse, antibody-catching IgM-ELISA.

Groups of pigs (N=6-10) were inoculated with tachyzoites of the RH-strain, tissue cysts of two complete strains and oocysts in two doses (10^3 and 10^4). Infection courses were followed by recordings of symptoms (fever, anorexia) and regular blood and faecal samplings. All inoculations were tolerated well. The presence of chronic tissue stages were tested by i.p. inoculation of pepsin/HCl-digested brain and muscle samples into mice.

Irrespective of strain and stage used for inoculation, specific IgG and anti-P₃₀ blocking activity appeared after one to two weeks with OD-values stabilising after three to four weeks and persisting throughout the study period (three to four months). Specific IgM appeared quickly, but was short-lived (approximately two weeks). After infection with complete strains, a strong correlation was found between seropositivity at slaughter and the presence of chronic stages in brain and heart. The RH-strain, however, also induced persistent IgG antibody despite the absence of chronic stages.

Further studies on assay specificity and the long-term persistence of reactivity are needed before the *Toxoplasma* ELISA may be integrated in running herd surveillance programs.



RECOMBINANT ANTIGEN ELISAs FOR EPIDEMIOLOGICAL STUDIES ON *TOXOPLASMA GONDII* INFECTIONS IN CATS

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Cats play an important role in the epidemiology of toxoplasmosis. Thus, there is a need for accurate data on the prevalence of *Toxoplasma gondii* infections in cats. Collection of such data involving serological testing of various cat populations requires the availability of a standardised test that allows the comparison of results among different laboratories and over time. Recombinant antigens are well-defined, reproducible and offer the potential to develop standardised tests that are easily adaptable to test large numbers of sera. Therefore, we tested two recombinant *T. gondii* antigens, termed H4 and H11, for their suitability as diagnostic antigens in enzyme-linked immunosorbent assays (ELISAs). The results obtained by ELISAs based on single H4, on single H11, or on a mixture of H4 and H11 (H4/H11-ELISA) were compared with results obtained by an ELISA based on traditional ELISA antigen (TEA-ELISA) and by an indirect fluorescent antibody test (IFAT). A total of 306 cats from a suburban cat population were tested of which about 45% showed serological evidence of *T. gondii* infection. Infection rates varied from about 32% for cats kept indoors to about 55% for stray cats. The specificity and positive predictive value of the H4/H11-ELISA were equal to those of the TEA-ELISA ($\geq 99\%$), and better than those of the IFAT (92% and 90%, respectively). The sensitivity and negative predictive value of the H4/H11-ELISA (95% and 96% respectively) were almost as high as those of the TEA-ELISA (both 98%), and again better than those of the IFAT (both 94%). Hence, the H4/H11-ELISA appears to be a very suitable test for epidemiological studies on *T. gondii* infections in cats.



ANTIBODY RESPONSES AGAINST EM18 AND EM16 IN HUMANS, WILD VOLES AND MICE INFECTED WITH *ECHINOCOCCUS MULTILOCULARIS*

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Most recently, we reported that antibody responses against single epitope, named Em18, of *Echinococcus multilocularis* were highly reliable for detection of active cases of alveolar hydatid disease (AHD) in humans (Ito *et al.* 1993, Trans. Roy. Soc. Trop. Med. Hyg., in press; Ito, Wang and Liu 1993, Am. J. Trop. Med. Hyg., in press): Most patients of AHD with advanced lesions recognized another epitope Em16 as well: Em18 was the major epitope recognized in AHD patients. In contrast, mice experimentally infected with protoscolices (Em-Ps) of this parasite showed different antibody responses: Em16 was the major epitope recognized in mice. So, we speculated that Em18 should be produced at the early stage of egg infection and not the major component of the Em-Ps (Ito *et al.* 1993). In order to evaluate this speculation, we analyzed antibody responses in wild voles naturally infected with this parasite using antisera against IgG of *Clethrionomys rufocanus bedfordiae* (Crb) comparing with those in AHD patients and mice.

SDS- PAGE and Western blot were carried out as described previously (Ito *et al.* 1993). All serum samples were diluted 1/50 with blocking buffer.

Antibody responses against Em18/Em16 were +++/+++ (Crb), +++/+++/+~+/- (patients) and +/+++ (mice). These antibody responses appeared to be divided into three groups: A, recognizes both Em18 + Em16 strongly as shown in the wild voles naturally infected with eggs and harbouring active lesions full of Em-Ps; B, recognizes Em18 more strongly than Em16 or recognizes Em18 only as shown in AHD patients accidentally infected with eggs and harbouring active lesions with poor proliferation of Em-Ps; C, recognizes Em16 more strongly than Em18 as in mice injected with Em-Ps and harbouring active lesions full of Em-Ps.

The present results appear to support our speculation that Em18 is produced at early stage of egg infection and minor component of Em-Ps, whereas Em16 is produced at late stage of infection and major component of Em-Ps.



DEVELOPMENT OF AN ELISA AND WESTERN BLOT FOR THE DIAGNOSIS OF *BABESIA CABALLI* INFECTIONS IN HORSES AND COMPARISON WITH IMMUNOFLUORESCENCE ANTIBODY TEST (IFAT) AND COMPLEMENT FIXATION TEST (CFT)

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Hitherto diagnosis of *B. caballi* infections in horses is routinely performed by the CF and IFA test. Both tests suffer from insufficient sensitivity and specificity. While our ultimate aim is to develop an ELISA based on defined recombinant antigens, we have now developed an ELISA and Western blot with crude antigen. From *B. caballi* *in vitro* cultures a preparation of 100% infected erythrocytes was obtained and antigens extracted with SDS-sample buffer for SDS-PAGE and Western blotting or with the detergent CHAPS for the ELISA. Control antigens of normal erythrocytes from the same donor horse were treated in an identical manner. The ELISA and Western blot were validated by testing of sera from horses experimentally infected with *B. caballi* or *B. equi* or not infected with *Babesia* spp. The sensitivity of the ELISA of 98.3% was superior to Western blot (94.9%), IFAT (96.6%) and CFT (28.8%). Cross-reactions of *B. equi*-positive sera did occur to a larger extent in the ELISA (20%) than in the IFAT (4%); no cross-reactions were observed with the Western blot or with the CFT. The higher sensitivity of the ELISA was also demonstrated by testing of 132 field sera when more positive results were obtained by ELISA (112) as compared to IFAT (92) or CFT (41). The validity of these results was confirmed by Western blotting. The ELISA provides the best method for the identification of carrier horses to prevent the introduction into non-endemic areas (testing for export). Positive ELISA results can be confirmed by Western blot, if a species-specific diagnosis is required.



DIAGNOSIS OF TAENIASIS-CYSTICERCOSIS BY WESTERN BLOT

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Taeniasis-cysticercosis is a clinical binomial very important in Mexico, due to the high incidence of neurocysticercosis. Diagnosis is difficult because there are no stage specific antigens. Proteins of metacestodes and adults of *Taenia solium* were studied by Western blot analysis. The antigens were revealed with sera from patients with confirmed taeniasis or cysticercosis, sera of hamsters with different times of infections with *T. solium* (infection was carried out by ingestion of cysticerci) and normal controls (sera from healthy people and from non-infected hamsters). Patients with confirmed cysticercosis recognised antigens from cysticerci with molecular weights (MW) of 170, 155 and 135 kDa. Antigens with MW of 107 and 90 kDa from *T. solium* and from cysticerci were recognised by all patients and all hamsters' sera. Therefore, we think these antigens might be cross-reactive. A 36 kDa antigen present only in *T. solium* extract was only recognised by taeniasis patients and by hamsters infected with the *Taenia*. We think this could be a *T. solium* stage specific antigen.



THE USE OF PCR AND DNA PROBE IN THE EPIDEMIOLOGICAL SURVEYS OF NATURAL TRYPANOSOME INFECTIONS

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The use of species-specific DNA probes in epidemiological surveys of African trypanosomiasis is being extensively tested in the field by several workers. Here we dissected over 3000 tsetse flies in three regions of Kenya and found a total of 156 flies infected with 71 *T. congolense* type and 85 *T. vivax*. Out of 71 *T. congolense* isolates, 38 were sub-inoculated into mice and only three of these infected the mice.

All the 71 infected tsetse midgut materials were spot blotted on Hybond Nylon filters and processed for DNA probe hybridization. We found 25 *T. congolense* savannah, two Killifi, 15 *T. simiae* and one immature *T. brucei* either as homogenous or simultaneous infections. At least 28 isolates either did not hybridize at all, or gave very weak and unclear hybridization signals to any of the probes examined.

These isolates were subjected to *Thermus aquaticus* polymerase chain reaction and amplified to sequence oligonucleotides specific to *T. simiae*, *T. brucei* and savannah *T. congolense*. The amplified products showed positive DNA probe hybridization signals in 22 isolates either as single or mixed infections of savannah *T. congolense* and *T. simiae*. Six isolates still remained unidentified even after PCR amplification.

This work was supported by IFS grant B/1206-2, ILRAD and ICIPE.



DNA PROBES FOR THE DETECTION OF *FASCIOLA HEPATICA* IN AND *DICROCOELIUM DENDRITICUM* IN INTERMEDIATE HOSTS

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Repetitive DNA probes have been shown to provide sensitive tools for the detection of a wide variety of parasites in their intermediate and final hosts and have thus proven to be particularly useful for epidemiological purposes. Monitoring the infection rate of snails and ants, which function as intermediate hosts and harbour larval stages of *Fasciola hepatica* (*F.h.*), and/or *Dicrocoelium dendriticum* (*D.d.*) is an important component of epidemiological studies on large and small liver fluke disease. For this purpose, DNA probes were generated which can be used for the detection of *F.h.* larvae in snails and *D.d.* in both snail and ant intermediate hosts. Highly repetitive DNA fragments were cloned in a plasmid vector and tested by Southern blot hybridisation to the DNA of various trematodes for specificity and sensitivity. Three probes termed Fhr-I, Fhr-II and Fhr-III hybridised only to *F. hepatica* DNA. Squash blot analysis showed that the different probes were able to detect the parasite larvae in trematode-infected snails even as isolated single larvae. One of the probes, derived from *D.d.* and designated as Ddr-IV, was found to be specific for *D.d.* Infected ants were easily identified by squash blot hybridisation with ³²p-labelled Ddr-IV. Probes Fhr-I, Fhr-II, Fhr-III and Ddr-IV are thus useful specific tools for studying the epidemiology of liver fluke infections.



GENUS SPECIFIC DNA PROBES FOR THE IDENTIFICATION OF STRONGYLE EGGS FROM CATTLE FECES

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DNA sequences specific for members of four common cattle nematode genera were sought in order to develop a semi-quantitative DNA based test for the identification of morphologically similar parasite eggs excreted within the feces. Genomic DNA libraries were developed from *Ostertagia ostertagi*, *Haemonchus placei*, *Cooperia oncophora*, *Oesophagostomum radiatum*. Sub-libraries consisting of 400 randomly chosen clones from each species were screened in quadruplicate with radiolabelled homologous DNA as well as radiolabelled heterologous DNA to identify specific sequences. One clone from each parasite DNA library was selected and its specificity verified by Southern blot and dot blot analyses. Clones were further tested for specificity within their own genus. Subsequently, the clones were used to analyze strongyle egg DNA blotted on nylon membranes. The results showed that the clones may be employed diagnostically to differentiate strongyle eggs isolated from cattle feces.



**ABSTRACTS OF
SUBMITTED PAPERS**

SESSION 13

**Protozoa - Antigens and
Immunity I**





RESTORATIVE EFFECTS OF A NEWLY SYNTHESISED PEPTIDE (OBIOPEPTIDE-1) IN CYCLOPHOSPHAMIDE-PRETREATED MICE INFECTED WITH OPPORTUNISTIC BACTERIA

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Large numbers of cyclophosphamide- or carrageenan-pretreated immunosuppressed adult ddY mice died within 10 days after inoculation with several species of opportunistic bacteria, including *P. aeruginosa*, *K. pneumoniae*, *E. coli*, *S. aureus* and methicillin resistant *S. aureus*. When immunosuppressed mice were administered bacteria in combination with two 100 µg/mouse intramuscular doses of a newly synthesised peptide, Obiopeptide-1 (OP-1), survival rates increased significantly. At 24 and 48 hours after intraperitoneal inoculation with *P. aeruginosa*, counts of viable organisms from the livers, spleens, lungs, hearts and kidneys of mice that were administered cyclophosphamide in combination with OP-1 were significantly lower than counts from mice that were administered cyclophosphamide alone. Bacteriocidal activity of peritoneal cells, neutrophils and monocyte-macrophages was higher in OP-1 pretreated mice than in non-OP-1 treated mice. This newly synthesised peptide, OP-1, is a potential immunomodulator which increases host resistance against bacterial infection. The peptide may also function as a nonspecific blood stimulating factor.



THE EFFECT OF IMMUNOSUPPRESSION CAUSED BY CYCLOPHOSPHAMIDE TO MICE CHRONICALLY INFECTED WITH *TOXOPLASMA GONDII*

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Mice chronically infected with *Toxoplasma gondii*, were treated with cyclophosphamide or anti-CD4 monoclonal antibody to identify the effect of these immunosuppressive reagents on the cysts in the brain of infected mice. The effect of Obiopeptide-1 as an immunomodulator treatment of the immunosuppressed mice was also examined.

In the brain of non-treated and cyclophosphamide-treated, chronically infected mice mainly typical large tissue cysts, and sometimes divided cysts, were detected after staining with hematoxylin-eosin and anti-*Toxoplasma* ABC technique. In contrast, the brain from anti-CD4 - treated, chronically infected mice, contained multiple degenerated *Toxoplasma* tissue cysts of different size in some partial regions in the brain. Mice chronically infected with *Toxoplasma* and treated with a combination of cyclophosphamide and Obiopeptide-1 showed a higher survival rate than those treated with cyclophosphamide alone. The percentage of neutrophilic leukocytes in mice treated with a combination of Obi-1 and anti-CD4, or Obi-1 and cyclophosphamide, was higher than that of mice treated with anti-CD4 or cyclophosphamide alone.

These results indicate that reactivation or rupture of tissue cysts in mice treated with cyclophosphamide, chronically infected with *Toxoplasma*, might be mainly mediated by CD4 positive cells rather than other immunocomponent cells. The increase of neutrophilic leukocytes might contribute to the induction of the resistance to *Toxoplasma gondii* in mice, after treatment with Obi-1 and cyclophosphamide in combination.



IMMUNISATION OF CATS WITH TISSUE CYSTS, BRADYZOITES AND TACHYZOITES OF THE T-263 STRAIN OF *TOXOPLASMA GONDII*

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Previous studies have demonstrated that oral administration to cats of tissue cysts of the oocyst negative mutant of *Toxoplasma gondii*, T-263, induces immunity to oocyst shedding following challenge. The experiments described herein were designed to compare the levels of protection induced by *T. gondii* T-263 when tissue cysts, bradyzoites released from tissue cysts and tachyzoites are administered to cats. In one experiment, groups of cats received two oral doses of intact tissue cysts or released bradyzoites of *T. gondii* T-263 and were challenged with the oocyst producing strain of *T. gondii*, T-265. All cats seroconverted following immunisation and all were protected from shedding oocysts following challenge. In a second experiment, groups of cats received tachyzoites of *T. gondii* T-263 as a single oral dose and either one or two intraduodenal doses; they were challenged with *T. gondii* T-265. All cats seroconverted following immunisation. None of the cats were protected from shedding oocysts except for two of seven cats that received two intraduodenal doses of tachyzoites. Thus, orally administered bradyzoites of *T. gondii* T-263, either contained in intact tissue cysts or liberated from cysts, induced immunity to oocyst shedding. In contrast, tachyzoites did not completely protect against shedding, even when delivered directly to the duodenum and despite the development of high antibody titers.



CHARACTERISATION OF DIAGNOSTIC ANTIGENS FOR *BABESIA CABALLI*

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The existing serological tests (CFT and IFAT) for *B. caballi* lack sufficient sensitivity and specificity. Thus, improved serological tests ideally based on defined recombinant antigens would be highly desirable. As a first step towards this aim diagnostic *B. caballi* antigens of apparent molecular weights of 141, 112, 70, 50 and 48 kDa were identified by SDS-PAGE and Western blotting. In this study three of these antigens (141, 50 and 48 kDa) were further characterised. Rabbits were vaccinated with gel-purified antigens and monovalent antibodies obtained except for the 50 kDa antiserum which cross-reacted with the 48 kDa antigen. The rabbit sera and a pool of equine *B. caballi* sera were used for Western analysis of *B. caballi* antigens after two dimensional electrophoresis. When probed with the pool of equine *B. caballi* sera, the 50 and 48 kDa antigens were present as horizontal bands over a pH range from approximately 5.0 to 7.0; probed with the rabbit serum directed against the 50 and 48 kDa antigens focussed spots at a pH of 5.5 and 5.9 respectively were observed. The 141 kDa antigen was not present after two dimensional electrophoresis. None of the antigens could be identified as a glycoprotein by labelling via the oxidised hydroxyl groups of sugar residues. Judging from the immunofluorescence staining pattern of the rabbit sera the 141 kDa antigen is present on the surface of infected erythrocytes. The 50 and 48 kDa antigens are located in the parasite itself, but not on the surface of infected erythrocytes. The punctate staining pattern suggests that the 50 and 48 kDa antigens might be located in or associated with the apicomplex of the parasite.



ANALYSIS OF *BABESIA EQUI* ANTIGENS BY TWO-DIMENSIONAL ELECTROPHORESIS AND WESTERN BLOTTING

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Only very limited information is available about the antigens of and antigenic differences between different strains of *Babesia equi*. We characterised *B. equi* antigens by two dimensional electrophoresis and Western blotting with equine sera from different geographical origins. In a lysate of *B. equi* infected erythrocytes (USDA strain) nine major antigens or antigen groups of mol. wts. from 43 to 19 kDa were recognised by sera from horses experimentally infected with the USDA strain and field-infected horses from Europe. This suggests, that most antigens demonstrated in the USDA strain are highly conserved among European strains. In contrast, only four of these nine antigens were also recognised by sera from field-infected horses from Brazil, reflecting considerable antigenic differences between the USDA or European strains and South American strains. Some antigens were also recognised by sera from horses not infected with *Babesia* spp. and appear to be of host rather than parasite origin. Of the antigens only recognised by immunesera, two antigens of 31 kDa and one each of 19 and 20 kDa were identified as diagnostic antigens for European strains and one of the 31 kDa antigens as diagnostic for all strains of *B. equi*.



**ABSTRACTS OF
SUBMITTED PAPERS**

**SESSION 14
Anthelmintics - Drug
Resistance II**



A FIELD SURVEY ON ANTHELMINTIC RESISTANCE IN SMALL STRONGYLES OF THE HORSE

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Purpose: to evaluate the presence of cyathostome resistance to the commonly used anthelmintics for horses in Norway, fenbendazole (FBZ), pyrantel (PYR) and ivermectin (IVM). Further, to compare the effect of IVM and PYR on the cyathostome larval stages.

Methods: 17 stables, from seven to 24 horses, of different races were studied. The horses within a stable were randomly grouped and treated with FBZ, PYR and IVM. Fecal samples for egg counts were taken just before treatment, at Day 14 and Day 90 post treatment. Fecal egg count reductions were calculated for each group at Day 14 post treatment (FECR) and at Day 90 post treatment (FECR3) for the PYR and IVM groups. Because of no parasite reinfection and EPG-values very close to 0 at Day 14 post treatment for both IVM and PYR groups, FECR3 was used to compare the effect of IVM and PYR on the cyathostome larval stages.

Results: According to the definition of resistance; FECR <95% and lower 95% confidence limit <90%; resistance to FBZ in cyathostomes was present in 14 of the 17 stables. For PYR and IVM no resistance was detected. No significant difference in FECR3 was found between IVM and PYR groups ($p=0.68$).

Conclusions: This study shows a similar frequency of benzimidazole resistance to cyathostomes as studies from Germany (Bauer 1986) and from Denmark (Bjørn *et al.*, 1991). The FECR-values in the resistant stables are, however, considerably higher in this study than in the two others. The reason could well be the different parasite control programs. Questionnaire study showed 3.8 treatments per year in this study compared to 7.1 in Denmark. Besides, alternation between anthelmintics of different modes of action was much more common among the stables in this study. The FECR3 values were used for comparison of IVM and PYR, but does not tell anything about the overall effect on cyathostome larval stages of the two anthelmintics.



SYNERGISTIC ACTIVITY OF ANTHELMINTICS FOR THE CONTROL OF SUSCEPTIBLE AND RESISTANT STRAINS OF *FASCIOLA HEPATICA* FOR THE PREVENTION OR MANAGEMENT OF ANTHELMINTIC RESISTANCE

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The efficacy of anthelmintics or their combinations against immature *Fasciola hepatica* populations which were susceptible or possessed resistance to one or more drugs was evaluated in controlled tests in merino sheep. The efficacy of triclabendazole, clorsulon and luxabendazole alone at the low dose rates of 2, 3 to 5 and 5 mg/kg were 79, 25 to 31 and 26% effective against a susceptible strain respectively. When triclabendazole at 2 mg/kg was given in combination with either the two low dose rates of clorsulon or that of luxabendazole, the high efficacy of 91 to 96% was achieved through an additive effect. When the efficacy was tested against a strain resistant to triclabendazole, the efficacy of triclabendazole at 2 mg/kg was only 30%. Clorsulon at 3 or 5 mg/kg and luxabendazole at 5 mg/kg gave efficacies of 30, 68 and 21% respectively. When triclabendazole was combined with either clorsulon or luxabendazole, high efficacies of 98 to 99% were achieved, showing a true synergistic effect. In the trials, conducted with a strain resistant to both closantel and luxabendazole, the resistance of the strain was shown when either closantel or luxabendazole at the dose rate of 7.5 mg/kg was ineffective. Triclabendazole at 2 mg/kg and clorsulon at 5 mg/kg were 30 and 68% effective respectively. When closantel was combined with luxabendazole, triclabendazole or clorsulon at the above dose rates, high efficacy of 93 to 97% was achieved through a strong synergistic effect. Some synergistic combinations with triclabendazole, clorsulon, salicylanilides and luxabendazole would reduce the cost of anthelmintics for the treatment against immature fluke. The combination products would prevent the development of resistance and would control strains already resistant to any of the anthelmintics used.

Some combinations would synergize for high efficacy against resistant and susceptible *F. hepatica* and will be effective against gastrointestinal nematodes, lungworms and tapeworms. The closantel + luxabendazole combination will synergize for efficacy against susceptible *F. hepatica* or against those resistant to either closantel, luxabendazole or both. Since the mode of drug action and the mechanism of resistance in liver fluke and nematodes are similar, synergistic effect would also occur against strains of *Haemonchus contortus* resistant to closantel, benzimidazoles or levamisole. They would also be effective against other gastrointestinal nematodes, lungworms, tapeworms and *Dicrocoelium dendriticum*.



ANTHELMINTIC DRUG RESISTANCE IN GOATS IN PENINSULAR MALAYSIA

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Ninety six randomly selected farms, located throughout Peninsular Malaysia, were surveyed for goat nematodes resistant to benzimidazoles (BZ). On 33 farms BZ resistance was demonstrated by means of an egg hatch assay (EHA) (LD₅₀ thiabendazole (TBZ) > 0.10 µg/ml). There was a clear association between frequency of anthelmintic treatments and the development of BZ resistance. To assess the value of the EHA, faecal egg count reduction (FECR) tests were also done on 20 farms. On seven farms the LD₅₀ TBZ was less than 0.10 µg/ml and the FECR higher than 95%; on 10 farms with an LD₅₀ TBZ of over 0.10 µg/ml a FECR of less than 95% was measured; on three farms the FECR was less than 95% although the EHA showed LD₅₀ TBZ values of less than 0.10 µg/ml. On two of these three farms a controlled efficacy test confirmed the presence of BZ resistant *Haemonchus contortus*. From these results it can be concluded that the EHA underestimated the true incidence of BZ resistance. Levamisole resistance was detected with a FECR test on two of 10 farms investigated. *H. contortus* was found to be the main species involved in anthelmintic resistance.



USE OF ANTHELMINTICS BY SWINE FARMERS IN DENMARK

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An epidemiological investigation of anthelmintic resistance in swine herds in Denmark is being performed. The first phase of the study of which the data are presented here was an interview with the farmers using a questionnaire on the use of anthelmintics. Eighteen specially trained veterinarians returned 123 questionnaires which were used in the following analysis.

In 1992, 87.1% of the farmers have been treating their sows while 77.4% of the farmers have not been treating their fatteners. When treated, Class I anthelmintics were used by 49.4% of farmers on the sows. Class II and Class III anthelmintics follow in preference being 27.1 and 15.3% respectively. The pattern among classes was similar within the past 6 years. Farmers preferred the currently used anthelmintics since they can be used as a feed dressing (61.3%). The anthelmintic preparation but not necessarily the class of anthelmintic was changed every two to three years by 42.1% of the farmers. A similar proportion of farmers (42.1%) changed the preparation with a frequency of less than once in four years. Dosage calculations were done by eye measurement of the bodyweight of sows by 63.4% of the respondents. Price of drugs in the market was involved in choice of the anthelmintic by 45.2% of the farmers.

Faecal egg count reduction tests and larval development assays are being performed on the same study material testing at least two classes of anthelmintic at each locality. One case of *Oesophagostomum* spp. resistance against Class II anthelmintics has already been detected during the study which will be completed in three months time.



**ABSTRACTS OF
SUBMITTED PAPERS**

**SESSION 15
Anthelmintics - Control I**





THE EFFECTS OF DIFFERENT METHODS OF ANTHELMINTIC SUPPRESSION IN CALVES ON PARASITOLOGICAL PARAMETERS

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The objective was to examine the effects of different methods of anthelmintic suppression for first year grazing calves on parasitological parameters during their first two years. Five groups of 15 calves were used. During year one each group grazed on a separate paddock within the same field; in year two all calves grazed together as one group. The treatment groups were (1) untreated controls, (2) ivermectin injection given at 3, 8 and 13 weeks after turnout, (3) ivermectin injection given at week 10 post turnout, (4) morantel flex bolus administered at turnout, (5) morantel flex bolus given at week 10 post turnout. Five calves were slaughtered at the end of both year 1 and year 2, and in addition at the end of year 2 the remaining calves were artificially infected with a single infection of *Ostertagia* and *Cooperia* spp.

During year one all anthelmintic methods protected the respective calves from clinical parasitism. There were marked differences between treatments in faecal egg output and in the percentages of *Ostertagia* and *Cooperia* spp eggs excreted during both years, in serum pepsinogen concentrations, and worm counts.



THE STRATEGIC CONTROL OF FASCIOLIASIS USING TRICLABENDAZOLE - A FOUR YEAR STUDY

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The object of this study was to assess the potential of a strategic dosing scheme to provide long term control of fascioliasis. The principle of the control programme was to prevent infection of the intermediate host snail, *Lymnaea truncatula*, and thus reduce subsequent pasture contamination by using triclabendazole to suppress *Fasciola hepatica* egg output at critical times of the year. During the first two years all stock were treated three times at eight week intervals starting in March with a fourth dose being given when the cattle were housed. Out-wintered sheep were given an extra dose in January. In years three and four the dosing intensity was reduced, sheep being treated in January, April and November and cattle prior to turnout and post housing. Stock infection levels were monitored by faecal sampling, pasture contamination by the grazing of fluke free tracer sheep and snail infection levels by the monthly collection and dissection of snails. Monitoring was also carried out on a neighbouring farm which continued with a more traditional dosing regime. After the first two years there was a reduction in the percentage infected stock, in fluke burdens, in the proportion of infected snails and in metacercarial pasture contamination. Results obtained in years three and four show that a reduction in dosing intensity did not lead to a re-emergence of the disease nor an obvious increase in infection levels. It appears therefore, that a strategic dosing scheme based on the use of triclabendazole could be used to lower the incidence of fascioliasis in areas where the disease is endemic so that clinical disease is not apparent and production losses are minimised.



THE DOSE AND MOVE SYSTEM FOR THE CONTROL OF CATTLE LUNGWORM

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In the Netherlands low patent lungworm infections have been shown to occur in spring in a small proportion of cows in almost all dairy cattle herds. This implies that calves almost certainly will acquire low infections when they are turned out on a pasture which has been grazed earlier by cows. Reinfection will occur from one week after the beginning of patency and disease may occur two to three months after turnout. It may be possible to prevent such disease outbreak by anthelmintic treatment within two months after turnout combined with a move to aftermath, a procedure which is well established as a preventive measure for gastrointestinal helminthiasis. This 'dose and move' concept for the control of lungworm has been studied in a grazing trial at the University of Utrecht using moxidectin 7 weeks after turnout. The results demonstrated an effective control of lungworm and *Ostertagia*. Despite a high anthelmintic efficacy of moxidectin *Cooperia* was not effectively controlled because initial faecal egg counts were extremely high (> 6000 EPG). Challenge infections at the end of the grazing season indicated that development of immunity of calves against lungworm was sufficient.



STRATEGIC CONTROL OF STRONGYLES IN PONY FOALS IN ONTARIO

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In mid-May 1992, 30 pony mares with foals were allocated to three treatment groups and each group was placed on a separate pasture. In late September, foals were weaned and mares removed from the trial. From mid-November, foals were in loose housing until April 1993. Mares in two treatment groups were given ivermectin paste orally at 200 µg/kg bodyweight at turnout to pasture and eight weeks later. Foals in one group of treated mares were given ivermectin paste orally at 2, 4, 6 and 8¹/₂ months of age. Every two weeks, a faecal sample was taken from each mare and foal and from May to November herbage samples from each pasture. Foals were weighed monthly.

Prior to treatment the mean strongyle epg for mares in all groups was similar and above 1100. Mean eggs for untreated mares then ranged from 1386 to 2200; for treated mares it was at or about zero except in September when it rose to 362 and 587 in the two groups. Herbage strongyle larval counts from the pasture with untreated ponies were high throughout the season; the highest, on 20th July was 69,420 larvae/kg dry herbage. Fewer larvae were on the other two pastures where the highest numbers were 16,530 and 17,920.

Untreated foals had strongyle, roundworm, threadworm and pinworm eggs in the faeces and from September the mean strongyle epg in each of the two groups was greater than 1000. Treated foals had few or no faecal parasite eggs except in January before the 8¹/₂ month treatment and in April when the mean strongyle epg was approximately 500. Mean weights of foals in the three groups ranged from 24 to 25 kg at the start of the trial and 106 to 120 kg at the end. Mean weight gain was similar in all groups until September after which it was similar in foals from treated mares and significantly higher than in foals from untreated mares. The 2-treatment strategy in mares resulted in less pasture contamination with strongyle eggs and infective larvae and allowed for increased weight gains in their foals.



THE BENEFITS OF AN ANTHELMINTIC CONTROL PROGRAMME BASED ON THE USE OF STRATEGIC ANTHELMINTIC TREATMENTS AND USING THE EPIDEMIOLOGICAL DATA FOR TRADITIONALLY MANAGED SHEEP IN THE MIDDLE ATLAS (MOROCCO)

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In a farm representative of the Timahdit area, two flocks containing 150 (Flock A) and 70 (Flock B) ewes of the same breed (Timahdit) and health conditions, two to seven years old and traditionally managed in the same conditions, were used to compare the economics of two anthelmintic control programmes (EPOM and ME) using strategic anthelmintic treatments during two consecutive years (August 1988–July 1990). EPOM was based on the results of our previous epidemiological investigations on the epidemiology of sheep parasitic disease in the Middle Atlas, while ME was based on some sparse observations and had been established several years before. EPOM comprised three treatments: Ivermectin (0.2 mg/kg b.w.) at the beginning of August, Albendazole (5 mg/kg b.w.) in late November and Fenbendazole (10 mg/kg b.w.) in late February. ME comprised four treatments: Ranisole (12.5 mg/kg b.w.) at the beginning of September, Ranisole (12.5 mg/kg b.w.) at the beginning of November, Tetramisole (15 mg/kg b.w.) in late December (ewes only) and Rafoxanid (12.5 mg/kg b.w.) for ewes and Tetramisole (15 mg/kg b.w.) for lambs in late February. As compared with ME, the EPOM programme showed more efficient prevention of clinical forms of mange, *Melophagus ovinus*, gastrointestinal and lungworm infections. It also showed an improvement of lamb weight gain (an additional 2.43 kg per lamb at six months of age) and of wool production (an additional 460 g per shearing). The yearly bank return showed an economical benefit after using EPOM of 7986 Dirhams (998.25 US \$) per 100 ewes when compared with the ME programme.



**ABSTRACTS OF
SUBMITTED PAPERS**

SESSION 16

Pathology I





EXPERIMENTAL *ASCARIS SUUM*-INFECTIONS IN CALVES

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The intensive pig industry in the Netherlands produces more than 19×10^9 kg slurry. Most of the slurry is spread on arable land, but a certain amount is brought onto pasture land. If the slurry contains viable eggs of *Ascaris suum* and sheep or cattle graze on these pastures, infections may occur in these hosts and give rise to clinical problems. To investigate the effect of *A. suum* in calves, experimental infections were carried out. Nine calves were infected with doses varying from 10^2 to 10^7 eggs, given as a single or repeated dose. Calves were slaughtered at 4, 9, 14 and 66 days after the first infection. Mild coughing was observed in calves dosed with at least 10^4 eggs. A high eosinophilia ($>40\%$) was found in all calves dosed with 10^4 eggs or more. In calves which were dosed repeatedly the eosinophilia remained at a high level. The ELISA-titre was also highest in repeatedly infected calves. After slaughter at 66 days no severe pathological lesions were seen. Lesions were most pronounced in the calf dosed with 10^6 eggs and slaughtered 9 days later. Larvae could be recovered from the lungs and the trachea of calves slaughtered after 4, 9 and 14 days.



ALLERGIC PARASITIC ABOMASITIS IN ZEBU CROSS CATTLE IN ARGENTINA

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An edematous abomasitic syndrome in Zebu cross cattle has been observed in the Humid Pampa of Argentina since 1987. This work describes five particular cases studied between 1987 to 1991 which tend to define this disease. The disorder starts with a progressive loss of weight and a general clinic bad condition. It is responsible for a high mortality rate when no proper measures are taken to control it. At necropsy, no food is found in the digestive tract and the most important lesion is a jelly-watery oedema in the mucosae-submucosae of the abomasum which in serious cases can measure 2 cm width. Signs occur in winter although some cases have been observed in autumn. It occurs in contaminated areas, while no cases have been observed in animals on clean or "safe" pastures. Apparently, food quality and quantity, different climatic conditions (frost, rain or cold temperature) have no special importance in the evolution of this disease.

No relationship between previous anthelmintic treatments or number of larvae on grass samples and the disease, was found. The most inclined to die from this disease, although it is not categorical, are young animals. Mortality ranges between 3 and 17% of total animals. In some herds mortality can range to 40%.

Disease is controlled by treating animals with ivermectin for all parasites, managing allergic signs (corticoids), preventing secondary bacterial infections (antibiotic) and avoiding ingestion of new L3 larvae from grass (management). In order to prevent this syndrome, pastures have to be free from larval infection.



