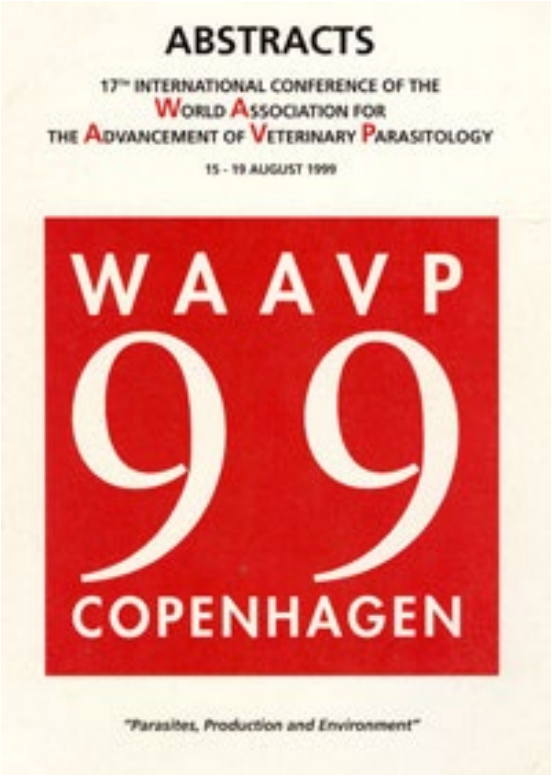
The 17th International Conference of the

WORLD ASSOCIATION FOR THE ADVANCEMENT OF VETERINARY PARASITOLOGY

"Parasites, Production and Environment"

August 15-19, 1999

COPENHAGEN, DENMARK



ABSTRACTS

17TH INTERNATIONAL CONFERENCE OF THE WORLD ASSOCIATION FOR THE ADVANCEMENT OF VETERINARY PARASITOLOGY

15 - 19 AUGUST 1999

"Parasites, Production and Environment"

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TABLE OF CONTENTS:

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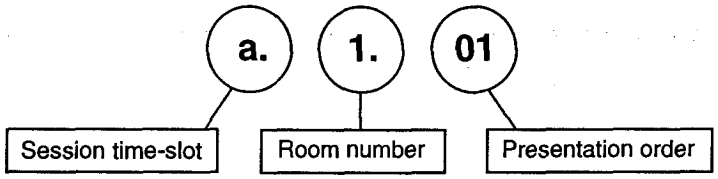
INTRODUCTION

This book contains the abstracts and posters of the 17th International WAAVP conference, held in Copenhagen, August 1999. The abstracts are organised to follow the occurrence of the abstract presentations during the conference. Each abstract has a programme number designated according to session time-slot, room number and order of presentation within the session. Please see the programme on the next pages for session designations:

Programme number example: a.1.01



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PROGRAMME OVERVIEW

Sunday 15 August

15:00-21:00	Registration	Registration at the Royal Veterinary and Agricultural University (KVL) Thorvaldsensvej 40
19:00-22:00	Reception	Welcome reception at KVL

Monday 16 August

08:00-	Registration	Registration at KVL
09:30-11:00	Opening (at Falconer) & Plenary paper	Opening Ceremony at Falconer Center, Falkoner Alle 9 Announcements of WAAVP Honorary Members and WAAVP/Pfizer Awards *B Perry:* Improving the assessment of the economic impact of parasitic diseases and of their control in production animals
11:00-11:30	Coffee	
11:30-12:30	Plenary papers (at Falconer)	*O Halvorsen:* Parasites, biodiversity, and population dynamics in an ecosystem in the high Arctic *SM Thamsborg:* Integrated and biological control of parasites in organic and conventional production systems
12:30-14:00	Lunch (at KVL)	Lunch in tent at KVL, Thorvaldsensvej 40

14:00-16:00	Oral session a (at KVL)	Room 1: a.1.01-08 Molecular studies of parasiticide resistance	Room 2: a.2.01-04 Pathology of parasite infection a.2.05-08 Immunity against helminth infections	Room 3: a.3.01-04 Epidemiology and control of horse parasites a.3.05-08 Epidemiology and control of protozoan infections	Room 4: a.4.01-08 Basic parasite biology	Room 5: a.5.01-08 Diagnosis of parasitic infections
16:00-16:30	Coffee					
16:30-17:30	Symposium	Room 1 and 2: NOVARTIS Corporate Symposium				
19:00	Reception Tivoli	Welcome Reception at Copenhagen Town Hall Tivoli Garden Visit				

Tuesday 17 August

08:00-	Registration	Registration at KVL

08:30-10:00	Plenary papers	Room 1: *T Geary:* Frontiers in anthelmintic pharmacology	Room 2: *JP Dubey:* Recent advances in Neospora and neosporosis
		RB Gasser: PCR based technology in veterinary parasitology	*T Schoitz:* Parasites in cultured and feral fish
10:00-10:30	Coffee		

10:30-12:30	Oral session b	Room 1: b.1.01-04 Parasiticide resistance and drug use b.1.05-08 Chemotherapy against horse and cattle parasites	Room 2: b.2.01-08 Immunity against protozoan and ectoparasitic infections	Room 3: b.3.01-08 Integrated and biological parasite control	Room 4: b.4.01-08 Parasites of wildlife	Room 5: b.5.01-05 Economic impact of parasitic infections b.5.06-08 Implementation of research findings to the end user
12:30-14:00	Poster session and lunch	Poster sessions c.6.00 to c.6.87 in room 6 Poster sessions c.7.00 to c.7.99 in room 7				

14:00-15:00	Oral session d	Room 1: d.1.01-08 Chemotherapy against livestock parasites	Room 2: d.2.01-08 Development of vaccines against parasites	Room 3: d.3.01-06 Biological parasite control using nematophagous fungi	Room 4: d.4.01-08 Parasites of fish	Room 5: d.5.01-08 Parasites of poultry
15:00-15:30	Coffee					

SESSIONS by TOPIC and PRESENTATION FORM

Session topic	Programme numbers	
	Oral	Poster
Economic impact of parasitic disease in production animals	b.5.01-05	c.7.00-01
Epidemiology and control		
Of ruminant parasites in temperate climates	e.1.05-08	g.6.09-22
Of ruminant parasites in the tropics and subtropics	f. 1.01-08	g.6.23-33
Of swine parasites	e.1.01-04	g.6.01-08
Of horse parasites	a.3.01-04	c.7.12-19
Of protozoan and ectoparasitic infections	a.3.05-08	c.7.02-11
Of companion animal parasites		g.6.34-41
Chemotherapy/Pharmacokinetics		
Chemotherapy against horse and cattle parasites	b.1.05-08	
Chemotherapy against horse parasites		c.7.20-27
Chemotherapy against livestock parasites	d.1.01-04	c.7.38-56
Chemotherapy against companion animal parasites		g.6.42-49
Chemotherapy against protozoan and ectoparsitic infection	e.3.01-08	g.6.50-83
Molecular studies of parasiticide resistance	a.1.01-08	c.7.34-37
Parasiticide resistance and drug use	b.1.01-04	c.7.28-33
Pharmacokinetics of parasiticides	e.2.01-04	g.7.01-14
Non-conventional treatment of helminths	f. 5.01-08	g.6.84-88
Immunity and pathology of parasite infection		
Immunity against helminth infections	a.2.05-08	c.6.11-24
Immunity aginst protozoan and helminth infections	b.2.01-08	
Immunity against protozoan and ectoparasitic infections		c.6.25-30
Development of vaccines against parasite infections	d.2.01-04	c.6.31-40
Pathology of parasite infection	a.2.01-04	c.6.00-10
Genetic resistance to parasitic infections	f. 3.01-08	g.7.42-48
Integrated and biological parasite control in conventional and organic farming		
Integrated and biological parasite control	b.3.01-08	c.7.57-63
Biological parasite control using nematophagous fungi	d.3.01-04	c.7.64-75
Nutrition-parasite interactions	f. 4.01-08	g.6.89-94
Implementation of research findings to the end-user	b.5.06-08	
Teaching of veterinary parasitology		g.7.81
Molecular and biochemical parasitology		
Biochemical parasitology	e.2.05-08	g.7.15-27
Use of PCR in molecular parasitology	e.5.01-08	g.7.28-33
Parasite systematics	f. 2.01-08	g.7.34-41
Diagnosis of parasitic infections	a.5.01-08	c.6.41-49
Parasitic zoonoses	e.4.01-08	
Protozoan parasite zoonoses		g.7.49-55
Helminth parasite zoonoses		g.7.56-69
General aspects of parasitic zoonoses		g.7.70-77
Further examples of zoonoses		g.7.79-81
Basic parasite biology	a.4.01-08	c.7.76-92
Parasite morphology		c.7.93-99
Parasites in wildlife	b.4.01-08	
Protozoan parasites of wildlife		c.6.50-58
Helminth parasites of wildlife		c.6.59-69
Parasites in cultured and feral fish	d.4.01-04	c.6.77-87
Parasites of poultry	d.5.01-04	c.6.70-76