SUDDEN CARDIAC DEATH OF LAMBS FOLLOWING EXPERIMENTAL INFECTION WITH *STRONGYLOIDES* *PAPILLOSUS*

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It has been demonstrated that a heavy percutaneous infection with *S. papillosus* larvae causes sudden cardiac death in calves. In the present study, we carried out experimental infections to elucidate the pathogenicity of the parasite in lambs. Thirteen Suffolk lambs were percutaneously infected with 1, 000-32,000 infective larvae/kg of body weight. Four lambs were intraduodenally implanted with 2,500-22,500 parasitic females/kg recovered from the small intestines of infected rabbits on Day 10 post-infection (pi). Electrocardiograms were monitored. Eleven lambs given 3,200 or more larvae/kg died on Days 11 to 20 pi. They showed no premonitory signs except for anorexia just before death. Sinus tachycardia was recorded from Days 6 to 9 pi up to terminal ventricular fibrillation preceded by atrioventricular block, premature ventricular contraction and/or ventricular tachycardia. The sequence of arrhythmias was identical to that in fatal strongyloidiasis in calves. No lesions were found at necropsy except for congestions in the small intestine and hemorrhages in the lung alveoli. Three out of the four lambs given parasitic females developed continuous sinus tachycardia immediately and died on Days 2 to 9 post-inoculation having arrhythmias as found in the cases of percutaneous larval infection. These results indicate that *S. papillosus* is pathogenic for lambs as much as for calves and the parasitic females in the small intestine can cause cardiac dysfunctions in the absence of migratory larvae.

EFFECT OF GASTROINTESTINAL NEMATODE INFECTIONS ON SERUM ALKALINE PHOSPHATASE IN SHEEP

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Purpose: Serum alkaline phosphatase (SAP; EC 3.1.3.1) activity originates mainly from bone, liver and intestine. Intestinal nematode infections in sheep reduce intestinal mucosal AP and affect mineral absorption, and hence bone formation. The aim of this study was to correlate SAP with levels of mixed nematode infections.

Methods: Groups of eight 5 month old lambs were on infected (I-groups) or clean (C-groups) pasture, respectively, from April at low, medium or high stocking rates. In September lambs were faecal sampled and bled. EPG, serum-pepsinogen, and SAP were measured.

Results of EPG and blood analyses (means of eight lambs per group)

	EPG	S-pepsinogen(Iu/l)	Weight gain (g/day)	SAP (μkat/l)
I-low	140	0.3	125	7.4
I-med	140	0.4	108	5.4
I-high	2040	0.6	52	3.5
C-low	0	0.2	194	10.5
C-med	0	0.2	109	7.7
C-high	0	0.1	110	7.3

Conclusions: The groups on infected pastures acquired moderate to severe parasitic gastroenteritis; the nematode infections significantly reduced SAP (t-test; $P < 0.05$), probably due to reduced bone formation or -remodelling. Data on bone CT-scanning at post mortem will be presented.



BODY COMPOSITION, WATER AND NITROGEN BALANCE IN CALVES INFECTED WITH *COOPERIA PUNCTATA*

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Because of the high prevalence of *Cooperia punctata* in Brazilian herds, an experiment was conducted to examine the water, nitrogen balance and body composition changes in calves infected with that nematode. Four helminth-free male Friesian calves, four-months-old, received a daily infection of 20,000 *C. punctata* infective larvae, for a two-week period. Five days before and at the third week after the beginning of infection, the calves were housed in metabolic crates for body composition and nutritional studies. They were all injected with 1 MBq of tritiated water per Kg body weight. Fecal, urine and body samples were daily collected during the metabolic observations. Egg counts and PCV examinations were conducted throughout the experiment. The calves were necropsied 24 days after the beginning of infection. The mean worm burden at necropsy was $26,643 \pm 12,028$ *C. punctata*. There were no changes in the PCV parameters. The first eggs were passed out in the faeces on the 15th day. The study showed a tendency for decrease in the half-life and fractional turnover rate as well as a significant ($p < 0.05$) decrease in the total body water and fat free weight. Infection did not appear to affect adversely the water balance, however the mean fecal excretion and the fecal water excretion were lower in the infected animals. A significant ($p < 0.05$) change in the nitrogen (N) balance was also observed, with a lower N intake and a reduction in the N retention by the infected calves. These changes were influenced by a significant urinary N loss and a decrease in the fecal N excretion.



**ABSTRACTS OF
SUBMITTED PAPERS**

**SESSION 17
Arthropod Control II**



EFFICACY OF IVERMECTIN AGAINST CATTLE MYIASIS CAUSED BY *COCHLIOMYIA HOMINIVORAX*

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Myiasis caused by *Cochliomyia hominivorax* is one of the main ectoparasitic problems in the tropical areas of South and Central America. To evaluate the prophylactic effect of ivermectin administered subcutaneously at a dose level of at least 200 mcg per kg b.w., eight trials were conducted in Argentina and Brazil. In two trials, newly born calves were treated within 24 hours of calving to investigate the prevention of navel myiasis. In the other six trials, two with two-month-old male calves and four with male calves four months or older, the animals were castrated immediately prior to treatment and the incidence of scrotal myiasis observed. In all trials, control and treated calves were maintained together on pasture where they were naturally exposed to *Cochliomyia hominivorax*. The navel or scrotal wound of each calf was examined frequently for the presence of myiasis for at least 14 days after treatment.

Calves treated with ivermectin had a significantly ($p < 0.01$) lower incidence of navel and scrotal myiasis than untreated control calves.

Ivermectin injection administered to calves immediately after birth or castration provided prophylactic control of myiasis caused by *Cochliomyia hominivorax* larvae of the navel and of the scrotal wound.



DERMATOBIA HOMINIS CONTROL BY FLUMETHRIN AND CYFLUTHRIN POUR-ON

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The A. A. report in this paper the field trials carried out in the province of Misiones (Argentina) during the *Dermatobia hominis* infestation seasons 1988-1989 and 1991-1992, testing Flumethrin 1% pour-on and Cyfluthrin 1% pour-on at the dosage rate of 10 ml/100 kg b.w. In these trials 386 cattle of different breeds, sex and age were treated and to evaluate the efficacy against larval stages of *Dermatobia hominis*, the animals were checked every 10 days for 100 and 105 days respectively. The results obtained for both products were highly effective for the control of the tropical warble fly. Flumethrin 1% pour-on killed *Dermatobia hominis* larvae up to 22 days after treatment and prevents new reinfestation for another 12 days. Cyfluthrin 1% pour-on also killed *D. hominis* larvae up to 30 days after treatment and prevents new reinfestation for another 14 days. In this area of the province of Misiones where trials were carried out the A. A. estimate that repeating the pour-on treatment with these products every 40/45 days, it is possible to control tropical cattle grubs during the *Dermatobia hominis* endemic season and also other harmful ectoparasites such as *Boophilus microplus* (common cattle tick) and *Haematobia irritans* (horn fly).



SUSCEPTIBILITY TO INSECTICIDES OF HORN FLY *HAEMATOBIA IRRITANS* RECENTLY INTRODUCED INTO LIVESTOCK REGIONS OF BRAZIL

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The horn fly *Haematobia irritans* was restricted to the northern region of South America by a natural barrier of the Amazon Forest for more than 20 years. However, the horn fly crossed the natural barrier and it is now present in the most important livestock areas of the country. *In vitro* studies conducted twice at five month intervals using filter paper impregnated with different concentrations of pyrethroids and organophosphorus showed the following lethal concentration (LC 50): deltamethrin 0.23 - 0.56 $\mu\text{g}/\text{cm}^2$; cyhalothrin 0.43 - 0.64 $\mu\text{g}/\text{cm}^2$; permethrin 0.68 - 1.10 $\mu\text{g}/\text{cm}^2$; diazinon 0.20 - 0.44 $\mu\text{g}/\text{cm}^2$. Field studies of the treatment of naturally infested cattle with pyrethroids and organophosphorus prescribed for the control of the tick *Boophilus microplus* showed that the actual population of *H. irritans* is very susceptible to deltamethrin, alphamethrin, cypermethrin, DDVP and coumaphos plus trichlorfon when applied either by spraying or pour-on at the concentrations recommended for control of *B. microplus*. A dose titration trial in the field using deltamethrin showed excellent control at the lowest level used 1.56 ppm.



MOXIDECTIN: EFFICACY AND DOSE TITRATION IN CATTLE EXPERIMENTALLY INFECTED WITH *BOOPHILUS MICROPLUS* (CAN.), IN ARGENTINA

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Twenty Hereford beef cattle of approximately 200 kg lw were experimentally infected twice a week from Day -25 to +14 with 70 mg of *Boophilus microplus* (Can.) larvae. At treatment (Day 0) the cattle were allocated to five similar groups based on tick counts and individually housed in cement floor stalls. Group 1 was considered the untreated control; Groups 2, 3 and 4 were treated with Moxidectin (1% injectable solution) at 0.1, 0.2 and 0.4 mg/kg lw, respectively, whilst Group 5 was treated with ivermectin SC at 0.2 mg/kg. From Day 1 until Day 35 all engorged female ticks dropping from the cattle were collected and counted. To determine the effects on egg production, 20 engorged female ticks were taken daily, at random, from each Group and incubated for 15 days to induce oviposition. A 1 gramme aliquot of the egg mass of ticks collected from each treatment group, was incubated for 30 days to study egg fertility.

Efficacy against engorged females ranged between 71 and 92%, with no differences among the four treated groups ($P>0.05$). All treated groups showed more than 90% inhibition of egg production ($P<0.05$), and hatchability was also diminished. No lesions at the injection site were seen or adverse reactions observed in any treated group during the experimental period. The trial proved that Moxidectin 1% injectable, at 0.1, 0.2 and 0.4 mg/kg is effective against *Boophilus microplus* in the different parameters studied.



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Monitoring studies based on *in vitro* and *in vivo* evaluation showed a decrease in the efficacy of several pyrethroids and organophosphorus compounds used for control of ectoparasites in cattle. Spray treatment of cattle naturally infested with larvae of *Dermatobia hominis* on two different farms with DDVP and trichlorfon at concentrations of 0.111675% and 0.392%, respectively, showed a larvae mortality rate ranging from 25.4% to 50.9% for DDVP, and 13.7% for trichlorfon. During an outbreak of biting lice *Damalinia bovis* which occurred during the winter of 1992, in the State of Rio Grande do Sul, the evaluation *in vitro* using the sandwich larval test and *in vivo* based on the mini-dip- test detected the resistance of *D. bovis* to pyrethroids. A retrospective study with *Boophilus microplus* the cattle tick, showed an increase of resistance to pyrethroids and amitraz. The treatment of dairy cattle twice a month will probably put more pressure for development of highly resistant strains in the future.



**ABSTRACTS OF
SUBMITTED PAPERS**

SESSION 18

Diagnosis II





APPLYING REGRESSION OF P.C.V. ON E.P.G. FOR SUGGESTING AN EFFECTIVE ANTHELMINTIC TREATMENT FOR OVINE HELMINTHOSIS

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To determine the degree of helminth pathogenesis and predict an intervention with anthelmintics, 64 six to twelve month old sheep were grazed for one year on an area predominantly infected with *Haemonchus contortus*. Every month eggs per gram of faeces (e.p.g.) by McMaster and packed cell volume (p.c.v.) by Microhaematocrit Centrifuge methods were used to estimate the worm burden and resulting helminthic pathology in the sheep. Simple and quadratic linear regressions of the p.c.v. on the e.p.g. were then analysed.

The quadratic regression was found to be more informative. It showed a gradual fall in the p.c.v. until it reached 25% with a corresponding e.p.g. of 1800. Thus it is concluded that anthelmintic treatment until an e.p.g. of 1800 and p.c.v. of 25% could possibly save the sheep. Treatment after this stage has been reached becomes ineffective to reverse the condition and the sheep continue suffering from progressive debility.



EPIDEMIOLOGICAL RISK FACTORS ASSOCIATED WITH CLINICAL CYATHOSTOMIASIS IN THE HORSE

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Multiple logistic regression was used to assess epidemiological risk factors associated with the diagnosis of cyathostomiasis in 87 cases of chronic diarrhoea in the horse. Bivariate analysis identified age, season and the length of time since last deworming as important parameters whereas access to grazing and recurrence of symptoms were only weakly associated with a diagnosis of cyathostomiasis. Multivariate analysis of these parameters using logistic regression was performed. The final model included age, season and time since last deworming. The model had a specificity of 86.0%, sensitivity of 66.7%, overall correct classification of 79.3%, a positive predictive value of 71.4% and a negative predictive value of 83.1%. The results of this study indicated that only the specified variables may be useful in the differentiation of clinical cyathostomiasis from other causes of chronic diarrhoea.

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NATURAL *ASCARIS SUUM* INFECTIONS IN SWINE MEASURED BY COPROLOGICAL AND SEROLOGICAL METHODS

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The purpose of this study was to examine the antibody response against *Ascaris suum* and to compare the results with faecal egg counts. In each of 20 medium to large size sow herds in Denmark faeces and blood were collected from 15 weaners (appr. 25 kg b.w.), 15 large fatteners (appr. 90 kg b.w.) and 10-15 lactating sows together with blood from two piglets of each litter. The samples were analyzed by a modified McMaster technique and an indirect ELISA, using *A. suum* L₂/L₃-excretory-secretory antigens and anti-swine-IgG. The results showed an increasing percentage of coprologically positive samples with age (weaners: 4%, fatteners: 20%), followed by a decrease in the sows (9%). The serological results on the other hand showed much higher numbers of positive cases, which clearly increased with age (weaners: 28%; fatteners: 63%; sows: 80%). The *A. suum* specific antibodies were to a large extent transferred to the suckling piglets, which sometimes had even higher antibody levels than their mothers. However, the 25 kg weaners were often totally seronegative, which reflected that the maternal antibodies had been catabolized and that the piglets in these herds didn't become infected in the farrowing pens. In some of the latter herds the fatteners continued to be helminth-free during the whole fattening period. In some other herds the pigs acquired infection at an early stage as illustrated by coprologically and serologically positive weaners, indicating that a heavy transmission had taken place in the farrowing pens. In individual pigs there was no significant correlation between egg counts and antibody level. It is concluded that serodiagnosis is superior to coprological examination in studies on prevalence rates and transmission patterns of *A. suum* in swine herds.



EVALUATION OF AN ELISA AND A HISTAMINE RELEASE TEST SYSTEM FOR THE DETECTION OF PIGS NATURALLY INFECTED WITH *ASCARIS SUUM*

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An indirect ELISA and a histamine release test system were evaluated for the detection of pigs naturally infected with *Ascaris suum*. Histamine, released by antigen provocation of blood leukocytes was selectively bound to glass microfibres and detected fluorometrically, following coupling to o-phthaldialdehyde. Two antigens were used in the two tests: Adult body fluid (ABF) and L2/L3 excretory/secretory antigens, obtained by in vitro cultivation of hatched infective larvae. The number of worms in the small intestines of the pigs and the number of eggs in the faeces were determined and the liver milk spots counted, together with differential blood leukocyte counts. A total of 150 pigs, weighing approximately 90 kg, from 23 farms were tested. Seventy one (47%) of the pigs had either adult worms, faecal eggs or liver milkspots. Twenty out of 23 farms (87%) delivered *A. suum* infected pigs to the slaughter-house. Liver milk spots were detected in 23.4% of the pigs. When the presence of three or more liver milkspots was considered evidence of an *A. suum* infection, the ELISA using L2/L3-ES as the antigen gave a test sensitivity of 97% and a specificity of 89%. Significant associations were achieved between the presence of milkspots and the results obtained in the tests using both antigen types in ELISA and using L2/L3 in the histamine release assay. No significant association was found between milkspots and the histamine release test using ABF as antigens, between number of milkspots and presence of intestinal *A. suum* worms and between the immunological test systems and the hematological data. The present material provided a model for calculating the probability of a pig having three or more liver milk spots originating from a natural *A. suum* infection. This model could be of value in epidemiological surveys as well as in combination with surveillance of other infections in pigs, which is regularly performed by blood sampling. Furthermore, it can be used as a tool to classify herds as *A. suum* free.



**ABSTRACTS OF
SUBMITTED PAPERS**

SESSION 19
Protozoa - Antigens and
Immunity II





IDENTIFICATION OF *BABESIA BIGEMINA* INFECTED ERYTHROCYTE SURFACE ANTIGENS CONTAINING EPITOPES CONSERVED AMONG STRAINS

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The presence of new antigens on the surface of intact erythrocytes infected with three strains of *Babesia bigemina* from Kenya and one each from Puerto Rico, Mexico, St Croix and Texcoco-Mexico was demonstrated by Indirect immunofluorescent antibody (IFA) reactions. These new antigens were not strain specific because antibodies in bovine immune serum to either the Mexico or Kenya isolates reacted with all seven strains tested. Immune serum antibodies bound a maximum of 83% of intact infected erythrocytes, but not uninfected erythrocytes and caused agglutination of only infected erythrocytes. Antibodies eluted from the surface of infected erythrocytes had IFA reaction patterns among strains similar to those of immune sera before elution. Eluted antibodies were used to determine if these antigens were protein and encoded by *B. bigemina*. Eluted antibodies bound seven parasite-encoded proteins of 240, 220, 66, 62, 58, 52 and 38 kDa in an erythrocyte surface-specific immunoprecipitation reaction of ^{35}S -methionine labeled proteins. It was concluded that the surface of *B. bigemina* infected erythrocytes had parasite-encoded proteins and that these proteins had surface exposed epitopes that were conserved among the seven strains examined which were from two continents.



FIELD IMMUNISATION OF CATTLE IN IRELAND AGAINST BOVINE BABESIOSIS WITH A GERBIL- DERIVED LIVE VACCINE

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Live *Babesia divergens* parasites in gerbil erythrocytes were used to vaccinate calves, yearling cattle and cows in County Clare, Ireland. Vaccination consisted of the subcutaneous injection of 10^6 - 10^7 infected gerbil erythrocytes in RPMI 1640 medium with 40% foetal calf serum, at least three weeks before turnout, with or without the prophylactic drug, imidocarb dipropionate (IMDP) at 0.5 mg/kg three days before or on the day of vaccination. In most trials these procedures resulted in no adverse reactions to the vaccine and protected the cattle from natural infections. So far 1281 cattle have been vaccinated and adverse vaccine reactions have occurred in only six (0.4%) animals, all of which were in high risk groups. The current vaccine is usable in calves and strong yearlings without IMDP, but pretreatment with IMDP in older cattle and especially cows is recommended. Attenuation, immunostimulation, storage and culture studies are in progress in order to improve the vaccine further.



TROPICAL THEILERIOSIS IN MOROCCO - STUDIES ON AN ATTENUATED CELL CULTURE VACCINE

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An attenuated schizont infected cell line known as *T. annulata* Doukkala has been the subject of investigation as a potential vaccine cell line for use in Morocco where tropical theileriosis represents a major threat to small holder dairy cattle.

Controlled *in vivo* experiments were performed to test the degree of attenuation of the vaccine and the level of immunity stimulated. Groups of calves were immunised with cells from early (10⁵-117) and late (257) passages and at low (10⁴) and high (10⁶) cell doses and were given a lethal heterologous sporozoite challenge at either one or six months post immunisation. The vaccine cell line was more, but not fully, attenuated at the higher passage level at which both low and high cell doses provided good immunity even after six months. Characterisation of the vaccine cell line using glucose phosphate isomerase isoenzyme analysis, monoclonal antibodies and DNA probes showed it to contain a single parasite population even at early passage levels (p45), indicating that attenuation involves more than just clonal selection. This single population appeared to be present as a subpopulation in several mixed field isolates examined. These methods of characterisation have provided several markers which remain stable on passage through the bovine host and provide a means for confirming the identity of the vaccine cell line. Analysis of breakthrough reactions in calves post challenge showed them to be due to components of the challenge parasite rather than recrudescence of vaccine parasite.



**ABSTRACTS OF
SUBMITTED PAPERS**

SESSION 20

Anthelmintics - Control II





DEVELOPMENTAL SAFETY OF ALBENDAZOLE IN CATTLE

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The developmental toxicity of albendazole was assessed in cattle by oral administration. Pregnant cows were exposed to 10, 15 or 25 mg/kg on Days seven and 14; on Days seven or 14; on Days 21, 31, 41, 51, 61; and on Days 7, 14, 28 and 48 of gestation. One of these groups of pregnant cows was exposed to 25 mg/kg 30 days prior to calving. The conception rate of cows dosed with 25 mg/kg on Days 7 and 14 of gestation was 63% (conception rate in concurrent controls was 85%); the conception rate of cows dosed with 25 mg/kg on Days 21, 31, 41, 51 and 61 of gestation was 85%; all calves born were normal and there were no abortions in cows dosed. Conception rates were significantly reduced when 25 mg/kg was administered to pregnant cows on Day 7 or 14 of gestation. The 14-day old embryo appeared to be much more susceptible to albendazole than the 7-day old embryo. Differences in conception rates for the controls and cows dosed with 15 mg/kg on both Days 7 and 14 of gestation were not statistically significant. The conception rates of presumed pregnant cows dosed on gestation Days 7, 14, 28 and 42 with 10 mg/kg were similar to those in the concurrent controls. These studies demonstrated that albendazole (1) 25 mg/kg was embryotoxic, but not teratogenic, (2) 25 mg/kg of albendazole was not abortifacient, and (3) 10 or 15 mg/kg was safe to pregnant cows even when administered at the time of high embryonic vulnerability.



EFFECTS OF MORANTEL SUSTAINED RELEASE BOLUS AGAINST GASTROINTESTINAL NEMATODES IN FIELD GRAZING CALVES IN KIAMBU DISTRICT, KENYA

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The efficacy of the morantel sustained release bolus (MSRB) (Paratec^R bolus) in reducing gastrointestinal nematodiasis in field grazing calves was evaluated using 12 steer calves of various European breeds aged approximately six months and randomly divided into two groups of six animals each. Calves in the treatment group each received a MSRB while the controls were left untreated.

Each group was placed on adjacent 2.5 acre paddocks obtained by subdivision of a 5.0 acre permanent pasture which had previously been grazed by adult cattle and all animals were thus exposed at the same time to a risk of infection from gastrointestinal nematodes.

All animals were weighed and fecal samples per rectum including jugular blood samples for serum extraction were collected from each animal, beginning on Day 0. Herbage samples were also collected for larval counts, live weight gain and serum pepsinogen levels were monitored at regular intervals until trial termination (Day 140). On Day 140, two control and two treated calves were removed from pasture, housed in isolation for three weeks then necropsied for recovery of gastrointestinal nematodes. Three sets of parasite naive tracer male calves were utilized to evaluate the initial, interim and final levels of pasture contamination by nematode larvae.

The use of the MSRB resulted in a reduction in fecal egg counts of trichostrongyles and numbers of gastrointestinal nematodes in the treated principal animals, as well as daily live weight gain advantage (0.5 kg^{-1} MSRB versus 0.34 kg^{-1} controls) over the 140 day period. Similarly there was a reduction in pasture larval nematode contamination of pastures grazed by the treated animals, as indicated by the parasite burden in tracer calves and pasture larval counts.



SUPPRESSION OF INDUCED INFECTIONS OF *DIROFILARIA IMMITIS* BY MONTHLY TREATMENT WITH IVERMECTIN (6 MCG/KG) BEGINNING AT FOUR MONTHS PI

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Clinical prophylactic treatment of dogs with 6 mcg/kg ivermectin (IVER) monthly for 13 months beginning four months after inoculation with heartworm suppressed infection, whereas treatment with milbemycin oxime (MIL) (500 mcg/kg) was only partially effective. In a preliminary trial, nine beagles were each given 50 L₃ of *D. immitis* and allocated to three groups. One group was given IVER (Heargard 30®), one group was given MIL (Interceptor®), and one group served as a nontreated control. Worm recoveries at necropsy one month after the last treatment (18 mos. PI) revealed that IVER was 98% effective in suppressing infection. One dog treated with IVER was cleared of worms, one had one dying female worm, and one had one live male worm only. MIL was 46% effective, but all dogs treated with MIL had heartworms (avg. 15/dog); it appeared that male worms were more sensitive. All control dogs had heartworms (avg. 27.7/dog). None of the dogs treated with IVER developed a patent infection, but two dogs treated with MIL had a few microfilariae (MF) at eight and nine months PI, respectively. All nontreated dogs had MF at eight months PI and monthly thereafter. Adult heartworm antigen (ASSURE/CH™ test) levels were negative to low in IVER-treated dogs and moderate to high in MIL-treated and nontreated dogs. At 17 months PI, two controls were negative for antibody to cuticular antigen(s) of MF on an IFA test, whereas two IVER-treated and all MIL-treated dogs were positive. This experiment is being repeated using five dogs per group. Also, some dogs are being treated monthly with these drugs beginning at three months PI.



WORLD CLINICAL DEVELOPMENT OF MELARSOMINE DIHYDROCHLORIDE FOR ADULTICIDE TREATMENT OF CANINE HEARTWORM

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Melarsomine dihydrochloride (code named RM340) has been developed as an adulticide therapy for the treatment of symptomatic and asymptomatic infestations of stage 5 larvae and adult *Dirofilaria immitis* in the dog.

After definition of the optional dose regime clinical studies have been carried out in veterinary practices in France, Italy, Australia, Japan and the USA.

The product has been launched for sale in Italy and Australia and request for marketing authorisation has been filed in France and Japan.

An NADA is expected to be filed in the USA in the second half of 1993.

Clinical studies have shown that local and general tolerance are acceptable given the indication for which the production is intended.

Efficacy of treatment determined by response to serum antigen tests and resolution of clinical signs is excellent.



RESIDUAL NEMATOCIDAL EFFECTIVENESS OF IVERMECTIN IN CATTLE

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A study was conducted to assess the duration of ivermectin anthelmintic persistence by measuring post-treatment nematocidal effectiveness of both the topical and injectable formulations of ivermectin. Thirty-five nematode-free calves were randomly allocated to one of five treatment groups (seven animals/group). The treatment group designations were: unmedicated control, IVOMEC® Injection at the rate of 0.2 mg/kg BW on trial Day 0, IVOMEC Injection at the above rate on trial Day 7, IVOMEC Pour-on at the rate of 0.5 mg/kg BW on trial Day 0, and IVOMEC Pour-on at the above rate on trial Day 7. All animals were subsequently given a standard dose of infective larvae comprised of *Haemonchus*, *Cooperia*, *Trichostrongylus* and *Oesophagostomum* genera on trial Day 21. One week later, each animal was additionally administered a standardized dose of *Dictyocaulus* and *Ostertagia* infective larvae. Trial animals were humanely sacrificed on trial Days 49-52 for nematode quantification. In animals infected 28 days after treatment with Pour-on, counts were reduced 89% for *Dictyocaulus* and at 21 days after treatment 99% and 97% respectively for *Oesophagostomum* and *Ostertagia* and at 14 days after treatment 94%, 98% and 98% respectively for *Haemonchus*, *Cooperia* and *Trichostrongylus*. For injection treated animals, highly significant persistent efficacy was shown: 28 days post treatment (PT) – *Ostertagia* and *Dictyocaulus*, 21 days PT – *Haemonchus* and *Oesophagostomum* and 14 days PT – *Cooperia* and *Trichostrongylus*. Similarly, for the pour-on treated animals, results were as follows: 28 days PT – *Dictyocaulus*, 21 days PT – *Oesophagostomum* and *Ostertagia*, and 14 days PT – *Haemonchus*, *Cooperia* and *Trichostrongylus*. Study results indicate that ivermectin persists at highly efficacious levels in cattle following routine treatment with either the injectable or pour-on formulation, and that this persistence is exhibited against the most prominent and deleterious pulmonary and gastrointestinal nematodes of cattle.



**ABSTRACTS OF
SUBMITTED PAPERS**

SESSION 21
Anthelmintics in Productivity





THE IMPACT OF A STRATEGIC PARASITE CONTROL PROGRAM WITH IVERMECTIN ON WEIGHT GAIN AND ASSOCIATED REPRODUCTIVE PERFORMANCE OF ANGUS HEIFERS IN ARGENTINA

C. M. ENTROCASSO, P. E. STEFFAN AND A. ALMADA

Vergara 1485, (1638) Vicente Lopez, Argentina

A strategic parasite control program for weaned Angus heifers in the Humid Pampa region of Argentina was designed with the aid of a computer model (PARABAN). A group of 156 newly weaned Angus heifers, approximately 7-9 months old, was allocated by restricted randomization on body weight to three treatments: 1) oxfendazole at 2.5 mg/kg orally, once on Day 0 (weaning = Apr. 26 1991); 2) oxfendazole at 2.5 mg/kg orally, on Day 0 and Day 132 (Sep. 5); 3) ivermectin at 200 mcg/kg SC, on Days 0, 35 (May 31) and 73 (Jul. 8). Four replicates of each treatment were formed and maintained on separate pastures until Day 196 (Nov 8). Weight gain and parasitological variables were monitored at approximately monthly intervals until Day 196, when all heifers ≥ 240 kg were run with bulls for six weeks. Pregnancy examinations for heifers that ran with bulls were conducted on Day 304 (Feb. 24). Cattle treated three times with ivermectin gained significantly ($p < 0.01$) more weight than cattle treated once or twice with oxfendazole. At the start of the mating season, 79% of the cattle in the ivermectin treatment group had reached the minimum weight for service, compared with 23% and 53% for the oxfendazole treatment groups. Considering all heifers in each treatment group at the start of the trial, 67% of the ivermectin group was pregnant, compared with 15% and 47% for heifers treated once or twice with oxfendazole. Pasture larval counts were similar for all groups from Day 0 through Day 101 (Aug. 5). On Days 132 and 185 (Oct. 28), counts on the ivermectin pastures were lower than on the oxfendazole pastures. Parasite control with ivermectin used according to a schedule designed with PARABAN allowed heifers to achieve optimum growth and reproductive performance under prevailing management conditions.



STUDY OF PRODUCTIVITY IN WEANER STEERS TREATED WITH ABAMECTIN IN THE NORTHERN HUMID PAMPA OF ARGENTINA

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S. GROSS, J. GUERRERO AND A. MASCOTENA

Vergara 1485, (1638) Vicente Lopez, Buenos Aires, CC 3686 (1000) Capital Federal RCA, Argentina

The impact of nematode parasite control in weaner steers was evaluated in experimental animals naturally infested with common gastrointestinal strains found in the Northern Humid Pampa of Argentina. Sixty Brangus weaned calves, seven to eight months old were chosen from a herd. Fifteen animals each were allocated to four treatment groups. Group 1 was treated with abamectin 1% w/v injection (DUOTIN, Merck) administered subcutaneously at 1 ml/50 kg bw (equivalent to a minimum dosage of 200 mcg abamectin/kg), at weaning and three times more at intervals not longer than five weeks each.

Group 2 was treated with abamectin at weaning, twice at two month-intervals during the winter, and in December. Group 3 was treated with fenbendazole 10% w/v (AXILUR, Hoechst) administered orally at a dose rate of 5 cc/100 kg bw (equivalent to 5 mg/kg bw) at weaning, twice during the winter (according to common farm practice), and later on in December at a dose rate of 7.5 cc/100 kg bw (equivalent to 7.5 mg/kg bw) to avoid *Ostertagiasis* type II.

Group 4 received fenbendazole orally at a dose rate of 5 cc/100 kg bw at weaning (equivalent to 5 mg/kg bw) and were kept untreated during the trial.

Every 30 days, the following procedures were carried out: weighing, faecal egg counts, larval culture, blood samples, monitoring of pasture herbage samples.

Large numbers of EL4 *Ostertagia ostertagi* were observed from August to December in tracer calves slaughtered. Evidence from tracer calves indicated that maturation of inhibited larvae occurs from December to January.



MOXIDECTIN: EFFICACY IN A CATTLE PRODUCTIVITY TRIAL UNDER RANGE CONDITIONS IN THE HUMID PAMPAS OF ARGENTINA

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A total of 45 Aberdeen Angus heifer calves, in three groups of 15 animals each, were grazed during a 12 month period in three homogenous separate paddocks on a single farm in the central area of the Humid Pampas, with improved pasture naturally infested with gastrointestinal nematodes. Group I was the untreated Control; Groups II and III were treated with Ivermectin and Moxidectin (0.2 mg/kg, i.w., SC), respectively, in June, November and March. Major objectives of the trial were to determine the effects on weight gain, pasture contamination and infectivity, using susceptible cattle treated with Moxidectin when compared against Ivermectin or non-treated controls. At the end of the trial average weight gain increased by 27 kg in both treated groups, compared with controls ($P < 0.05$); no significant difference ($P > 0.05$) in weight gain was observed between the treated groups. High egg counts (EPG) were present on commencing the trial (June 1989) in the three groups, decreasing after the first treatment in Groups II and III, and after September (spring) in the untreated control. High levels of L_3 on the pasture were recovered in Group I from July to early October 1989 (winter and early spring), then decreasing until the end of the trial. Main parasites recovered in faecal cultures were *Ostertagia* spp., *Trichostrongylus* spp., *Haemonchus* spp., *Cooperia* spp. and *Oesophagostomum* spp., *Ostertagia* being the most frequent of the larvae found. The new macrocyclic lactone, Moxidectin, showed excellent activity and efficacy, both in decreasing faecal egg counts and improving weight gains, whilst the recovery of L_3 from the pasture was kept consistently at a low level. Ivermectin showed comparable results in the evaluated parameters. No adverse effects were observed with the use of either of these two endectocides.



STUDY OF PRODUCTIVITY IN WEANER STEERS TREATED WITH ABAMECTIN IN THE PROVINCE OF CORDOBA, ARGENTINA

M. E. MUÑOZ COBENAS, C. EDDI, J. CARACOSTANTO GOLO, J. NOLAZCO,
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The impact of nematode parasite control in weaner steers was evaluated in experimental animals naturally infested with common gastrointestinal strains found in the Northern Humid Pampa of Argentina. Sixty Zebu x Hereford weaned calves, seven to eight months old were chosen from a herd. Fifteen animals each were allocated to four treatment groups.

Group 1 was treated with abamectin 1% w/v injection (DUOTIN, Merck & Co) administered subcutaneously at 200 mg/kg at weaning, and twice again at intervals not longer than five weeks each, and lastly in December.

Group 2 was treated with abamectin at weaning, twice at two month-intervals during the winter, and in December.

Group 3 was treated with fenbendazole 10% w/v (AXILUR, Hoechst) administered orally at a dose rate of 5 mg/kg bw at weaning, then at the beginning of spring and later on in December at a dose rate of 7.5 mg/kg bw to control *Ostertagiasis* type II.

Group 4 received fenbendazole orally at a dose rate of 5 mg/kg bw and were kept untreated during the trial.

Every 30 days, the following procedures were carried out: weighing, faecal egg counts, larval culture, blood samples, monitoring of pasture herbage samples.

An increase in weight gain ($p < 0.05$) was observed in calves which received systematic treatment with abamectin during autumn/winter and during the *Ostertagiasis* type II infective period (Group 1).

Mean plasma pepsinogen values were higher in those groups receiving fenbendazole (Group 3 and 4), while levels of plasma pepsinogen were always lower in groups treated with abamectin even during *Ostertagiasis* type II infective period during which an increase in plasma pepsinogen values was observed in all the experimental groups.



STUDY OF THE EPIDEMIOLOGY AND CONTROL OF OSTERTAGIASIS IN THE NORTHERN HUMID PAMPA OF ARGENTINA

M. M. COBENAS, C. EDDI, J. CARACOSTANTOGOLO, J. NOLAZCO, S. GROSS,
J. GUERRERO AND A. MASLOTENA

Vergara 1485, (1638) Vicente Lopez, Argentina

The impact of nematode parasite control in weaner steers was evaluated in experimental animals naturally infested with common gastrointestinal strains found in the Northern Humid Pampa of Argentina. Sixty Brangus weaned calves, 7-8 months old were chosen from a herd. Fifteen animals each were allocated to four treatment groups. Group 1 was treated with abamectin 1% w/v injection (DUOTIN, Merck & Co. Inc.) administered subcutaneously at dosage of 200 mcg. abamectin/kg., at weaning, and three times more at intervals not longer than five weeks each. Group 2 was treated with abamectin at weaning, twice at two month-intervals during the winter, and in December. Group 3 was treated with fenbendazole 10% w/v (AXILUR, Hoescht) administered orally at a dose rate of five mg/kg b.w. at weaning, twice during the winter, and later on in December at a dose rate of 7.5 mg/kg b.w. to control *Ostertagiasis* type II. Group 4 received fenbendazole orally at a dose rate of 5 mg/kg b.w. at weaning and were kept untreated during the trial. Every 30 days, the following procedures were carried out: weighing, fecal egg counts, larval culture, blood samples, monitoring of pasture herbage samples. Large numbers of EL4 *Ostertagia ostertagi* were observed from August to December in tracer calves slaughtered. Evidence from tracer calves indicated that maturation of inhibited larvae occurs from December to January.



**ABSTRACTS OF
SUBMITTED PAPERS**

SESSION 22

Pathology II





BABESIA MICROTI AND TRYPANOSOMA MUSCULI INFECTIONS IN MICE CONCOMITANTLY INFECTED WITH TRICHINELLA SPIRALIS

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Naturally occurring parasitic diseases of tropical livestock are often the result of concurrent infections with several species of helminth and/or protozoan parasites. The host parasite relationship in such polyparasitoses is complex and most conveniently studied using laboratory animal models.

Experiments were carried out to examine the influence of concurrent infection of inbred mice with the nematode, *Trichinella spiralis*, on the kinetics of parasitaemia of two blood protozoa namely, *Babesia microti* and *Trypanosoma muscui*. Concomitant nematode infection had no significant influence on the parasitaemia profile of *B. microti* in NIH mice but produced a small increase in peak parasitaemia in C57BL/10. In contrast, a single dose of 300 larvae, given from seven days before to seven days after intraperitoneal inoculation of 1.5×10^4 or 3.0×10^4 trypanosomes, gave rise to significant ($P < 0.05$) increase in peak parasitaemia and to delayed immune elimination of the trypanosome in both strains of mice but particularly in the NIH, which is naturally refractory to the trypanosome. The outcome of *T. spiralis* - *B. microti* interaction was therefore essentially neutral, especially in the NIH, while that between the nematode and *T. muscui* was synergistic. The latter effect was strongly influenced by 1) host genotype 2) the timing of nematode infection.



EFFECT OF PSOROPTIC MANGE IN HOUSED SHEEP IN PATAGONIA, ARGENTINA

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One of the difficulties in eradicating *Psoroptes ovis* (Hering) is convincing the farmer and the government that sheep scab is of economic importance. Therefore, the objective of this study was to measure comparatively the effect on production and quality of the wool in *P. ovis* affected sheep. Two groups of 28, clean uninfested eight-month-old, male Merino sheep were allocated separately in covered pens. Sheep from Group 1 (G1) were each infested by placing 20 to 30 living *P. ovis* mites on to the skin of the back. Sheep from Group 2 (G2) were set aside as uninfested controls. The sheep were weighed individually on Day 0, 24, 31, 38, 54, 63, 70, 89 and 96. The disease followed its normal course in G1, showing a general deterioration in health, failure to compete for food and loss of body condition. At Day 32 and 39 all animals (G1 and G2) were dosed with Ivermectin 200 mcg/kg, in order to prevent unnecessary suffering in G1. The result of the treatment was a complete control of *P. ovis*. In G1, five sheep died exhausted after three to five days prostrated. The average body weight in G1 decreased up to 10% during the trial, while in G2 was maintained with minor fluctuations as is seen in normal farming winter conditions in Patagonia. The evolution of mean body weight between groups were statistically different ($P < 0.05$). At shearing, majority of G1 sheep showed depilating on the back, neck and legs, and 64% of them showed felted wool. Wool characteristics such as wool weight, finess, staple length and style were measured, but only the last two were statistically different ($P < 0.05$) between groups. Despite the deteriorated production induced by sheep scab, the main financial loss in winter outbreaks, however, would be by mortality as a number of sheep would certainly die if the animals are not treated or if they are treated by dipping.



CLINICAL PICTURE AND SERUM ANTIBODY RESPONSE IN EXPERIMENTAL *SARCOPTES SCABIEI* VAR. *VULPES* INFECTION IN THE RED FOX (*VULPES VULPES*)

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A devastating *Sarcoptes scabiei* epizootic hit the Swedish wild red fox (*Vulpes vulpes*) population in the mid 1970's reducing it drastically in just a few years. The infection also affected foxes on fur-farms. The aim of this investigation was to study the clinical picture of the infection and to determine if serum antibodies to *S. scabiei* in red foxes could be detected by an ELISA employing crude *S. scabiei* antigen and a monoclonal anti-dog immunoglobulin G antibody (Mab- α -dog).

Materials and methods: Three red foxes were infected on their backs with a small number of *S. scabiei* mites from a piece of skin of a naturally infected wild red fox. One fox was left non-infected. An indirect ELISA was run on sera obtained before the application of the infection and then once every week for 18 weeks. ELISA-plates were coated overnight with a crude antigen of *S. scabiei* var. *vulpes*. Mab- α -dog with rabbit- α -mouse conjugated to HRPO was employed as conjugate.

Results: The first signs of sarcoptic mange were seen on the infected foxes about four weeks after the infection (wpi) and developed to typical symptoms of mange on two of them. The third fox developed chronic localised lesions on the back. Antibody to *S. scabiei* was first recorded at three - four wpi in all infected foxes. Antibody levels progressively increased during the period of observation.

Conclusion: Symptoms of sarcoptic mange in red foxes can take more than a month before being recognisable. Serum antibodies can be detected earlier, from wpi three, by an ELISA.



DEMODICOSIS IN CATTLE

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Cattle demodicosis occurs frequently and mostly as a chronic subclinical folliculitis. Its importance is due to the degradation of raw skin quality which results in leather damage. Clinical signs and diagnostic possibilities in nodular and squamous cattle demodicosis are shown on living cattle, raw skin, intermediate products in leather manufacturing and on finished leather. Eight main defects are distinguished: at fleshside - crater; at grain - deepenings, colour clearings, glitter spots, small gaps, great holes, cicatrices and coarse grain. These defects separately or together present the damage picture in the leather. Developmental stages of *Demodex bovis* and their anatomy are shown. There is an annual dynamics in extensity and intensity of cattle demodicosis with maximum during summertime. The pathogenesis of a single *Demodex* nodule includes four stages: primary stage = penetration of mites into hair follicle, secondary stage = development of a mite population and emigration of infectious *D. bovis* onto the skin surface, tertiary stage = occlusion of connection between nodule i.e. the mite population and skin surface, end stage = elimination of mites by host defence. *D. bovis* is spread through contact between animals, the spread is favoured by group housing. First success in control has been achieved by Ivomec Pour-on (3 x 0.5 mg Ivermectin/kg KM - reduction of nodule number by 30%) and Bayticol Pour-on (3 x 0.2 mg Flumethrin/kg KM - reduction of nodule number by 49%).



WORKSHOP PAPERS

SUSTAINABLE PRODUCTION SYSTEMS

Sustainability of intensive livestock systems with particular reference to parasite control.
F. Borgsteede, The Netherlands.

Sustainability of animal production systems - an industry perspective. *M.D. Soll, U.S.A.*

PARASITE AND INTERMEDIATE HOST POPULATION MONITORING

Intermediate host population monitoring.
J.B. Malone, G. Smith, D.J. Rogers, K. Khallaayoune and H. Ouhelli, U.S.A.

PARASITIC ZONOOSES - NEW ISSUES

Ehrlichiosis: an emerging zoonosis in the U.S.A.
S.A. Ewing, U.S.A.

Molecular characterisation as an aid to determining the zoonotic potential of *Giardia* and *Cryptosporidium*. *U.M. Morgan, B.P. Meloni and R.C.A. Thompson*

PATHOPHYSIOLOGY OF GASTROINTESTINAL PARASITES

Ostertagiasis: fiberoptic exploration of the abomasum of sheep. *H. Hertzberg, L. Kohler, F. Guscelli and J. Eckert, Switzerland.*

HYDATID - NEW APPROACHES

Application of alternative host model for *Echinococcus multilocularis* in coproantigen detection. *M. Kamliya, Y. Oku, H.-K. Ooi, H. Sakai and N. Nonaka, Japan,*

Application of ultrasound (US) and serology for screening human cystic echinococcosis in Uruguay. *P.S. Craig, E. Paolillo, R. Bonifacina, H. Cohen, B. Botta, K. Snowden and M.T. Rogan, U.K.*

Resumption of *Taenia ovis* control in New Zealand? *J.R. Lawson, New Zealand.*

Possibilities for regional collaboration on hydatid research in North Africa. *A.L. Willingham, Denmark.*

ANTIPARASITIC TESTING GUIDELINES

Anthelmintic pharmacology research. Status and future directions in relation to anthelmintic resistance. *D.R. Hennessy, Australia.*

Efficacy of moxidectin inj. (Cydectin) against mixed *Psoroptes/Sarcoptes* infestation in sheep. *J. Corba, M. Varady, J. Praslicka O. Tomasovicova and G. Gasparik, Slovak Republic.*

WAAVP Guidelines for Evaluating the Efficacy of Anthelmintics for Dogs and Cats. *D.E. Jacobs, U.K.*

WAAVP Second edition of Guidelines for Evaluating the Efficacy of Anthelmintics in Ruminants (Bovine and Ovine). *I.B. Wood, N.K. Amaral, K. Balrden, T. Kassal, J.B. Malone, Jr., J.A. Pankavich, R.K. Relnecke, S.M. Taylor and J. Vercruysse, U.S.A.*

Anti-parasitic guidelines of The European Community. *G.C. Coles, U.K.*

Evaluation of prophylactic anticoccidial drugs for broiler chickens in Canada. *G. Blanchard, Canada.*

Antiparasitic testing for animals in Japan. *N. Taira and M. Ishikawa, Japan.*

The use of electrophysiological techniques to examine the actions of anthelmintics. *R.J. Martin, U.K.*

Antiparasitic testing guidelines. *T. Letonja, U.S.A.*

Potentiation of ionophorous anticoccidials with a new antioxidant. *I. Varga, P. Laczay, J. Lehel, Z. Mora, A. Romvary and J. Fekete, Hungary.*

Internal parasites of working donkeys in Morocco. K. Khallaayoune, A. Mahboub and A. Belemilh, Morocco.

Ruminant Health Research Project, a north-south, south-south collaboration. H.O. Bogh and P. Nansen, Denmark.

Strategies and economics of East Coast Fever control in the Eastern Province of Zambia.
D.L. Berkvens, D.M. Geysen, J.R.A. Brandt and G.M. Luyen, Belgium.

An integrated control strategy for gastrointestinal nematode infections of ruminants in the Gambia and other West African countries. *J. Zinsstag, M. Ndao, B. Bonfoh, L. Ouattara, A. Ouedraogo, Ph. Ankers, J. Kaufmann, H. Wagner, T. Fritzsche and K. Pfister, The Gambia.*

Progress in the understanding of non-tsetse transmitted animal trypanosomes (NTTAT).
L. Touratier, France.

The present status of anthelmintic resistance in *Fasciola hepatica*. J.C. Boray, Australia.

Anthelmintic resistance in South Africa: an update. J.A. van Wyk and J.S. van der Merwe, South Africa.

Anthelmintic Resistance (AR) in Australia.
P. Rolfe, Australia.

Simulation models of anthelmintic resistance - the good news. R. Dobson and E.H. Barnes, Australia.

**Indication of benzimidazole resistance in
Osophagostomum spp. of plgs in Germany.
C. Bauer, S. Gerwert and H.-J. Burger, Germany.**

First stage larval reduction test (L₁RT) for detection of nematode resistance to anthelmintics in cattle. *R.K. Reinecke, Brazil.*

The prevalence of benzimidazole resistance in the small strongyles of horses in Ireland.
K.L. Strickland, S.L. Parr and D.J. O'Brien, Ireland.

Benzimidazole resistance in *Haemonchus contortus* from sheep in Malaysia.
V.S. Pandey and S. Sivaraj, Belgium.

The detection of anthelmintic resistant nematodes. G.C. Coles, U.K.

Paracox^R - a new live, attenuated vaccine against coccidiosis in chickens. A.C. Bushell.

Livacox^R - an attenuated live vaccine against
coccidiosis of domestic fowl. *P. Bedrník,*
A. Firmanová and J. Kucera.

Transfer of immunity against *Elmeria* from laying hens to offspring chicks. M. Wallach, N.C. Smith, C.M.D. Miller, R. Morgenstern, R. Braun, J. Eckert, A. Halabi, G. Pillemer, O. Sar-Shalom, D. Mencher, M. Gilad, U. Bendheim, H.D. Danforth and P.C. Augustine, Switzerland, Israel, U.S.A.

Development of a genetically engineered vaccine against poultry coccidiosis. F.M. Tomley, U.K.

Prospects of precocious *Elmeria* strains for vaccination of rabbits against coccidiosis.
P. Coudert, France.



SUSTAINABILITY OF INTENSIVE LIVESTOCK SYSTEMS WITH PARTICULAR REFERENCE TO PARASITE CONTROL

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The Netherlands is not only one of the most densely populated countries in the world, but also has a very intensive livestock production. This causes several problems with regard to diseases, environment and welfare. The sustainability of the production methods used depends on how we will cope with these problems. Control of parasites is of highest importance. In the poultry industry, which takes place almost exclusively indoors, coccidiosis is a major threat. Change to free range systems offers new opportunities for parasites such as *Capillaria* and tapeworms. In the pig industry, *Ascaris*-infections are now causing less than 5% liver condemnation. However, in free ranging systems this will be much higher, simultaneously giving much better chances for nematodes like *Hyostrogylus rubidus*, *Oesophagostomum* spp. and lungworms. In the cattle industry, modern treatment systems do not always allow the build up of sufficient immunity against gastrointestinal nematodes and lungworms thus producing more damage at a later age. In the sheep industry, anthelmintic resistance becomes more and more widespread. Possible solutions for sustainable production in intensive systems will be discussed.



SUSTAINABILITY OF ANIMAL PRODUCTION SYSTEMS - AN INDUSTRY PERSPECTIVE

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Parasitism remains an important factor limiting the ability of animals to realise their full genetic potential. Sustainability of production systems is, therefore, influenced by the ability to control this threat. Chemotherapy will continue to serve as a cornerstone of parasite control, but effective antiparasitic compounds constitute a limited resource. Their future availability will be impacted by continued escalation of research costs, increased regulatory requirements, development of resistance and attrition of older products which fail to meet more stringent modern regulatory requirements. Approval and usage of antiparasitic products may be further impacted by socio-economic justification for usage, animal welfare issues, environmental impact and human food safety concerns. Limited growth or reduction in animal numbers in many markets and integration and consolidation of production systems can also be expected to result in fewer, more sophisticated users. In the absence of new chemical classes, emphasis will be on formulation diversification and optimisation of product usage in integrated control strategies. There will be increased interest in exploiting non-chemotherapeutic approaches and selection of tolerant/responder hosts can be expected as integrated control strategies are implemented, but widespread commercial application of non-chemotherapeutic approaches may be slow to develop. Implementation of effective and rational parasite control programs in an environment of evolving production systems and socio-economic conditions will be the challenge of the future.



INTERMEDIATE HOST POPULATIONS MONITORING

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Recent mathematical and geographical models that run on microcomputers have provided new possibilities for monitoring the landscape epidemiology of intermediate host populations and vector borne diseases. The workshop will address the potential for integrating mathematical models with climate and computer map databases generated by remote sensing and geographic information systems (GIS): 1) Dr Gary Smith, University of Pennsylvania, will introduce a new mathematical model for *Fasciola hepatica* to illustrate principles of models and data requirements for their development and use; 2) Dr David J. Rogers, Oxford University, will review recent work on 'Satellite Imagery, TseTse and Trypanosomiasis in Africa' to illustrate methods of evaluating environmental factors that determine the geographic distribution of intermediate host populations and prevalence of disease; 3) Dr Khalid Khallaayoune and Dr Hammou Ouhelli will review the current status of *Fasciola* and *Theileria* in Morocco. The latter disease scenarios will be the focus of a panel discussion on model construction methods, data collection needs and the potential for use of GIS in national control programs.



EHRlichiosis: AN EMERGING Zoonosis IN THE USA

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When human ehrlichiosis was first differentiated from Rocky Mountain spotted fever in the New World it was thought to result from *Ehrlichia canis* (Order Rickettsiales: Tribe Ehrlichieae), causative agent of classical canine ehrlichiosis. More recently a new species, *E. chaffeensis*, has been isolated from human beings, and it has been shown to infect dogs experimentally. Several other ehrlichial species are associated with dogs in North America, either naturally or experimentally: *E. ewingii*, causes canine granulocytic ehrlichiosis; *E. platys*, canine cyclic thrombocytopenia; *E. risticii*, equine monocytic ehrlichiosis (Potomac horse fever); and *E. equi*, equine granulocytic ehrlichiosis, but the last is now thought to be identical to *E. phagocytophila* of the Old World. The zoonotic potential of these several species remains to be determined; and the role of *E. chaffeensis*, putative cause of human ehrlichiosis, as a pathogen of dogs under natural conditions is unknown. The epidemiologic puzzle is likely to be quite complex, and the role of various acarines in transmission of ehrlichial species from domestic and wild animals to human beings will require considerable research. Attempts to transmit *Ehrlichia* spp. via selected laboratory-reared ticks that feed on human beings - as well as on dogs and wildlife hosts - will be discussed in connection with the impact of acarine host preference on zoonotic potential of species in the genus *Ehrlichia*.



MOLECULAR CHARACTERISATION AS AN AID TO DETERMINING THE ZONOTIC POTENTIAL OF *GIARDIA* AND *CRYPTOSPORIDIUM*

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Cryptosporidium and Giardia are both protozoan parasites responsible for diarrhoeal illness in immunocompromised and immunocompetent individuals. Understanding the cross-transmission potential of these organisms is of great importance to the study of the epidemiology of both cryptosporidiosis and giardiasis, particularly with respect to the role of animals as sources of infection to humans. The lack of appropriate morphological markers and problems associated with *in vitro* amplification have made it difficult to define the zoonotic potential of both parasites. The application of molecular characterisation procedures, in particular, PCR-based techniques, has great potential for furthering our understanding of some of these problems. We have applied RAPD (Random Amplified Polymorphic DNA) analysis to both *Giardia* and *Cryptosporidium*. This technique uses single arbitrary primers to produce simple reproducible polymorphisms. As the technique is PCR-based, only minute quantities of material are required. Preliminary data suggests the potential for cross-transmission in both organisms is greater than originally suspected.



OSTERTAGIASIS: FIBEROPTIC EXPLORATION OF THE ABOMASUM OF SHEEP

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Follow-up studies on alterations of the gastrointestinal mucosa of ruminants during trichostrongylid infections are usually performed by serial post-mortem examination of animals at different intervals after exposure to a uniform infection regime. As an alternative, fiberoptic technique have been employed to enable long-term investigations of the abomasum of sheep. Hereby, the abomasum is reached with an endoscope via a rumen fistula to avoid any surgical damage to the abomasum. In a preparatory study, parameters that are known to be altered during trichostrongylid infections were investigated in two naive sheep and two sheep that had been previously immunised against *Ostertagia leptospicularis*. The sampling of mucosal biopsies and abomasal contents at intervals of three to four days during a period of four weeks did not influence serum pepsinogen and gastrin levels which did not exceed 318 mU tyrosine and 37 pmol/l respectively. Electrolytes in the abomasal fluid were not found to be affected by the sampling procedures and showed mean values of Na⁺:64 mmol/l, K⁺:29 mmol/l and Cl⁻: 106 mmol/l. The absence of a major influx of rumen fluid during the fiberoptic examination was confirmed by continuous measurements of the acidity of the abomasal fluid using pH-probes connected with a computer-assisted recording system. During the observed period of seven days the acidity remained within the physiological range, fluctuating between pH 2 and 3. An influence of feeding on the abomasal pH was not observed. The described technique seems to be well suited for the investigation of mucosal reactions after challenge infections, and its possible use for immunohistochemical studies is currently under investigation. The present observations showed that sampling intervals of three to four days are well tolerated by the animals with no impairment of the food intake and regurgitation.



APPLICATION OF ALTERNATIVE HOST MODEL FOR *ECHINOCOCCUS MULTILOCULARIS* IN COPROANTIGEN DETECTION

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The production of coproantigen in prednisolone-treated golden hamster (GH) serving as an alternative definitive host for *Echinococcus multilocularis* was examined with the aim of developing a diagnostic method for the natural canid definitive host. Using polyclonal antibodies raised in rabbit against excretory/secretory antigen and a mouse monoclonal antibody against the adult tapeworm of *E. multilocularis* in a sandwich ELISA, specific coproantigen was detected in the feces of experimentally infected GH. The monoclonal antibody used is an IgG, and it was found to recognize a 26 kD band on a Western blot using adult cestode somatic antigen partially purified by immunoprecipitation with the polyclonal antibodies. The dynamics of the shed coproantigen from the GH was compared with that of experimentally infected dogs. The diagnosis of the infection can be made even during the early phase of the infection, that is, during the prepatent period. This diagnostic method was further successfully applied to the testing of wild fox feces.



APPLICATION OF ULTRASOUND (US) AND SEROLOGY FOR SCREENING FOR HUMAN CYSTIC ECHINOCOCCOSIS IN URUGUAY

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The comparative roles of ultrasound and serology for screening and prevalence studies on human cystic echinococcosis are frequently discussed. The results of a collaborative study in Uruguay are an important contribution to this debate. In November 1991, 1620 people from La Paloma township were screened by portable US and ELISA. Abdominal cystic lesions (mostly asymptomatic) were recorded in 74 persons (60 hepatic and 14 renal). On US alone 33% (20/60) of hepatic hydatid cases were diagnosed (laminated layer, daughter cysts and/or multiple cysts). Of 40/60 (67%) suspected hepatic cases (univesicular, solid mass and/or calcified cysts) on US 62.5% (25/40) were serologically confirmed. Surgical confirmation in 31 of 60 hepatic cases resulted in a serologic sensitivity of 74.2% (23/31) and of US sensitivity (diagnostic image) of 71% (22/31). Thus nine patients were operated on the basis of serologic confirmation. All single kidney lesions were seronegative and considered non-hydatid. Of eight strongly seroreactive but US negative persons (0.52%, 8/1546) followed up by X-ray/CT, two additional cases (lung, liver) were confirmed. The optimal approach to screening should therefore combine US and serology. The prevalence of hydatid was at least 3.7% (60/1620) for this community.



RESUMPTION OF *TAENIA OVIS* CONTROL IN NEW ZEALAND?

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In New Zealand control of *Echinococcus granulosus* and *Taenia hydatigena* led to an unexpected upsurge in the prevalence of *T. ovis*. From 1969 it was officially included in the control campaign and untreated sheep meat was added to untreated offal as prohibited food for dogs. The programme, which from 1972 employed 6-weekly dog dosing with cestocide, was very successful against *E. granulosus* and also initially against *T. ovis*. However, from 1976 the prevalence in sheep started to increase and in 1989 the campaign against it and *E. granulosus* were dissociated and non-discriminatory dosing of dogs was halted. Between 1989 and 1992 there was a 4-fold increase in the prevalence of *T. ovis* in lambs. The meat and farming industries have recognised the increased risks to our sheep meat markets and are currently examining future options. They are not confident that eradication of *T. ovis* in New Zealand is possible with the control tools currently available. Permanent control funded by a levy on all sheep carcasses is therefore being canvassed.



POSSIBILITIES FOR REGIONAL COLLABORATION ON HYDATID RESEARCH IN NORTH AFRICA

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The Maghreb Countries of North Africa have long been recognized as a very important endemic focus of hydatidosis/echinococcosis. The economic impact of hydatidosis on these countries in terms of public health costs and reduced agricultural production is considered to be very high although its assessment is limited by inadequate information on the prevalence and clinical significance of the disease. No serious attempts at effective control have been attempted in the region. Development of a regional strategy for control of the disease would be economically desirable and may be practical due to common sociocultural factors including ethnic groups, religion, languages and agricultural practices. A Center for Zoonoses should be established in North Africa to provide training for veterinary and medical health workers involved in control of hydatidosis as well as support basic and applied parasitological research, standardize diagnostic techniques and develop intervention strategies. The international scientific community and development agencies need to support efforts by these countries to deal effectively with hydatidosis by providing technical and financial assistance.



ANTHELMINTIC PHARMACOLOGY RESEARCH. STATUS AND FUTURE DIRECTIONS IN RELATION TO ANTHELMINTIC RESISTANCE

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With the paucity of new compounds with unique modes of action, helminth chemotherapy necessitates more efficient presentation of existing drugs. Anthelmintic activity principally results from the duration of parasite exposure rather than absolute concentrations which are attained in the host; formulations which extend availability will increase potency. While the activity of moxidectin against ivermectin resistant nematodes is suggested to relate to binding site availability, the relative difference in potency *in vivo* may also be due to changes in pharmacokinetic behaviour. Co-administration of methimazole altered netobimin metabolism increasing its availability while combining levamisole and benzimidazoles produce a synergistic action in addition to removing parasites susceptible to either drench component. Short-term extended presentation can be achieved with multiple dosing and feed-induced reduction in digesta flow rate, whereas controlled-release technology and drench impregnated feed blocks provide long-term action against the establishment of resistant parasites. More refined formulations, which specifically target the drug to sites of parasitism also add to our chemotherapeutic arsenal. Integrated use of these modified formulations with epidemiologically-based treatment programs will continue to maintain activity against resistant strains of parasites.



EFFICACY OF MOXIDECTIN INJ. (CYDECTIN) AGAINST MIXED PSOROPTES/SARCOPTES INFESTATION IN SHEEP

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A total of 45 merino ewes naturally infested with psoroptic and sarcoptic mange were included in a trial to study the efficacy of moxidectin injected subcutaneously. The animals were allocated into three groups. The first group of 15 animals received 0.2 mg/kg^{-1} moxidectin on Day 0; the second group of 15 animals were treated with the same dose of moxidectin on Days 0 and 7, while the third group of 15 animals remained untreated control. Mites were counted in epidermal scrapings made prior to treatment and at 7, 14, 21 and 28 days thereafter. In both treated groups the signs of itching disappeared within seven days of treatment and rapid clinical improvement was apparent. In skin scrapings several live mites of both species were present; the reduction in numbers of mites compared with initial score was over 90%. The repeated injection of moxidectin removed all living mites and the skin scrapings from this group were negative on Day 28. Untreated animals suffered from intensive scratching, anorexia and moist active lesions of skin.



WAAVP GUIDELINES FOR EVALUATING THE EFFICACY OF ANTHELMINTICS FOR DOGS AND CATS

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These guidelines were compiled by seven veterinary scientists and reviewed by eight others. Authors and reviewers were from nine countries with experience spanning academia, industry and regulatory affairs. The general format of earlier publications in the series was adopted - i.e. introduction; methods of evaluation; selection, allocation and management of animals; definition of the main therapeutic targets; dose titration, dose confirmation and clinical trials. Animal welfare is emphasised throughout. Statistical advice was obtained from several sources. An appendix and tables outline techniques and methodologies in current use for major parasitic infections, including *Echinococcus*, other cestodes, ascarids, other gastrointestinal nematodes, and heartworm (treatment and prophylaxis). It is hoped that these guidelines will provide a basis for uniform international standards. Sincere thanks are extended to the authors, the reviewers, the WAAVP officers and all others who contributed.



WAAVP SECOND EDITION OF GUIDELINES FOR EVALUATING THE EFFICACY OF ANTHELMINTICS IN RUMINANTS (BOVINE AND OVINE)

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The first edition of the W.A.A.V.P. anthelmintic guidelines for ruminants was published in 1982 (*Vet Parasitol* 10, 265-284). Since then: 1) improved parasitological procedures have been developed; 2) new therapeutic and prophylactic delivery systems have evolved requiring different test methods; and 3) registration authorities are requesting more detailed record keeping and means to validate data. This second edition addresses these changing factors to fulfill the original goal of publishing guidelines for high quality, scientifically valid testing standards for trials that would be accepted as proof of efficacy by registration authorities regardless of country of origin.

This edition includes updated guidance on: 1) general standard parasitological procedures, Dose Titration, Dose Confirmation and Clinical Field Trials; 2) specific guidelines for the determination of efficacy against anthelmintic resistant parasites, persistent anthelmintic activity; 3) guidelines to evaluate prophylactic anthelmintics. Tests for efficacy against nematodes, cestodes and trematodes are included.



ANTI-PARASITIC GUIDELINES OF THE EUROPEAN COMMUNITY

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The Efficacy Working Group (EWG) is a subcommittee of the Committee of Veterinary Medicinal Products (CVMP) established under the Veterinary Medicine Directive 81/851-2. The CVMP is made up of representatives from the member states and has a secretariat from the Commission. The EWG drafts guidelines which are passed to the CVMP to issue for consultation with the European veterinary pharmaceutical industry. The guidelines are then reconsidered by the EWG and forwarded to the CVMP for final approval and formal publication by the Commission. Various antiparasitic guidelines have been either finalised (i.e. are in the public domain) or are in draft stages. Where relevant EC guidelines will compare with those of the WAAPV.



EVALUATION OF PROPHYLACTIC ANTICOCCIDIAL DRUGS FOR BROILER CHICKENS IN CANADA

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Before any new drug can be sold in Canada, the sponsor of that new drug must file a New Drug Submission (NDS) with the Bureau of Veterinary Drugs, Ministry of Health and Welfare, Canada. The NDS must provide evidence that use of the drug in animals presents no demonstrable risk to human health. In particular, foods that are derived from treated animals must be free of potentially harmful residues. In addition, the NDS must provide substantial information concerning the manufacturing of the new drug, as well as substantial evidence of its efficacy and safety in the target animal species.

To establish the safety of a prophylactic anticoccidial new drug in the absence of coccidiosis, it must be demonstrated that, at the recommended dosage rate, it does not interfere with growth and feed conversion. Efficacy data have to be generated from controlled battery trials (single and mixed coccidial infections), controlled floor pen studies (under simulated use conditions) and field trials (under actual use conditions) conducted in Canada, or in geographical areas of the United States of America where climatic conditions, management methods and feeding techniques are similar to those in Canada. Specific registration requirements and guidelines will be discussed in detail.



ANTIPARASITIC TESTING FOR ANIMALS IN JAPAN

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In Japan, antiparasitic drugs for animals are under the jurisdiction of the Ministry of Agriculture, Forestry and Fishery (MAFF). All the experimental data of a given antiparasitic drug are included in the registration dossier and sent to MAFF for its approval.

The experiments should be designed to assess the efficacy of a compound against given species of parasites. Generally, two types of tests are performed 'basic test' and 'field test'; these tests must be conducted under G.L.P. 'Basic tests' are preliminary tests which required use of experimentally infected laboratory animals and/or target animals. When significant data are obtained with 'basic tests', then 'field test' with target animals are performed. However, sometimes 'basic tests' are not conducted when it is difficult to produce experimental infections. In these tests the standardization of the parasitological technique is required, and several treatment groups are involved: treatment groups at different dose rates and untreated control group. Parameters include: counts of eggs per gram in the feces and number and species of worms recovered at necropsy. Adverse reaction as well pathological findings must be assessed in a special safety trial as well as in field trials. The results of trials must be analyzed statistically.



THE USE OF ELECTROPHYSIOLOGICAL TECHNIQUES TO EXAMINE THE ACTIONS OF ANTHELMINTICS

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A number of anthelmintics exert their therapeutic effect by acting selectively on membrane ion-channels of parasites to produce changes in current and membrane potential. These changes may be monitored by a variety of electrophysiological techniques including the use of intracellular micropipettes for current-clamp and voltage-clamp and the use of patch clamp for recording single ion-channel currents. The anthelmintics which act in this way include the nicotinic anthelmintics like levamisole, pyrantel and morantel, the GABA mimetic piperazine and the Cl channel opener ivermectin. Effects on parasite tissues may be compared using the same techniques on host tissues.

A proper knowledge of the mode and site of action of anthelmintics may allow toxic effects in the host animals to be predicted and controlled: pyrantel and morantel have nicotinic actions in nematodes and their toxic effects in host animals arise from stimulation of host nicotinic receptors. Electrophysiological techniques can also allow a comparison of the relative merits of competing analogues which act at the same site. The relative merits of the avermectins and milbemycins might be assessed with these techniques. There are other possible benefits which may ensue from electrophysiological studies including a better understanding of the development of resistance in parasites.



ANTIPARASITIC TESTING GUIDELINES

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In the United States, the administration of laws and regulations pertaining to drugs intended for use in animals is the responsibility of the Food and Drug Administration's Center for Veterinary Medicine (CVM). Section 512 of the Federal Food, Drug and Cosmetic Act mandates a demonstration of safety and effectiveness of each new animal drug before approval and marketing. The accompanying regulations are found in Title 21 of the Code of Federal Regulations Part 58, Part 511 (New Animal Drugs for Investigational Use) and Part 514 (New Animal Drug Application, NADA). Part 58 includes general provisions, organization and personnel, facilities, equipment, testing facilities operation, test and control articles, protocol and conduct of a nonclinical laboratory study, records and reports, and disqualification of testing facilities. Nonclinical laboratory studies should be conducted in accordance with good laboratory practice (GLP) regulations. Part 511 provides information on the requirements for proper shipment of investigational new animal drugs, qualifications of clinical investigators, authorization for use of edible products derived from treated food-producing animals, and appropriate withdrawal times for investigational new animal drugs. Part 514 describes the requirements to demonstrate the safety and effectiveness of new animal drugs. Section 514.1(d) includes provisions for minor use applications for new animal drugs intended for use in animals other than cattle, horses, swine, chickens, turkeys, dogs and cats. In the near future they will be considered minor species in all respects. Several guidelines provide principles regarding the collection of scientific data to demonstrate effectiveness of: 1) anticoccidial drugs alone, 2) anticoccidial drugs in combination with an antibiotic and/or arsenical drug for use in poultry feeds, and 3) anthelmintics in equine, canine and feline, porcine, and bovine species. CVM's policy is specific about the acceptance of data collected in foreign countries in support of drug safety and effectiveness. In addition, CVM's Policy and Procedure Manual Guide establishes that foreign non-clinical safety studies may be used to satisfy NADA requirements, but they must comply with GLP regulations. The current anticoccidial drugs guideline is organized into three sections: a) experimental procedures that should be implemented in studies designed to demonstrate the efficacy of an anticoccidial drug, b) discusses specific considerations for evaluating the effectiveness of the compounds, and c) specific considerations for evaluating the effectiveness of an anticoccidial drug in combination with antibiotic(s) and/or arsenical(s).



POTENTIATION OF IONOPHOROUS ANTICOCCIDIALS WITH A NEW ANTIOXIDANT

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The interaction between four ionophorous anticoccidials (monensin, salinomycin, narasin and maduramicin) and a dihydroquinoline type new antioxidant (Duokvin^R, Material Chemical Co., Budapest) was studied in chickens. Seven battery tests on a total of 1344 wingtagged Hybro cockerels were carried out. The birds were infected at the age of seven days with oocysts of *Elmeria tenella* and *E. mitis*, and the survivors were killed eight days later. The diet contained anticoccidials at various dose rates in combination with Duokvin. Parameters to measure the anticoccidial efficacy were as follows: mortality, weight gain, faecal scores, oocyst production, macroscopic lesions in the caeca, presence of coccidia in the mucosal scraping of the caeca. A significant, non-selective toxic interaction was established, resulting in growth depression and improved anti-coccidial efficacy. In the presence of 120 ppm Duokvin, the adverse effects could be eliminated by reducing the dietary level of monensin, salinomycin and narasin to approximately 12%, whereas those of maduramicin only to approximately 50%.



INTERNAL PARASITES OF WORKING DONKEYS IN MOROCCO

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The gastrointestinal tracts of 168 donkeys originating from different regions of Morocco were examined to evaluate the degree and prevalence of internal parasites in working donkeys.