15:30-17:00	Workshops	Room 1: BAYER: Diseases related to protozoa and possibilities for treatment	Room 2: Disease control in integrated herd health management	Room 3: Genetic resistance to parasites	Room 4: Control of parasites in fish production	Room 5: New techniques in experimental parasitology	Room 6: Standardized nomenclature on parasitic diseases
17:00-18:00	Symposium	Room 1 and 2: MERIAL Corporate Symposium					
18:20-	Bus	Bus transfer to Holmens Kirke					
19:00-20:00	Concert	Concert in Holmens Kirke					

Wednesday 18 August

08:00-	Registration	Registration at KVL					
08:30-10:00	Plenary papers	Room 1: ENT Meeusen: Immunology of helminth infections, with special reference to immunopathology	Room 2: GA Schad: Chemo- and thermosensory neurons: Structure and function in animal parasitic nematodes				
		RL Coop: Nutrition-parasite interaction	MD Ruff: Important parasites in poultry production systems				
10:00-10:30	Coffee						
10:30-12:30	Oral session e	Room 1: e.1.01-04 Epidemiology and control of swine parasites e.1.05-08 Epidemiology and control of ruminant helminths I	Room 2: e.2.01-04 Pharmacokinetics of parasiticides e.2.05-08 Biochemical parasitology	Room 3: e.3.01-08 Chemotherapy against protozoan and ectoparasitic infection	Room 4: e.4.01-08 Parasitic zoonoses	Room 5: e.5.01 -08 Use of PCR in molecular parasitology	
12:30-13:00	Get ready	Lunch box hand out and departure for afternoon excursions					
13:00-18:30	Excursions	Afternoon excursions					

Thursday 19 August

08:00-10:00	Oral session f	Room 1: f.1.01-08 Epidemiology and control of ruminant helminths II	Room 2: f.2.01-08 Molecular and biochemical parasitology: Systematics	Room 3: f.3.01-08 Genetic resistance to parasitic infections	Room 4: f.4.01-08 Nutrition - parasite interactions	Room 5: f.5.01-08 Non-conventional treatment of helminths, and product development	
10:00-10:30	Coffee						
10:30-11:30	Awards	Room 1 and 2: Presentations by recipients of the WAAVP/Pfizer Awards					
11:30-12:30	Symposium	Room 1 and 2: PFIZER Corporate Symposium					
12:30-14:00	Poster session and lunch	Poster sessions g.6.01 to g.6.94 in Room 6 Poster sessions g.7.01 to g.7.78 in Room 7					
14:00-15:00	WAAVP	Room 1: WAAVP General Assembly					
15:00-15:30	Coffee						
15:30-17:00	Workshops	Room 1: 15:15-16:45 FORT DODGE: Control of horse and dog parasites	Room 2: Understanding and solving the problems of parasiticide resistance	Room 3: Emerging parasitic zoonoses	Room 4: Systematics of the cyathostomes in horses	Room 5: New approaches for teaching veterinary parasitology	
19:30-	Dinner	Conference Dinner at Falconer					

Friday 20 August

10:00-12:00	University Tour

ABSTRACTS

a.1.01-08 Molecular studies of parasiticide resistance

a.1.01 LEVAMISOLE AND PYRANTEL RESISTANCE RESOLVED AT THE SINGLE-CHANNEL LEVEL

Martin[1], R.J., Robertson[1], A.P. and Bjorn[2], H.

[1]Department of Preclinical Veterinary Sciences, R.(D.)S.V.S., University Edinburgh, EH9 1QH. [2]Danish Centre For Exp. Parasitology, Royal Vet and Agricultural University, Bulowesvej 13 D-K1870, Frederiksberg C, Denmark.

Background: Levamisole and pyrantel are nicotinic anthelmintics that act as agonists on nematode nicotinic acetylcholine receptors (nAChRs). Resistance to these anthelmintics is now a wide-spread problem. We have investigated mechanisms of resistance in *Oesophagostomum dentatum* using patch-clamp techniques to compare properties of individual receptors in sensitive and resistant isolates of this nematode.

Method: Channel currents were recorded from nAChRs of *O. dentatum* muscle vesicles of levamisole-resistant, pyrantel-resistant and sensitive isolates with levamisole as the agonist. Numbers of active receptors, channel conductances, open-times and Popen values were determined.

Results: Comparison of the biophysical properties of individual nAChRs from sensitive isolates with the nAChRs of anthelmintic-resistant isolates revealed significant reductions in the numbers of active receptors and mean Popen values in resistant isolates. The nAChRs showed heterogeneity with different subtypes of receptor and wide variations in biophysical properties of receptors.

Conclusion: The study showed that averaged properties of nAChRs changed during development of anthelmintic resistance in such a way as to reduce the ability of the whole muscle membrane to carry current in response to levamisole or pyrantel. Interestingly the presence of receptor heterogeneity allowed some receptors in resistant-isolates to be indistinguishable from some receptors in sensitive isolates.

a.1.02 GABA RECEPTORS AS TARGETS FOR AVERMECTINS/ MILBEMYCINS IN NEMATODES

Prichard[1], R., Blackhall[1], W., and Beech[1], R.

Institute of Parasitology[1], McGill University, Ste.-Anne-de-Bellevue, Quebec, Canada H9X 3V9

Background: Gamma-aminobutyric acid (GABA) A receptors are inhibitory chloride channels in membranes of vertebrate and invertebrate neuromuscular cells. Gating of the channels by GABA leads to an influx of chloride ions into, and hyperpolarization of, the cells. Various drugs are known to act as agonists or antagonists at GABA receptors in brain cells of vertebrates, some insecticides are antagonists in insects, and the anthelmintic piperazine is a GABA-receptor agonist in nematodes. Avermectins act as agonists at GABA receptors in insects, causing ataxia and paralysis. Avermectins also paralyse nematodes.

Method: We have analysed by SSCP the genetic variation of a gene encoding a GABA-receptor subunit from two sets of unselected and anthelmintic-selected strains of the parasitic nematode *Haemonchus contortus*.

Results: Significant differences in allele frequencies were detected between one unselected strain and its derived ivermectin-selected strain and between the other unselected strain and its derived ivermectin- and moxidectin-selected strains. In each set of strains, one allele increased substantially in frequency in the drug-selected strains relative to their respective unselected strains. The selected allele, however, differed between the two sets of strains. Sequence analyses of the second transmembrane domains of the alleles detected at this locus indicate that resistance is not due to mutations in this region.

Conclusion: These results implicate the GABA receptor as a site of action for avermectins and milbemycins and indicate that GABA-receptor allele-specific probes may be used as markers for avermectin/milbemycin resistance

a.1.03 MOLECULAR CLONING OF THE AVERMECTIN RECEPTORS FROM *Haemonchus contortus*.

Wolstenholme, A.J., Delany, N.S., Jagannathan, S., Laughton, D.L. & Skinner, T.M.

Department of Biology & Biochemistry, University of Bath, Bath BA2 7AY, U.K.

The avermectins and milbemycins exert their effects by acting as agonists at glutamate-gated chloride channels (Glu-Cl), opening the channel and leading to hyperpolarisation of the muscle or nerve cell. This results in paralysis of both pharyngeal pumping and movement and it is likely that different forms of the Glu-Cl are present at the 2 sites. Eight subunits of Glu-Cl, coded by 6 genes, have been identified in *C. elegans* using molecular cloning techniques and as a result of the genome sequencing project. We used RT-PCR methods to amplify 5 cDNAs (HG1-5) from purified *H. contortus* eggs. HG1 is most likely a component of the neuromuscular GABA receptor and is orthologous to the *C. elegans* gene ZC482.1. HG2 & HG3 are alternatively spliced subunits orthologous to *gbr-2/avr-14*: the pattern of alternative splicing is conserved between the two species. HG4 is orthologous to the Glu-Cl β subunit. HG5, though closely related to *gbr-2/avr-14* and to the Glu-Cl α subunits, does not seem to have an exact orthologue in the *C. elegans* genome. Synthetic peptides corresponding to unique sequences in the N-terminal domain of the subunits were used to raise specific antibodies. These were used in immunolocalisation studies on adult worms. HG1 was located on isolated muscle cells and on a few neurons in the nerve ring. HG4 was expressed on motor neuron commissures in the anterior portion of the worm. This result is consistent with the pattern of paralysis observed after avermectin treatment but differs from that seen for *C. elegans* Glu-Cl β subunit, which is found only on pharyngeal muscle cells. The antibodies used did not distinguish between HG2 and HG3; they stained the nerve cords and the nerve ring. Many aspects of the avermectin receptors are conserved between *C. elegans* and parasitic species, but there are differences both in the subunits present and the expression patterns seen.

a.1.04 P-GLYCOPROTEINS: MOLECULAR MARKERS FOR DRUG-RESISTANCE?