All donkeys were infected with *Trichostrongylus axei* and *Habronema* spp. with average numbers of 50 and 239, respectively. *Habronema musca* and *H. microstoma* were the only species of spirurids encountered. *Gasterophilus intestinalis* and *G. nasalis* larvae were found in most of the examined donkeys, and *G. haemorrhoidalis* in some.

Parascaris equorum was found in the small intestine of 37% of the donkeys. Of these donkeys 82% were less than five years old. *Strongylodes westeri* was found only in a few examined donkeys. Three species of tapeworm were found as adult parasites in the small intestines of donkeys in Morocco: *Anoplocephala perfoliata*, *A. magna* and *Paranoplocephala mamillana*. The helminth species identified in the large intestine were *Strongylus vulgaris*, *S. edentatus*, *Oxyuris equi* and small strongyles. *S. equinus* was not encountered in the present study. *Strongylus vulgaris*, the most pathogenic of the equine nematodes, was found in 98% of the donkeys. Severe verminous aneurysms were observed in 87% of animals.

Fasciola hepatica and hydatid cysts were encountered in 4% of the livers. *Dictyocaulus arnfieldi* was found and lung hydatidosis in 23 and 4% of the donkeys. *Setaria equina* was present in the peritoneal cavity of 29% of the animals.

No significant difference in gastrointestinal worm counts among animals of different origins was found.



RUMINANT HELMINTH RESEARCH PROJECT, A NORTH-SOUTH, AND SOUTH-SOUTH COLLABORATION

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Parasitic worms can cause disease and death, resulting in severe losses to the livestock industry in Africa and elsewhere. However, the main economic losses are due to chronic infections in which several parasites are present simultaneously (polyparasitism). This causes unthriftiness, poor utilization of feed and slow growth rates together with decreased meat quality and milk production. Furthermore, decreased fertility and a reduced resistance against other diseases are often observed. Financial support for a research assistance project on parasitic worms in ruminants was in 1991 granted by Danida. The aims of the research assistance project are to strengthen the research performed at some African universities and to recruit researchers in the area of parasitic worm infections in livestock. The research assistance project is a long term project which will perform collaborative research in the above mentioned area involving researchers from Veterinary Faculties in Tanzania, Kenya, Zimbabwe, Zambia and Denmark. However, the collaboration will be concentrated around specific research areas in Tanzania and Kenya. From the beginning of the project, it has been an objective to ensure a close scientific collaboration between the participating laboratories. One way of doing this is to organize yearly seminars in one of the four African countries. Two such seminars have been held with success in Morogoro, Tanzania and Kabete, Kenya. Initially, prevalence and epidemiological studies will be necessary together with studies on pathogenicity and clinical evaluations. Several different management systems including both large and small scale farmers in different geo-climatic regions will be included in the initial surveys. The research project comprises M.Sc. as well as Ph.D. students.



STRATEGIES AND ECONOMICS OF EAST COAST FEVER CONTROL IN THE EASTERN PROVINCE OF ZAMBIA

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East Coast Fever (caused by the protozoon *Theileria parva* and transmitted by the three-host tick *Rhipicephalus appendiculatus*) was responsible for a calf mortality of up to 50% in traditional cattle herds in the endemically unstable areas of the Eastern Province of Zambia. Control of the disease was attempted by intensive dipping. This method was fraught with problems, the most important being the reluctance of cattle owners to bring their animals in the absence of an immediate disease threat. It was therefore decided to initiate an immunisation program, aimed at the calves in the traditional herds. To date over 80,000 calves have been immunised successfully and the mortality has been reduced to below 3% in the immunised calves and, because of an improved veterinary service and judicious use of theilericides, to around 20% in the non-immunised calves. Veterinary services have been provided free-of-charge so far, but the Zambian Government has decided to gradually introduce a self-supporting and privatised veterinary service. The obvious approach is to determine the equilibrium between an acceptable income for the private veterinarian and the farmer's expenses. Whereas it may be possible to determine this point easily on purely economical grounds, it is argued that a live animal has lots of extra benefits for its owner, which are not readily quantified. These benefits include extra draught power, extra manure output, improved nutrition and extra financial security for the owner's family. Furthermore, it is impossible to quantify the renewed faith kindled by the improved veterinary service, spreading the above benefits to a large slice of the population which up to now did not keep cattle. The majority of owners in the Eastern Province are in a position to raise the necessary initial investment required for the purchase of a limited number of animals. However, recurrent costs cannot be met, as the idea "sell one animal to keep another ten alive" obviously does not hold for these small-scale farmers. On the other hand, it is equally obvious that the government cannot continue to provide support in the form of a free veterinary service.



AN INTEGRATED CONTROL STRATEGY FOR GASTROINTESTINAL NEMATODE INFECTIONS OF RUMINANTS IN THE GAMBIA AND OTHER WEST AFRICAN COUNTRIES

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Epidemiological studies revealed a high prevalence of gastrointestinal nematode infections of more than 90% in cattle and small ruminants. The predominant species are *H. contortus* and *Cooperia spp* in cattle and *T. colubriformis* in small ruminants. A distinct seasonal pattern was recorded, the peak worm burdens occur during the rainy season. During the dry season, no new infections occur and animals dewormed at the end of the rains with a larvicidal drug are being reinfected only during the following rainy season. Appropriate management methods of the night holding places during the rainy season have resulted in a significant increase of the weight gain of risk animals without any drug treatment.

Strategic anthelmintic control trials on cattle and sheep (private farmers) kept under traditional managements conditions were carried out with Fenbendazole (one or two applications) during the rainy season. Improved weight gains of 11 - 20% were achieved in cattle up to three years of age (risk group). Sheep productivity measured as kg bodyweight at 90 days produced per ewe per year rose by 30% using the same treatment scheme as for cattle.

An alternative scheme with one treatment at the end of the rains, i.e. November, and a second one during the rainy season, i.e. end of August has also been tested. It revealed improved weight gains from November to April which reflects that a persisting worm burden, although in an inhibited and/or hypometabolic stage, has still a pathologic effect during the dry season. More than two treatments per year appear not to be economic under the given management systems.

A pilot deworming campaign with the national extension services demonstrated a high degree of acceptance by the farmers.



PROGRESS IN THE UNDERSTANDING OF NON-TSETSE TRANSMITTED ANIMAL TRYPANOSOMOSE (NTTAT)

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Initially limited to the study of *Trypanosoma evansi* infections the relevant international working Group - set up in 1983 - accumulated a considerable amount of scientific (laboratory and field) data in agreement with the defined purposes at its creation. In particular it gave rise to (i) more detailed information (and consequently better awareness) of zones infected by *T. evansi*; (ii) refinement of diagnostic techniques and the development of test kits suitable for field use; (iii) development of a new synthetic trypanocide (melarsomine). This work was developed thanks to many contacts between qualified research workers on the occasion of annual meetings at the OIE Headquarters in Paris and joint meetings in Africa (ISCTRC/OAU/IBAR) and Asia. Considering the work carried out the OIE Permanent Committee decided to extend the scope of this working into an 'OIE ad hoc Group on Non Tsetse-Transmitted Animal Trypanosomoses (NTTAT) in May 1991 with the following terms of reference: 'To study, discuss and inform OIE Member Countries of the following points:

- (i) the pathological and economic impact of NTTAT in Africa, Asia and America
- (ii) the possible interaction of NTTAT with other diseases and immune responses to vaccination for other diseases (e.g. foot and mouth disease, haemorrhagic septicaemia, brucellosis)
- (iii) the reliability of diagnostic tests, the cost involved and the ease with which trypanosomes may be differentiated from each other (e.g. *T. evansi*/*T. brucei*, *T. evansi*/*T. equiperdum*; *T. evansi*/*T. vivax*, etc.)
- (iv) the similarities between strains, isolates and stabilates of different origin, so that possible differences in their genetic, immunological and biochemical characteristics may be determined
- (v) the problem of chemoresistance to trypanocidal drugs
- (vi) the current research into new drugs and drug evaluation
- (vii) new means of control of NTTAT.

A First International Seminar on NTTAT was held in Annecy, France in October, 1992 under the sponsorship of OIE, FAO, WHO, IAEA with the material support of the Alphonse Laveran and Marcel Mérieux Foundations. It gave rise to Conclusions related to: General aspects of these infections; their impact and epidemiology; the chemotherapy; the basic research.

A 2nd Seminar is scheduled to be held in P. R. China in 1995.



THE PRESENT STATUS OF ANTHELMINTIC RESISTANCE IN *FASCIOLA HEPATICA*

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Recent studies confirmed that several Australian strains of *Fasciola hepatica*, resistant to salicylanilide and benzimidazole carbamate drugs, did not change their resistant status when they were maintained through laboratory passages.

In collaboration with Dr W. Maurice Allen of Compton Paddock Laboratories, Newbury, UK, we found one isolate from western England and one from Wales to show a degree of resistance to salicylanilides.

Triclabendazole was previously found significantly less effective against a selected strain of *F. hepatica* aged 4 weeks at a reduced dose rate of 6.75 mg/kg compared to susceptible strains. Recent studies showed that after further selections the efficacy of the drug was reduced from 98% to 60% at the recommended dose rate of 10 mg/kg against flukes aged two weeks. The efficacy of clorsulon did not change against the fluke aged six weeks after several laboratory selections at the dose rate of 10 mg/kg.

There is no appreciable advance in developing a simple method for the diagnosis of drug resistance in *Fasciola*, although in a recent study some correlations were found between the level of glutathione S-transferases and susceptibility to salicylanilide drugs (Miller, C.M.D., Howell, M.J. & Boray, J.C. unpublished data).

It can be concluded that resistance in *F. hepatica* can develop to most anthelmintics and wide use of a single drug is not desirable. More work is needed to develop simple methods for the detection of resistance and the resistance status of fluke should be monitored. Comprehensive strategic programs with alternating drug use may prevent resistance and manage existing resistance.



ANTHELMINTIC RESISTANCE IN SOUTH AFRICA: AN UPDATE

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During the past two years faecal egg count reduction tests were done on sheep by Onderstepoort (JSvdM *et al.*) and Logos AGVET (Kloeck, unpublished) on 60 farms (situated in the Eastern Transvaal Highveld, Northern Transvaal, Lebowa, Orange Free State, Natal, Karoo, and Eastern Cape), using the methods of Presidente (1985). The principal helminths were *Haemonchus contortus* and *Ostertagia circumcincta*.

The results indicate that 90% of the properties house resistant helminth strains, and that on 40% the strains are resistant to three or more anthelmintic groups.

The farms investigated in the surveys were not selected at random, but on all but a few of them there was no history of resistance and the farmers did not complain of reduced anthelmintic efficacy. The results indicate serious resistance in South Africa.



ANTHELMINTIC RESISTANCE (AR) IN AUSTRALIA

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Recent surveys (1992) on sheep farms (1139) indicate 85% of farms have resistance to benzimidazoles (BZs) (FECR <95%, <95% CL). Severe AR (reduction <70%) was present on 45% of farms (28% and 11% for levamisole and combinations respectively). AR was most often found in *O. circumcincta* and *Trichostrongylus* spp. Resistance to levamisole was found on 75% of farms; the extent less than for BZs and most often in *Trichostrongylus* spp. Increasing the dose rate (2x) had a significant effect on efficacy. Combination AR has increased from 9% (1984-1989), to 34% (1991/1992) and 44% (1992/1993), coinciding with an increase in the proportion of farms using combinations (2.5% in 1988 to 15% in 1991). The degree of AR is significantly lower than that for other classes (11% of farms <70% FECRT). Ivermectin resistance has been found in two parasite species in three separate locations. Resistance in *H. contortus* in sheep was detected in unusual circumstances from a research farm. *Ostertagia circumcincta* from goats has been confirmed to be resistant in detailed slaughter studies (94-95% efficacy at 200 µg/kg) but not in *H. contortus*, *T. axei* and *T. colubriformis*. A similar level of resistance has been confirmed in *O. circumcincta* in sheep on a single farm. No resistance was found on 1139 farms surveyed throughout Australia from 1991 to 1993. In New Zealand there have been four reports of ivermectin resistance in *Ostertagia* spp. in goats. Closantel resistance in *H. contortus* has been confirmed on approximately 1% of farms in summer rainfall areas and has been increasing since 1987. Resistance to BZ's (and recently oxbendazole) in cyathostomes in horses is widespread (no current data). BZ resistance in *T. axei* is the only confirmed resistance in cattle.



SIMULATION MODELS OF ANTHELMINTIC RESISTANCE - THE GOOD NEWS

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How do you simulate the evolution of anthelmintic resistance when you don't know the type and number of worm phenotypes, genotypes or the efficacy of an anthelmintic against them? Details of how this is incorporated into a helminth parasite model will be discussed. The model was used to examine the relative importance of anthelmintic treatment, drug efficacy and grazing management in controlling *Trichostrongylus colubriformis* in grazing sheep under Australian conditions. In brief, the good news is that excellent worm control was obtained when both grazing management and drug treatment were used together, however, the level of drug efficacy (or anthelmintic resistance) was insignificant in obtaining this result. Using either grazing management or anthelmintic treatment alone led to unacceptable levels of host mortality in the Armidale (New South Wales, Australia) environment.



INDICATION OF BENZIMIDAZOLE RESISTANCE IN *OESOPHAGOSTOMUM* SPP. OF PIGS IN GERMANY

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A representative survey was conducted in sow herds of about 750 pig farms in three districts of Northrhine-Westphalia, Germany, in early 1993 to detect anthelmintic resistant strongyle populations using a modification of the larval development test described by Coles *et al.* (1) An inhibition of the development of third stage strongyle larvae of less than 95% by 0.0113 $\mu\text{g/ml}$ thiabendazole as compared to controls was considered from preliminary studies to indicate benzimidazole resistance. Inhibition was only 28%, 30% and 39%, respectively, in three replications performed on separate occasions in one of 79 sow herds surveyed. In a subsequent faecal egg count reduction test, 5 mg/kg bwt. flubendazole reduced the *Oesophagostomum* egg counts in nine sows of this herd by only 53% seven days after treatment as compared to untreated controls. A controlled test with piglets experimentally infected with this *Oesophagostomum* isolate (GIBZ-1) is under way.

References: Coles, G.C., Tritschler, J.P., Giordano, D.J., Laste, N.J. and Schmidt, A.L. (1988) *Res. Vet. Sci.* 45, 50.



FIRST STAGE LARVAL REDUCTION TEST L_1 RT FOR DETECTION OF NEMATODE RESISTANCE TO ANTHELMINTICS IN CATTLE

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Visser filters are an efficient method of separating nematode eggs from 15 ml faeces, from which volume it is possible to detect mild infections common in cattle. Such diagnosis is impossible with standard egg counts.

L_1 incubated in clear water at 37°C hatch in 18-20 h when genera can be diagnosed. FECRT diagnosis utilizes infective larvae, only present in co-procultures after seven days - an unnecessary delay.

Grids on the top surface of the lower, modified McMaster slide enable accurate counting of recently hatched L_1 and unhatched eggs and results expressed as eggs+ L_1 15ml⁻¹, which is impossible with standard egg counts.

Absence of faeces facilitates accurate counting of small, semitransparent embryonated eggs of *Strongylodes*, which is difficult with conventional egg counts due to the presence of faeces.

Cooperia resistance to levamisole and *Ostertagia* resistance to albendazole, previously unknown in Brazil, has been established by L_1 RT.

Conclusion: L_1 RT is a better ante mortem test for NRA than is FECRT.



THE PREVALENCE OF BENZIMIDAZOLE RESISTANCE IN THE SMALL STRONGYLES OF HORSES IN IRELAND

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In 1992 a survey was carried out to estimate the prevalence of benzimidazole resistant strongyles in horses in Ireland. A total of 30 establishments including stud farms, riding stables and private yards were selected according to set criteria. The resistance status of these properties was determined by assessing the efficacy of fenbendazole (FBZ) using the faecal egg count reduction assay. An efficacy of less than 85% was recorded at 19/29 sites, the thirtieth having been discarded as unsuitable. With reference to establishment type, FBZ was less than 85% effective at 9/9 thoroughbred studs, 5/6 non-thoroughbred studs and 5/9 riding stables. The efficacy exceeded 85% at all five private yards. Cyathostomes accounted for over 95% of larvae recovered from faecal cultures both before and after treatment. Examination of data regarding dosing regimes indicated that there is a correlation between efficacy and dosing frequency. In Ireland, benzimidazole resistant cyathostomes are widespread in stables that have a minimum of nine horses and which use or have used, benzimidazole anthelmintics regularly. The implications of this are serious for certain establishment types, particularly the stud farms.



BENZIMIDAZOLE RESISTANCE IN *HAEMONCHUS* *CONTORTUS* FROM SHEEP IN MALAYSIA

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Albendazole, fenbendazole, oxfendazole, levamisole, closantel and ivermectin were administered at manufacturer's recommended dose rates to sheep on three farms and their efficacies assessed by faecal egg count reduction test (FECR). Great disparity in the FECR was found according to the method of calculation used, which depended whether the arithmetic or geometric mean was used and whether the pre- and post-treatment egg counts of control group were taken into account in the calculations. Post-treatment faecal cultures had only *Haemonchus contortus* larvae. On all farms high level of resistance of *H. contortus* was found to benzimidazoles, the highest being to fenbendazole. There was no direct relationship between the frequency of treatment with a compound and the level of resistance. Side resistance within the benzimidazole compounds was observed even in the absence of use of a compound on a farm. No resistance to levamisole, closantel and ivermectin was detected. These results indicate that the anthelmintic resistance to benzimidazoles is a serious and probably a widespread problem in *H. contortus* in sheep in Malaysia. Besides the better use of available anthelmintics other strategies of worm control, such as breeding of animals for resistance to nematodes, merit serious consideration.



THE DETECTION OF ANTHELMINTIC RESISTANT NEMATODES

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The only definitive way of determining the presence of anthelmintic resistant nematodes is a dose and slaughter trial but this is too expensive to use except for research. WAAVP methods for detecting anthelmintic resistance have been published for the faecal egg count reduction test (FECRT) and egg hatch test (EHT) but tests are not reliable if less than 25% of the worms are resistant. The FECRT is considered diagnostic for levamisole resistance in Australia, but is not reliable in the UK yielding false positives. The FECRT has given a false negative in Scotland with ivermectin. The EHT is a fast inexpensive method of detecting benzimidazole resistance and can be used with anaerobic storage of eggs. Further evaluation of three variations of the larval development test should be undertaken. More sensitive tests for the detection of anthelmintic resistance are needed.



LIVACOX: AN ATTENUATED LIVE VACCINE AGAINST COCCIDIOSIS OF DOMESTIC FOWL

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LIVACOX is based on lines of coccidia, attenuated either by their adaptation to growth and passaging in chicken embryos or by selection for premature development (precociousness). The attenuation is stable for at least five consecutive relaxed passages in chickens. The epidemiological studies have indicated that out of seven coccidial species parasitizing domestic fowl, only *E. tenella*, *E. acervulina*, *E. maxima* and probably *E. brunetti* are important in the field from the economic point of view. LIVACOX is therefore so far available in two variants: LIVACOX D (*E. tenella* and *E. acervulina*) for vaccination of caged chicks and LIVACOX T (*E. tenella*, *E. acervulina* and *E. maxima*) for chickens housed on litter. Each ml of the vaccine contains 100 vaccination doses with approximately 500 oocysts of each species present in the vaccine in a 1% solution of trihydro-Na-benzenesulfochloroamide. It is administered in a single dose in drinking water, when chickens are 7–10 days old. Routine vaccination of more than 4,000,000 of both the layers and the heavy breeders and experimental vaccination of more than 4,000,000 broilers showed that it can be a viable alternative to using anticoccidials.



TRANSFER OF IMMUNITY AGAINST *EIMERIA* FROM LAYING HENS TO OFFSPRING CHICKS

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Newly hatched chicks are very susceptible to infection with *Elmeria*. Thus, it is necessary to ensure that chicks are protected against infection from their day of hatching. Several studies have demonstrated that maternally transferred antibody may play an important role in resistance against coccidial infection in young chicks and, therefore, maternal immunisation may be both the most effective and cost efficient (since each hen produces 150 to 200 chicks) approach for protecting hatchlings against coccidiosis.

In our recent work using live oocysts to immunise laying hens, we were able to strongly protect (ie a greater than 90% reduction in oocyst output) offspring chicks against infection with *Elmeria maxima*. Furthermore, we showed that this immunity can last for at least five months in the hens and up to three weeks in their offspring chicks. We believe that inhibition of the excretion of oocysts by over 90% for the first two to three weeks of the rearing period may reduce disease transmission to levels that can still induce resistance against reinfection without producing any of the pathological effects associated with coccidiosis.

We have also used affinity purified gametocyte glycoproteins to immunise laying hens and have found that these antigens induce highly significant levels (70 to 80% reduction in oocyst output) of protection against *E. maxima* in offspring chicks. In addition, we analysed the antigen specificity of yolk from hens immunised by infection with *E. maxima* and sera from their progeny. Although some of these chicks had relatively low antibody titers, they were still strongly immune to infection with *E. maxima*, and only a few antigens from both the asexual and sexual stages of development were recognised by this type of antisera. Furthermore, these antigens appear to be well conserved between *Elmeria* species. Thus, it seems feasible that these putative protective antigens used alone or in combination with live immunisation, may lead to the development of a maternally based, subunit vaccine for coccidiosis.



DEVELOPMENT OF A GENETICALLY ENGINEERED VACCINE AGAINST POULTRY COCCIDIOSIS

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Recently, live-attenuated vaccines for the control of coccidiosis in poultry have been developed and shown to be effective. The manufacturing costs of these products are high and the shelf-life is limited so the development of a recombinant vaccine remains a desirable goal. To date, many attempts have been made to induce protective immunity by vaccinating poultry with antigens, either derived from parasites or expressed from recombinant DNA clones and recently some experiments have shown that partial protection against severe coccidiosis can be achieved. However, the levels of protection that have been reported are not generally considered sufficient for the effective control of disease in the field. The performance of recombinants might be improved by increased research in several areas of coccidian biology. For example, further knowledge of early events in the establishment of infection might pinpoint the targets of the immune response and help to more clearly define both the cells that mediate immunity and those which process and present parasite antigens. In addition, if a recombinant vaccine is to mimic the effects of natural infection, one of the most important keys to its successful development is likely to be the design and use of suitable vectors for the presentation of antigens to the immune system of the chicken.

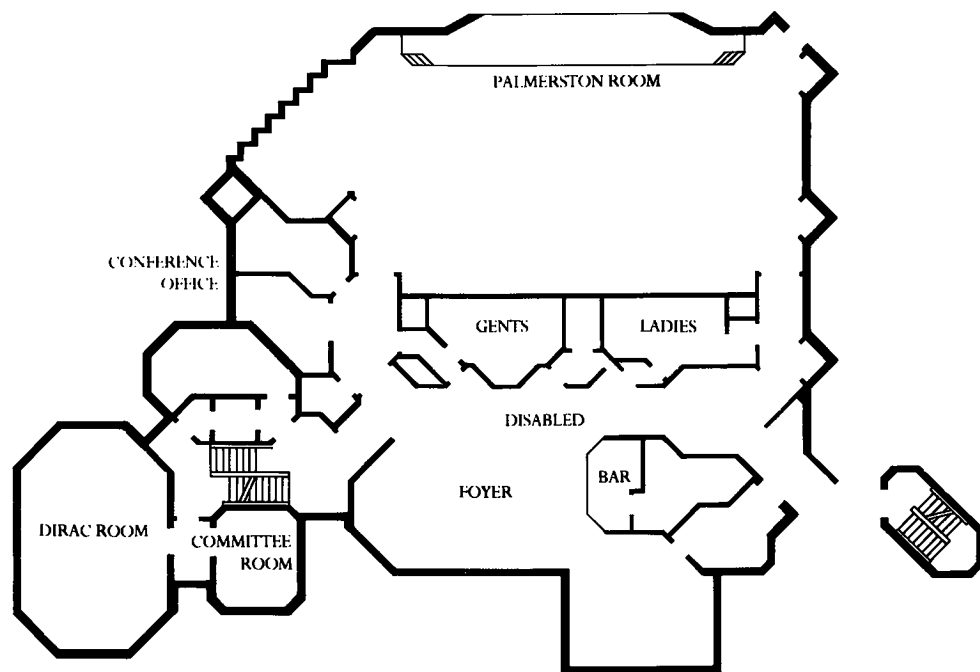
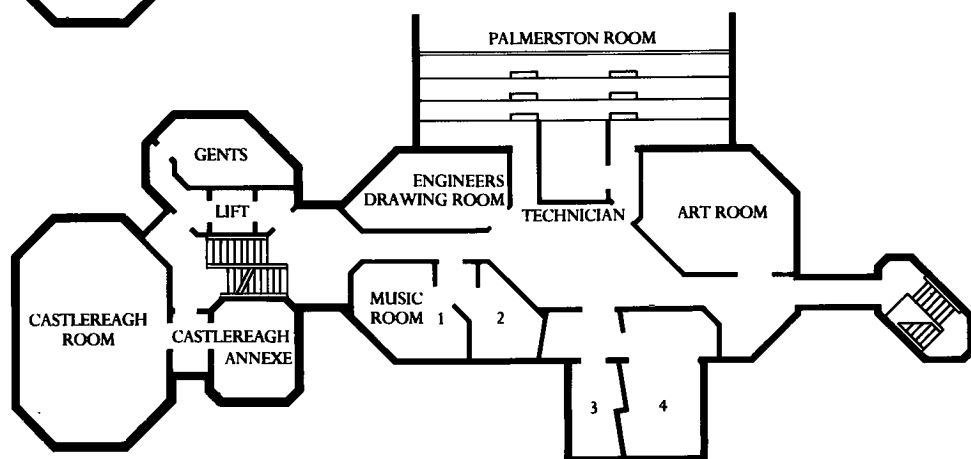
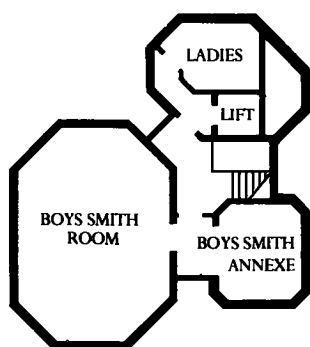


POTENTIALS OF PRECOCIOUS *EIMERIA* STRAINS FOR VACCINATION OF RABBITS AGAINST COCCIDIOSIS

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For more than ten years, coccidiosis has been controlled by the use of Robenidine (Cyanamid) in commercial rabbitries. Now, four chemico-resistant *Eimeria* spp. are very frequently identified: *E. magna*, *E. media*, *E. perforans* and *E. coecicola*. Precocious strains of *E. magna* and *E. media* were obtained and their potential as a vaccine in laboratory conditions was described (D. Licois, P. Coudert, 1992, 1993). The potential in the field of industrial rabbit production has yet to be evaluated by monitoring the efficacy, the mode of administration, the innocuity and the epidemiology of the vaccine strains. Some aspects of these problems are discussed, taking into account distinctive features of rabbit breeding, of rabbit coccidiosis and of precocious strains. In rabbit breeding, all prophylactic or therapeutic actions should concern not only the young growing rabbits but also the nursing females because it is essentially during the days preceding and following weaning that contamination of sucklings progressively takes place. Contamination of young rabbits with precocious strains must occur just before weaning but as long as they only consume their mother's milk there is little or no multiplication of the parasite. On this and other accounts, it seems necessary to control the eimerian pattern in the whole rabbit unit (eradication of the wild strains) rather than individual situations. Therefore it is necessary to study not only systematic individual vaccination upon weaning or bulk vaccination at regular intervals (monthly?) but also the survival of the vaccine and of the wild strains. We have also to consider that the does are constantly the epidemiological source of diffusion of pathogenic agents. If the excretion of the vaccine strain oocysts were sufficient to vaccinate the litter, then the most elegant way of vaccination would have been found. Theoretically there is no technical problem to obtaining enough oocysts to vaccinate all the industrially produced rabbits: one or two rabbits produce enough oocysts to vaccinate 10^4 rabbits (i.e. the annual production of one French breeder). Moreover the purity of the precocious strain is easy to achieve because of morphological differences from the wild strains.



- 20 Influence of technologies of sheep breeding on spreading of cestodosis in Moldova. A.S. Bessonov and L.F. Bondar, Russia.
- 21 Regional differences in the prevalence of cysticercosis in Slovakia and their reasons. J. Corba, P. Dubinsky and A. Stefancikova, Slovak Republic.
- 22 Evidence for the long distance dispersal of cysticercosis. P.R. Torgerson, J. Pilkington, F.M.D. Gulland and M.A. Gemmell, Ireland, U.K.
- 23 *Taenia polyacantha* (Cestoda: Taeniidae) in foxes in the U.K. A. Jones, U.K.
- 24 Behaviour and pathogenicity of *Toxocara canis* Werner 1782 (Anisakidae) second stage larvae in mice of different strains. C. Epe and T. Sabel, Germany.
- 25 Ecological aspects of the occurrence of zoonoses and their control in urban districts. V. Letkova, M. Goldova and G. Csiszarova, Slovakia.
- 26 Study of the prevalence of intestinal parasitism in dogs in country areas in Galicia (NW Spain). P. Diez Banos, M. Prieto Novoa, P. Morondo Pelayo and O. Fernandez Perez, Spain.
- 27 Frequency of *Trichinella spiralis* in pigs sacrificed at ABC slaughterhouse of Los Reyes, La Paz State of Mexico, using two techniques of diagnosis. N.V. Alarcon, Mexico.
- 28 The occurrence of *Isospora suis* and *Strongyloides ransomi* in nursing piglets in The Netherlands. W. Hollanders, G.A. Boerdam, M. Eysker and J.H.M. Verheyden, Belgium.
- 29 A comparison of nematode parasites of horses from two management schemes. R.C. Krecek, A.J. Guthrie and L.C. van Nieuwenhuizen, South Africa.
- 30 *Dictyocaulus eckerti* in the respiratory tract of reindeer. S. Nikander and S. Saari, Finland.
- 31 Distortion of the gizzard of Cyprus pigeons associated with *Hadjella truncata* infestation. L.M. Gibbons, E.C. Appleby and K. Georgiou, U.K., Cyprus.
- 32 Helminth parasites of wild rodents in Khuzestan Province, South-West of Iran. M. Sasljadi and J. Massoud, Iran.
- 33 Development of *Neostongylus linearis* (Nematoda: Protostrongylidae) larvae in *Cermea* (*Cermea*) *virgata* (Mollusca: Helicidae), infected and kept in a natural environment. C. Lopez Sanz, P. Morondo Pelayo, M. Prieto Novoa, P. Diez Banos and R. Panadero Fontan, Spain.
- 34 The epidemiology of helminth infections of growing sheep in Argentina's western Pampas. V.H. Suarez and M.R. Busetti, Argentina.
- 35 Natural infection with gastroenteric nematodes in helpers of second year grazing in North-West Spain. P. Morondo, M. Mezo, P. Diez and N. Diez, Spain.
- 36 Epidemiology of bovine (*Bos indicus*-Guzera) nematode parasites in cerrado, Mato Grosso do Sul State, Argentina. M.C. Zocoller, R.Z. Machado, W.A. Starke and W.V. Valerio Filho, Brazil.
- 37 Prevalence and levels of infection with helminths in sheep in Nyandarua district of Kenya. N. Maingi, W.K. Munyua and V.G. Gichohi, Denmark.

BIOLOGY

- 38 Selection of Brazilian isolates of *Bacillus thuringiensis* with nematocidal activity against free living stages of trichostrongylid nematodes. T.P. Charles, C. de P. Santos, J.O. Silva-Werneck, J.M.C.S. Dias, D.M. Capalbo, L. Rabinovitch and T.P.V. Guaycurus, Brazil.
- 39 Diseases of farmed snails in the Czech Republic. D. Lukesova and A. Cizek, Czech Republic.
- 40 Isoenzymatic profiles of *Fasciola hepatica* and *Dicrocoelium dendriticum* (Trematoda) adult specimens. R. Campo, M.Y. Manga-Gonzalez and D. Rollinson, Spain.
- 41 Aspects of the site distribution of strongyles in the large intestines of horses in Portugal. Emphasis on small strongyles. L.M. Madeira de Carvalho, M.M. Afonso-Roque and M. Carvalho-Valera, Portugal.

- 42 *Strongylodes stercoralis*: control of development by amphidial neurons. G.A. Schad, F.T. Ashton, V.M. Bhopale and A.E. Fine, U.S.A.
- 43 The effects of sugars on invasion of *Elmerta tenella* sporozoites in primary chicken kidney cells. A. Arakawa, N. Sadano, T. Fukata and E. Baba, Japan.
- 44 Feeding of *Dermacentor nuttalli* (Acari: Ixodidae) through a silicon membrane. B. Habedank and Th. Hlepe, Germany.
- 52 Skin delayed hypersensitivity in buffaloes induced by *Toxocara vitulorum* antigens. W.A. Starke, R.Z. Machado, M.C. Zocoller and G.H. Bechara, Brazil.
- 53 A role of surface and ES proteins of adult flukes during *Dicrocoelium dendriticum* infections in cattle. H. Wedrychowicz, D. Ducommun and K. Pfister, Switzerland.
- 54 Reactive oxygen intermediate production in *Fasciola hepatica* infected hosts. E.A. Kerr and K.S. Ovington, Australia.
- 55 Cellular and humoral responses to *Fasciola hepatica* primary and secondary infestation in sheep. A. Chauvin and C. Boulard, France.
- 56 *Schistosoma bovis* in goats: the antibody response to egg and adult worm antigens. J. Monrad, M.V. Johansen and P.E. Simonsen, Denmark.

GENETIC BASIS OF HOST RESISTANCE

- 45 Susceptibility of Corriedale and Crioula breeds to *Haemonchus contortus*. M.F.S. Borba, F.A.M. Echevarria and A.C. Pinheiro, Brazil.
- 46 Responder classification of sheep: comparative assessment of distinct *Haemonchus* and *Trichostrongylus* infections. T. Sreter and T. Kassai, Hungary.
- 47 Larval migration inhibitory activity of the ileal contents of goats infected with *Trichostrongylus colubriformis*. W.E. Pomroy and W.A.G. Charleston, New Zealand.

HELMINTHS - ANTIGENS AND THE IMMUNE RESPONSE

- 48 Vaccination of sheep against *Haemonchus contortus* with sub-fractions of the parasite's intestinal microvillar integral membrane protein H11. E.A. Munn, T.S. Smith, H.F. Smith and M. Drewitt, U.K.
- 49 Effect of protein supplementation upon the cellular responses towards *Nematodirus battus* infection in lambs. D.A. Israfi, R.L. Coop, F. Jackson, L.M. Stevenson, D.G. Jones and J.F. Huntley, U.K.
- 50 Effect of state of immunity on the establishment of *Toxocara canis* infection in puppies. O. Fernandez Perez, M. Prieto Novoa, P. Diez Banos and P. Morondo Pelayo, Spain.
- 51 Eosinophilic responses in Beagle puppies infected with *Toxocara canis* embryonated eggs. M. Prieto Novoa, O. Fernandez Perez, P. Diez Banos and P. Morondo Pelayo, Spain.
- 52 Skin delayed hypersensitivity in buffaloes induced by *Toxocara vitulorum* antigens. W.A. Starke, R.Z. Machado, M.C. Zocoller and G.H. Bechara, Brazil.
- 53 A role of surface and ES proteins of adult flukes during *Dicrocoelium dendriticum* infections in cattle. H. Wedrychowicz, D. Ducommun and K. Pfister, Switzerland.
- 54 Reactive oxygen intermediate production in *Fasciola hepatica* infected hosts. E.A. Kerr and K.S. Ovington, Australia.
- 55 Cellular and humoral responses to *Fasciola hepatica* primary and secondary infestation in sheep. A. Chauvin and C. Boulard, France.
- 56 *Schistosoma bovis* in goats: the antibody response to egg and adult worm antigens. J. Monrad, M.V. Johansen and P.E. Simonsen, Denmark.

PROTOZOA

- 57 Polypeptide profiles and antigenic characterisation of cell membrane and flagellar preparations of different stocks of *Trypanosoma evansi*. V. Singh, A. Singh and M.B. Chhabra, India.
- 58 Effects of extracts from *Sarcocystis gigantea* (SGE) on HIV-target T-cells. F. Pötzsch, H.-J. Tietz, R. Gantenberg, D. Scholz, E. Brose, U. Dröbigk, D. Jakob and Th. Hlepe, Germany.
- 59 Studies of antibody reaction in experimental *Theileria hirci* infection in sheep. I. Leemans, P. Hooshmand-Rad and A. Ugglä, Sweden.
- 60 Parasitic infections of cattle in Fulani nomadic herds. S.U. Abdullahi, S.O. Okalyeto and Y.U. Abubakar, Nigeria.
- 61 *Elmerta* infection in mouflon (*Ovis montanus*). M. Gomez-Bautista, M. Luzon-Pena and A. Meana, Spain.
- 62 Studies on parasitaemia, packed cell volume, total serum protein and antibody levels in naturally acquired babesiosis and anaplasmosis in buffalo calves. A. Dell Porto, T.U. Fujili and P.S. Baruselli, Brazil.
- 63 Seroepidemiological studies on *Toxoplasma gondii* infections in sheep and cats in northern Germany. K. Simon, A.M. Tenter, M. Rommel and W. Lehmacher, Germany.

- 64 Course of endogenous developmental phase of selected *Elmeria* species in pheasants. M. Goldova, V. Letkova, G. Cslzsmarova, J. Kocis and L. Kolodzleyski, Slovakia.
- 65 On the epidemiology of coccidian infections (*Isospora suis*, *Cryptosporidium parvum*) in pigs. J. Mathea, R. Schuster, G. Werner and U. Dröbige, Germany.
- 66 Epizootiology and experimental studies of goat sarcocystosis in Romania. E. Suteu, V. Cozma, D. Turcu, O. Rotaru, O. Negrea and C. Gherman, Romania.

DIAGNOSIS

- 67 Faecal egg counts by a new method using tube filters. F.S. Malan and P.S. Visser, South Africa.
- 68 A species specific recombinant antigen for the rapid diagnosis of *Dictyocaulus viviparus* infections in cattle. T. Schnieder, Germany.
- 69 Ante-mortem diagnosis and drug treatment trial for *Taenia saginata* cysticercosis. L.J.S. Harrison, J.A. Onyango-Abuje and G. Hughes, U.K.
- 70 Molecular markers for species identification and genetic variation analysis of filarial worms at various stages in their life cycles. C. Genchi, C. Bandi, B. di Sacco, G. Damiani and S. Comincini, Italy.
- 71 Detection of *Cryptosporidium parvum* using the polymerase chain reaction. K. A. Webster and M. Gilles, U.K.
- 72 The development of specific ELISAs for use in epidemiological studies of bovine babesiosis in Brazil. L.M.F. Passos, C.G.D. Brown, T.W. Jones, L. Bell-Sakyl and R. Bold, U.K.

PATHOLOGY

- 73 Internal parasites in dairy heifers born in autumn in Buenos Aires province (Argentina). I. Effect on body weight gain. II. Epidemiological study. C.A. Flel, A.S Fernandez, E.M. Roderiguez, L.A. Fuse and P.A. Somlinson, Argentina.

- 74 Comparative effects of nematode infection on *Bos taurus* and *B. indicus* crossbred calves grazing on Argentina's western Pampas. V.H. Suarez, M.R. Busetti and R.G. Lorenzo, Argentina.
- 75 Milk production and live weight variation in heifers treated against gastrointestinal nematodes. N. Diez Banos, M. Mezo, P. Diez and P. Morondo, Spain.
- 76 The pathogenesis of cryptosporidia in chickens, mice and calves. M.A. Taylor, J. Catchpole, R.N. Marshall and J. Green, U.K.
- 77 Pathophysiological studies on *Dicrocoelium dendriticum* infection in sheep. J.M. Maclean, Y. Theodoridis and J.L. Duncan, U.K.
- 78 Comparative histopathological study of hepatic changes induced by *Fasciola hepatica*/Schistosoma bovis and *Schistosoma bovis*/Fasciola hepatica. Y.M. Gonzalez, M.C. Ferreras, M.Y. Manga, Y. Mizinska, V. Ramajo, R. Campo and J.M. Martinez, Spain.
- 79 Electronmicroscopic and histopathological studies in experimental eimeriosis in lambs. V. Cozma, E. Suteu, D. Turcu and O. Rotaru, Romania.

COST 89

- 80 Potassium metabolism in *Elmeria bovis* infected calves. A. Al-khaled, A. Gahr and H.-J. Burger, Germany.
- 81 Molecular cloning of *Elmeria tenella* alpha-tubulin cDNA. M. Yamage, A. Hemphill, D. Silva, M. Wallach, N. Smith, R. Braun and I. Roditi, Switzerland.



**PROCEDURES AND STANDARDS FOR THE APPROVAL
OF ANTIPARASITIC DRUGS FOR THE CONTROL OF
ECTOPARASITES IN SOUTH AFRICA**

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In view of the extreme importance of parasite control, basic protocols to ensure that efficacy, safety to man and animals, residues in animals in the feed chain and environmental impact have been drawn up in South Africa. The steps taken ensure that products for sale in South Africa comply with the unique demands of the area and include all steps that have to be taken to ensure that products for sale are efficacious, safe and used correctly.



ALTERNATIVE BLOWFLY TREATMENTS AND CONTROL METHODS

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The future of organophosphate sheep dips is uncertain due to safety and environmental considerations. This research reports on an alternative blowfly treatment and possible biological control by trapping. Pairs of twin lambs were challenged with *Lucilla sericata* under controlled environmental conditions. Groups of lambs were treated with varying concentrations of high cis-cypermethrin, 0.16-1.25% HCC pour-on; others received solvent alone. Flies were stimulated to oviposit by liver attached to an area of moistened fleece. All concentrations of HCC delayed the onset of egg laying and greatly reduced the numbers of eggs laid. At the highest concentration oviposition was restricted to the liver and no eggs were laid in the fleece suggesting it could prevent blowfly attack under natural conditions. This has been confirmed by recent field trials. A target trap for blowflies was evaluated. Trap catches of 3% *L. sericata* and 15.2% *L. caesar* increased to 34% and 22.6% respectively in the proximity of sheep. Trap catches were further increased using baited fly tables on which the dominant species was *L. caesar*, 52.6%, with *L. sericata* comprising 2.7%. This particular attractiveness to *L. caesar* could be an advantage in Scotland where this species is associated with recent outbreaks of strike.



EFFICACY OF MOXIDECTIN AGAINST *SARCOPTES BOVIS* IN EXPERIMENTALLY INFECTED CALVES

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Twenty-four female cattle calves were artificially infested two to three times within 22 days with a total of 3,000 to 4,000 *Sarcoptes bovis* mites each. After the development of a clinical infection on Day 31 to 38 post inoculation, four trial groups (I and IV) of six calves each were made, housed separately and treated as follows: I) no treatment; II) 0.2 mg moxidectin/kg body weight as a subcutaneous injection; III) 0.4 mg moxidectin/kg body weight by subcutaneous injection; and IV) 0.5 mg moxidectin/kg body weight as pour-on. Four skin scrapings (4 by 4 cm each) were taken from each calf on Days -1, 6, 13, 20, 27 and 55 and quantitatively analysed for live and dead mites. Based on live mite counts all treatments gave complete control from Day six post treatment except for one living mite on one animal in Group II on Day six. These decreases in mite counts in each treated group were significantly different from controls on Days 6 and 13. Itching stopped on Day three, after three weeks there was a clear improvement or cure of the mange lesions. Although the mite counts in the controls decreased and no mites were recovered from Day 27, all control animals continued to have typical mange lesions, even up to Day 55 for four out of six controls. It was concluded that moxidectin in all tested doses and formulations was effective against *S. bovis* experimentally infested on calves, well tolerated with no local or general adverse reactions.



CONTROL OF SARCOPTIC MANGE OF CATTLE

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Sarcoptic mange results in reduced productivity and poor food conversion in calves and reduction in the quantity of milk in older animals and also produces significant skin damage.

Twelve female cattle, 11 months of age, were experimentally infested with 4,000-10,000 mites of *Sarcoptes bouis* (Cameron 1924). After successful establishment, abamectin 1% solution (AVOMEK) was administered subcutaneously behind the shoulder to six animals at 1 ml/50 kg bodyweight. The remaining animals served as untreated controls. Six skin scrapings were taken from each animal at weekly intervals for eight weeks and any mites recovered were counted.

The success of treatment was confirmed by comparing the following parameters in treated versus control-animals.

a. Disappearance of skin lesions.

Within two weeks in five animals and within seven weeks in the sixth animal the acute skin lesions were healed.

b. Disappearance of live *Sarcoptes* mites.

Treated cattle had significantly ($p < 0.05$) fewer mites present in scrapings than controls at all post-treatment examinations. Five of the six medicated cattle became mite free by Day seven and the sixth by Day 14.

c. Improved bodyweight gain.

During the trial period of 56 days the treated animals gained at an average 7.1 kg while the controls lost 5.9 kg per head.



CURRENT STATUS OF THE FREQUENCY OF *SARCOPTES SCABIEI* VAR. *SULS* AND STUDY OF THE MICROSCOPIC INJURIES FOUND ON PIGS SACRIFICED AT SEVERAL SLAUGHTERHOUSES OF THE MEXICAN REPUBLIC

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The aim of the present work was to find the frequency of *Sarcoptes scabiei* var *suls* in pigs sacrificed at slaughterhouses of the Mexican Republic. The material consisted in pig ears from day of sacrifice in eight slaughterhouses of the Mexican Republic. Samples were taken from a total of 965 sacrificed pigs at the states of Chihuahua, Sonora, Aguascalientes, Zacatecas, Guanajuato, Michoacán, State of México and Yucatán.

At the laboratory, scrapings from the ears were made and watched to write down whether they were positive or negative to *Sarcoptes*. In 15 positive cases an histopathological study was carried.

The results obtained were: out of 965 animal samples, 53 were positive (5.4%). They presented mites *Sarcoptes scabiei* var *suls*.

As the microscopic injuries at the 15 ears studied, pervascular infiltration by white cells in superficial and deep dermis was found, and coagulative necrosis in epidermis, micro abscesses with subcorneal and intraepidermic spaces with the aspect of a tunnel where there were abundant mites were watched.

The conclusion is that up to this date we have found *S. scabiei* in 5.4% of pigs coming from eight states of the Mexican Republic. Microscopic injuries were found in 15 positive cases. We shall, therefore, continue studying the frequency of this mite and its microscopic injuries in other slaughterhouses of the Mexican Republic in order to have a general panoramic view so as to apply the preventive measures for the control of this mite.



PREVALENCE OF INFECTION WITH *HYPODERMA* SPP. IN CATTLE IN LUGO PROVINCE (GALICIA, NW SPAIN)

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In autumn 1991, 1460 serum samples were collected from cows older than a year, from four zones in Lugo province (Coast, Mountain, Centre and South), with notable climatic and geographic differences. In this province situated in Northwest Spain, we have frequently identified *Hypoderma bovis* and *Hypoderma lineatum*; however, hypodermosis eradication programmes have not been carried out. Samples were processed using the indirect ELISA test, as described by Prieto *et al.* 1990 (*Vet. Parasitol.*, 1990, 35, 211), using as antigen the hypodermine C collagenase, which was obtained from soluble extracts of first-stage larvae of *Hypoderma lineatum*, purified by HPLC. Optic density was measured at 492 nm. Positive and negative control sera against *H. lineatum* were used, and test samples were considered positive if absorbance values were higher than 1.75.

The prevalence of seropositive animals to *Hypoderma* in Lugo province was high (76%), similar to results obtained by Prieto *et al.* (1990) in Asturias (region close to Lugo); whilst Martinez (1992) found lower percentages (42.07%) in Andalucia (Southern Spain). This prevalence varied in the different zones studied, being 87.9%, 78.1%, 59.9% and 56.9%, in the Southern, Coastal, Mountain and Central regions, respectively. The average figures for positive absorbances varied from 2.9 ± 0.02 in bovines in the Southern zone, to 2.53 ± 0.03 in those in the Central area. By means of variance analysis, statistically significant differences were found between the four areas studied ($F = 114.1$; $p = 0.000$).

Taking into account the climatic parameters (temperature, rainfall and wind strength) corresponding to months when theoretically adult *Hypoderma* flies could have laid the eggs (June-September), and using the Spearman test of range correlation, we found a negative correlation between the percentage of animals positive against *Hypoderma* in the different zones, and wind strength.



SCANNING OF *OESTRUS OVIS* LARVAE (LINNÉ 1761)

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O*estrus ovis*, the sheep nasal bot-fly, is a well-known cosmopolitan myiasis producing Diptera. Larvae induce irritation of pituitary mucosa by their cuticular spines and oral hooks together with their antigens which are responsible for the development of local hypersensitivity and secondary lung lesions.

Larval instars have been well studied (Zumpt 1965) but no Scanning Electron Microscopy (SEM) has been performed until now. The aim of this paper is to describe the general aspect of larval stages with emphasis on hooks and spines which allow larvae to stay and to move on mucosa of the upper respiratory tract.

Scanning of the three larval instars of *Oestrus ovis*, the sheep nasal bot fly, allowed a study of spines and hooks. The first instar larvae has many strong spines and hooks to catch on pituitary mucosa and to resist the air stream in nasal cavities. The second instar has only weak spinulosity and lives in a quiet place: middle meatus or sinuses. The third instar larvae has ventral spines to move and leave the upper respiratory tract. All these structures show a very good adaptation to the larval environment. A ventral orbicular hole with prominent rims is also described on the first larval instar, its significance is now known. This poster is illustrated with original slides.



CTENOCEPHALIDES FELIS BOUCHÉ: LUFENURON AND FENTHION BASELINES IN AN ARTIFICIAL FEEDING SYSTEM

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The benzoylphenyl urea lufenuron is a potent insect development inhibitor being developed by Ciba. The organophosphate fenthion is used in commercial products against fleas on cats. The efficacy of both products, following oral uptake by adult *Ctenocephalides felis*, was checked on an artificial feeding systemss containing cattle blood.

Lufenuron had no effect on the adult fleas but prevented development of the F₁ generation. The IC₅₀ (Inhibition Concentration) in blood was as follows: egg hatch 0.119 ppm: cocoon production 0.076 ppm; F₁ adults 0.077 ppm. The main effect was therefore in preventing egg hatch; there was supplementary effect against larvae, but insects reaching the cocoon stage survived.

Fenthion has been tested from time to time over the last four years. Results were consistent. The LC₅₀ in blood, for a 24 hour exposure period, is 3.4 ppm.

These experiments showed the system to be most useful for comparison of oral toxicants against cat fleas, under close observation and without use of host animals.



EVALUATION OF ORAL MOXIDECTIN (MOX) AGAINST GI NEMATODES AND OF TOPICAL FORMULATIONS OF MOXIDECTIN AND/OR TEMEPHOS (TEM) AGAINST IMMATURE HEARTWORMS, ADULT GI NEMATODES, TICKS, AND FLEAS IN DOGS

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Efficacy of MOX against GI nematodes was studied in 30 naturally infected dogs with superimposed infections of *A. caninum*, *U. stenocephala*, and *T. leonina*. Dogs (5/gp) were given MOX orally at 0, 25, 50, 100, 150 or 300 mcg/kg BW. Faecal samples were collected daily for seven days for recovery of worms; the GI tract was examined. MOX was 100% effective against *A. caninum* at 25 mcg/kg and cleared 21 of 25 dogs at higher dose levels. All dogs were cleared of *U. stenocephala* at 150 and 300 mcg/kg. MOX eliminated all *T. vulpis* from two of five dogs given 300 mcg/kg. The drug appeared to have some activity against *T. leonina* and *T. canis*. Separately, 33 beagles were infected with *D. immitis*, *A. caninum*, *T. leonina*, *R. sanguineus*, and *C. felis*. Dogs were given vehicle or single spot-on formulations of MOX, TEM or both. MOX (0.5 mg/kg) and TEM (5 or 10 mg/kg) plus MOX (0.01 mg/kg) were 100% effective against one-month-old *D. immitis*. MOX (0.5 mg/kg) and MOX plus TEM (5 or 10 mg/kg) were 99-100% effective against *A. caninum*, but lower levels of MOX plus TEM (10 mg/kg) were less active. Activity of MOX, TEM, or MOX plus TEM against ascarids and ticks could not be evaluated. TEM at 20 mg/kg was 100% effective against fleas by seven days after each of four weekly infestations; after the 5th, efficacy was about 90%. At 10 mg/kg, TEM (alone or plus MOX) was 97-100% effective against fleas after the first two infestations; thereafter, efficacy declined. At 5 mg/kg, TEM plus MOX (0.5 mg/kg) was highly but not completely effective. MOX (0.5 mg/kg) had little efficacy against fleas.



EFFECTIVENESS OF MELARSOMINE DIHYDROCHLORIDE (RM 340) AGAINST *DIROFILARIA IMMITIS*

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A two-phase study using 35 heartworm-infected dogs housed outdoors at a heartworm enzootic site (Glennville, GA, USA) was done to determine the effectiveness of RM 340 for chemotherapy and clinical prophylaxis. In Phase I, 21 dogs were given RM 340 (2.2 mg/kg IM x two doses, three h apart) in August and December 1990. Fourteen dogs served as sham-treated controls. In December 1990, seven RM 340-treated and seven control dogs were brought indoors and later necropsied in May 1991. In the second phase, seven RM 340-treated dogs were again treated with RM 340 in August and December 1991. The seven other RM 340-treated dogs were given conventional monthly prophylaxis with ivermectin from January 1991 to December 1991. The remaining controls were sham-treated in August and December 1991. All 21 dogs were brought indoors in December 1991 and later necropsied in May 1992. Three sets of heartworm-naïve beagles (17 dogs) were placed in trial conditions for different periods to assess the risk of infection. Testing for adult heartworm antigen was done on all dogs at treatments with RM 340 and at necropsy. RM 340 was 99.7% effective in eliminating existing infections with adult *D. immitis*. Circulating antigen was greatly reduced or eliminated by four months after the first treatment series with RM 340. For clinical prophylaxis, RM 340 was 98.2% effective in preventing new infections with adult *D. immitis*. The use of ivermectin following chemotherapy with RM 340 was 100% effective. Antigen concentrations remained low or at zero for these treated dogs. Heartworm-naïve dogs acquired *D. immitis* during August to December 1990 (one of five dogs) and April to December 1991 (five of five dogs).



EFFECTIVENESS OF LIPOSOMES WITH ANTHELMINTICS AGAINST EXPERIMENTAL TRICHINELLOSIS IN MICE

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Liposomes with mebendazole (MBZ), albendazole (ABZ) and methyl-2-benzimidazole carbamate (MBC) at the dose-rates of 15 and 75 mg/kg (according to AS) were administered to white mice intraperitoneally on the 2nd, 3rd, 4th; 6th - 11th and 25 - 30th days after infection with *T. spiralis* larvae which corresponded to intestinal, migration and muscle stages of development of parasite. According to the same scheme the same anthelmintics were administered orally in the form of suspension tween-21 at the dose-rate of 75 mg/kg. Thirty five days after infection the results of the therapy were registered by the method of compressor trichinelloscopy and digestion of diaphragm in artificial gastric juice. MBZ, ABZ and MBC included in liposomes at the dose-rate of 75 mg/kg were 100% effective against all stages of *Trichinella*. Analogous effect of MBZ in liposomes was observed at the dose-rate of 15 mg/kg including the muscle stage. Effectiveness of liposomal forms of ABZ and MBC at the dose-rate of 15 mg/kg varied from 90 to 100%.

In all cases activity of the three anthelmintics administered orally in the usual form was lower than that used in liposomes and varied from 52 to 96%. So liposomal forms of MBZ, ABZ and MBC are more effective and perspective for treatment of trichinellosis.



SPECIFIC PROPHYLAXIS AGAINST DICTYOCAULOSIS, FASCIOSIS AND ECHINOCOCCOSIS

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Polyvalent vaccine was created on the basis of P-62 and P-93 antigens and highly molecular immunostimulant. The vaccine administered twice corrected immunologic insufficiency that occurred two to three weeks after infection. The vaccine intensified proliferation and differentiation of lymphoid cells, helper and cytotoxic activity of T-lymphocytes, caused activation of normal and antibody-dependent killers, correction of secondary immunologic insufficiency and T- and B-systems of immunity. Increase of antibody production led to a 4-5-fold rise in the level of antibodies. Protective effect of the vaccine consisted in 5-10-fold decrease of survival of helminths.



LUNGWORM INFECTION IN MOUFLON AND THE RESPONSE TO TREATMENT

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Lungworm infection in mouflon (*Ovis musimon*) from a cinegetic reserve and the efficacy of Netobimin (Hapasil®) and Ivermectin (Ivomec®) were analysed. Ten female mouflons (G₁: five 1-2 years and G₂: five 3-4 years old) were isolated from the other specimens during the study period (February - May 1993). Rectal faecal samples were individually collected and analysed by the Baermann-Wetzel method. After quantification and identification of lungworm larvae, all animals were treated with Netobimin (7.5 mg/kg lw) and two months later with Ivermectin (200 µg/kg lw). The prevalence was 100% for Protostrongylidae and 70% for Dictyocaulidae (100% in G₁ and 40% in G₂). The species were identified as *Dictyocaulus filaria*, *Müllerius* sp, *Protostrongylus* sp, *Cystocaulus* sp and *Neostrongylus* sp. The intensity of Protostrongylidae larval excretion was higher in older than in young mouflons (126 L₁/g versus 68 L₁/g). The predominant species were *Müllerius* sp and *Protostrongylus* sp. Netobimin was 100% effective against *Dictyocaulus filaria* infection whereas a reduction in the intensity of larval excretion in mouflons less than three years old was the only effect against Protostrongylidae. Ivermectin was 77% effective against Protostrongylidae infection (100% in G₁ but only 25% in G₂) with a 97% of reduction in larval excretion in the mouflons still infected three weeks post-treatment. There was a high prevalence and intensity of lungworm, multiple infection involving the four species of Protostrongylidae and *Dictyocaulus filaria* and a high resistance of the former to Netobimin and Ivermectin treatment.



OESOPHAGEAL GROOVE CLOSURE AND EFFICACY OF FEBANTEL AGAINST *MUELLERIUS CAPILLARIS* IN GOATS

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Oesophageal groove closure is known to reduce bioavailability of oxfendazole and fenbendazole in sheep and then reduce also the efficacy against digestive tract strongyles. This phenomenon seems to be of a great concern in goats. Comparison was made of the relative efficacy of a probenzimidazole drug, febantel, administered to goats at 7.5 mg.kg^{-1} either as an oral drench ($n=23$) or as a paste formulation ($n=22$) against *Muellerius capillaris* in dairy goats. Faecal samples were collected on days 0, 7 and 21 post-treatment to assess larval counts. Both formulations were equally potent as the faecal larval count depressions were 63% and 56% on Day 7 and 54% and 50% on Day 21 for drench and paste respectively.

Direct intraabomasal and intraruminal febantel administrations (7.5 mg.kg^{-1}) were then performed to assess the eventual difference in disposition in goats ($n=2$) for each route of administration. The pharmacokinetics of the two most active metabolites (fenbendazole-FBZ, oxfendazole-OFZ) were studied. Peak plasma FBZ and OFZ concentrations occurred sooner in intraabomasal route (2 and 4 hours respectively) than in intraruminal (6 and 9 hours). Nevertheless the calculation of the areas under plasma concentration curves (AUC) gave divergent results according to the metabolites. In intraabomasal route, the AUC was reduced for FBZ (30%), increased for OFZ (37%) and slightly increased for the sum of both products (9.6%) when compared with intraruminal administration. The pharmacokinetic behaviour of febantel may account for the absence of effect of oesophageal groove closure on the efficacy of febantel in goat.



CONTROL OF BENZIMIDAZOLE-RESISTANT NEMATODES IN SHEEP BY A CONTROLLED RELEASE ALBENDAZOLE DEVICE

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An earlier field study (*Vet. Rec.* 1992 **130**, 351) indicated that the use in grazing sheep of a controlled release intraruminal capsule (CRC) containing albendazole had prevented the establishment of patent BZ-resistant *Haemonchus contortus* infection. A second field study was conducted on premises known to harbour BZ-resistant *Ostertagia circumcincta* and *H. contortus* and where thiabendazole treatment had given a 48% reduction in faecal egg-count. Three groups of 13 parasite-naïve lambs were turned out to graze together: untreated controls, those given a CRC and a group treated at monthly intervals with ivermectin. Maximum mean faecal egg-counts over 100 days for the three groups were 2655, 127 and 1875 epg, respectively, indicating that the CRC had provided a high degree of protection. Faecal culture and egg-hatch assays confirmed exposure to BZD-resistant strains of the two species.



IVERMECTIN ADMINISTRATION TO REINDEER

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Adult reindeer hinds ($n=61$) were allocated into five groups 11 December, 1990. Animals of Group C were left untreated as controls, while Group D received injectable ivermectin subcutaneously at a dose decimal of that recommended ($20\mu\text{g/kg}$), Group I got the standard injection ($200\mu\text{g/kg}$), Group O was treated orally with the paste registered for horses at a dose of $200\mu\text{g/kg}$, and Group T was treated topically with Ivomec pour-on at a dose of $500\mu\text{g/kg}$. The antiparasitic effect was evaluated with monthly examination of faeces until June. For each animal, nematode egg output means were calculated for the whole follow-up period. After logarithmic conversion the means were compared between the groups. Warbles (*Oedemagena tarandi* larvae) were counted by visual examination and digital palpation and throat bots (*Cephenomyia trompe* larvae) by pharyngoscopy in May. The trichostrongyle egg output of the standard injection Group I differed significantly from all the other groups, being lower. No significant differences could be seen regarding other nematodes. All the control group animals harboured both warbles and throat bots. All the treatments were 100% efficient against warbles and all but the decimal injection (85% reduction) against throat bots, too. No difference in weight development between the groups could be seen, probably because they were on natural pasture with a limited amount of food available. The standard injection treatment is recommend, the results indicating it has best efficacy against seasonally inhibited trichostrongyle larvae. Thus it should also minimise the risk of development of ivermectin resistance.



RESISTANT STRAIN OF SMALL STRONGYLES (CYATHOSTOMINAE) OF HORSES ON FENBESAN

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The studies were carried out on 10 selected mares at the age of four to 14 years, highly infected with small strongyles. The extent as well as the intensity of infection was established on the basis of coprological examination (Willis-Schlaaf and McMaster methods) and larvae culture. The drug resistance was ascertained by the faecal egg-count depression test. The mares were treated with Fenbesan (the synonym of Fenbendazol - Hoechst) a 4% powder in a single 7.5 mg/kg dose. The coprological examinations had been done four days before and 4, 7, 14; 21 days after treatment. The efficacy of the drug was estimated by using the Eysker (1988) model.

Before the administration of Fenbesan all mares were very infected with the small strongyles. The results are presented in the table.

Before treatment	4 days	Days after treatment x (EPG)			
		7 days	14 days	21 days	
1535	505	790	1240	1640	

The faecal egg-count depression test had showed low efficacy against small strongyles of Fenbesan. Four days after administration the efficacy was 67%, seven days - 48% and 14 days only 19.2%. After 21 days the infection rate was 6.8% higher as compared with the infection in mares before treatment.

It is worthy to note that in the experimental stable it is necessary to change the drug for nematode control.



STUDIES ON BENZIMIDAZOLE RESISTANCE IN CYATHOSTOMES OF HORSES IN SOUTHEAST ENGLAND

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A survey in 1990 in the southeast of England (*Vet. Rec.* 1992 130, 135) based on the faecal egg-count reduction test (FECR) showed benzimidazole (BZD)-resistance to be widespread. Five of the stables were revisited in 1992 in order to reassess their level of BZD resistance. The results of FECR tests were compared with those of in-vitro tests. The presence of marked BZD-resistance was confirmed on all premises. Moreover, the level of resistance appeared to have increased on all sites, particularly at those that had continued to use fenbendazole. Although in-vitro tests confirmed resistance, correlation with FECR results was otherwise poor. At least 90% of larvae cultured from faeces pre- and post-treatment at each site were cyathostomes. A non-BZD anthelmintic (pyrantel) was used as a positive control and gave an overall efficacy of more than 90%.



EPIDEMIOLOGY OF FASCIOLOSIS IN EASTERN NEPAL

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Among the diseases of ruminants, fasciolosis is probably the most common and possibly one of the most important causes of poor livestock productivity in Nepal. However, despite the importance of the disease, there is a shortage of epidemiological information and little understanding of the relationship between fasciolosis and the animal management systems practised in the country. An epidemiological survey was therefore conducted covering almost all the physiographic and agro-ecological zones of Eastern Nepal. Generally the prevalence of fasciolosis was greater in the hills than in the Terai with the highest prevalence in animals occurring during February and March and in rice cultivation areas. New infections in animals tend to occur between November and April. Infected snails are found all year round, but the highest numbers occur in November and December.



INFLUENCE OF TECHNOLOGIES OF SHEEP BREEDING ON SPREADING OF CESTODOSES IN MOLDOVA

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Dogs and sheep were examined for cestodoses in order to reveal connection between technology of sheep breeding and spreading of diseases. The obtained data were compared and analysed. Technological process of sheep breeding in Moldova includes four main campaigns: winter lambing (January - February), spring shearing (May - June), insemination of sheep (August - September) and driving to winter stable keeping (November). Traditionally veterinary treatments of animals (dehelminthizations, etc) are fulfilled at the same periods. Technological and veterinary measures are accompanied by crowding of a great number of sheep and watchdogs on limited territories which promotes infection of sheep with larval forms of cestodes and dog - with imaginal ones. This was confirmed by examinations of 370 pasture dogs. The rate of infection of dogs with *E. granulosus* especially increased at the second quarter ($21.25 \pm 0.55\%$) after lambing of sheep when some weak animals died and fell prey to dogs. According to the slaughter data of 9506 animals the rate of infection of sheep with *E. granulosus* larvae in Moldova was 63.1% and in sheep older than one year 60.78% cysts were fertile. With regard to peculiarities of technology of sheep breeding dehelminthizations of dogs against echinococcosis and other cestodoses are recommended in January, May, August and November timing them to the beginning of each of the mentioned campaigns.



REGIONAL DIFFERENCES IN THE PREVALENCE OF CYSTICERCOSIS IN SLOVAKIA AND THEIR REASONS

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An analysis of the human morbidity rate due to *Taenia saginata* and of *Cysticercus bovis* prevalence in slaughtered cattle shows that while the human morbidity rate is a permanent falling tendency, the prevalence of cysticercosis remains at an almost unchanged high level. The territory of Slovakia was divided into four areas, depending on the character of cattle raising and on the presence of attractive tourist localities in the area. The highest human morbidity rate caused by *T. saginata* was found in the region with the predominance of pastoral cattle raising. The highest prevalence of cysticercosis was recorded in the region with attractive tourist localities. The analysis is also indicative of the primary (direct) life cycle of *T. saginata* in the region with pastoral cattle raising, while other regions can be characterized by the secondary (indirect) life cycle of *T. saginata* with regional but especially transregional infection cycles.



EVIDENCE FOR THE LONG DISTANCE DISPERSAL OF CYSTERCERCOSIS

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St Kilda is an isolated archipelago some 60 km west of the Outer Hebrides off the west coast of Scotland. The last permanent human population was evacuated in 1932 and there are known carnivores on these islands.

The population of feral Soay sheep on St Kilda shows an instability that is characterised by periodic population crashes that are the result of overpopulation and parasitism. *Taenia hydatigena* cystercercosis has been noted in these Soay sheep for a number of years. Necropsy examination of victims of a recent population crash provided the opportunity to study the epidemiology of this parasite more closely. Despite the absence of dogs, *T. hydatigena* was found in all ages of sheep with both an increasing prevalence and intensity of infection with age. This suggested that there was no regulation, by immunity, of the establishment of the metacestode which is in contrast to geographical regions where dogs are present. It is concluded, therefore, that the most likely explanation of the presence of *T. hydatigena* in the Soay sheep on St Kilda is persistent but low levels of environmental contamination with taeniid eggs due to long distant dispersal by wildlife from the Hebrides or Scottish mainland.



TAENIA POLYACANTHA (CESTODA: TAENIIDAE) IN FOXES IN THE UK

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T*aenia polyacantha* Leuckart, 1856 is a fairly common parasite of foxes in the Northern Hemisphere, with a holarctic distribution. Although it is frequently reported from the fox (*Vulpes vulpes* L.) in Europe, its occurrence in foxes in Britain has only recently been confirmed. Adults have now been found in nine (4.57%) of 197 foxes from Powys, mid-Wales, and in one fox from Devon. The larval stage, which develops in the peritoneal cavity of rodents, has been recovered from 4 of 40 long-tailed field mice, *Apodemus sylvaticus*, in Cheshire. *T. polyacantha* may have been overlooked in Britain by being confused with other species of *Taenia* from canids. These utilise sheep as intermediate hosts. Current information suggests that the species most commonly found in foxes utilise rodents as intermediate hosts and have no veterinary significance.



BEHAVIOUR AND PATHOGENICITY OF *TOXOCARA CANIS* WERNER 1782 (ANISAKIDAE) SECOND STAGE LARVAE IN MICE OF DIFFERENT STRAINS

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In the present study behaviour and pathogenicity of second stage larvae of *Toxocara canis* were examined in different mouse strains with special emphasis on the MHC. Mice of the inbred strains BALB, C3H, C57BL and DBA and the outbred strain NMRI were infected orally with 1000 second stage larvae of *T. canis*. Clinical behaviour of the animals, numbers of larvae in liver, lungs, brain and musculature, hematological, serological parameters and histological sections were examined. The following results were obtained:

The infected and uninfected mice of BALB strain had an almost identical pattern of body weight development, the highest larval counts in the brain, an increasing percentage of eosinophils, and lesser histological changes than the other strains.

In infected mice of strains C3H, C57BL, DBA and NMRI deaths occurred from the 4th week p.i. on, and mice lost weight compared to the controls. Lower numbers of larvae were found in brains compared to the BALB mice, but clinical symptoms developed strongly. Histologically, perivascular round cell infiltrations, glia cell proliferation, edema of the medullary sheaths, glia scars and crystalized cholesterol could be demonstrated in the brains of the infected mice.

There is no evidence that mechanical damage caused by migrating larvae in the brain tissue is mainly responsible for symptoms of central nervous toxocarasis. Likewise, the assumption that the MHC is involved in the allergic-inflammatory response in the brain, could not be proved: infected mice of the BALB and the DBA strains reacted completely differently, although they are both equipped with the same MHC.



ECOLOGICAL ASPECTS OF THE OCCURRENCE OF ZONOSSES AND THEIR CONTROL IN URBAN DISTRICTS

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The number of dogs and cats per household considerably increased in this country, thus the frequency of parasitic diseases in these pets also increased. The problems caused by gastrointestinal helminths and protozoa are extremely important in view of the changes in our understanding of their biology and significance as causes of diseases in animals and man. In 1991 - 1993 384 dogs and 63 cats were evaluated. *Canis familiaris* 384 ex./216 pos. EI 56,25%, *Felis domestica* 63 ex./41 pos. EI 65,1%.