Sangster, N.C., Bannan, S.C., Weiss, A.S., *Nulf, S.C., *Klein, R.D. and *Geary T.G.

Department of Veterinary Anatomy and Pathology; Department of Biochemistry, University of Sydney, 2006 Australia.

*Animal Health, Pharmacia and Upjohn, Kalamazoo MI 49001 USA

Background:: P-glycoproteins are transmembrane proteins associated with acquired multidrug resistance in mammalian cells and some protozoan parasites by a process of active drug export. The project was designed to assess the role of P-glycoproteins in anthelmintic resistance in *Haemonchus contortus*.

Method: Each P-glycoprotein contains two nucleotide binding domains. These regions were PCR-amplified from adult and larval cDNA libraries using degenerate primers. Six distinct clones were isolated, sequenced and used in the following hybridization experiments. The clones were of unique regions of at least four Pgp genes. Northern blots of several developmental stages were performed. Further, the role of Pgps in resistance was assessed by Southern blotting of *Eco*RI-digested DNA from several field isolates of *H. contortus* resistant to a range of anthelmintics.

Results: Northern blotting revealed that P-glycoprotein genes are transcribed in a developmentally regulated fashion in *H. contortus*. Depending on the probe used, Southern blots could be grouped into three patterns. Some genes appeared to have no link to any resistance while others provided complex patterns. A third group showed an RFLP with avermectin/milbemycin, but not other, resistances.

Conclusions: A genetic marker for AM resistance has been described. Work continues to determine if this is related to a mechanism of resistance in this and other trichostrongyloid nematodes.

a.1.01-08 Molecular studies of parasiticide resistance

a.1.05 EXPERIMENTAL SELECTION OF RESISTANCE TO MOXIDECTIN IN AN IVERMECTIN RESISTANT ISOLATE OF *HAEMONCHUS CONTORTUS*

Ranjan, S., Wang, G.T., Hirschlein, C.A. and Simkins, K.L.

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An ivermectin resistant isolate of *Haemonchus contortus* was exposed to subtherapeutic levels of moxidectin through 14 generations. For each passage, moxidectin dosage was increased or maintained on the basis of the previous passage fecal egg count (EPG) reductions to project 80-95% efficacy. After selection through 14 generations, the ivermectin resistant strain became resistant to the recommended dose of 0.2 mg moxidectin/kg b.w. A dose titration study was conducted with the parent ivermectin resistant strain and the moxidectin selected ivermectin resistant strain using 5 dosage points and 5 lambs per point to determine ED_{50}, ED_{90} and ED_{95} for ivermectin and moxidectin. Based on necropsy worm burdens ED_{50}, ED_{90} and ED_{95} for the parent strain were 0.04, 0.11 and 0.15 mg moxidectin/kg b.w., respectively, and all were found to be >0.8 mg ivermectin/kg b.w. Based on necropsy worm burdens ED_{50}, ED_{90} and ED_{95} for the moxidectin selected strain were 0.32, 0.66 and 0.81 mg moxidectin/kg b.w., respectively, and all were found to be >1.6 mg ivermectin/kg b.w. The results indicate moxidectin is more potent than ivermectin and though resistance development to moxidectin is slow that side resistance exists within macrocylic lactones.

a.1.06 EVOLUTION OF THE BENZIMIDAZOLE RESISTANCE IN A POPULATION OF *TELADORSAGIA CIRCUMCINCTA* SUBMITTED TO DIFFERENT ANTHELMINTHIC TREATMENTS.

Leignel, V.[1], Humbert J.F.[2] & Cabaret, J.[1]

1: INRA, Station P.A.P., 37380 Nouzilly, France.
2: INRA, Station S.H.L., 74203 Thonon, France.

Background: To find new strategies of control for benzimidazole-resistant populations, we have tested different protocols of treatment based on the use of Fenbendazole (Bz) and Levamisole (Lev), alone or alternatively. These tests were performed on a population of *Teladorsagia circumcincta* which is in temperate climates, a highly prevalent nematode of the small ruminants.

Methods: Four groups of ten of animals were placed on four contiguous pastures, contamined beforehand with a *T. circumcincta* population in which the proportion of resistant worms (rr) was 0.25, susceptible heterozygous (rS) 0.50, and homozygous worms (SS) 0.25. Four treatment protocols were tested on these pasture: (1) control (without treatment), (2) only Lev treatments, (3) only Bz treatments, (4) Bz and Lev alternatively. The estimation of the mutant resistant proportions was realised using PCR at different periods of the year.

Results: After a year of experiment, it appeared that the proportions of resistant worms did not vary on paddocks 1 and 2. On the paddock 3, a very strong increase of the resistant worm proportion was observed, while only a light increase of the proportion was recorded on the paddock 4 in which two anthelminthics were alternatively used.

Conclusion: A pursuit of these protocols is planned for a second grazing season so as to confirm or infirm the results observed during the first year.

Acknowledgements: The financing of the study by the Region Centre is gratefully acknowledged.

a.1.07 BREEDING MANAGEMENT AND DEVELOPMENT OF BZ-RESISTANCE ON GOAT NEMATODE SPECIES DIVERSITY.

Boudsocq A., Chartier C., Cabaret J.

INRA, Station P.A.P., 37380 Nouzilly, France

Background : Helminth infections are responsible for production losses in goats. Anthelminthic treatment are the main solution adopted to control infection. Benzimidazoles (BZ) have been intensively used because they are well adapted to production constraints. BZ-resistance soon appeared in the three major species of small ruminant nematodes : *Teladorsagia circumcincta*, *Haemonchus contortus* and *Trichostrongylus colubriformis*. We studied the effects of breeding management and acquisition of BZ-resistance on species diversity. Our hypothesis was that development of BZ-resistance should result in a decrease of species diversity due to the loss of susceptible species after anthelminthic treatment.

Results : We found that goats housed during winter, after anthelminthic treatment, increased BZ-resistance development. When goats went back on pastures in spring, contamination was essentially realised by resistant larvae which survived after anthelminthic treatment. Effects of anthelminthic treatments on species diversity seemed to be more complex. Species diversity decreased when all species of the community were susceptible or when BZ-resistance was appearing in only one species among the community. Species diversity was then reduced, due to the loss of the less abundant species in one case or to the elimination of susceptible species in the other case. There was no species diversity decrease after anthelminthic treatment, in resistant communities. This is due to the preservation of each species which acquired BZ-resistance.

Acknowledgement : This work was supported by a PhD grant from INRA and "Bureau des Ressources Génétiques".

a.1.08 INHERITANCE OF IVERMECTIN RESISTANCE IN A FIELD ISOLATE OF *TRICHOSTRONGYLUS COLUBRIFORMIS*

R.M. Gopal, W.E. Pomroy, D.M. West and D.J. Garrick

Institute of Veterinary, Animal and Biomedical Science, Massey University, Palmerston North, New Zealand

Background: An ivermectin resistant isolate of *Trichostrongylus colubriformis* was isolated from a goat farm in Northland, New Zealand. This is the first physical identification of such a field isolate worldwide. This isolate was found to be equally infective for sheep and ivermectin resistance was confirmed in sheep. For this trial, this field isolate was passaged three times through goats, once with single dose of ivermectin and twice with a double dose of ivermectin. The aim of this trial was to investigate the heritability of this isolate, in particular, whether it is a dominant single trait as found with other ivermectin-resistant isolates of related nematodes.

Method: Three lambs were infected with 25000 larvae of ivermectin-resistant larvae (89% *Trichs*, 11% *Ostertagia*) and three other lambs were infected with 25000 larvae of ivermectin susceptible strain of *T. colubriformis*. Thirteen days post infection (DPI), all the lambs except one lamb with susceptible strain (SS) were killed. Immature adult *T. colubriformis* worms were recovered and transferred surgically into another three lambs as follows: Resistant males×Resistant females (RR), Susceptible males×Resistant females (RS(M)), Resistant males×Susceptible females (RS(P)). Larval development assays (LDAs) were conducted on 23, 27, 31 and 35 DPI using ivermectin. The experiment was repeated in the following year.

Results: The LD_{50} values of RS(M) and RS(P) were slightly less than those of RR and greater than those of SS suggesting that ivermectin resistance in *T. colubriformis* is inherited as an incompletely dominant character.

Conclusions: Ivermectin resistance in *T. colubriformis* is inherited as an incompletely dominant character.

a.2.01-04 Pathology of parasite infection

a.2.01 MANAGEMENT OF THE NEUROPATHOLOGY OF AFRICAN TRYPANOSOMOSIS

Rodgers, J.[1], Kennedy, P.G.E.[3], Jennings, F.W.[1], Bradley, B.[1], Eckersall, P.D.[1], Williams, A.[2], Murray, Max[1].

Departments of Veterinary Clinical Studies[1], Veterinary Pathology[2] and Neurology[3], University of Glasgow, Glasgow.