437 coprological analyses and 10 helminthological sections revealed the following endoparasites: *Elmeria* sp., *Isospora* sp., *Sarcocystis* sp., *Cryptosporidium* sp., *Giardia* sp., *Taenia* sp., *Dipylidium caninum*, *Strongylodes canis*, *Toxocara canis*, *Toxascaris leonina*, *Ancylostoma caninum*, *Trichuris vulpis* (dogs), *Isospora* sp., *Sarcocystis* sp., *Toxocara cati*, *Toxascaris leonina*, *Ancylostoma tubaeforme* (cats). The necessity of accurate diagnosis is underscored by the increasing public awareness of the potential for human infection by canine and feline parasites. The recognisable syndromes of both visceral and cutaneous larva migrans, toxoplasmosis, giardiasis, hydatidosis are now well defined in humans.

The presence of the above mentioned parasite species in dogs and cats is considered to be extremely important.



STUDY OF THE PREVALENCE OF INTESTINAL PARASITISM IN DOGS IN COUNTRY AREAS IN GALICIA (NW SPAIN)

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In rural areas in Galicia systematic anthelmintic treatment of dogs is not a common practice; moreover, most of them live in poor hygiene conditions. Thus, 172 faecal samples were collected from dogs of different ages and aptitudes. For each case, we did the anamnesis, paying special attention to hygiene and the state of health. The samples were processed using different concentration and flotation techniques, in order to determine the presence of intestinal parasites.

We observed that 74.4% of the animals carried some kind of intestinal parasite, and found the following species in decreasing order of frequency: *Trichuris vulpis* (52.3%); *Ancylostomatidae* (50%); *Toxocara canis* (27.3%); *Taenia spp.* (9.9%); *Isospora spp.* (5.8%); *Dipylidium caninum* (4.1%); *Sarcocystis spp.* (1.2%); *Toxascaris leonina* (0.6%). Infections by only one parasite species were the most predominant (26.2%), especially the whipworms (11.6%). Prevalence of double infections was also considerable (24.4%), and the most frequent association was whipworms and hookworms (13.9%). 19.8% of dogs were infected by three different parasites, commonly, *T. canis*, *T. vulpis* and *Ancylostomatidae*.

Taking into account the age of the animals, we observed that the frequency of intestinal parasitism was higher in adult dogs than in puppies (69.2%; 5.2%, respectively). This difference was proved to be statistically significant by means of the χ^2 squared test ($\chi^2 = 6.29$; $p < 0.025$). With reference to the animals' aptitudes, the prevalence of parasitism was similar in companion (20.3%), hunting (22.1%) and guard dogs (32%).



FREQUENCY OF *TRICHINELLA SPIRALIS* IN PIGS SACRIFICED AT ABC SLAUGHTERHOUSE OF LOS REYES, LA PAZ, STATE OF MEXICO, USING TWO TECHNIQUES OF DIAGNOSIS

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The aim of the present study was to determine the frequency of *Trichinella spiralis* using the methods of trichinoscopy and artificial digestion in pigs sacrificed at the ABC Slaughterhouse of Los Reyes, La Paz State of Mexico. Eight thousand muscular samples of the pillars of the diaphragm with an approximate weight of 25 g each were used for this purpose. They were first examined by trichinoscopy after the technique of Nemeseri. Later groups of approximately ten (pigs channels) were formed in a total of 800 in order to practise artificial gastric digestion following the technique of Koheler and Grisselaus preparing the gastric juice with 5 g of pepsin, 2 g of sodium chlorid, 10 ml of HCl and water 1000 ml. The results obtained were the following: through the technique of trichuinoscopy: all were negative and through the second method: 10 groups presented positive results (1.25% of the animals) these were submitted to the proof of interval of confidence at 9.5% where the result fluctuates round 3% of the population whereby we conclude that artificial gastric digestion constitutes a more precise method for the diagnosis of *Trichinella spiralis*.



THE OCCURRENCE OF *ISOSPORA SUIIS* AND *STRONGYLOIDES RANSOMI* IN NURSING PIGLETS IN THE NETHERLANDS

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The prevalence of *Strongyloides ransomi* and *Isospora suis* in nursing piglets in The Netherlands was assessed in two studies. In 1989, the prevalence of *S. ransomi* and *I. suis* was evaluated in a survey comprising 113 litters (2-5/farm) from 25 farms around Utrecht. No *Strongyloides* was found. Patent *Isospora* infections were observed in pooled rectal faecal samples from 41 litters, representing 18 of the 25 farms surveyed. Oocyst counts generally were low, counts above 1000 oocysts/gram faeces (OPG) being observed in only seven litters.

In 1990, rectal faecal samples were taken twice weekly between 4-6 and 16-23 days after parturition from all piglets of five litters and from their dam on 10 farms. Samples from piglets were pooled per litter prior to examination. Patent *I. suis* infections in piglets were observed on nine farms: three with 1-2 positive litters, four with 3-4 positive litters and two high prevalence farms with all litters positive. In most litters, oocysts were not seen before the second week after birth, suggesting a slow build-up of infections. Early patency and high (>1000 OPG) oocyst counts were mainly observed at high prevalence farms. *I. suis* oocysts were also observed in faecal samples from two sows.

It is concluded from the above that *I. suis* infections are frequent in nursing piglets in The Netherlands.



A COMPARISON OF NEMATODE PARASITES OF HORSES FROM TWO MANAGEMENT SCHEMES

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Internal parasites of horses, comprised mainly of nematodes, are one of the causes of colic. Little information exists concerning the presence of these parasites in horses in South Africa. No information is available which compares horses housed under differing management schemes. The aim of the present study was to compare the internal parasites of two groups of horses housed at the Faculty which had been managed differently. Group 1, chiefly ponies, were comprised of cycling or early pregnancy mares. They were maintained in paddocks, given supplemental feed and treated four times a year with antiparasitic remedies. Group 2 horses, chiefly thoroughbreds, grazed daily on irrigated kikuyu pastures and received antiparasitic remedies twice a year.

The two groups were each divided into conventional and strategic subgroups. The conventional subgroups were treated as they had been in previous years. The strategic subgroups were treated with an anthelmintic if the nematode egg count was larger or equal to 300 eggs per gram of faeces.

Monthly faecal analyses were carried out which included nematode egg counts for each horse and larval cultures for each subgroup. The nematode eggs which were recovered included those of strongyles, and *Parascaris equorum* and *Strongylodes*. Differentiation of third-stage larvae (L₃) from cultures distinguished between small strongyles (cyathostome) and large strongyles. Statistical analyses were performed on the total mean nematode egg counts for conventional and strategic subgroups within each group of horses.

Differences between subgroups were significant in Group 1 but not in Group 2. A considerable financial saving was evident, however, for the strategic subgroup as compared to the conventional, particularly in Group 1. In addition to financial advantages, is the avoidance of development of resistance to the antiparasitic remedies. Further, a strategic programme of control can be developed with basic management that includes monitoring of internal parasite levels through faecal examination.



DICTYOCAULUS ECKERTI IN THE RESPIRATORY TRACT OF REINDEER

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Light and scanning electronmicroscopical studies of the lungs of reindeer were performed in order to describe the normal morphology of the lower respiratory tract and the damage caused by lungworm *D. eckerti*. The material was collected in Finnish Lapland. It consisted of tissue samples from lungs of clinically healthy parasite-free reindeer, and animals with lungworm infection. Specimens of lungworms were collected from the infected animals.

Light microscopy: The tissue specimens were fixed in 4% neutral formalin and routinely processed and stained.

Scanning electron microscopy: Samples were fixed in a solution containing 2.5% paraformaldehyde and 0.1% glutaraldehyde, dehydrated, critical point dried in liquid CO₂ and coated with gold. The paraformaldehyde-glutaraldehyde fixative was also used for the lungworms.

D. eckerti is a nematode which is distinguished from *D. viviparus* by the characteristic oval oral opening and the shallow, thick walled buccal capsule. The longitudinal ridges of the cuticle are also of diagnostic value.

The respiratory surface of the reindeer generally resembles that reported previously in similar studies of other mammalian species. Ciliated epithelial cells, goblet cells, microvillous cells, Clara cells, alveolar epithelial cells of type 1 and type 2, and alveolar macrophages could be distinguished by their universally characteristic surface morphology. The rarity of the pores of Kohn in the alveolar walls of reindeer was considered to be the most striking difference in comparison with most other species of ruminants.

The main lesion in acute *D. eckerti*-infection was the obstruction of alveoli and small bronchioli by inflammatory cells mainly eosinophils. In more chronic stages a bronchitis caused by the adult worms and a nonsuppurative granulomatous pneumonia caused by aspirated eggs and larvae was a typical finding. Another typical feature was proliferation of the lymphoreticular cells.



DISTORTION OF THE GIZZARD OF CYPRUS PIGEONS ASSOCIATED WITH *HADJELIA TRUNCATA* INFESTATION

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Gross examination of infested gizzards reveals that the areas of distortion are limited to the junction with the proventriculus and at the opposite caudal pole. The major, biconate, muscle mass appears to be comparatively unaffected. Histologically the parasites lie between the acellular horny lining of the gizzard and the layer of secretory glands. There is an inflammatory response limited to infiltration with eosinophils and other cells. There is a small amount of fibrosis and some of the secretory glands show cystic dilatation. No granulomata have so far been detected by us. The relatively non-muscular areas of gizzard wall appear to show some thickening compared with the normal perhaps indicating reactive hyperplasia due to the decreased efficiency of the grinding process as the cornified layer becomes softened in the neighbourhood of the parasites. Studies of the parasite with the aid of the light and scanning electron microscope confirms its identity as the spirurid nematode *Hadjella truncata*. Further work is required with regard to various aspects of this condition and one of us (Dr Georgiou) is actively testing the efficacy of various anthelmintics in the hope of controlling this parasitic disease.



HELMINTH PARASITES OF WILD RODENTS IN KHUZESTAN PROVINCE, SOUTH-WEST OF IRAN

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A total of 64 wild rodents including 60 *Tatera indica*, two *Nesokia indica* and two *Rattus rattus* were collected from different parts of Khuzestan province, South-West of Iran and examined for helminth parasites. The prevalence and infestation intensities are outlined and morphologic details were drawn using camera lucida. The following species are reported:

Trematode: *Entosiphonus* sp.*

Cestodes: *Cysticercus fasciolaris*, *Hymenolepis nana*, *H. diminuta*, *H. sp.**, *Catenotaenia* sp.* and *Skrjabinotaenia lobata**.

Nematodes: *Trichuris rhomboides**, *Trichuris mofidi*, *Nematospiroides* sp., *Physaloptera* sp.* and *Rictularia* sp.*

Acanthocephala: *Moniliformis merionis*.

The species marked with (*) are new geographic records and are reported for the first time in Iran.



DEVELOPMENT OF *NEOSTRONGYLUS LINEARIS* (NEMATODA; PROTOSTRONGYLIDAE) LARVAE IN *CERNUELLA (CERNUELLA) VIRGATA* (MOLUSCA; HELICIDAE), INFECTED AND KEPT IN NATURAL ENVIRONMENT

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This study was carried out between November 1990 and October 1991. Snails were collected in a locality in the province of Lugo (North-West Spain) near the place where this experiment was performed. 10% of the molluscs were examined to determine that they were not naturally infected by Protostrongylidae. Once a month, sheep faeces with first-stage larvae (L-1) of *N. linearis* and 100 snails were placed together on 0.5 x 0.5 plots in natural conditions (with a total of 12 plots in the experiment). Every 14 days, from two to six snails from each plot were killed to study *Neoststrongylus linearis* larval development. This development was studied by taking into account these parameters: days required for the appearance of the first L-2 and L-3, and also the number of L-1, L-2 and L-3 found in molluscs.

Larval development of *N. linearis* was faster in the warmer months (spring and summer); in these snails the first L-2 and L-3 were found 28 and 42 days, respectively, after placing. However, in the colder months (autumn and winter), larval development was slower, the appearance of the first L-3 varying from 70 days after placing, in March, and 210 days, in November. In the molluscs placed in October, the first L-2 were found 154 days after placing, and no L-3 were found throughout the study. In no case did we find L-3 before May, and it is thereafter that the higher risk of definitive host infection exists. The number of L-3 per mollusc varied from one to 460 ($x = 106.13$).



THE EPIDEMIOLOGY OF HELMINTH INFECTIONS OF GROWING SHEEP IN ARGENTINA'S WESTERN PAMPAS

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The seasonal population trends of helminth parasites of growing lambs were investigated during four years. Successive worm-free lambs were grazed together with untreated lambs for 20-30 days and then slaughtered for helminth counts two weeks after their removal from pasture. Also untreated lambs from the same flock were slaughtered from two to 15 months of age for worm counts in the same way as tracer lambs. The predominant parasites were *Haemonchus*, *Nematodirus* and *Trichostrongylus*. *Haemonchus* was found to be of major importance, flock acquired massive worm burdens from summer to mid-autumn. Minimum burdens were seen from winter to early spring and maximum L4 stages were found from mid-autumn to early winter. *Nematodirus* burdens increased from December with a peak in late summer and then decreased to low values. Maximum larvae availability was in autumn-early winter. *Trichostrongylus* (mainly *T. colubriformis*) populations increased in autumn and peaked in June-July while the highest larvae availability was in autumn. The minor genera recovered were *Ostertagia*, *Cooperia*, *Trichuris*, *Oesophagostomum* and *Moniezia*. *Dictyocaulus*, *Chabertia* and *Teladorsagia* were seen occasionally and liver flukes were not seen. No important and pathogenic numbers of L4 stages were seen and all predominant species were able to survive over summer or winter in pasture.



NATURAL INFECTION WITH GASTROENTERIC NEMATODES IN HEIFERS OF SECOND YEAR GRAZING IN NORTH-WEST SPAIN

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This study was carried out from December 1989 to November 1990, at the Agricultural Research Centre in Mabegondo (La Coruña, Spain). Thirty frisian heifers of first lactation with parturitions grouped in winter (being February the average data of parturition) were used. At the second trimester of pregnancy (December 1989), half of the animals were orally administered a single dose of 7.5 mg/kg bw of Albendazole (G-T), the rest of the heifers remained untreated and formed the control group (G-C). After treatment, both batches of cows grazed separately in plots of similar vegetable composition, which had been grazed the previous year by the other two groups of treated and control heifers, respectively. Faecal and blood samples were taken monthly from each animal, so as to determine the number of eggs of gastrointestinal nematodes per gram of faeces (epg), and serum pepsinogen (expressed in milliunits of Tyrosine, mU Tyr). At the same time, the grass of the meadows of both groups was sampled, and the number of 3rd-stage larvae per kg of dry matter was calculated (L-3/kg DM).

Figures of epg were higher in G-C than in treated animals, the most remarkable differences being during the four months following treatment, especially at parturition time (Max = 366, $x = 40.2$ in G-C and Max = 29, $x = 6.7$ in G-T). From May onwards, until the end of the study, we observed a reduction in the number of epg in both groups (Max = 80.5, $x = 9.6$ in G-C and Max = 34, $x = 5.4$ in G-T). The nematode species found in the corresponding coprocultures were, in decreasing order of frequency: *Trichostrongylus*, *Ostertagia*, *Oesophagostomum* and *Cooperia*. From January to May the figures of L-3/kg DM were scarce in plots of both the control (Max = 200.4, $x = 114.7$) and the treated animals (Max = 255, $x = 115$). The flow rainfall from May to September limited the larval development in the autumn (Max = 209.5 $x = 135.3$ in G-C and Max = 81.3, $x = 49.5$ in G-T). Serum pepsinogen levels were lower in the treated (Max = 2588.8, $x = 889.4$) than in the control (Max = 3047.7 $x = 1308.6$) heifers. Statistically significant differences were shown using variance analysis. From the results we can deduce that heifers may have had low parasitic burdens; treatment at the last trimester of pregnancy significantly reduced the passage of eggs, and consequently contamination in plots.



EPIDEMIOLOGY OF BOVINE (*BOS INDICUS*-GUZERA) NEMATODE PARASITES IN CERRADO, MATO GROSSO DO SUL STATE, BRAZIL

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Availability of infective gastrointestinal bovine nematode larvae was studied on cerrado pasture in Selviria, Mato Grosso do Sul, from 1988 to 1991. Two tracer calves were necropsied monthly and grass samples were collected from the experimental paddock at two-week intervals. The contamination of the experimental paddock was maintained by naturally infected calves (permanent calves). The following genera from the tracer calves and grass samples, respectively, were found: *Cooperia* 72.80 and 83.20%; *Haemonchus* 23.20 and 11.78%; *Oesophagostomum* 2.90 and 3.24%; *Trichostrongylus* 0.80 and 1.75%; *Trichuris* 0.30 and 0.00%; *Bunostomum* 0.00 and 0.30%. The driest (June, July and August) and the rainiest (December, January and February) periods of the year were less suitable to most of the genera from the tracer animals. There was no statistical significant difference between periods for all nematode larvae genera measured by grass sampling. Outbreaks of haemonchosis were observed in permanent calves in early August, 1988 (dry period). The climate of the region proved to be suitable for the development and survival of larvae through the year, especially of the genera *Cooperia* and *Haemonchus*.



PREVALENCE AND LEVELS OF INFECTION WITH HELMINTHS IN SHEEP IN NYANDARUA DISTRICT OF KENYA

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Prevalence and levels of infection with gastrointestinal helminths in sheep were monitored monthly for one year on farms in Nyandarua district of Kenya through examination of faecal and herbage samples and gastrointestinal tracts. Genera of nematode larvae recovered from faecal cultures were *Haemonchus* (55.4%), *Trichostrongylus* (23.1%), *Oesophagostomum* (11.8%), *Cooperia* (9%), *Bunostomum* (0.6%) and *Strongylodes* (0.13%). Nematode and liver fluke eggs were most prevalent in adult sheep (over 12 months old) and immature (4 - 12 months old), while tapeworm eggs were most prevalent in the young sheep (under 4 months old). Nematode eggs per gram (EPG) of faeces were also significantly higher ($P < 0.01$) in adults and immature sheep compared to the young sheep during all periods of sampling. EPG counts and/or prevalence of nematode and tapeworms eggs were highest during the wet seasons which coincided with the highest levels of pasture contamination with nematode larvae. Prevalence of liver fluke eggs was however, highest during the dry seasons. *Haemonchus*, *Oesophagostomum*, *Trichostrongylus* and *Cooperia* were encountered in that order of prevalence in most guts examined. There was a significant difference ($P < 0.01$) in the worm burdens in the abomasum and total worm burdens between the dry and wet seasons. This study provides information that may be used in the formulation of helminth control programmes for sheep in the area.



SELECTION OF BRAZILIAN ISOLATES OF *BACILLUS THURINGIENSIS* WITH NEMATICIDAL ACTIVITY AGAINST FREE LIVING STAGES OF TRICHOSTRONGYLID NEMATODES

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The nematicidal activity of *Bacillus thuringiensis* and the possibility of its use as a biological control agent against plant and animal nematodes is in the process of investigation. Brazilian isolates of *B. thuringiensis* are being tested in our laboratory against trichostrongylid nematodes of ruminants. A bioassay to check efficiency of microbial products (dried culture sediment or a 48 to 72 h culture broth) using faeces positive for *Haemonchus contortus* is being conducted to evaluate levels of reduction of the free living stages in treated faeces. Reduction levels observed in the preliminary bioassays conducted on seven strains were not statistically significant when compared with controls. Tests are being continued using other isolates.



DISEASES OF FARMING SNAILS IN THE CZECH REPUBLIC

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Little information is available on diseases and pathology of snails. At present there is a trend to keep snails *Helix aspersa* in captivity. In the Czech Republic it is necessary to consult veterinarians about snail health, welfare and processing. Snails can be euthanased by physical methods. Post-mortem examination, laboratory tests, food inspection, and water analyses, etc were carried out.

Samples from 96 snail farms were examined. Nematodes, particularly of the genera *Nemhelix*, and many other free-living saprophytes were found as well as white mites (*Riccardoella limacum*) and their eggs. Smears of the alimentary canal of 67 *Helix aspersa* were examined bacteriologically (for aeromonads isolation). Strains of *Aeromonas hydrophilla*, *A. sobria*, *Pseudomonas aeruginosa*, *Klebsiella oxytoca*, *Kl. planticola*, *Morganella morganii*, *Kluyvera ascorbata*, and other species were detected.

These results show that *Nemhelix baceri*, *Riccardoella sp.*, *Aeromonas hydrophilla*, *A. sobria*, and *Pseudomonas aeruginosa* are the most important pathogens of *Helix aspersa* snails in Czech farms.



ISOENZYMATIC PROFILES OF *FASCIOLA HEPATICA* AND *DICROCOELIUM DENDRITICUM* (TREMATODA) ADULT SPECIMENS

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The liver flukes *Fasciola hepatica* LINNAEUS, 1758 and *Dicrocoelium dendriticum* (RUDOLPHI, 1819) LOOS, 1899 are the parasites responsible for fasciolosis and dicrocoeliosis in ruminants in Spain, with a high percentage of double infections. Control methods can be applied more effectively if we obtain more detailed information about the parasites' genetic diversity. A wide range of adult worms was therefore recovered from the livers of cattle, sheep and goats (different breeds) and from various places. Water soluble extracts of individual worms were analyzed for enzyme activity in thin-layer polyacrylamide gels using a conventional isoelectric focusing technique. The enzymatic systems used were: glucose phosphate isomerase (GPI), lactate dehydrogenase (LDH), phosphoglucumutase (PGM), acid phosphatase (AcP) and α -glycerophosphate dehydrogenase (α -GPDH). Our results showed GPI (between 6.5-7.5 pH), PGM (7.0-8.0 pH) and α -GPDH (6.5-7.0 pH) activity in *F. hepatica* and LDH (6.5-8.0 pH), GPI (6.5-7.5 pH), PGM (6.0-6.5 pH) and AcP (5.5-6.0 pH) activity in *D. dendriticum*. In general, little variation was observed between worms recovered from a single host, between those from hosts of the same species and between those from hosts of different species, for both flukes. The differences are mainly in band intensity.

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ASPECTS OF THE SITE DISTRIBUTION OF STRONGYLES IN THE LARGE INTESTINES OF HORSES IN PORTUGAL. EMPHASIS ON SMALL STRONGYLES

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During 1991, the large intestine of 11 horses were examined at necropsy and processed for parasite recovery. The organs involved in the microbiotopes study of adult and larval stages were the caecum, large colon (ventral and dorsal) and small colon.

A total of 28 species of adult strongyles (six of *Strongylinae* and 22 of *Cyathostominae*), was found in the large intestine, as well as the larval stages (L₄) of *Cyathostomum* (*sensu lato*) and *Tridontophorus* spp. In the caecum we registered 24 species, in the large colon 28 species and in the small colon eight species.

The species *Strongylus vulgaris*, *Cyathostomum coronatum*, *Cyllocylus elongatus*, *Cyllostephanus callicatus* and *C. poculatus* were found mostly in the caecum.

Species like *S. edentatus*, *Tridontophorus brevicauda*, *T. serratus*, *Cyathostomum labratum*, *Cyllocyclus ashworthi*, *C. nassatus*, *C. radiatus*, *Cyllostephanus minutus* and *Gyalocephalus capitatus*, prefer the ventral colon.

Cyllocyclus insigne, *Cyllostephanus goldi* and *C. longibursatus* showed a marked preference for ventral colon.

These data are similar to those reported by other authors and point out the possibility of a behaviour pattern of strongyles in Portuguese horses similar to those of horse strongyles, specially *Cyathostaminae*, known in other geographic areas.



STRONGYLOIDES STERCORALIS: CONTROL OF DEVELOPMENT BY AMPHIDIAL NEURONS

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The life cycle of *Strongyloides stercoralis*, an important nematode parasite, is controlled by chemical signals from the host or environment. Under unfavourable environmental conditions *Caenorhabditis elegans*, a related free-living nematode, can interrupt normal development by including a resistant resting stage, the dauer larva, in its life cycle. Entry into and exit from this stage is controlled by four amphidial neurons, as demonstrated by ablation studies (Bargmann and Horvitz 1991, *Science* 251, 1243). The *S. stercoralis* infective larva, which is similar to the dauer larva of *C. elegans*, resumes development on receipt of signals from a host. We have traced each of the 13 amphidial neurons, which are likely to include the relevant receptors, to its cell body, and, by comparing the positions of the cell bodies in the two species have found possible homologs with neurons in *C. elegans*. Consequently, microlaser ablation studies can be conducted to determine which neurons are involved in the infective process.



THE EFFECTS OF SUGARS ON INVASION OF *EIMERIA TENELLA* SPOROZOITES IN PRIMARY CHICKEN KIDNEY CELLS

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Effects of sugars on the invasion of *Eimeria tenella* sporozoites into primary chicken kidney cell culture were investigated. Of five different sugars (D-mannose, D-galactose, α -L-fucose, α -methyl-D-galactose, methyl-D-mannophyranose), only D-galactose inhibited sporozoite invasion when added to culture medium at infection, and the effect of D-galactose was dose dependent. Cell invasion of sporozoites also was inhibited when kidney cells or sporozoites independently were treated with D-galactose. No adhesion of sporozoites onto the kidney cells was observed whether sporozoites were treated with D-galactose or not. Presence of D-galactose was recognised not only on kidney cell surface but also on sporozoite under a laser scanning microscope. These results suggest that D-galactose is directly involved in invasion of *E. tenella* sporozoites into the cell culture.



FEEDING OF *DERMACENTOR NUTTALLI* (ACARI:IXODIDAE) THROUGH A SILICON MEMBRANE

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To date, only one team of scientists has successfully fed female hard ticks to repletion using a Baudruche membrane. No report is available regarding the hatching of larvae after oviposition of the *in vitro* fed ixodid ticks.

Adult *Dermacentor nuttalli* of a laboratory strain were fed on an artificial membrane made from silicon rubber with a thickness of about 0.16-0.20 mm. Heparinised cattle blood was taken from slaughtered animals, deep frozen and stored at -21°C for 1 - 12 days and 16 - 20 weeks. The feeding device was placed in an incubator to simulate natural conditions as provided by the host (temperature: 36.7°C; relative humidity: 90%; CO₂ concentration: 4.5%) . For olfactory and tactile stimulation further cotton-wool fibres with ether-diluted secretions of sebaceous and sweat glands, cattle hair and tick faeces were used.

Female ticks detached from the membrane for the first time on Day 6. 37.9% of the female *D. nuttalli* were sucking blood to repletion and ranged an average weight of $0.304\text{g} \pm 0.064\text{g}$ (0.1961 - 0.4268g). Oviposition was recorded in 100% of gravid females. Normal developed larvae hatched from 49.83% of all laid eggs.



SUSCEPTIBILITY OF CORRIEDALE AND CRIOULA BREEDS TO *HAEMONCHUS CONTORTUS*

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Considering the results obtained in other parts of the world, where local sheep breeds have been found to be resistant to natural nematode infections and the need of new alternatives for the control of internal parasites, a research study has started in Brazil looking for possible differences between Corriedale, the most important breed in the region, and a local breed, the Crioula. Resistance to primary and secondary infections with 10000 L₃ of *Haemonchus contortus* in worm-free lambs are compared on the basis of worm egg counts (EPG), packed cell volumes (PCV) and worm counts. Partial results of primary infection showed similar EPG in both breeds but lower reduction in PCV in the Crioula. The results will be presented and their possible implications on the epidemiology and control of sheep nematodes discussed.



RESPONDER CLASSIFICATION OF SHEEP: COMPARATIVE ASSESSMENT OF DISTINCT *HAEMONCHUS* AND *TRICHOSTRONGYLUS* INFECTIONS

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It has been shown that lines of sheep either resistant (high responder, HR) or susceptible (low responder, LR) to gastrointestinal nematodes can be selected. Little is known, however, on the specificity of the type of responsiveness. Therefore, in a pen experiment involving 99 animals, comparative assessment of responder classifications of sheep based on distinct *H. contortus* and *T. colubriformis* infections were attempted.

Faecal egg counts (epg) after double artificial infections were used as parameter of responsiveness. After infections with 2x7000 L₃ of *H.c.* sheep were ranked into three categories: epg < 1000 = HR, epg 1000-3000 = IM (intermediate), epg > 3000 = LR. After deworming, the same animals were infected with 2 x 15000 L₃ of *T.c.* and classified as: epg < 400 = HR, epg 400-900 = IM, epg > 900 = LR.

The majority of animals (69.7%) were classified identically when tested by both methods. In 23.2% marginal variation only occurred. Marked difference was found in 7.1% of sheep only.

In conclusion, results of responder typing obtained by test infections with different trichostrongyle species show remarkable conformity implying this feature to be of characteristic to the host animal in general sense.



**LARVAL MIGRATION INHIBITORY ACTIVITY OF THE
ILEAL CONTENTS OF GOATS INFECTED WITH
*TRICHOSTRONGYLUS COLUBRIFORMIS***

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Goats generally appear less able to mount an effective immune response to gastrointestinal nematodes than sheep. In investigating possible reasons for this a study was designed to examine the larval migration inhibition activity of ileal contents of goats infected with *T. colubriformis*. Activity was measured by the number of exsheathed L3 of *T. colubriformis* migrating out of an agar gel containing ileal contents, relative to those migrating out of control gels. Six Angora goats, raised on pasture, were infected with 35,000 L3, treated with an anthelmintic after 28 days, reinfected with 35,000 L3 20 days later and then slaughtered after a further 28 days. Ileal contents, collected from cannulae inserted into the terminal ileum, were assayed for activity every two to three days. Inhibitory activity was significantly increased ($p<0.05$) from preinfection levels on Days 17, 20, 23 and 28 for the first infection and Days 7, 9, 11, 13, 15, 18, 20 and 27 for the second infection. These findings show that, as has been described for sheep, the ileal contents of infected goats can contain substances that are inhibitory to larval movement.



VACCINATION OF SHEEP AGAINST *HAEMONCHUS* *CONTORTUS* WITH SUB-FRACTIONS OF THE PARASITE'S INTESTINAL MICROVILLAR INTEGRAL MEMBRANE PROTEIN H11

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The protective, 'hidden' protein H11 runs on SDS-PAGE as a doublet. H11 fractionated by re-cycling free flow isoelectric focussing yields H11-U, M_r ca. 112,000 at pH 4.5, H11-L, M_r ca. 108,000 at pH 6.5 and both bands in intermediate fractions. To test whether the protective capacity is associated with any one component, sheep about six months old in five groups of six were injected with a total of 150 μ g of either H11-L, H11-U, recombined H11-L and H11-U, the unseparated bands obtained at intermediate pHs, or a control protein, in three equal doses over a period of 54 days. The sheep were challenged with 10,000 IL_{III}, three weeks after the third injection and the experiment terminated 29 to 31 days later. Injection of either component of the doublet induced the formation of antibodies which cross-reacted with the other. Injection of any of the fractions reduced parasite egg out-put throughout the trial by 90% and total worm numbers by 63 to 84%. There were no significant differences between the H11-vaccinated groups, but all showed a significant difference ($p < 0.05$) to the controls. As with the unfractionated doublet, reductions (70 to 88%) in the numbers of female worms were greater than the reductions of the males. It is concluded that the individual components of the H11 doublet are equally effective in protecting against *Haemonchus*.



EFFECT OF PROTEIN SUPPLEMENTATION UPON THE CELLULAR RESPONSES TOWARDS *NEMATODIRUS* *BATTUS* INFECTION IN LAMBS

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Two groups of lambs were fed a basal diet (16.2% CP) (Groups 2 and 4) and two groups the diet supplemented with 10% fish meal (Groups 1 and 3). One group from each dietary treatment (Groups 1 and 2) was continuously infected over a seven week period with a trickle infection of *N. battus* (1000 L₃/day at week 1 to 4000 L₃/day at week 3 and declining to 1000 L₃/day at week 7). The two uninfected groups (3 and 4) served as challenge controls. All groups were treated with anthelmintic at week 8, given a single challenge dose of 30,000 *N. battus* at week 9 and killed nine days later. Peripheral blood eosinophil counts were monitored throughout the trial and samples taken at post-mortem for worm population analysis and evaluation of cellular responses. Worm burdens in Groups 1 and 2 were significantly reduced in both number and size. Intestinal eosinophilia was observed in Groups 1 and 2, and the number of these cells as well as intestinal mast cells and globule leucocytes were significantly elevated in the mucosa of Group 1 lambs. Peripheral blood eosinophilia was detected towards the end of the trickle infection and following challenge in Group 1 and to a lesser extent in Group 2 although the effect was less pronounced after challenge. There were no significant differences in sheep mast cell protease (SMCP) levels in the intestinal mucosa between groups although the levels in Group 1 were more than 1.5 times that of the other groups.



EFFECT OF THE STATE OF IMMUNITY ON THE ESTABLISHMENT OF *TOXOCARA CANIS* INFECTION IN PUPPIES

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Three groups of eight Beagle puppies each were experimentally infected with 3000 *Toxocara canis* embryonated eggs at 2.5 months of age. Animals of the first group (G-P) had been infected in uterus and during early life, by the galactogenic route; the other two groups received an immunosuppressive (G-D) or immunostimulant therapy (G-E), respectively, from 15 days prior to infection until 20 days post-infection (p.i.). Every third day a faecal sample was collected from each puppy, examined for *Toxocara* eggs by flotation with zinc sulphate and the number of eggs per gram of faeces (e.p.g.) was determined.

The first eggs appeared in faeces from 21 to 33 days p.i. in the G-D group. This prepatence period was shorter than in G-E (24-51 days p.i.) and G-P (30-57 days). Several puppies of each group spontaneously eliminated different numbers of adult worms, which affected the passing of eggs in faeces. The number of e.p.g. varied throughout the period of study, and notable differences were observed between the three groups of animals. At the end of the experiment, the highest figures of e.p.g. were detected in G-D (250-10250, $x = 3391$) and in G-P (100-9050, $x = 2881$) and the lowest in G-E (450-5400, $x = 1368$). Using the Kruskal-Wallis test, highly significant statistical differences were observed in the passing of *T. canis* eggs in the groups studied. These results can be confirmed by their relationship with the number of worms which developed to maturity in each puppy.



EOSINOPHILIC RESPONSES IN BEAGLE PUPPIES INFECTED WITH *TOXOCARA CANIS* EMBRYONATED EGGS

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Twenty-four Beagle puppies aged 2.5 months were infected orally with 3000 embryonated eggs of *Toxocara canis*. The animals were divided into three groups: the first one (G-1) was made up of puppies that had previously acquired natural infection during prenatal life and from the bitch's milk, and later treated with piperazine citrate at 30 and 45 days of age. The animals of the second (G-2) and third groups (G-3) had received a corticosteroid and immunostimulant treatment, respectively. In order to assess the eosinophilic responses in these animals a blood sample was collected from the cephalic vein at weekly intervals, from 20 days previous to infection, until 100 days post-infection (p.i.).

During the phase of larval migration an increase in % of circulating eosinophils was observed, and this peaked at 14 days p.i. The eosinophilic response was higher in puppies of G-1 ($\bar{x}$ = 43.6%; Máx. = 58.0, Mín = 27.3) and G-3 ($\bar{x}$ = 40.0%; Máx. = 56.8, Mín = 19.3) than those of G-2 ($\bar{x}$ = 15.5%; Máx = 31.6, Mín = 3.1). The eosinophilic count progressively decreased, at the same time that adult worms developed in the intestine. Finally it remained lower than 6% in puppies of the first and third groups, whilst in the corticosteroid-treated group eosinophilia remained between eight and 12%. By means of the Kruskal-Wallis test, statistically significant differences were found (χ^2 = 14.41; p = 0.0000) in the eosinophilic response of the three groups.

More studies are needed to establish a direct effect of eosinophil levels on *T. canis* infection; however, the % of larvae which underwent tracheal migration was higher in depressed animals.