Background: The protozoan parasites *Trypanosoma brucei* spp can infect animals and man resulting in major disease problems. This species is highly invasive affecting all tissues including the CNS causing pathological changes that lead to severe neurological consequences. The CNS pathology can be exacerbated by trypanocidal drug treatment, a consequence believed to be related to subcurative therapy. This study investigated the pathogenesis of the CNS reaction and identified novel methods of managing the inflammatory response.
Method: A mouse model simulating all phases of African trypanosomiasis was used in this study. Histological and immunocytochemical staining methods together with RT-PCR were employed to map the kinetics of the neuropathological changes and cytokine production within the brains of *T.b.brucei* -infected mice subject to various chemotherapeutic regimens.
Results: At day 7 post infection, mRNA transcripts for TNF-α and IL-6 appeared although no neuropathological changes were found. IL-1α was constitutively expressed. By day 14 post infection astrocyte activation and some CD4 and CD8 cell infiltration were apparent. Treatment with eflornithine, an ornithine decarboxylase inhibitor, both prevented and ameliorated the neuropathology while the substance P receptor antagonist, RP-67,580 reduced the inflammation but could not prevent its onset.
Conclusion: These findings could lead to novel treatments for trypanosomosis and other inflammatory mediated CNS conditions.
Acknowledgement: We wish to thank the Sir Jules Thorn Charitable Trust for financial support of this project

a.2.02 EXCRETORY/SECRETORY PRODUCTS OF HAEMONCHUS CONTORTUS SUPPRESS STIMULATION OF PARIETAL CELLS BY INHIBITING SECRETORY ACTIVITY OF ENTEROCHROMAFFINE-LIKE (ECL) CELLS

Hertzberg[1], H., Lindström[2], E., Chen[2], D., Hakanson[2], R.

Institute of Parasitology[1], University of Zurich, Switzerland; Institute of Pharmacology[2], University of Lund, Sweden.

The pathophysiology of haemonchosis in sheep includes a decreased secretory capacity of the acid producing stomach (abomasum). This functional impairment is currently explained by a destruction or loss of differentiation of functional parietal cells in the fundic part of the abomasum. The aim of the study was to investigate possible interference of parasite derived excretory/secretory (e/s) products with the pathway regulating acid secretion. Under physiological conditions proton secretion of parietal cells is stimulated by histamine released from enterochromaffine-like (ECL) cells in the fundic mucosa. Therefore, ECL cells were cultivated in vitro and then challenged with e/s products of adult *H. contortus*. Their secretory capacity was measured by determining basal and gastrin-stimulated pancreastatin secretion. Both, basal and stimulated secretion were significantly inhibited in the presence of the e/s products in a dose-dependent manner. The results indicate, that adult *H. contortus* may change their microenvironment by suppressing acid secretion which most likely improves their survival in the host. This strategy represents a classical example for active interference of parasites with regulatory pathways of the host.

a.2.03 MORPHOLOGICAL CHANGES INDUCED BY ADULT *OSTERTAGIA CIRCUMCINCTA*

Simpson, H.V., Scott, I. Simcock, D.C., Reynolds, G.W. & Pomroy, W.E.

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Background: The presence of adult worms, either following larval infection or direct transfer from donor sheep, raises abomasal pH and serum gastrin and pepsinogen levels and causes mucous cell hyperplasia and loss of parietal cells in non-nodular areas. This study aimed to relate functional impairment after infection to morphological changes in a series of gastric biopsies.
Methods: Fundic biopsies were obtained by endoscopy through surgically implanted abomasal cannulae before and at 1-2 day intervals after infection with either 40,000 L3 or 18,000 adult *O. circumcincta*. Sheep receiving adult worms were treated with ivermectin 12 days after adult worm transfer and studied for a further 8 days. Epithelial cell abnormalities and inflammation were scored on scales of 1-4 of increasing severity. Abomasal pH and serum gastrin and pepsinogen were estimated twice daily.
Results: Raised abomasal pH and serum gastrin and pepsinogen, accompanied by parietal cell loss and inflammation, were apparent within 1 day of adult worm transfer or 5-6 days after larval infection. Over 12 days when adult worms were present, median scores for epithelial cell changes and inflammation were 3.0 and 2.25 respectively, significantly different from control sheep ($P<0.001$). Although abomasal pH began returning to normal almost immediatly, morphological changes did not resolve in the 8 days following drenching.
Conclusions: Adult worms caused almost immediate generalised parietal cell loss and inhibition of acid secretion. The physiological effects were rapidly reversible after worm removal, implicating worm chemicals as mediators.
Acknowledgments: We wish to thank the C. Alma Baker Trust and E.&C.Thoms Bequest for financial support.

a.2.04 EFFECT OF A *ASCARIS SUUM* REINFECTION ON LAMBS.

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Fertilization of the ruminant pastures with *Ascaris suum* - contaminated pig slurry poses a risk of infection to nonspecific hosts. Change in the organism were studied on two groups of four-month-old lambs infected for 23 days with a daily dose of 100 or 1000 infective *Ascaris suum* eggs. The lower infective dose elicited no clinical changes in the lambs. The higher infective dose caused dispnoe on days 9-20 and eosinophilia on days 14-28 post infection. Day 19 after the last infection marked an insignificant lymphocytic infiltration in the area of bile ducts and major blood vessels in the liver of both the animal groups. Numerous disseminated grey nodules, 1-2 mm in size were observed in all pulmonary lobes. Histologically they consisted largely of lymphocytes. Immunohistochemically, CD_3^+ cells were mainly observed in these lymphoid nodules. Lambs infected with the higher dose exhibited a marked peribronchial lymphocytic infiltration reaching even interalveolar septa. Lambs examined on day 33 after the last infection showed only sporadical grey pulmonary nodules, but pale greyish foci were observed. Like in pigs, migrating *Ascaris suum* larvae in the organism of nonspecific hosts elicited inconspicuous clinical manifestations and pathomorphological changes characteristic of verminous pneumonia.

a.2.05-08 Immunity against helminth infections

a.2.05 Immunity to Strongylus vulgaris Involves a Type 2 Cytokine Response

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A Type 1 cytokine response involves the production of interleukin (IL)-2, interferon-gamma and tumor necrosis factor-α and is characteristic of cell-mediated immunity directed against intracellular parasites. A Type 2 response is characterized by the production of IL-4, IL-5, IL-10 and IL-13 and typifies the humoral immune and cellular response generated against helminth parasites. While these dichotomous responses have been well described in various murine models for infectious disease, the possible role of Type 1 and Type 2 cytokine responses in other species remains unclear. We have addressed the possible contribution of these responses in equids using the Strongylus vulgaris-naive pony model. Immunization with radiation attenuated L_3 produces a strong protective resistance to challenge infection which reduces larval burdens, eliminates clinical signs of infection, and prevents colic. This protective immune response is associated with both *in situ* production of IL-4 in colonic lymph modes (CLN) and peripheral blood mononuclear cells (PBMC) and an anamnestic Type 2 cytokine response by $CD4^+$ T cells collected from both sites. By contrast, parenteral immunization with somatic worm antigens with RIBI adjuvant results in exacerbation of the lesions, lack of protection and a Type 1 cytokine response both *in situ* and by $CD4^+$ T cells isolated from CLN and PBMC of vaccinated ponies.

a.2.06 THE EFFECT OF HOST ON DEVELOPMENT AND INFECTIVITY OF *T. COLUBRIFORMIS*

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Background: A variation between hosts on the developmental success of the free-living stages of trichostrongylid infections of sheep has recently been demonstrated. These results suggested that eggs from less immune animals had a higher developmental success. The objective of the present experiment was to test this hypothesis on the common nematode *T. colubriformis*.

Method: Immune and non-immune lambs were trickle-infected with *T. colubriformis*. Faecal samples were analysed for FEC and developmental success in faecal cultures. Blood samples were analysed for circulating eosinophils and serum-IgG to *T. colubriformis*. At day 28 PI all lambs were killed and worm burdens, worm lengths and in-utero egg counts were determined. In an infectivity trial larvae from the above mentioned groups of animals were used to infect an immunologically homogenous group of lambs. At day 28 PI all lambs were killed and establishment rates determined.

Results: The groups differed significantly with regards to FEC, developmental success, eosinophils and serum IgG. Worm burdens, female worm lengths and in-utero egg counts were also found to differ significantly between the two groups. However, the larvae obtained from the immune and non-immune groups of animals did not differ in infectivity.

Conclusion: This study showed an effect of host immunity on the development of eggs to third stage larvae of *T. colubriformis*.

Acknowledgements: Thanks to Shirley Calder and Barbara Adlington for their technical assistance and to Meat NZ for their financial support.

a.2.07 PROTECTION IN YOUNG SHEEP VACCINATED AGAINST *HAEMONCHUS CONTORTUS* IS IGE RELATED

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Background: Sheep under the age of 6 months are difficult to immunise to *Haemonchus contortus* by natural infection or vaccination. IgE plays an important role in immunity against helminths. Therefore, we investigated the role of IgE in unresponsiveness after vaccination.

Method: Sheep 9, 6 and 3 months of age were vaccinated 3 times with 15 and 24 kD proteins obtained from excretory/secretory (ES) products of *H. contortus* with DDA as adjuvant. 2 weeks after the last vaccination the sheep were challenged with 20,000 L3 of *H. contortus* and worm counts were done 4 weeks later. Age matched control sheep were only challenged. ES specific IgE and IgG1 and total IgE levels were measured in serum and differential cell counts in blood and abomasal tissue were made.

Results: The vaccinated 9, 6 and 3-months-old sheep had a reduction in worm burden of 83, 77 and -34 % respectively. The IgG1 response of all the vaccinated animals were high, independent of their age. However, the IgE response were high in the vaccinated and protected 9-month-old sheep but absent in the vaccinated and unprotected 3-months-old sheep. The 6-month-old were intermediate. The peripheral blood eosinophils and mucosal mast cell counts were also higher in the protected, older sheep than in the unprotected younger ones.

Conclusions: Increased serum IgE levels, eosinophilia and mucosal mast cell hyperplasia, hallmarks of the Th2 response, are observed in protected older sheep but not in unprotected younger sheep.

a.2.08 CELLULAR IMMUNE RESPONSES OF BALB/C MICE INFECTED WITH THE FILARIID *LITOMOSOIDES SIGMODONTIS*

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Litomosoides sigmodontis infected BALB/c mice allow - in contrast to all other filariae-host systems - detailed analyses of immunological events. In the current study BALB/c mice were infected with 80 L_3 or 160 L_3. Spleen cells isolated from infected mice were used in stimulation assays. The mitogen Con A and parasite antigens were used as stimulants. Using the method of semi-quantitative RT-PCR mRNA levels of IL-2 and IFNγ as Th1-associated cytokines and IL-4, IL-5, IL-10, IL-13 as Th2-associated cytokines were determined throughout a period of 200 days p. i. Synthesis of both Th1- and Th2-associated cytokines was stimulated by filarial antigens during early prepatency in infected animals. Shortly before the beginning of patency (day 45 p. i.) the transcription of IL-2-, IL-4-, IL-5-, IL-13- and IFNγ-mRNA was completely downregulated, accompanied by an upregulation of the IL-10-mRNA transkription. These reactions turned out to be specific for filarial antigens as strong responses to Con A were not affected. With the onset of patency the reactivity of T cells to filarial antigens was restored with respect to transcription of IL-4-, IL-13- and IFNγ-mRNA. These were also the predominant transcripts found after specific stimulation in lymphocytes isolated during postpatency. Infection with 160 L_3 resulted in a higher and longer lasting microfilaremia than infection with 80 L3. However, the latter infection induced stronger reactions compared with the heavier infection. These data show an independant regulation of cytokines that does not fit in a general Th1/Th2-paradigm.

a.3.01-04 Epidemiology and control of horse parasites

a.3.01 SEASONAL PATTERN OF A POPULATION OF HORSE STRONGYLES IN PORTUGAL*

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Background: From January 1995 until May 1998 a study was performed on a group of young horses (1-3 years) on pasture, harbouring a mixed natural infection of strongyles. The animals were in a horse farm located in Ribatejo, 50 km far from Lisbon. The research aimed a better knowledge of the seasonal pattern of EPG peaks, L3 population in faecal cultures and L3 fluctuations on pasture, without interfering with the normal deworming scheme, management and animal husbandry procedures.
Method: Faecal samples were collected on a pooled basis for EPG counts and cultures. Herbage sampling was performed using the double W technique and the collections were on a fortnightly (1995/96) and monthly basis (1997/98).
Results: There were 2 marked EPG peaks on Spring and Autumn: 4356 on April and 2921 on October. Summer values are lower but ranging in constant limits of 1730-2867. Winter values are the lowest ones (January/February), but December and March EPG reached 2000. In the faecal cultures, several genera and species were identified: *Cyathostomum s.l., Gyalocephalus capitatus, Oesophagodontus robustus, Poteriostomum, Strongylus equinus, S. edentatus, S.vulgaris* and *Triodontophorus*. The predominant genus was *Cyathostomum s.l.*, which prevalence/culture/month ranged 77-100%, with an aproximate global prevalence/year of 98-100%. The main peaks of L3 on pasture were recorded on May, and in the months from October till January. This last period showed the highest values of L3/kg dry herbage.
Conclusions: The main seasons for horse infection in Portugal are Spring and Autumn/Winter, specially in those months with a heavy rainfall and higher relative humidity (RH). Start and end of Winter, when compared to northern countries, may be critical in what concerns the host infection since EPG output is superior to 2000 and coincides with high levels of rainfall and RH, together with poor feed intake in the pasture during this season.

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a.3.02 STRONGYLE INFECTIONS OF HORSES IN SWEDEN

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Objective: A survey was carried out to determine the occurrence of strongyle egg positive horses in Sweden and investigate the influence of geographic location, type of farm, herd size, horse age and pre-sampling treatment on the output of strongyle eggs.
Methods: During the first quarter of 1995 faecal samples from 1183 horses on 110 farms in different regions of Sweden were examined by McMaster technique for strongyle eggs. Larval cultures were performed to identify farms infected with *Strongylus vulgaris*. In connection with sampling a questionnaire on herd size, horse identity, age and anthelmintic usage was completed by the horse owner.
Results: In total 922 (78%) individuals were found to shed strongyle eggs and 15 (14%) of the farms had at least one horse infected with *S. vulgaris*. The output of strongyle eggs was highest in young horses from stud farms in the south of Sweden. Horses treated with ivermectin in the previous autumn showed significantly lower EPG values than untreated horses and those treated with pyrantel pamoate.
Conclusion: It appears that problems with strongyles are most likely to occur at stud farms in the south of Sweden. Despite frequent usage of anthelmintics, *S. vulgaris* is still a parasite that deserves attention in Sweden.

Acknowledgement: Financial support was obtained from the Swedish Horse Race Totalisator Board (ATG) and Agria Insurance Co, Stockholm, Sweden.

a.3.03 A NOVEL CONTROL STRATEGY FOR HORSE CYATHOSTOMES USING MOXIDECTIN

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Background: Anthelmintic strategies for preventing equine cyathostomosis aim to maintain low pasture contamination by controlling faecal egg output.
Method: *Study 1*: the cyathostome egg re-appearance time (ERT) was measured in naturally infected horses given a single dose of moxidectin 2% equine gel (0.4 mg/kg) (MOX) on day 0, at 3 stables in SE England. Pyrantel (PYR) treated controls were included. *Study 2*: 82 naturally infected horses at 6 stables were allocated to MOX and PYR groups grazing together on contaminated pastures. MOX was given every 91 days (Jul, Oct, Jan, Apr); PYR when 50% of group had faecal egg counts (FECs) ≥ 200 epg. FECs were monitored each month and larvae cultured and identified at start of study.
Results: *Study 1*: FECs in MOX were below limit of detection (50 epg) up to and including day 84; 3% of horses had FECs of 50 epg at day 98. *Study 2*: 98% of samples collected over 12 months from MOX group had zero FECs. No eggs were detected at any time after the start of the study in 85% of MOX horses. Just one animal was positive more than once, a foal with counts of 50-450 epg. Otherwise, only one FEC >50 epg was recorded in the MOX group. *In both studies*: controls confirmed exposure to challenge. 66% of PYR FECs were positive in Study 2. >99% of cultured larvae were cyathostomes.
Conclusion: The prolonged ERT of MOX indicated that treatment at three-monthly intervals should prevent eggs being dropped onto the pasture thereby fulfilling the control objective. This was confirmed in the field experiment.

a.3.04 ALTERNATIVE METHODS FOR HELMINTH PARASITE CONTROL IN WORKING DONKEYS IN SOUTH AFRICA

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Background: In South Africa most donkeys are worked by resource-limited people who are unable to purchase dewormers. Donkeys harbour a wide variety of worms and most of them can adversely affect the host's health. Thus, there is an urgent need for alternative control strategies for helminth parasites in this host.
Method: Data was collected from 24 male and female donkeys for 15 months (October 1997 to January 1999) at Onderstepoort. The animals were allocated to 8 camps (ie 3 animals/camp). Four management systems, with repeats, were tested: control, monthly faecal removal, prewinter (May) deworming and monthly faecal removal with a prewinter deworming. Faecal samples, blood samples, body condition scoring and live weights were recorded monthly from each animal. Herbage samples were collected from the camps, washed and the third-stage larvae (L_3) separated, identified and counted using standard techniques.
Results: The most abundant worms were cyathostomes, *Strongyloides westeri, Strongylus edentatus, Strongylus vulgaris* and *Trichostrongylus axei*. During the summer rainfall months (October - March) there was a significant difference ($p < 0.1$) between the numbers of L_3 per kg dry matter recovered from the camps with faecal removal as opposed to the control camps (no faecal removal). During the lower rainfall months the numbers of L_3 per kg dry matter decreased in all the camps.
Conclusion: This data suggests that regular faecal removal from grazing areas could be an effective means of reducing worm burdens, improving the health of donkeys and therefore performance and work output.