SKIN DELAYED HYPERSENSITIVITY IN BUFFALOES INDUCED BY *TOXOCARA VITULORUM* ANTIGENS

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This work conducted in Ilha Solteira, São Paulo State, Brazil, from 1991 to 1992. *Toxocara vitulorum* soluble brute antigen was obtained from extracts of second-stage larvae (infective larvae). 0.1 ml of antigen containing 315 to 630 μ g protein was injected intradermally on the depilated neck area of 16 buffalo calves, 11 to 124 days old and under of the tail of five buffalo cows, 27 to 115 days after calf-birth. As control, saline 0.85% was used. These calves, 87.5% were naturally infected with *Toxocara vitulorum*. Nodular dermal formation was measured by caliper ruler, while induration was estimated and arbitrarily graded from + to +++++. Skin tests done at 24, 48 and 72 hours after injection of the antigen showed dermal reactions in 81.25% calves and in 100% cows examined. Dermal nodules at 72 hours after injection of the antigen were up to six times larger (65 mm mean diameter, +++++ Induration) than those initial reactions in calves. Furthermore, the nodular reactions were larger with antigen containing 630 μ g protein. Histologic preparations of such reactions showed a typical mononuclear cell infiltration, with occasional eosinophils and perivascular cuffing. There was also infiltration of polymorphonuclear cells in one cow.



A ROLE OF SURFACE AND EXCRETORY/SECRETORY PROTEINS OF ADULT FLUKES DURING *DICROCOELIUM DENDRITICUM* INFECTIONS IN CATTLE

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Pathogenicity and immunogenicity of *Dicrocoelium dendriticum* are poorly recognised. In the present experiments we attempted to analyse local humoral responses of naturally infected cattle to surface and excretory/secretory (ES) proteins and glycoproteins of adult flukes.

Surface proteins and glycoproteins of freshly isolated and careful purified flukes were labelled with NHS-biotin and Biotin Hydrazide (BH) respectively then extracted using Tris-buffered saline. ES products were obtained during 24 hours incubation of undamaged worms at 37°C in Tyrod's salts solution enriched with streptomycin and penicillin. Bile samples were collected at slaughter from cattle harbouring 120-280 lancet flukes. The fluke antigens inducing bile antibody response were immunoprecipitated and analysed by Western-blot technique.

Bile of non-infected cattle did not precipitate any fluke proteins. One to eight polypeptides were found in immune complexes formed by individual bile of infected cattle and surface proteins while immune complexes formed by bile and surface of flukes labelled with BH contained 2-5 fluke glycoproteins. On average three polypeptides were recognised in ES products. One polypeptide of molecular weight 150 kDa seems to be released from the fluke surface to the environment.

It is suggested that the surface and ES antigens may be of particular importance in the host parasite relationship.



REACTIVE OXYGEN INTERMEDIATE PRODUCTION IN *FASCIOLA HEPATICA* INFECTED HOSTS

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There has been extensive research on the immune responses of sheep to *F. hepatica* but it is still not clear why even heavily infected sheep remain susceptible to the parasite. In contrast, infected rats are resistant to reinfection. In a rat which has adult flukes in its bile duct, incoming juvenile flukes (JF) are killed in the intestine or peritoneal cavity and therefore do not reach the liver. We examined the mechanisms by which JF are killed in the peritoneal cavity of rats. The hypothesis that highly destructive reactive oxygen intermediates (ROI) released by peritoneal cells kill JF was tested. Following stimulation with fluke antigens, peritoneal cells from infected rats produced more ROI than the cells of uninfected rats. ROI production was increased by plasma from both uninfected and infected rats. Peritoneal cells and plasma from infected rats were also shown to be capable of killing juvenile *F. hepatica* *in vitro* through their production of ROI. Peritoneal cells from infected sheep produced fewer ROI than cells from infected rats. The response of sheep cells was not increased by plasma. The results of this study suggest that sheep may not develop resistance to *F. hepatica* because their peritoneal leukocytes produce only low levels of ROI in response to the parasite.



CELLULAR AND HUMORAL RESPONSES TO *FASCIOLA HEPATICA* PRIMARY AND SECONDARY INFESTATION IN SHEEP

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The general immune response to *Fasciola hepatica* was studied in sheep, after a primary infestation (150 metacercariae) and a secondary infestation six weeks later. The animals were necropsied 12 weeks after primary or secondary infestation for counting and measuring flukes. Cellular and humoral responses were detected by peripheral blood lymphocyte proliferation with excretory-secretory antigens and IgG or IgM specific ELISA.

The number of flukes with respect to the infestation dose showed no difference between the monoinfested (MG) and biinfested group (BG). The number of flukes measuring less than 15 mm was significantly lower in BG than in MG. Primary infestation induced a specific lymphocyte response from week 2 post infection (W2 PI) to W5 PI and specific IgM and IgG responses from W2 PI; IgM response was biphasic with peaks in W3 PI and W11 PI; IgG levels increased up to W6 PI and became stable afterwards. After secondary infestation, the level and the duration of the specific lymphocyte response was reduced by comparison with a primary response. IgG and IgM levels showed no difference between primary and secondary infestation.

Specific lymphocyte and humoral responses to *F. hepatica* primary infection in sheep are similar to those in rats or cattle. The decrease of the number of small flukes in biinfested animals shows that the flukes resulting from the secondary infestation have a quicker growth. The decrease of the lymphocyte proliferation associated with the stability in a high level of IgM and IgG after secondary infestation suggest that the immune response of sheep is turned away, in a non protective way that facilitates the installation of flukes resulting from secondary infestation.



SCHISTOSOMA BOVIS IN GOATS: THE ANTIBODY RESPONSE TO EGG AND ADULT WORM ANTIGENS

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The aim of the present study was to elucidate the antibody response (total Ig and specific IgG) in goats to *S. bovis* egg and adult worm antigens during early infection and following treatment with praziquantel. West African dwarf goats were randomly divided into four groups. Two groups were infected with *S. bovis* (2,000 cercariae each). Thirteen weeks post infection (p.i.) one infected and one uninfected group were treated with praziquantel (60 mg/kg body weight). Faecal egg excretion (EPG) was determined using a vacuum filtration technique. Crude worm and egg antigens were prepared and an ELISA technique was applied to detect circulating antibodies (Ig and IgG).

Faecal egg excretion commenced six weeks p.i., culminated eight weeks p.i., and declined evenly for both groups until week 12 p.i. Subsequently EPG of the untreated group levelled out, whereas EPG of the treated group declined to nearly zero. The antibody response to *S. bovis* worms rose significantly in the infected groups two weeks prior to the onset of faecal egg excretion, whereas the Ig and IgG responses to egg antigen were not elevated until two weeks after initial egg excretion. Treatment resulted in a significant increase in the antibody response to worm antigens, probably due to release from dead worms, but dropped only one week later. Throughout the study period antibodies against egg antigens continued to increase in the untreated group in spite of the steeply declining egg excretion from week eight p.i. This could be due to egg accumulation in the tissues. In the treated group a steep egg antibody decline was seen immediately after treatment in accordance with elimination of adult worms.



**POLYPEPTIDE PROFILES AND ANTIGENIC
CHARACTERISATION OF CELL MEMBRANE AND
FLAGELLAR PREPARATIONS OF DIFFERENT STOCKS
OF *TRYPANOSOMA EVANSI***

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Polypeptides and antigens of cell membrane (CM) and flagellar (FI) preparations of seven different stocks of *Trypanosoma evansi* were studied by employing SDS-polyacrylamide gel electrophoresis and by western blotting. The stocks were collected from Bareilly, Bikaner, Hisar, Karnal, Lucknow and Ludhiana localities of northern India, isolated from buffalo, equids and camel. Polypeptides numbering two to five in the molecular weight (MW) range from 48.4 to 80.2 kD (except one stock) were detected in the CM preparations and four to 12 polypeptides ranging from 17.6 to 80.2 kD MW were found in the flagellar preparations of different stocks. It was interesting to note that 78.3 kD polypeptide was common to the CM preparation of all the seven stocks. The number of antigens found in the CM of different stocks by using homologous and heterologous hyperimmune sera (HIS) varied between three to 11 in the MW range of 25 to 98 kD. The flagellar antigens of different stocks varied in number between three to 14 mostly in the MW 17 to 98 kD, by using homologous and heterologous HIS. Antigenic cross reactivity among polypeptides of the CM and FI preparations detected by various HIS was also high indicating the possibility of less antigenic variability among these seven stocks.



EFFECTS OF EXTRACTS FROM *SARCOCYSTIS GIGANTEA* (SGE) ON HIV-TARGET T-CELLS

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S*arcocystis* spp. are widely spread protozoans in humans and livestock. Extracts from *S. gigantea* (Syn. *S. ouifells*) contain a powerful mitogen for human mononuclear cells (MNC). This mitogen activity is due to a lectin. In the present study we investigated the reactivity of SGE on H9 and MT4 cells. These artificial tumor T-cells are used as target cells for HIV in vitro. In this study, experiments were performed using lymphocyte transformation assay with incorporation of radioactive thymidin. We found that the *S. gigantea* lectin is a powerful activator for MNC as well as for H9. H9 cells showed a similar proliferation rate as the human MNC. In contrast, no stimulation effect on MT4 cells was observed. These findings show that attention should be paid more intensely towards the molecular level of host-parasite-interaction including aspects of virulence against a specific defence mechanism of the host.



STUDIES OF ANTIBODY REACTION IN EXPERIMENTAL *THEILERIA HIRCI* INFECTION IN SHEEP

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T *helleria hirci* is a tick-borne protozoan parasite which is highly pathogenic to sheep. The parasite primarily infects lymphocytes, and at a later phase of infection erythrocytes. *T. hirci* is closely related to *T. annulata* and *T. parva*, both causing serious disease in cattle.

Level and duration of the antibody response in experimental *T. hirci* infection were studied in six adult sheep which were inoculated with *in vitro* cultured lymphoid cells infected with schizonts. Antibodies were measured by the indirect fluorescent antibody test (IFAT). Sera, added to *T. hirci* infected cells on antigen slides, were tested in two-fold dilutions in PBS, starting at 1:10. Negative control sera were obtained from the experimental animals prior to inoculation. Serum from a sheep experimentally infected with *T. hirci* and subsequently reinoculated with autologous infected cells, was used as positive control serum.

Bright fluorescence specific to the intracellular schizonts could be demonstrated in all experimental animals from Day 15 onwards and attained maximum levels 20 to 61 days after inoculation. Maximum titre reached was 1:2560. All animals were still positive at the end of the examination period, 99 days after inoculation. No fluorescence was detected in sera collected from the animals prior to infection.



PARASITIC INFECTIONS OF CATTLE IN FULANI NOMADIC HERDS

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Over a period of six years (1986-1992), the Fulani Ambulatory Section of the Ahmadu Bello University Veterinary Teaching Hospital handled the following cases of parasitic infections in cattle belonging to nomadic Fulani of northern Nigeria: trypanosomiasis (8,071); fascioliasis (1,139); theileriasis (550); parasitic gastroenteritis complex (451); louse infestations (256); thelaziasis (67); ascariasis (37); and coccidiosis (11). Few animals had ticks but as Fulani herdsman routinely remove ticks from their animals on a daily basis not much attention was given to tick infestation. Diagnosis in all cases was established through physical and laboratory evaluations.

Trypanosomes were the most prevalent blood parasites constituting about 93.3% of all blood parasites while *Fasciola gigantica* and *Linognathus vituli* were the commonest helminth and ecto-parasites respectively. Trypanosomes also produced the severest form of disease in the cattle. The most common trypanosomes were *Trypanosoma vivax* and *T. congolense*. From the results of this study and interviews with Fulani herdsman, it is concluded that parasitic infections are the most common important conditions of Fulani cattle.



EIMERIA INFECTION IN MOUFLON (*Ovis musimon*)

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Eimeria infection and the species involved have been studied in mouflons (*Ovis musimon*) living together with deer and Spanish ibex in a cynegetic reserve (400 Ha) for Iberian forest repopulation. Ten adult female mouflons were isolated from the other specimens during the study period. From February to March of 1993, rectal faecal samples were collected from these adult females and their offspring for oocyst detection. The species involved were identified after sporulation at environmental temperature in 2.5% potassium dichromate solution. The oocysts counts were higher than 10,000 oocysts/g (10,000-25,000) in 40% of the adults, which did not show diarrhoea. The average oocyst shedding in young animals was 16,800 oocysts per gram at one month and at 1.5 months of age, associated with a transient diarrhoea. The species involved in adult infections were *E. caprovina/caprina*, *E. crandallis*, *E. faurei*, *E. granulosa*, *E. intricata*, *E. ovina*, *E. ovinoidalis*, *E. parva* and *E. punctata*, with a remarkable predominance of oocysts of *E. ovina*. In young animals *E. intricata* and *E. punctata* were not detected but *E. ahsata* was. Morphological characteristics of oocysts from these species are described, with special reference to those not previously identified in mouflon. The intensity of *Eimeria* spp. infection supported by adult mouflons, multiple infections involving ten species and the detection of *E. caprovina/caprina*, *E. granulosa*, *E. intricata* and *E. punctata* are the remarkable aspects extracted from the study.



STUDIES ON PARASITAEMIA, PACKED CELL VOLUME, TOTAL SERUM PROTEIN AND ANTIBODY LEVELS IN NATURALLY ACQUIRED BABESIOSIS AND ANAPLASMOSIS IN BUFFALOE CALVES

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Babesia bigemina, Babesia bovis and Anaplasma marginale occur either in bovines or in buffaloes, but in the latter the infectious process generally passes unnoticed and they become an important reservoir of these parasites to the bovines. In São Paulo State, Brazil, mixed infection with Babesia spp. and A. marginale has been observed, however no parasites were found in the blood and organ smears. An epidemiological survey using the indirect fluorescent antibody test (IFAT) revealed positive results to B. bigemina, B. bovis and A. marginale. The aim of the present study was to observe the dynamics of Babesia spp. and A. marginale infection in buffaloe calves. Twenty-five buffaloe calves were used. Blood samples were taken monthly, from one month until one year of age, and analysed for the presence of parasites. The packed cell volume (PCV), total serum protein (TSP) and antibody levels by IFAT to the three parasites, were measured. The blood smears showed no parasites during all the trial. The PCV values were within the normal range, the same occurring with the TSP values. The IFAT revealed that buffalo calves acquired maternal antibodies anti-B. bigemina, anti-B. bovis and anti-A. marginale via colostrum, which persisted for three, two and four months, respectively. After this time, they disappeared, reappearing at six months of age to B. bigemina and A. marginale and at ten months to B. bovis, showing acquired infection in the livestock.



SEROEPIDEMIOLOGICAL STUDIES ON *TOXOPLASMA GONDII* INFECTIONS IN SHEEP AND CATS IN NORTHERN GERMANY

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Toxoplasmosis is one of the more common parasitic zoonoses. If first contracted during pregnancy, *Toxoplasma gondii* can cause perinatal death or foetal abnormalities in animals and humans. In addition, *T. gondii* is gaining importance as an opportunistic protist in immunocompromised patients, such as those with AIDS. Hence, there is an urgent need for accurate data on the epidemiology of *T. gondii* infections. Such data are needed to find the importance of the different sources of infections in animals and humans, to identify populations at risk from the infection and to develop measures for prevention or control of the disease. To obtain information on the prevalence and incidence of *T. gondii* infections in sheep and cats in Northern Germany, animals from different areas were sampled at random and tested serologically by enzyme-linked immunosorbent assays based on recombinant *T. gondii* antigens, termed H4 and H11, or on traditional antigen derived from *T. gondii* endozoites. *T. gondii* infections were present in all areas investigated. In total, about 69% of adult sheep and 61% of cats showed serological evidence of infection, and 49% of sheep and 58% of cats had antibodies to H4 or H11. Follow-up sera were collected from 22 ewes and their offspring selected at random from a flock of 450 sheep. Relations between the prevalence of antibodies to the different *T. gondii* antigens and a range of epidemiological parameters were analysed by logistic regression. Analyses included breed, age, flock size and management of sheep, and abortion in sheep, as well as breed, age, type of keeping and feed of cats.



COURSE OF ENDOGENOUS DEVELOPMENTAL PHASE OF SELECTED *EIMERIA* SPECIES IN PHEASANTS

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Coccidiosis is a frequent finding at post-mortem examinations of shot or dead pheasants. The present study focussed on the endogenous phase of development of *Eimeria colchici* and *E. duodenalis* from the blind sacs and duodenum of pheasants, respectively. Eighteen two-week-old pheasants were experimentally infected with a suspension of *E. colchici* and *E. duodenalis* oocysts at a dose of 5000 oocysts per bird. The endogenous phase of development was observed in 24 h intervals, i.e., 24, 48, 72, 96, 120 and 144 h after infestation; morphology of the single developmental stages was determined in histological sections of the duodenum, jejunum, ileum, caecum and colon. In addition to the posterior part of the small intestine, great numbers of 1st generation meronts of *E. colchici* were mainly seen in the intestinal crypts of the blind sacs. 72 h after infection numerous divisions of 2nd and 3rd generation meronts could be recognized. Numerous macro- and microgametocytes were seen in the epithelium of the blind sacs and on the peaks of the large intestinal crypts, thus indicating the peak of gametogony. The endogenous phase of *E. duodenalis* starts in the epithelial cells of the duodenum in the near proximity of duodenal villi. In short 24h intervals three generations of merogonies and on Day four after infection the first oocysts occur. In this study the localization of the endogenous developmental stages of *E. colchici* has been made more accurate and knowledge of the endogenous development of *E. duodenalis* confirmed. Studies are also being conducted into ultrastructure of the developmental stages of the above mentioned *Eimeria* species.



ON THE EPIDEMIOLOGY OF COCCIDIAN INFECTIONS (*ISOSPORA SUI*S, *CRYPTOSPORIDIUM PARVUM*) IN PIGS

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Carbofuchsin stained piglet faecal smears originating from two farms of various size and structure were checked for *Isospora suis* and *Cryptosporidium parvum*. In the large farm A *Isospora suis* occurred in piglets for the first time at Day seven. The highest prevalence was noted between Day 19 and 22. Piglets originating from older sows were infected more often than those from gilts. The highest prevalence of *I. suis* in the conventional farm B was established between Day seven and 10. *C. parvum* occurred more often between Days 34 and 39.



EPIZOOTOLOGY AND EXPERIMENTAL STUDIES OF GOAT SARCOCYSTOSIS IN ROMANIA

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Investigations were carried out on epizootology and life-cycle aspects through experimental infections, histology and electronmicroscopy of muscular cysts.

Gross lesions revealed that disease incidence varied between 11.1–16.6% in adults and it was sporadic in youngsters and lambs.

Transmission attempts by meat consumption were successful in dog but not in cats. The identified species was *S. capracanis* and its sporocysts were 14.5/11.2 μm big.

Histologically, a granulomatous myositis and cysts well delimited by a circular fibrous capsule were observed.

Electronmicroscopy showed an apical conoid which gives birth to rhoptrial channels. The anterior third presented lots of micronemae while the nucleus and several vesicles were seen in the posterior third.

Under the pelicular membrane microtubuli were present. The fundamental substance was amilopectin with ribosomal elements.



FAECAL EGG COUNTS BY A NEW METHOD USING TUBE FILTERS

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A new technique, consisting of the washing of a faecal sample through a set of three tube filters that fit into one another, has been developed for quantifying eggs of nematodes, trematodes and cestodes in a single process. Trematode eggs (washed through the inner, 110 μm filter and retained in the middle, 70 μm filter) and nematode and cestode eggs (retained in the outer, 25 μm filter), together with faecal particles of similar size, are drained into separate, previously calibrated containers. Granulated sugar is ladled only into the bottle containing the contents of the outer, 25 μm filter and both samples are made up to 60 ml with water. While nematode and cestode eggs are counted in a McMaster counting chamber, those of the trematodes are examined in a modified McMaster chamber, in which the eggs sediment onto a grid scored on the upper surface of the bottom slide. Faecal samples can each be processed and counts made in five minutes by the new method.



A SPECIES SPECIFIC RECOMBINANT ANTIGEN FOR THE RAPID DIAGNOSIS OF *Dictyocaulus viviparus* INFECTIONS IN CATTLE

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The *Dictyocaulus viviparus* gene fragment Dv3-14 codes for a species and adult stage specific, water soluble, excretory/secretory antigen. DNA sequence analysis revealed that Dv3-14 is a major sperm protein. Due to its high specificity and sensitivity in ELISA and Western blot, a dipstick immunoassay was developed using recombinant antigen labelled nitrocellulose strips. The test can be used with serum or EDTA blood. *D. viviparus* infections in cattle can be detected with >99% specificity and >99% sensitivity between Day 30 and 85 after experimental infection. Results can be obtained within 90 min after blood sampling.



ANTE-MORTEM DIAGNOSIS AND DRUG TREATMENT TRIAL FOR *TAENIA SAGINATA* CYSTICERCOSIS

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The drug trial presented here was carried out as part of a helminthology link project between the Centre for Tropical Veterinary Medicine, Scotland and the National Veterinary Research Centre (NVRC), Kenya. The trial was carried out on the NVRC estate. It was designed to evaluate the use of a monoclonal antibody based antigen capture ELISA assay for the detection of cattle naturally infected with *Taenia saginata*, to investigate the efficacy of treating such infections with praziquantel (Bayer) and to monitor the cattle serologically throughout the period of the trial. The study clearly demonstrated the efficacy of praziquantel and that *ante-mortem* diagnosis and drug treatment for this parasite under Kenyan conditions, is feasible. A logical extension to the work is to transfer the trial to the field situation.



MOLECULAR MARKERS FOR SPECIES IDENTIFICATION AND GENETIC VARIATION ANALYSIS OF FILARIAL WORMS AT VARIOUS STAGES IN THEIR LIFE CYCLES

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Characters selected for taxa (e.g. species) identification should meet three basic criteria: distinguishable inter-taxa variation, minimal intra-taxa variation, temporal and environmental stability. However, for the analysis of genetic variation within species and populations, characters showing a certain degree of intra-taxa variability should be studied. In order to standardize a molecular approach suitable for both species identification and populational studies on filarial worms, Random Amplified Polymorphic DNA (RAPD) fingerprinting followed by endonucleasic digestion with restriction enzymes was applied to individual specimens of *Dirofilaria immitis*, *Dirofilaria repens* and *Setaria labiatopapillosa*. Several parameters of the amplification reaction and different arbitrarily chosen oligonucleotides were tested to select primers and conditions yielding reproducible results starting from single microfilariae (L1). The stability of RAPD fingerprints was verified by analyzing individual adult worms and microfilariae of *D. immitis* recovered from the same domestic dog. The third stage larvae recovered from artificially infected mosquitoes were also analyzed. The patterns obtained were species-specific with a high degree of species differentiation and a very low amount of intra-species variation. Therefore, these patterns provide a molecular key for species identification. The high number of comigrating bands (15-30 for each primer) within each species provides an internal control to monitor the reliability of the amplification patterns. The identity of patterns obtained from both adult worms and larvae indicates that the procedure can be applied to different life cycle stages. Endonucleasic digestion of the amplified DNAs was applied to increase the number of scorable polymorphisms, providing a tool for the study of genetic variation within species and populations. Preliminary results obtained on individuals of *D. immitis* recovered from domestic dogs and red foxes (*Vulpes vulpes*) failed to demonstrate differences between the parasites collected from the two hosts.



DETECTION OF *CRYPTOSPORIDIUM PARVUM* USING THE POLYMERASE CHAIN REACTION

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Polymerase Chain Reaction primers and probes were designed using sequence data obtained from a *Cryptosporidium parvum*-specific clone originating from a genomic library. Sensitive and specific amplification of a 329 bp product was demonstrated by ethidium bromide staining and hybridisation of labelled probes. These reagents were then applied to faecal samples from humans, cattle and sheep which came from two different regions of the UK. Positive PCR results were obtained from both geographical regions and all species of host animal tested. Several samples were negative using conventional microscopic examinations, but positive PCR.



THE DEVELOPMENT OF SPECIFIC ELISAS FOR USE IN EPIDEMIOLOGICAL STUDIES OF BOVINE BABESIOSIS IN BRAZIL

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B *abesia bovis* and *B. bigemina*, the causative agents of bovine babesiosis in tropical areas where the tick vector (*Boophilus microplus*) occurs, are responsible for important economic losses in both milk and beef production in Brazil.

Following previous work on the identification of *B. bovis* and *B. bigemina* specific antigens using immunoblotting and immunoprecipitation of biosynthetically labelled parasite components (Passos, L.M.F. 1992 *Trans. R. Soc. Trop. Med. Hyg.* **87**, 122), the present study reports the purification of selected parasite proteins from acrylamide gels and their use in the development of antibody-detection ELISAs for these two protozoans to use in epidemiological studies in Brazil. Crude antigen extracts of each parasite were compared in ELISAs with one candidate purified protein from each parasite to test against a panel of calf sera experimentally raised to three geographically different isolates of *B. bovis* and three of *B. bigemina*. In addition, these ELISA antigens were tested against a panel of sera from cattle in Brazil, Malawi and Mozambique which had been infected naturally by ticks in the field.

The purified *B. bovis* protein detected specific antibodies and is a potential candidate to replace the crude parasite extract in immunodiagnostic assays. However the purified *B. bigemina* protein cross-reacted with *B. bovis* antisera and other candidate proteins will need to be assessed.



INTERNAL PARASITES IN DAIRY HEIFERS BORN IN AUTUMN IN BUENOS AIRES PROVINCE (ARGENTINA).

I. EFFECT ON BODY WEIGHT GAIN

II. EPIDEMIOLOGICAL STUDY

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This work was carried out between December 1991 and November 1992 in Tandil county. The production trial involved 50 Holando Argentino female calves born in autumn, approximately five to six months of age, allocated in three groups: Group 1: monthly anthelmintic treatment; Group 2: tactical anthelmintic treatment (March, June, August, October); Group 3: untreated control. The anthelmintic used was Fenbendazole at a dose rate of 5 mg/kg. The total body weight gains were: Group 1: 157.3 kg; Group 2: 149.5 kg; Group 3: 115.5 kg. The daily body weight gains, in 341 days of trial, were 0.461 kg, 0.438 kg and 0.339 kg for each group, respectively. Eggs per gram (Epg) counts of Group 3 reached a peak in June and decreased markedly until the end of the trial. The identified helminth genera by faecal cultures were: *Ostertagia*, *Cooperia*, *Haemonchus*, *Trichostrongylus* and *Oesophagosomum*.

In the epidemiological study, at the slaughter of tracer calves, which grazed the pasture successively for one month in order to determine the infectivity of the pasture, the higher adult worm burden was found in July. The principal genera were *Ostertagia* in January, in the period between April and July and in September; *Haemonchus* in February, March and July; and *Cooperia* largely in the period between December and March, and in July. *Ostertagia* showed inhibition mainly in spring (October and November). There were few *Dictyocaulus viviparus*, the high number of this genus was detected in August.



COMPARATIVE EFFECTS OF NEMATODE INFECTION ON *BOS TAURUS* AND *B. INDICUS* CROSSBRED CALVES GRAZING ON ARGENTINA'S WESTERN PAMPAS

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Breed differences in resistance or tolerance to naturally acquired gastrointestinal nematodes were compared between Aberdeen Angus AA and Santa Gertrudis SG weaned steer calves grazing in a temperate environment. Within the breeds, one half of the calves were untreated: AAU (n=18) and SGU (n=18) while the others were treated monthly with moxidectin or levamisole: AAT (n=14) and SGT (n=14). All calves grazed the same contaminated pasture from autumn to winter, when each group was separated to uninfected paddocks until the end of the study. Faecal egg counts, plasma pepsinogen levels (PPL), herbage larval counts and live weight (LW) were recorded monthly. Egg counts and PPL of AAU and SGU increased from autumn to early winter when calves showed heavy parasitism (mainly *Trichostrongylus axei*) and severely sick calves needed emergency treatment. On the clean paddocks parasitological parameters progressively decreased. Late winter egg counts were significantly ($P<0.05$) higher in SGU than in AAU. PPL of SGU were higher than AAU ones, but no significant differences were found. There were no breed differences in genera recovered on faecal culture. Numbers of severely sick and dead SGU calves (61%) were significantly ($P<0.01$) greater than those numbers of AAU calves (17.5%). Cumulative LW gains differences ($P<0.0001$) within SG breed were higher than those differences ($P<0.003$) within AA breed. LW gains of AAU during autumn-winter period were ($P<0.002$) greater than those of SGU. At the end of the study the LW gains of AAU, AAT, SGU and SGT were 139.8, 174.4, 72.3 and 182 kg respectively. The differential breed responses suggest that AA (*B. taurus*) may be more tolerant and resistant to nematode infections than SG (5/8 *B. indicus* crossbred).



MILK PRODUCTION AND LIVE WEIGHT VARIATION IN HEIFERS TREATED AGAINST GASTROINTESTINAL NEMATODES

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In this work undertaken from December 1989 to November 1990, we studied the effect of Albendazol treatment on milk production in heifers naturally infected with gastroenteric nematodes, during the 2nd year of grazing. At the second trimestre of pregnancy 15 heifers received a dose of 7.5 mg/kg b.w. of Albendazole (G-1), and the same number of animals remained untreated (G-2). Both groups of heifers grazed separately in similar plots, in rotation. In order to assess the milk production, we measured the individual production into the two daily milkings for three days a week. The data was grouped in three periods: at four weeks after delivery; from parturition to May, and from May to the dry period. Likewise, animals were weighed at parturition and monthly onwards, two consecutive days, and the average weight was calculated. These results were classified in four periods; weight at parturition; four weeks post-partum; from post-partum to May and from May to the dry period.

The daily production was similar in the treated and in the control heifers, being 12.1, 14.5 and 9.3 kg/cow in G-1, and 12.4, 15.7 and 9.3 in G-2, for each of the three periods considered, respectively. By means of variance analysis, no statistically significant differences were found in the milk production between the two batches. The daily variation of live weight did not show notable differences among the treated and the control animals, being in the four periods of study; 395.7, -495.8, 93 and 164.5 gr/cow in treated heifers and 392.5, -504.8, 54.9 and 142.6 in the controls. Using variance analysis statistically significant differences were not observed in relation to the weight. These results can be explained taking into account that the infection levels in heifers studied were not high, furthermore, treated animals went on grazing, and so were exposed to continuous reinfection.



THE PATHOGENESIS OF CRYPTOSPORIDIA IN CHICKENS, MICE AND CALVES

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Experiments were undertaken to investigate the effects of challenge dose on the pathogenesis and pathology associated with infections of *Cryptosporidium baileyi*, *C. muris* and *C. parvum* in chickens, mice and calves respectively.

Groups of parasite naive animals were infected with low (400), medium (20,000) or high (1,000,000) numbers of oocysts by the oral route. Infections were monitored by performing faecal oocyst counts and tissue smears/histopathological examinations of tissues on post mortem. The low dose rates were capable of initiating pathological lesions in all species. Clinical signs were only seen in calves infected with *C. parvum*, severity increasing with the size of challenge dose.

Histopathological findings and oocyst output data are provided for each of the species studied.



PATHOPHYSIOLOGICAL STUDIES ON *DICROCOELIUM* *DENDRITICUM* INFECTION IN SHEEP

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Dicrocoelium dendriticum, the lancet fluke found in the bile ducts, is considered to be an important parasite in sheep, goats and cattle in many countries in Europe and Asia. Infections can cause liver scarring, severe cholangitis and biliary fibrosis. Apart from this aspect little is known of the pathogenic effects of this parasite. Radioisotopic techniques have been widely used to elucidate the pathogenic effects of other liver and gastrointestinal parasites. In three experiments carried out in Greece, ^{51}Cr labelled red cells, ^{59}Fe -ferric citrate, ^{125}I -labelled albumin and $^3\text{H}_2\text{O}$ were used to compare red cell kinetics, plasma protein metabolism and total body water volumes in sheep with low to high, naturally acquired, *Dicrocoelium* burdens and in fluke-free controls. The results suggest that this parasite, in the range of infections found in the field, has no significant effect on either red cell kinetics, plasma protein metabolism or total body water volume.



COMPARATIVE HISTOPATHOLOGICAL STUDY OF HEPATIC CHANGES INDUCED BY *FASCIOLA* *HEPATICA*/*SCHISTOSOMA BOVIS* AND *SCHISTOSOMA* *BOVIS*/*FASCIOLA HEPATICA*

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The aim of this study was to investigate the long-term histopathological effects of *Fasciola hepatica* and *Schistosoma bovis* diachronic infections in lamb liver. Animals in Group I were primarily infected with 80 *F. hepatica* metacercariae administered by oesophageal probe and challenged after 10 weeks with a single percutaneous exposure to 400 *S. bovis* cercariae for 30 minutes using the leg immersion technique. Group II animals were exposed to 400 *S. bovis* cercariae and challenged six weeks later with 220 *F. hepatica* metacercariae. Necropsies were carried out 24 weeks after the last infection. Later on the macroscopic test, small pieces from the liver were processed for light and electron microscopy. Histologically the hepatic architecture was significantly altered in the two groups studied, though the lesions presented in Group I were more severe. Fibrosis affected portal tracts and was extended to connective septa and the parenchyma. The epithelial lining of smaller bile ducts was degenerated and the larger bile ducts had severe desquamation and even necrosis of the epithelium, glandular hyperplasia, intense infiltration of lymphocytes, plasma cells and eosinophils and thick concentric fibrous wall. Bile ductular proliferation was also observed. Hepatocytes were locally degenerated (feathery and acidophilic degeneration, lipid vacuoles). Near the dilated sinusoids, haemozoin were seen within Kupffer cells. A vessel reaction for the presence of eggs of *S. bovis* in branches of the portal vein were observed. There were thrombosis and a granulomatous inflammation in and around the vessels. Typical microgranulomas contained a central egg which was surrounded by histiocytes, giant cells, lymphocytes, eosinophils with a peripheral layer of fibroblasts and collagen fibers. Macrogranulomas with necrosis followed by calcification and two adult worms in the parenchyma were also seen. Ultrastructural analysis revealed abnormalities of the hepatocyte cytoplasm and nuclei; lysosomes were the predominant cell organelle. Perisinusoidal spaces were enlarged and Kupffer cells increased in number and are activated. According to the results, we suggested that the lambs should be a model for investigation into the interaction between schistosomiasis and fascioliasis infections in the pathogenesis of chronic liver disease.

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ELECTRONMICROSCOPIC AND HISTOPATHOLOGICAL STUDIES IN EXPERIMENTAL EIMERIOSIS IN LAMBS

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Eimeriosis in lambs was experimentally reproduced by using a mixture of eimerian species isolated in the western part of Romania.

Gross lesions consisted of either catharal, haemorrhagic-necrotic or necrotic-proliferative enteritis. This was associated with mesenteric serous lymphadenitis. Lymph-node medulla had white-grey zones.

Histologically, the intestinal epithelium was either present or mortified with the presence in the cytoplasm of enteric cells of either schizogonic (trophozoites, meronts) or gametogonic forms (macro-gametocyte, zygote) of *Eimeria*.

Lymph nodes showed lymph stasis and hyperplazia. In the cortex red particles (probably trophozoites) were noticed.

Electronmicroscopy revealed that besides a degeneration of enteric cells, schizonts or microgametes were present. The ultrastructure of eimerial schizogonic and gametogonic forms was established.



POTASSIUM METABOLISM IN *EIMERIA BOVIS* INFECTED CALVES

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Diarrhoea due to *Elmeria bovis* infections may affect mineral metabolism. This investigation was made to differentiate the influences of 1) developmental stages, 2) infection schedules and 3) challenge infection on K⁺ metabolism in the different parts of the gut.

A total of 16 male Black and White calves was used. After implantation of an ileocaecal re-entrant cannula, the animals were kept in metabolic cages and matched into four groups according to their bodyweight. Three groups were infected along different schedules with oocysts of *E. bovis*. Four individuals remained uninfected.

The total and post-ileal apparent digestibilities (AD) of K⁺ were reduced throughout patency in all animals after a single dose or split primary infection; the pre-caecal AD was only diminished in calves with split infection. The ADs were not or only modestly reduced in non-infected pair-fed individuals. None of the ADs was altered after challenge infection.