a.3.05-08 Epidemiology and control of protozoan and ectoparasitic infections

a.3.05 TRYPANOSOMOSIS IN HORSES AND DONKEYS IN THE GAMBIA

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In order to study the prevalence and incidence of trypanosomosis in horses and donkeys in the Gambia, a survey was carried out in two regions, Niamina East with a high and Bansang South with a low to moderate tsetse challenge. Eleven horses and 67 donkeys were sampled monthly from August 1997 till September 1998. The blood samples were examined using the buffy coat (BC) method and PCR. Three primer sets were used, specific for either *T.vivax* (TVW), *T.congolense* (GOL) or *T.brucei* (ORPHON5J).
The BC results showed that the prevalence (August 1997) and the average monthly incidence (Sept. '97 - Sept. '98) of trypanosome infections in horses (45.5 and 16 % respectively) were significantly higher than in donkeys (6.2 and 9 % respectively). Using PCR the number of positive cases was 7 times higher than using the BC. *T.congolense* was the most frequently observed species, followed by *T.vivax* and *T.brucei*. This study confirms earlier observations by other authors that donkeys, which are exposed to a similar tsetse challenge as horses, are much less infected with trypanosomes than the latter.

a.3.06 OVINE THEILERIOSIS IN SAUDI ARABIA

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Background: sheep production is an important part of Saudi Arabian agriculture and culture. Lamb and Mutton are the preferred meat of Saudi consumers, and sheep play an important role in social and religious events. Production is affected by a number of problems, including tickborne diseases. Little, however, is known about such diseases, reason why a survey was carried out among local sheep in the western (Jeddah) and central (Bureida) part of Saudi Arabia, during March-October 1995.
Method: The survey was carried out at Veterinary Diagnostic Laboratory. Sheep attended for treatment or autopsy were examined for ticks, and blood and lymph node samples were collected. A total of 305 blood samples- and 61 lymph node smears were stained with Giemsa and examined for parasites.
Results: Theileria spp were found in lymph node smear of 1 out of 36 sheep in Jeddah and 6 out of 25 sheep in Bureida. Based on the clinical signs of the affected sheep, and on the morphology of the schizont, the causative species was considered to be T. hirci. Two out of the infected sheep showed clinical signs typical for ovine theileriosis. Ticks, were found in relatively low numbers on 17/180 and 26/125 in respectively Bureida and Jeddah. All Theileria infected sheep carried Hyalomma impeltatum ticks except one that carried H. anatolicum.
Conclusion: The occurrence of malignant theileriosis in the Middle East is well known, although not specifically recorded in central Saudi Arabia. H. anatolicum is generally considered the vector. Although transmission through H. anatolicum can not be excluded, the finding ofH. impeltatum (and not H. anatolicum) on the majority of infected animals may indicate a role of H. impeltatum in the transmission of T.hirci in small ruminants on the Arabian peninsula.
Acknowledgement: Authors thank Dr. Schillhorn van Veen, the World Bank for his interest.

a.3.07 A SEROEPIDEMIOLOGICAL CASE - CONTROL STUDY OF NEOSPORA CANINUM INFECTION IN BELGIUM

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Background : *Neospora caninum* is a recently described tissue - cyst forming parasite which causes fetal and neonatal mortality in livestock and companion animals. A case - control study was conducted in cattle to assess the potential impact of *N. caninum* as an abortifacient in Belgium.
Method : The study was conducted in the province of Liège (Belgium) during the winter 1997. 370 sera from aborted cows were taken by local practionners within 24 h of abortion and constitued our case population. 341 control samples (165 dairy cows and 176 beef cows) were collected by a systematic sampling in the population of cows above 2 years of age (annual brucellosis screening). Sera were tested with both a commercial ELISA and a home made IFAT (dilution of sera 1/200).
Results : A very good agreement was found between the commercial ELISA and the home made IFAT (Kappa = 0.94). The *N. caninum* prevalence was significantly higher in case cows (17.5 % IFAT, 18.6 % ELISA) than in control cows (6.4 % IFAT, 7.5 % ELISA). The estimated odds ratio was 3.09 (IFAT) or 2.98 (ELISA). The attributable proportion was 11.9 % (IFAT) or 12.2 % (ELISA). The attributable proportion in the exposed population was 67.6 % (IFAT) and 65.5 % (ELISA). The *N. caninum* seroprevalence was significantly higher in beef cows (10.8 %) than in dairy cows (3.6 %).
Conclusion : *N. caninum* should be considered as a major cause of abortion in Belgium. The seroprevalence in beef cows is significantly higher than in dairy cows (unexpected observation considered published litterature).
Acknowledgment : This work was supported by a grant (grant 5774 A) from the Ministry of Agriculture, Belgium. Our thanks to Dr D. Buxton (Moredun Institute, Scotland) who provided us with the NC - 1 strain.

a.3.08 EFFICACY OF FLUAZURON AGAINST INFESTATIONS WITH ARGENTINEAN *B. MICROPLUS*

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Background: Fluazuron, an new IGR, has shown high efficacy against Australian strains of *Boophilus microplus* when applied at a dose rate of 1.5 mg/kg bw. Latin American strains of *B. microplus* are known to be larger and heavier than Australian ones and their control requires a higher dose. An in-vivo trial was carried out with an Argentinean strain to evaluate the efficacy and the persistence of the Latin American dose rate of 2.5 mg/kg bw.
Method: To evaluate the efficacy twenty Hereford calves were artificially infested (3 times a week, from day -6 to day 18 post treatment). Ten calves were treated with a 5% ready-to-use pour-on formulation of fluazuron at a dose rate of 2.5 mg/kg bw. Engorged *B. microplus* females dropping from the animals were collected until day 40 post treatment and subsequently analyzed for oviposition and egg viability. Efficacy was measured by calculating the percentage inhibition of reproduction achieved by fluazuron on treated vs. untreated calves. To evaluate the persistence, ten calves were artificially infested (3 times a week, from day 0 until day 91). Five calves were treated with fluazuron as described before, and dropping engorged female ticks were collected until day 95. Persistence was measured by the number of days elapsed between treatment and dropping of the first engorged *B. microplus* females laying viable eggs.
Results: In the efficacy trial fluazuron achieved an inhibition of reproduction of >99.9%.The persistence trial showed that the first engorged *B. microplus* female tick laying viable eggs dropped on day 63 post treatment.
Conclusion: Fluazuron applied at 2.5 mg/kg bw efficiently control infestations with Argentinean *B. microplus* ticks. Persistence lasts for about sixty days.

a.4.01-08 Basic parasite biology

a.4.01 *SARCOCYSTIS DUBEYI* - A NEW SARCOCYSTIS SPECIES OF THE WATER BUFFALO

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Background: Previously, three Sarcocystis species were described from the water buffalo *(Bubalus bubalis)*, namely, *S. fusiformis* (Raillet 1897) Bernard and Bouche 1912, *S. levinei* (Dissanaike and Kan 1978) Huong, Dubey and Uggla 1997, and *S. buffalonis* Huong, Dubey, Nikkilä and Uggla 1997.
Method: By means of light and transmission electron microscopy, the shape and ultrastructure of thick-walled microscopical sarcocysts found in skeletal muscles and oesophagus of water buffaloes slaughtered in Vietnam were studied.
Results: Sarcocysts ultrastructurally distinct from those of previously described Sarcocystis species of the water buffalo were detected. The new species was named *S. dubeyi* Huong and Uggla 1999. Sarcocysts were 60-600 µm long and 60-200 µm wide with a 4.5-9 µm thick cyst wall consisting of tightly packed, palisade-like villar protrusions containing longitudinally oriented microfilaments. The definitive host of *S. dubeyi* was not established.
Conclusion: Four Sarcocystis species are now recognised in the water buffalo, namely, *S. fusiformis* and *S. buffalonis* forming macroscopically visible sarcocysts, and *S. levinei* and *S. dubeyi* forming microscopic sarcocysts.
Acknowledgement: The study was financially supported by the International Foundation for Science (IFS).

a.4.02 CHARACTERIZATION OF CRYPTOSPORIDIUM PARVUM-LIKE PARASITES IN FEEDLOT STEERS

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Background: *Cryptosporidium parvum* is frequently observed in young calves but is rarely observed in older cattle. Chronic shedding of large numbers of Cryptosporidium parvum-like protozoan parasites were recorded in 5% of feedlot steers.
Methods: Oocyst excretion patterns were recorded for over 2 years. The immune status and virus exposure of cattle was evaluated by performing CBC, serology and lymphocyte typing (fax scan). Morphological features of the parasite and host tissue reactions were determined by histology and by light and electron microscopy. The parasite was cultured *in vitro* and immunosuppressed rats were challenged with the parasite. Ribosomal DNA sequences were compared to other Cryptosporidium isolates.
Results: Cryptosporidium-infected cattle had a normal immune function and no viral infections (BVD, IBR, BLV, BIV). These cattle shed large numbers of oocysts ($10^6 - 10^8$/g) for up to 2 years. The parasite had morphological and pathological features of *C. parvum* but was genetically similar to *C. muris*.
Conclusion: Feedlot and adult range cattle can be infected with a *Cyrptosporidium parvum* –like coccidian parasite. It's significance is yet to be determined.
Acknowledgements: Natural Sciences and Engineering Research Council, Alberta Agriculture Research Institute.

a.4.03 TRANSPLACENTAL TRANSMISSION OF *BABESIA EQUI* IN HORSES: THE RULE RATHER THAN THE EXCEPTION?

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Background: Abortion due to *Babesia equi*, a major problem in Thoroughbred breeding in South Africa, is assumed to occur whenever the parasite crosses the placenta. We attempted to ascertain whether carrier mares could give birth to infected but clinically normal foals.
Method: A newly developed DNA probe was used to confirm the presence of *B. equi* parasites in the spleens of 6 foetuses that were mechanically aborted from known parasite carrier mares between 4 and 7 months of gestation, and in blood samples collected from 7 healthy new-born foals of known carrier mares. The progress of congenital *B. equi* infections was followed in 3 foals born in a tick-free facility and kept there with their dams for 2-3 weeks.
Results: The probe detected *B. equi* in all 6 foetuses and in all 7 new-born foals. The probe further detected *B. equi* in the blood of the 3 foals from birth to termination of the trial, thus demonstrating the persistence of congenital infections, at least to 3 weeks *post partum*, even in the presence of colostral antibodies.
Conclusions: We conclude that transplacental transmission of *B. equi* in carrier mares is the rule rather than the exception, and that it probably takes place during the first 4 months of gestation. Unfortunately, we are no closer to understanding the processes which may lead to abortion.

a.4.04 CHALLENGE INFECTION OF PRENATALLY *SCHISTOSOMA JAPONICUM* INFECTED PIGLETS

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Background: The current knowledge on congenital schistosomiasis is very scarce, hence, the aim of the study was to investigate the impact of a prenatal *Schistosoma japonicum* infection on the establishment and fecundity of a postnatal challenge infection.
Methods: Twenty piglets were used of which 10 were prenatally infected with *S. japonicum*. Group A (5 pigs) received a challenge infection with *S. japonicum* upon the prenatal infection (prenatal+challenge); group B (5 pigs) was followed without intervention (prenatal control) and the group C piglets (10 piglets), born by unexposed sows, were given a challenge control infection (challenge control). The study lasted 11 weeks after the challenge infection and variables included: worm burdens, tissue egg counts as well as pathological examination.
Results: Worm establishment and tissue egg counts were significantly higher in group A as compared to the two other groups. Additionally, worm establishment was significantly higher in group C compared to group B. No difference in worm fecundity was seen comparing the 3 groups. Liver pathology (periportal + septal fibrosis) was most severe in group C compared to the other groups whereas egg induced lesions in the gut mucosa were comparable between the 3 groups.
Conclusion: A prenatal *Schistosoma japonicum* infection in piglets did not prevent establishment of a homologous challenge infection, neither did it reduce worm fecundity. However, the challenge infection did not cause additional pathology.
Acknowledgment: The Danish National Research Foundation funded this study.

a.4.01-08 Basic parasite biology

a.4.05 THE EFFECT OF EXPOSING SOWS TO *ASCARIS SUUM* ON WORM DISTRIBUTIONS IN THEIR PIGLETS

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Background: While it has been shown that immunity against migrating *Ascaris suum* larvae can be transferred from sows to piglets, the effect of maternal exposure on development of patent *Ascaris* infections in piglets is unknown. Duration of exposure has recently been shown to influence worm distribution.
Method: Sows were exposed to *A. suum* trickle infection before and during gestation (6 months) or during gestation only (3 months). Piglets born to the exposed sows or to helminth naive control sows were infected with low doses of *Ascaris* eggs within the first week of life, and stayed with the sows for 10 weeks, whereafter worm burdens were recorded at necropsy.
Results: Worm burden distributions in piglets from both exposed and control sows were overdispersed, but the shapes of the distribution curves were significantly different. Short-term exposure of sows resulted in a higher number of lightly infected piglets compared to controls, while the majority of piglets from long-term exposed sows harboured light to moderate worm burdens. A non-linear relationship was found between prevalence and mean *A. suum* worm counts in piglets from both exposed and control sows.
Conclusion: The duration of exposure of sows to *A. suum* infection influenced worm distributions in their piglets, reducing parasite aggregation.
Acknowledgement: This study was supported by the Danish National Research Foundation.

a.4.06 ORAL TRANSFER OF ADULT *ASCARIS SUUM* TO PIGS

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Background: Attempts to establish patent intestinal *A. suum* infections under experimental conditions are often in vain. Therefore information on the *in vivo* reproductive biology of *Ascaris* and the effects of adult worms on the protective immunity to reinfection has so far been very limited.
Method: A technique for oral transfer of adult worms to pigs was developed, whereby infections with known numbers and sex of adult worms could be attained. This method was used to study the egg production of female worms with or without presence of adult male worms, and to study the effects of intestinal adult worms on the host resistance to reinfection.
Results: Of a total of 310 female and 148 male worms transferred to 48 pigs, 46% of the females and 42% of the males were recovered from the small intestine 5-8 weeks after transfer. Female worms transferred to previously parasite-naive pigs ceased producing eggs 2-3 weeks after transfer. However, these females readily resumed excretion of fertilised eggs a few days after oral transfer of adult male worms. Presence of adult *Ascaris* in the small intestine was found to be without influence on the host response against migrating larvae and had no effect on the survival of larvae from a single challenge inoculation. This was found irrespective of whether the challenged pig host was parasite-naive or had been immunized previous to the transfer of adult worms.
Conclusions: These results show that egg production in *Ascaris* is dependent on frequent mating, and that an existing patent *Ascaris* infection has little interference with the establishment of new parasite generations in the pig host.

a.4.07 BEHAVIOURAL DIAPAUSE IN *RHIPICEPHALUS APPENDICULATUS* (ACARI: IXODIDAE).

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Introduction: *Rhipicephalus appendiculatus* is the most important vector of the protozoan *Theileria parva*, the causative agent of East Coast fever (ECF). In southern Africa with monomodal rains, the ticks life cycle is synchronised with the rainy season by means of a behavioural diapause in the adult stage. Because of the impact on the epidemiology of ECF, diapause was studied in a variety of tick stocks.
Method: Three laboratory stocks and four newly isolated field stocks of *R. appendiculatus* were subjected to short (12:12 Light:Dark), intermediate (13:11) and long day lengths (14:10) under laboratory conditions. Diapause initiation, intensity and termination were determined with an *in vitro* assay.
Results: In ticks from eastern Africa (Kenya) diapause was completely absent allowing a multivoltine life cycle. With increasing latitude diapause intensity, induced by short day lengths, increased, becoming virtually obligatory in Zimbabwe. Diapause termination was regulated by increasing physiological age in Zambian populations. Zimbabwean ticks became active by exposure to an increasing day length.
Conclusion: Diapause characteristics are stock specific and adapted to local environmental conditions, to allow an optimal survival and reproduction of the population. Probably this trait is under constant environmental selection and modified if necessary.

a.4.08 WAS *OESTRUS OVIS* FIRST A PARASITE OF GOATS OR OF SHEEP? FACTS FOR FURTHER DISCUSSION.

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An extensive study of *Oestrus ovis* in its natural hosts, sheep and goats, has provided much information on differences between these hosts in their relationships with *O. ovis*. In the field, the prevalence of *O. ovis* and the parasitic burdens are usually higher in sheep than in goats. In the same way, mast cells and eosinophils in the mucosæ of the upper respiratory tract are more prevalent in sheep than in goats. The clinical signs and lesions of oestrosis are usually more severe in sheep than in goats. The higher prevalence and greater impact of infestation in sheep could be linked, i.e., if sheep harbour more larvæ, then humoral and cellular reactions are increased. However, this simplistic argument requires deeper analysis. It is well known that when a parasite is developing in a non-natural host, reactions are stronger than in a natural one : the latter host tolerates the parasite better because of the older established relationship between host and parasite. As it has been demonstrated that goats appeared earlier on earth (1.5 million years ago) than sheep (600,000 years ago), the following question arises: « Was *Oestrus ovis* first a parasite of goats or of sheep? ». The answer is partly given by RAPD of larval stages from sheep and goats : the average value of Nei's genetic distance is 0,08. Moeover, the artificial infection of goats is possible only with L1 from caprine origin and not with L1 of an ovine strain, whereas the artificial infection of sheep is possible with L1 from both sheep and goats. It is possible that further observations will modify our knowledge of the relationship of *Oestrus ovis* with sheep and goats : the latter could be the original natural host and sheep only a secondary one.

a.5.01-08 Diagnosis of parasitic infections

a.5.01 IgG AVIDITY - A TOOL TO ESTIMATE RECENCY OF *NEOSPORA CANINUM* INFECTION

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Background: Epidemiological data suggest that external or point source *Neospora caninum* infections have been the cause of bovine abortion outbreaks. However, the evidence is usually cirumstancial as sera are most often not available from the time before abortion. The aim was to evaluate if an IgG avidity ELISA could be used to differentiate between acute and chronic *N. caninum* infections in cattle.