The changes in K⁺ metabolism occurred concurrently with the clinical symptoms during patency and were attributable to the 2nd schizonts, gametes and oocysts in the post-ileal part of the gut. The effects seemed to be more pronounced after split infections than after single dose infection. Challenge inoculation did not affect K⁺ metabolism.



MOLECULAR CLONING OF *EIMERIA TENELLA* ALPHA-TUBULIN cDNA

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E *imeria tenella* has recently captured considerable attention due to its devastating effects on the poultry industry. In the realm of molecular biology, most research has been aimed at identifying protective antigens. The house-keeping genes of this organism, on the other hand, remain largely unexplored.

Preliminary screening for house-keeping genes with heterologous DNA probes was unsuccessful, possibly owing to a bias in codon usage. To circumvent this difficulty, we decided to use antibodies to identify clones from an expression library. A monoclonal antibody directed against *Trypanosoma brucei* α -tubulin was selected because it not only bound to the sub-pellicular microtubules of *E. tenella* sporozoites and merozoites, but also recognised the denatured protein on Western blots. The same antibody was used to isolate cDNA clones from an *E. tenella* expression library and these clones were then used as hybridisation probes to isolate a full length clone containing the 5' and 3' flanking sequences. The entire gene has been sequenced and shown to be most closely related to *Toxoplasma gondii* α -tubulin (97.8% identity at the polypeptide level). In contrast the 5' and 3' untranslated regions show little similarity to those of other organisms. We are currently attempting to clone the α -tubulin promoter with a view to establishing a transfection system for *Eimeria*.



ABSTRACTS OF

OPEN SESSION

ON

COCCIDIOSIS

Organised by the COST 89 Group





***EIMERIA ALABAMENSIS* COCCIDIOSIS IN YOUNG GRAZING CATTLE IN SWEDEN**

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E *imeria alabamensis* is distributed worldwide and has been shown to be pathogenic in experimental infections, but natural coccidiosis due to this species has been reported only from Germany. However, the parasite has recently been identified as a cause of diarrhoea in young grazing cattle in Sweden. Typically, a watery diarrhoea develops four to six days after the animals are first turned out on to pastures grazed in previous seasons by calves. Their appetite declines and they may lose up to 8% of their bodyweight within three weeks. Severely affected animals become reluctant to rise and may die. Eight to ten days after turn-out oocysts in numbers of 800 000 to 15 million/g faeces are being excreted. Decreases in the activities of alkaline phosphatase and glutamate dehydrogenase, and decreases in the concentrations of bile acids in serum have been recorded in affected animals. Overwintered sporulated oocysts on the pasture have been shown to be the source of infection. Delaying turn-out had no sufficient preventive effect. A single oral dose of 20 mg toltrazuril/kg bodyweight did not prevent coccidiosis in calves infected experimentally with 150 million oocysts daily for three days.



INFECTION RISKS IN BOVINE EIMERIOSIS

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Repeated investigations of approximately 500 cattle over extended periods (up to one year) revealed some factors of risk of infection: *Elmeria* infections of calves are not acquired prenatally; they originate exclusively from the environment of the neonate. A rise in the oocyst output of *Elmeria bovis* in cows in the periparturient phase (periparturient oocyst rise - POR) contributes to the infection risk of the young calf. Significant increases in the prevalence and the intensity of oocyst output of virtually all *Elmeria* species was observed within four weeks after assembling individually kept calves into small groups at an age of approximately one month. A second regrouping of calves two months later was not an essential risk factor as the oocyst output of the majority of *Elmeria* species remained unchanged. However, after turnout on pasture the oocyst output of at least *E. alabamensis* and *E. zuerni* increased substantially. Housing after grazing on pasture during the summer did not seem to be a risk factor for the yearlings in the present investigations. These results suggest that prophylactic measures should be envisaged at the time of delivery, at first assembling of calves into loose groups and at turnout on pasture.



ABSORPTION OF VITAMIN A IN *EIMERIA SEPARATA* INFECTED RATS

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The effects of *E. separata* on vitamin A (^3H -retinol) absorption from the intestine were determined in vivo. Experiments were performed in Lewis rats 2, 4 or 7 days after infection (a.i.) with 5,000 oocysts and in non-infected pair-fed as well as non-infected, ad libitum fed animals in order to discriminate between effects due to inappetence or to parasite. Disappearance from the gut lumen (D), retention in gut tissue (R) and transmural transport (T) were measured. Caecum and colon of non-infected rats were capable of absorbing vitamin A. *E. separata* caused malabsorption particularly in these sites on Day 4 a.i. This seems to be due to destruction of enterocytes by replicating parasites. R as well as T were highly affected reducing absorption by almost 50%. Despite the absence of parasites in the ileum, D decreased in this location with time of infection as a consequence of reduced T. Diminished food intake, due to the infection, had a positive effect on vitamin A retention in the intestinal tissue.



***Isospora suis* INFECTIONS IN PIGLETS: OOCYST SHEDDING FOLLOWING LOW LEVEL DOSING**

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Experimental infections with *Isospora suis* in piglets have been reported from several countries. So far, however, patterns of oocyst shedding induced by such infections have been studied only in piglets inoculated with > 10.000 oocysts per animal.

In a study in Denmark during the period 1988-1991, 47 coccidia free piglets were inoculated with single doses of sporulated oocysts (Christensen 1992). Seven of the piglets, aged four to eight days, were dosed with as few as 100 oocysts. The subsequent excretion of oocysts among those piglets was registered during the following three to four weeks, applying a modified McMaster technique (Henriksen & Christensen 1992).

Considering the OPG-figures recorded, we should like to point out the following observations:

1. The ultimate OPG-level was at the 10^5 level.
2. The shedding of oocysts followed a cyclic pattern, which was characterised by a periodicity of five days, including three peaks often separated by subpatent periods.

Based on the reported observations, it seems reasonable to conclude that experimental infections of piglets with single doses of 100 sporulated oocysts of *I. suis* will result in:

- shedding of oocysts at a high level, in less than two to three weeks,
- a cyclic excretion of oocysts characterised by a conspicuous regularity. This pattern may support the hypothesis of the existence of some extraintestinal developmental stages of *I. suis*, as it has been postulated previously by Harlemann & Meyer (1984).



CHARACTERIZATION AND LOCALIZATION OF RHOPTRY ANTIGENS IN *EIMERIA*

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Sporozoites and merozoites of *Coccidia* (*Eimeria*, *Toxoplasma*, *Sarcocystis*) are intracellular parasites of various tissues and cells of vertebrates. Secretory organelles (rhoptries, micronemes, dense granules), located in the apical region of the invasive stages, are involved in recognition, invasion and adaptation to the host cell. We have studied the fate of rhoptry proteins during host cell invasion and schizogony in *Eimeria tenella* (parasite of *Gallus domesticus*) and *Eimeria nieschulzi* (parasite of *Rattus norvegicus*) with monoclonal antibodies (McAb).

McAb A4C6 was raised against fractions of *Eimeria tenella* sporozoites. The McAb reacted on western blots with several polypeptides of approximately 40/45/60/99 kDa relative molecular weight. Antigens recognised by this McAb were localised on rhoptries and granules and detected by means of IFAT and immunoelectron microscopy. Redistribution of rhoptry antigens has been examined in primary chicken kidney cells between 15 minutes and 120 hours post infection in all schizogonic generations. Apical contact of sporozoites seemed to trigger the secretion of the contents of rhoptries forming the parasitophorous vacuole.

McAb D12A4 was raised against *Eimeria nieschulzi* third generation merozoites. On western blots a polypeptide of about 20 kDa was identified. The fate of this rhoptry protein was followed by IFAT during in vitro development of the parasite in a rat enterocyte cell line. The protein was first detected in immature first generation schizonts as multiple dots distributed in the undivided cytoplasm. The labeled structures were then integrated apically into budding merozoites and elongated during merozoite differentiation. Two rhoptry organelles were usually observed within each merozoite. After reinvasion of first generation merozoites into host cells, the 20 kDa protein could be localised as an intense patch within the vacuolar space and a fainter outline surrounding the merozoite. Development did not proceed beyond mature second generation merozoites.

The comparative study of rhoptry proteins shows that stage specific and stage cross reactive rhoptry proteins exist in *Eimeria* and that rhoptry proteins are secreted into the parasitophorous vacuole at the time of invasion.



AMPLIFICATION AND CHARACTERISATION OF *EIMERIA BOVIS* DNA FRAGMENTS

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Previously we described the isolation of a cDNA clone and a genomic clone derived from first generation merozoites of *Eimeria bovis* (1,2). The size of these clones was approximately 230 bp and 175 bp, respectively.

Sequencing of these clones by dideoxy chain termination revealed that they contained repetitive elements. Oligonucleotides homologous to nonrepetitive regions of these clones were synthesised and were used as primers for the polymerase chain reaction with genomic *E. bovis* DNA as a template. The resulting DNA fragments up to 2000 bp in size were isolated and cloned into the plasmid pUC19.

Results of restriction fragment analysis, sequencing and homology research will be presented.

1) Homrighausen, C. *et al.*, 2nd Conf. COST-Action 89, Münchenwiler, April 1989.

2) Barbisch, B. *et al.*, VIth International Coccidiosis Conference, Guelph, June 1993.



SARCOCYSTIS MURIS: CLONING OF A cDNA ENCODING A REPETITIVE ARGININE RICH REGION OF A PUTATIVE MICRONEME ANTIGEN

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Micronemes are organelles in the apical cell region of members of the phylum Apicomplexa and play an important role in the invasion of host cells. As described in previous studies, a mAb directed against a *S. muris* microneme antigen (>70 kDa) was used to isolate cDNA clones from an expression library.

DNA sequencing of a 1.85 kb cDNA insert (SL1.2) revealed an ORF encoding a protein of 229 aminoacids. The predicted size is 25.8 kDa. The polypeptide consists of a tandemly repeated decamer and is very rich in Arg, Thr, Ser, and Gly. It is predominantly hydrophilic. When searching the EMBL data base we found homologies to Arg- and Thr-rich proteins.

Fusion proteins derived from the recombinant pSL1.2 showed retarded electro-phoretic mobility in SDS-PAGE. This might be attributable to the unusual amino acid composition.

Southern blot analysis suggests that the gene coding for this microneme protein exists only as a single copy in the genome of *S. muris*.

In Northern blot analysis a single mRNA species of approximately 10 kb was detected indicating that the molecular weight of the putative translation product is higher than 250 kDa.

This is the first report on a repetitive protein of *Sarcocystis*. Its localisation in the micronemes and function, however, will have to be verified by further experimenting.



MOLECULAR CHARACTERISATION OF A DENSE GRANULE ANTIGEN (32 kDa) FROM *SARCOCYSTIS* *MURIS* CYSTOZOITES

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The dense granules are characteristic organelles of the Apicomplexa and play an important role in host-parasite interactions. In *Sarcocystis* and related species, the contents of the dense granules is exocytosed into the lumen of the developing parasitophorous vacuole. In the present study we used a monoclonal antibody (2f4) previously characterised as directed against a *S. muris* dense granule antigen with a molecular weight of 32 kDa. There are indications that this protein might be a substrate for a cysteine-protease which is also located in the dense granules. After an immunoscreening of a I-ZAP cDNA expression library, we isolated four positively reacting clones of 0,8-1,5 kb in length (BF1-BF4). A fusion protein derived from bacteria harbouring the recombinant plasmid BF4 (1,4 kb) reacted with mAb 2f4. This shows that the cDNA insert is very likely to encode the dense granule antigen.

We hope to get more information on the dense granule protein by sequencing the cDNA clone BF-4, which probably comprises the whole nucleotide sequence, and by subsequent data base searching.



CLONING OF GENE FRAGMENTS ENCODING ANTIGENIC POLYPEPTIDES OF *SARCOCYSTIS TENELLA*

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Current methods to diagnose infections with *Sarcocystis tenella* in sheep require refinement because crude antigen preparations from *S. tenella* cross-react with the non-pathogenic species *S. gigantea*. In addition, the preparation of antigen is complicated by the fact that *S. tenella* is obligatorily intracellular and, hence, antigen preparations are usually contaminated with host tissue. To improve diagnostic methods for sarcocystioses in sheep, we attempt to develop recombinant antigens that can be used to standardise serological tests. A cDNA-library was constructed in λ gt11 from cystozoite-derived poly(A)⁺ RNA of *S. tenella*, and immunoscreened with polyclonal sera; 23 positive clones were identified. In order to exclude identical clones from further study, amplification of DNA by polymerase chain reaction (PCR) using λ gt11 primers was performed on 10 of the positive clones. Four different sized inserts in the range of 170-700 bp were obtained. Single-stranded DNA fragments were generated by asymmetric PCR from eight positive clones, sequenced and classed into five groups by DNA homology. We are currently subcloning a representative of each group into the vector pGEX-3X in order to express them as glutathione S-transferase fusion polypeptides which are already proven to be useful for application in enzyme-linked immunosorbent assays (ELISAs) for a range of other parasites. One fusion polypeptide, termed STC-29, is currently being tested for suitability as a diagnostic antigen in an ELISA for diagnosis of *S. tenella* infections in sheep.



HOW DOES *IN VITRO* EXCYSTATION AFFECT MEMBRANES OF *SARCOCYSTIS* SPOROZOITES? A TEM STUDY

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Sporocysts of *Sarcocystis capracanis* were subjected to *in vitro* excystation without any or following different pretreatments: formic acid, NaOC1 w/o or combined with ultrasonication. Excystation rates and viability of sporozoites were determined and the ultrastructures of sporozoite membranes were studied by transmission electron microscopy. After NaOC1-pretreatment of sporocysts the excysted sporozoites had conserved integrity of their surface membranes. Intracellular features were intact. Formic acid and sonication pretreatment resulted in superficial damage of membranes which was also noticed after combined NaOC1-sonication use. However, sporozoites excysted by any of the above mentioned methods proved to be infective for their appropriate intermediate host, the goat.

Purification of sporozoites by passage through a modified DE 52 anion exchange column had no deleterious effect on surface membranes. It can be concluded that any of the tested procedures for *in vitro* excystation may be applied to obtain large amounts of pure and infectious sporozoites. To avoid alteration of membranes, sporocysts should not be sonicated if sporozoites are used for immunological/membrane protein studies.



ASSESSMENT OF ANTIBODIES TO RECOMBINANT GLUTATHIONE S-TRANSFERASE IN SERA OF SHEEP INFECTED WITH CYST-FORMING COCCIDIA

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Recently, a range of recombinant parasite polypeptides fused with glutathione S-transferase (GST) were found to be suitable antigens for use in enzyme-linked immunosorbent assays (ELISAs). However, a disadvantage of the use of GST fusion polypeptides in ELISAs may be the high prevalence of antibodies directed to GST in some parasite hosts, such as humans and cats. Because we intend to express a range of recombinant polypeptides of *Sarcocystis tenella* and *Toxoplasma gondii* as GST fusion polypeptides for use in ELISAs to detect infections with these parasites in sheep, we examined the prevalence of antibodies directed to recombinant GST in experimentally infected sheep. Antibody titers to GST in *T. gondii*-infected sheep were always low ($\leq 1/10$) and did not increase after infection. By contrast, antibody titers to GST in *S. tenella*-infected sheep increased from four weeks after infection onwards and remained elevated ($1/80$) up to the end of examination (14 weeks after infection). Antibody titers to GST were lower when ELISA plates were coated with less than $0.1 \mu\text{g}/\text{well}$ of GST or when sera were absorbed with GST before use in ELISA. The results of this study suggest that the high level of antibodies to GST in *S. tenella*-infected sheep may mask reactions with the *S. tenella* part of GST fusion polypeptides if antibodies directed to this polypeptide are not highly abundant or of low avidity. Therefore, the use of GST fusion polypeptides in diagnostic ELISAs for *S. tenella* should be restricted to target antigens that are highly antigenic and have high affinities for their corresponding antibodies, or cleavage of the GST fusion partner should be considered.



***EIMERIA MAXIMA*: ELISA AND WESTERN BLOT ANALYSES OF PROTECTIVE SERA**

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Infection of chickens with *Eimeria maxima* induces the production of parasite-specific antiserum which can be used, by passive immunisation, to protect naive chickens against infection. Similarly, intramuscular injection of hens with soybean lectin affinity purified gametocyte antigens of *E. maxima* in Freund's Complete Adjuvant induces production of antibodies which are maternally transferred and thereby protect hatchlings against *E. maxima*. ELISA analyses of serum pools having varying protective capacities revealed good correlation between passive protection and levels of anti-unsporulated oocysts, anti-sporulated oocyst, anti-merozoite and anti-gametocyte antibodies. Western blotting demonstrated that the sera mainly recognised a number of high molecular weight antigens in all developmental stages and that the intensity of the reactions reflected the degree of protection induced by the sera. Sera from birds immunised with gametocyte antigens also recognised high molecular weight antigens from all the developmental stages, with banding patterns remarkably similar to those observed for sera from infected birds. Taken together, these results demonstrate that antibodies can protect against infection with *E. maxima* and that these protective antisera recognise very similar antigens from both the asexual stages of the parasite.



SEROLOGICAL RESPONSE AGAINST POULTRY COCCIDIA, EVALUATED BY ELISA AND IFAT

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Seven day old chickens were immunized with doses of either 500 or 5000 oocysts of attenuated lines of *Eimeria tenella*, *E. acervulina* and *E. maxima* (LIVACOX T). The oocysts were administered via drinking water or orally. Sera from infected chickens were collected between five and 41 days after immunization (DAI), and tested by IFAT and ELISA. Sporozoites of *E. tenella* were used as antigen for IFAT and saline extract of broken sporulated oocysts of *E. tenella*, *E. acervulina* and *E. maxima* for ELISA.

Low levels of antibodies were detected in sera collected five DAI examined by IFAT. The highest titres were found 14 DAI (1:1280). Titres between 1:300 and 1:400 (average of 20 sera) were still observed 41 DAI. No marked differences in serological response were found after the various immunizations by ELISA. The highest level of IgG antibodies was found five DAI. These antibodies were possibly of maternal origin. Many cross reactions were observed between species of *Eimeria* in ELISA. This indicates the existence of common antigens in oocysts and sporocysts of different species. Soluble oocyst antigen is not suitable for determination of immunological response against various species of poultry *Eimeria* in ELISA.



INTRAEPITHELIAL LYMPHOCYTES OF THE SMALL INTESTINE IN LAMBS INFECTED WITH *EIMERIA* SPP.

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We have previously shown that infection with *Elmeria* spp. in lambs is associated with a significant increase in the area occupied by cytotoxic/suppressor T cells (CD8+) in the villous epithelium as measured by image analysis. Further studies showed that the epithelium containing parasitised cells had a significantly smaller area occupied by $\gamma\delta$ + T cells than epithelium containing cells without parasites. Double- and triple immunofluorescence were used to examine co-expression of different surface molecules on intraepithelial lymphocytes (IEL) of the small intestine. The IEL population in infected and uninfected lambs showed a similar expression of surface molecules. The majority of IEL of the villous epithelium in both groups was represented by CD3+, CD8+ T cells. Intraepithelial $\gamma\delta$ + T cells were almost always negative for CD8 both in infected and control lambs, whereas a proportion of lamina propria $\gamma\delta$ + T cells were CD8+. Intraepithelial T cells of the villous epithelium did not express MHC class II molecules, indicating that these cells are non-activated T cells. Absence of $\gamma\delta$ + T cells in areas of infected epithelium may reflect elimination of intraepithelial lymphocytes *in situ* in the epithelium or emigration of lymphocytes from infected epithelium.



THE EFFECT OF CHOSEN IMMUNOSTIMULATORS ON THE IMMUNITY OF THE CHICKENS VACCINATED WITH COCCIVAC

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The aim of the studies was to determine the effect of chosen immunostimulators - Tolpa's preparation (peat derived), selenium and combination of selenium with vit. C (Selcewet) on the immunity of chickens after the application of Coccivac.

The experiment was carried out on 180 Astra S chickens kept in the floor pen system. The studies comprised humoral and cell-mediated immunity and birds' sensitivity to control infection with invasive coccidia oocysts. Histologic and histochemical examinations of spleen as well as histologic examinations of bursa Fabricius and thymus were included in the experiment. During the rearing period the chickens were observed clinically, and body weight gain and feed consumption were recorded.

The results obtained showed a positive effect of applied preparations on the chickens post-vaccination immunity against coccidiosis. It was expressed as less intense lesion scores in the alimentary tract and lower production of oocysts in the chickens infected with invasive oocysts of *Elmeria* sp.

Tolpa's preparation affected favourably higher cell-mediated immunity increasing both phagocytic activity of neutrophils and proliferatic capability of mitogen-stimulated lymphocytes.

Selenium in combination with vitamin C resulted in increased neutrophil phagocytosis. Whereas selenium itself improved proliferatic capability of mitogen-stimulated lymphocytes and higher concentration of IgG in the serum of immunised chickens.



**ABSTRACTS OF
SUBMITTED PAPERS**

SPECIAL SESSION

Sponsored by Hoechst Animal Health

**New Formulations for the
Treatment of Helminths in Cattle**



PHARMACEUTICAL DEVELOPMENT AND QUALITY CONTROL OF THE PANACUR SR BOLUS

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Galenic studies have been undertaken in order to develop a sustained-release dosage form (bolus) for fenbendazole. Its intended purpose is to deliver the drug at a controlled rate of about 0.2-0.3 mg/kg bodyweight/day, for at least 130 days.

Utilising a process of continuous galvanic erosion of bolus components which exhibit different electrochemical potentials, such a device has been developed and this study describes some of the features of its design which are critical to performance. Emanating from the study are data which demonstrate correlation between *in vitro* and *in vivo* results. This can be used as a measure of efficacy and as a quality control tool. Other critical design features have been found to include the effects of compressional pressure and moisture content on the quality of bolus components during manufacture. These design features have led directly to quality control specifications which are performance related.

In conclusion, the unique concept of drug release in this bolus by galvanic action has had to be carefully validated in order to reap the clinical benefits of consistent, long-term performance and proven efficacy.



PHARMACOLOGICAL PROPERTIES OF THE PANACUR SR BOLUS, A DEVICE FOR SUSTAINED RELEASE OF FENBENDAZOLE IN THE RETICULORUMEN OF CATTLE

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Various studies indicate that at least 0.2 mg fenbendazole per animal per kg b.w. are required for therapeutic and prophylactic effect in long term treatment. The development of a slow release system, which meets this requirement, was accompanied by a number of controlled efficacy trials.

The efficacy of the final slow release device versus gastrointestinal and lung nematodes and the uniformity of pay out was investigated in more than 100 cattle, including rumen fistulated animals. 80 mg fenbendazole/animal/day on average (range 67–103) was released for at least 130 days resulting in elimination of existing worm burdens and prevention of new worm burdens after several artificial reinfections with *Ostertagia*, *Trichostrongylus*, *Cooperia*, *Oesophagostomum* and *Dictyocaulus*.

The investigation of serum kinetics over 25 weeks resulted in relatively low concentrations. While fenbendazole and fenbendazole-sulphoxide showed only occasionally measurable levels, fenbendazole-sulphone was detectable in almost all samples collected over 21 weeks.

The Panacur SR Bolus has been tolerated well. The examination of carcass and meat quality of bulls after first pasture season at slaughter 170 days after bolus application did not reveal any adverse effects. Because of its pharmacological properties the Panacur SR Bolus containing fenbendazole is capable of protecting cattle from infections with gastrointestinal nematodes and lungworms for a prolonged period.



THE SAFETY PROFILE OF FENBENDAZOLE AND THE PANACUR SR BOLUS

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The Panacur SR Bolus releases continuously over a period of several months, the benzimidazole anthelmintic, fenbendazole (FBZ). FBZ has low acute toxicity in a variety of animal species after oral, subcutaneous and intraperitoneal administration. However, in repeated dose studies, species differences occur; for example, rats tolerated dose rates of up to 1000 mg/kg/day for 90 days with no treatment related effects whereas the no-observed effect level in a six month study in beagle dogs was 4 mg/kg/day. FBZ has no sensitising properties in guinea pigs nor is it locally irritating to the eyes of rabbits. Studies in several species (rat, dog, horse, cattle, pig, sheep) showed FBZ to be free from embryotoxic or teratogenic effects. Embryotoxic but not teratogenic effects were observed in rabbits because of maternal toxicity in the high dose group (63 mg/kg). In-vitro and in-vivo mutagenicity tests (Ames-Test, Mouse Lymphoma Forward Mutation Assay, UDS-Test, Cytogenetics in vivo, Micronucleus-Test) demonstrated that FBZ had no genotoxic potential. Long term studies in mice and rats confirmed that FBZ was not carcinogenic. Based on the full toxicological profile, the acceptable daily intake (ADI) for FBZ for man is 5 µg/kg. The Panacur SR Bolus did not cause any adverse effects in treated cattle. The administration of 3 boli each to heifers had no adverse effects on fertility and the calves born to these heifers showed no abnormalities.



RESIDUES IN TISSUES AFTER ADMINISTRATION OF THE PANACUR SR BOLUS

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There is growing concern on the part of the consumer regarding drug residues in meat. This has prompted the introduction of maximum residue limits (MRLs) in tissues. Provisional MRLs have been determined for fenbendazole (FBZ) and its metabolites. For the calculation of these MRLs it is the combined concentration of FBZ and its metabolites oxfendazole (OFZ) and fenbendazolesulfone (FBASO₂) in tissues which is considered. The provisional MRLs in the UK are 10 ng/g in muscle, kidney and fat and 1,000 ng/g in liver. An analytical method was validated involving extraction of FBZ and its metabolites from tissues, oxidation of the material to convert the drug to the sulfone metabolites, separation using HPLC and detection and quantitation using fluorescent detection. The limit of quantitation for this method was validated as 5 ng/g of combined residue converted to FBZSO₂ in kidney, liver, muscle and fat. For the calculation of a withdrawal period after administration of the Panacur SR Bolus, tissues were examined at intervals after bolus administration. FBZ and its metabolites accumulated in the liver but concentrations were below the MRL by Day 162 or Day 179 in two separate trials. In all other tissues concentrations of FBZ and its metabolites were low. The provisional MRLs for these tissues are very low and residues fell below these concentrations in kidney, muscle and fat by Day 178, Day 199 and Day 199, respectively. The withdrawal period for the Panacur SR Bolus should be around 200 days. The bolus will be administered to cattle during their first grazing season when they are of insufficient weight for meat production and therefore the long withdrawal period should be of little practical significance.



THE PANACUR SR BOLUS IN THE CONTROL OF TRICHOSTRONGYLE AND LUNGWORM INFECTIONS IN GRAZING CATTLE

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More than 25 controlled trials were conducted in first season cattle in seven European countries to investigate the efficacy of the fenbendazole slow release bolus (Panacur SR Bolus, Hoechst) in the control of trichostrongyle and lungworm infections. In the majority of these, similar numbers of bolus-medicated and control cattle were maintained on separate pastures. The anthelmintic efficacy of the bolus was assessed by monitoring faecal egg and larval output, pasture larval counts, blood pepsinogen levels and worm counts at necropsy. The incidence of parasitic disease was also recorded in addition to regular monitoring of liveweight gain. Administration of the Panacur SR Bolus to all cattle of a herd at turnout resulted in a substantial reduction in trichostrongyle egg output for at least four months followed by a diminished pasture infestation with trichostrongyle larvae in the late summer and autumn. The bolus treatment prevented parasitic gastroenteritis throughout the grazing season and winter ostertagiosis during the subsequent winter. Lungworm infections and the faecal excretion of lungworm larvae were also largely suppressed during the first months and severe outbreaks of clinical lungworm disease during the whole grazing season were prevented.



**ABSTRACTS OF
SUBMITTED PAPERS**

SPECIAL SESSION

Sponsored by Pfizer Animal Health Group

**New Developments in the Control of
Parasites of Domestic Animals**



DISCOVERY AND PRE-CLINICAL BIOLOGY OF THE NOVEL ENDECTOCIDE, DORAMECTIN

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Doramectin is a novel, highly potent avermectin prepared by mutational biosynthesis. Following early indications of its antiparasitic spectrum and potency in a range of *in vitro* assays, doramectin was profiled *in vivo* using rats infected with the nematode, *Trichostrongylus colubriformis* and rabbits infested with ear mites, *Psoroptes cuniculi*. In each case the compound was highly effective and compared favourably to dilhydroavermectin B1a.

In initial cattle studies utilising an experimental micelle formulation, doramectin proved extremely efficacious against infections with *Ostertagia ostertagi*, *Cooperia oncophora* and *Dictyocaulus viviparus* following subcutaneous administration at 0.2 mg/kg. This experimental vehicle was also used to provide preliminary information on persistent activity of the compound against a variety of gastro-intestinal nematodes in cattle.

Optimisation of an injectable formulation was achieved by evaluating efficacy and pharmacokinetics of doramectin following administration of the compound to cattle in various oil-based formulations. Sesame oil with added ethyl oleate was the best parenteral vehicle tested, allowing the expression of good therapeutic and persistent efficacy combined with excellent injection site toleration.



THE EFFICACY OF DORAMECTIN IN THE THERAPY AND PREVENTION OF NEMATODE INFECTIONS OF CATTLE

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A series of 28 controlled anthelmintic studies involving 634 cattle was conducted throughout North America and Europe to evaluate the therapeutic efficacy of doramectin at a dosage of 200 $\mu\text{g}/\text{kg}$ against a broad range of gastrointestinal parasitic nematode species and against lungworms in naturally and experimentally infected animals. A further three studies were conducted using experimental infections to determine the duration of activity of doramectin at the same dosage in the prevention of infection of calves with *Ostertagia ostertagi*, *Cooperia oncophora* or *Dictyocaulus viviparus*. In the therapy studies, doramectin was >99% effective ($P < 0.0002$) in eliminating the immature and adult stages of 14 species of nematodes including *Ostertagia* spp., *Cooperia* spp. and *Dictyocaulus viviparus*. Efficacies against adult and L_4 stages of *N. helvetianus*, the dose limiting species, were 73.3 and 75.5%, respectively. In the studies designed to evaluate efficacy in the prevention of infection, accumulated burdens of *C. oncophora* in doramectin-treated cattle resulting from a daily challenge of infective larvae for 14 days following treatment were reduced by 99.2% in comparison with those of untreated control animals. Accumulated burdens were reduced by 90.7% after a 21 day challenge. For *D. viviparus*, burdens were reduced by 100% and 99.9% after a 21 or a 28 day challenge, respectively. The corresponding values for *O. ostertagi* were 99.9% after a 21 day challenge and 93.7% after a 28 day challenge.



THE SPECTRUM OF ACTIVITY OF DORAMECTIN AGAINST NEMATODE AND ARTHROPOD PARASITES OF SWINE

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The efficacy of doramectin against nematodes and ectoparasites of swine was assessed in a programme of 32 studies conducted across North America and Europe. 22 studies tested efficacy against immature and adult stages of the following nematode species: *Ascaris suum*, *Hyostrongylus rubidus*, *Metastrongylus* spp., *Oesophagostomum dentatum*, *O. quadrispinulatum*, *Strongyloides ransomi*, *Trichuris suis* and *Stephanurus dentatus*. Efficacy against *Sarcoptes scabiei* and *Haematopinus suis* was determined in four and six studies, respectively. In each study, animals were randomly assigned to a control or a treated group. The control group received no medication while each animal in the treated group was given an intramuscular injection of doramectin at a dosage of 300 µg/kg. Worm burdens were determined for each animal at slaughter 14 to 15 days after treatment. Lice and mites were counted immediately before treatment and then at weekly intervals until four weeks post-treatment. The efficacy of doramectin against all the nematode species tested was 98% or greater except for *T. suis* which was 87% for adults and 79% for immature stages. Efficacy against *S. scabiei* and *H. suis* was 98% and 100%, respectively.



THE EFFICACY OF DORAMECTIN AGAINST TEMPERATE CLIMATE ARTHROPODS OF CATTLE

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Forty-three studies involving more than 800 treated cattle across North America and Europe were conducted to evaluate the efficacy of doramectin against lice, mites or *Hypoderma* spp. In 16 studies, the efficacy of doramectin was evaluated against one or more of the following louse species: *Damalinia bovis*, *Haematopinus eurysternus*, *Linognathus vituli* and *Solenopotes capillatus*. Nine studies were conducted against *Psoroptes bovis* and two against *Sarcoptes scabiei*. Efficacy against first instar *Hypoderma lineatum* or *H. bovis* was determined in a total of 16 studies. In each study, animals were randomly assigned to a control or a treated group. The control group received no medication while each animal in the treated group was given a subcutaneous injection of doramectin at a dosage of 200 µg/kg. Lice and mites were counted immediately before treatment and then at predetermined intervals throughout the study. Efficacy against first instar *Hypoderma* was determined by counting numbers of warbles appearing in the backs of cattle. Doramectin was 100% efficacious against *H. eurysternus*, *L. vituli*, *S. capillatus*, *P. bovis*, *S. scabiei* and first instar *H. lineatum* and *H. bovis*. Doramectin reduced infestations of *D. bovis* by a mean of 28%.



EFFICACY OF DORAMECTIN AGAINST *BOOPHILUS* *MICROPLUS* IN CATTLE

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Efficacy studies were conducted in Brazil to evaluate doramectin at a dose rate of 200 µg/kg against induced infestations of the single host tick, *Boophilus microplus*. In the first study, 12 calves were each infested three times a week with recently hatched larvae, for four weeks before treatment. Six calves were treated with doramectin and six were untreated. From Day -3 to Day 21 post-treatment, individual collections of engorged female ticks that detached were made from each calf. In the second study, 12 calves were allocated to two groups of six animals. Six calves were treated with doramectin and six were untreated. From Day 1 to Day 17 post-treatment, each animal was infested three times a week with recently hatched *B. microplus* larvae. Post-treatment, daily collections of detached engorged female ticks were made from each animal. In the first study, reduction of collected engorged female ticks was 99% or higher after three days post-treatment. For the first six days after treatment, only a few detached engorged ticks were collected from treated animals, and their oviposition and hatchability declined rapidly. In the second study, doramectin treatment was highly efficacious in preventing the establishment of *B. microplus* populations for 20 days after the first ticks completed their cycle in the untreated group. The oviposition and hatchability of the few ticks that completed their life cycle in the doramectin group were severely reduced.



THE ACTIVITY OF DORAMECTIN AGAINST *COCHLIOMYIA HOMINIVORAX* IN CATTLE

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Studies were conducted in Brazil to evaluate doramectin at a dose rate of 200 $\mu\text{g/kg}$ against induced infestations of the New-World screwworm, *C. hominivorax*. In each of two efficacy experiments, six calves were treated with doramectin and six were left untreated. All animals received two incisions, one in the shoulder and one in the rump, and each incision was infested with 60 first instar larvae. In a single study to establish the duration of protection afforded by doramectin, 24 calves were allocated to six groups (T1 to T6) of four animals. Three groups were treated with doramectin and three paired groups were left untreated. All animals received four incisions, one in the shoulder and one in the rump of each side. Each incision received 30 first instar larvae of *C. hominivorax* with the following schedule: T1 and T2 at Day 3, T3 and T4 at Day 7, and T5 and T6 at Day 14 post-treatment. Calves were observed daily and incisions evaluated for eight days post-infestation (p.i.). Doramectin was 100% effective in preventing screwworm infestation by field isolates of *C. hominivorax* in all three experiments. In the doramectin-treated calves, infesting larvae were eliminated within the first 48 hours p.i. and no third instar larvae were recovered at any time. Healing started at 24 hours and was completed at 96–120 hours p.i. Severe screwworm strikes developed on all untreated calves, and healing did not begin until all larvae had completed their life cycle and exited as third instar larvae.