Method: An IgG avidity ELISA utilising *N. caninum* iscom antigen was used. After antibodies were allowed to react with the antigen, low affinity antibodies were eluted with urea. An avidity index was calculated by dividing the titres with and without urea treatment.

Results: In experminentally infected calves the IgG avidity increased during the course of infection. In a beef herd experiencing an abortion outbreak all the aborting cows had low IgG avidities in serum collected within 1 month after abortion. Further, the IgG avidity increased during the year following the abortion outbreak.

Conclusion: The results indicate that the avidity ELISA can discriminate between recent and chronic *N. caninum* infections, and that IgG avidity measurements may be a valuable complement to standard IgG assays in epidemiological studies of *N. caninum* in cattle.

a.5.02 CONDITIONAL DEPENDENCE OF SEROLOGIC TESTS FOR TOXOPLASMOSIS IN PIGS

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Background: Dependence between the sensitivities and specificities of tests when used in combination alters the gains expected in serial and parallel testing schemes. To our knowledge, the extent and implications of dependence for tests for parasitic diseases have not been evaluated.

Method: Pairwise sensitivity and specificity covariances were calculated for 5 serologic tests for toxoplasmosis in pigs: MAT, LAT, IHAT, ELISA and dye test. Using data for the MAT and ELISA, a Bayesian approach with the Gibbs sampler was used to determine whether similar estimates could be obtained using both tests in 3 populations of different prevalence and no "gold standard" information. Mean estimates and 95% confidence intervals were determined.

Results: The analysis indicated positive sensitivity and specificity covariances from 40 - 100% and from 30 - 70% of their maximum values, respectively. All covariances were significantly ($P < 0.05$) different from 0. The Bayesian approach yielded mean estimates that were within 5% of the true values and the confidence intervals bounded the true values.

Conclusion: The moderate dependence in test sensitivities for toxoplasmosis means that use of multiple tests in parallel will not yield the gain in sensitivity expected if tests were conditionally independent. We recommend that authors evaluating multiple tests should report test covariances so that end-users can be guided as to whether inclusion of more tests will substantially increase the certainty of diagnosis or just the cost of testing.

a.5.03 PERFORMANCES OF RAPID DOCTOR TESTS FOR THE DIAGNOSIS OF CANINE LEISHMANIASIS

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Two new kits intended for the serological diagnosis of canine leishmaniasis have recently become available to the veterinary profession for «in practice» testing. These assays are based on either a rapid indirect ELISA format or the rapid immunomigration technology and overall test implementation only needs 20 and 5 minutes respectively.

A trial was designed to compare the performances of tests with those of well-established methods (indirect immunofluorescence assay and dot ELISA) more expensive on time and equipment.

For this purpose, 150 sera were selected , including 70 sera collected from Leishmania-infected dogs with various antibody end point titers in the conventional tests. This panel also included sera from animals living in leishmaniasis- free areas, from supposed non infected animals from endemic leishmaniasis areas, and from dogs being infected with other confirmed infectious diseases.

Roughly this study demonstrated a good agreement between the four serological methods. Some samples providing discrepant results were further tested by Western blotting and results will be fully reported and discussed during the meeting

a.5.04 EPIDEMIOLOGY OF PSOROPTIC SHEEP SCAB: SERODIAGNOSTIC AND MOLECULAR ASPECTS

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Sheep scab caused by *Psoroptes ovis* was increasing in recent years in Switzerland despite compulsory control measures raising the question of how this epizootic disease is spreading. Subclinically infested sheep or other animal species acting as reservoir hosts could play an important role. We have addresses these questions by establishing an ELISA for the detection of specific antibodies against psoroptic antigen aiming at improving the traditional diagnosis which is based on clinical signs and the microscopical detection of mites in skin scrapings, and by genetically characterizing mites from different host species from the same area. Positive ELISA results appeared before clinical signs became obvious at about 10 days after experimental infestation of 4 sheep. The specificity of the test was 96% as assessed with 258 sheep from 36 flocks without proven scab. The sensitivity was 100% with 31 sheep with parasitologically proven infestation and 94% with 117 sheep with clinical symptoms from 18 naturally infested flocks. We detected 26 seropositive sheep among 42 clinically inconspicuous animals from flocks with scab suggesting that asymptomatically infested sheep could be responsible for the spread of psoroptic mites. Sequence analysis of the ITS2 of rDNA of psoroptic mites from sheep and rabbits revealed two genotypes. The 5 psoroptic isolates from natural outbreaks of sheep scab in Switzerland had the identical sequence as two laboratory isolates originating from sheep from Ireland and the UK, differing in one nucleotide position (0.4% of 223 bp) from the sequence of 8 isolates from naturally infested rabbits from Switzerland. Therefore, psoroptic mites from sheep and rabbits are genetically closely related but natural transmission between these two host species has to be considered as epidemiologically not relevant in the investigated area.

a.5.01-08 Diagnosis of parasitic infections

a.5.05 MOLECULAR CLONING AND EXPRESSION OF A MAJOR ANTIGEN FROM *SARCOPTES SCABIEI*

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Background: Scabies or sarcoptic mange caused by the parasitic mite *Sarcoptes scabiei* is a widespread highly contagious disease of animals and man. The objective of this study was to isolate genes coding for immunodominant antigens from *S. scabiei* for the use in serological screening of infected animals.

Method: A cDNA library was made and screened for clones expressing immunodominant antigens. One cDNA clone was used for the construction of an expression vector. After expression in *Escherichia coli* of the recombinant antigen, the protein was purified and used in Western blot experiments as well as in an ELISA.

Results: The partial cDNA coded for a 49 kDa *S. scabiei* protein that was readily produced as a fusion protein in *E. coli.* Sera from experimentally as well as naturally infected dogs reacted with the recombinant protein in Western blot analysis. Furthermore, high levels of antibodies could be demonstrated in an experimental ELISA with sera from infected dogs. Rabbit antibodies specific for the recombinant protein recognised a 164 kDa protein in a mite body extract. This protein size corresponds to one of the major antigens inducing a strong IgG response in infected animals.

Conclusion: We have shown that it is possible to demonstrate *S. scabiei* antibodies employing an ELISA based on recombinant antigens. This could be a powerful alternative to available diagnostic techniques for the detection of *S. scabiei* infections.

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a.5.06 IMPROVED DIAGNOSIS OF MANGE IN PIGS

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Pig mange is a major problem in pig health management despite the availability of effective chemical treatments and control programmes. Current diagnostic techniques enable the detection of mite infections but are insufficiently sensitive to allow mite eradication and are unsuitable for use by most herd managers. The development of tests that can detect mite antigens in the skin may allow detection of mites at levels below those currently available. Separation and analysis of *Scabies* and *Dermatophagoides* antigens by column chromatography, SDS-PAGE and western blotting, skin testing in pigs and utilising phage display libraries identified four antigen preparations, all of which showed improved reactivity in ELISA's for serum antibody or in skin tests for hypersensitivity. Three of these antigen preparations were purified to a single protein and this was identified and specifically trialed in ELISA assays. The other preparation was purified to a single class of compounds that are very active in skin testing. These antigens allowed the differentiation of mange infected from uninfected pigs at various levels of certainty, with the best giving a 100% correlation with positive pigs. Work is now required to determine the sensitivity and specificity of all of these assays in large numbers of pigs, under experimental and field conditions.

a.5.07 DEVELOPMENT OF A COPROANTIGEN CAPTURE ELISA FOR THE DETECTION OF *TELADORSAGIA (OSTERTAGIA) CIRCUMCINCTA* IN SHEEP

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Background: *Teladorsagia (Ostertagia) circumcincta* is a highly pathogenic gastrointestinal nematode of sheep, causing major production losses in the agricultural industry throughout the world. Selective anthelminthic treatment of affected animals would be more cost effective and may reduce the spread of resistance, but only if simple, accurate and inexpensive tests were available to identify those animals harbouring infections liable to cause reduced fitness.

Method: An ELISA has been developed for the detection of *T. circumcincta* specific coproantigens. The assay is based on a sandwich ELISA in which affinity-purified polyclonal catching antibodies, and alkaline phosphatase conjugated detecting antibodies are used. The antibodies used in the assay are obtained from rabbits immunised with the excretory-secretory products of adult *T. circumcincta.*

Results: The assay allows the detection of 180 ng *T. circumcincta* antigen per gram of faeces as the lower resolving limit The assay is specific for *T. circumcincta,* giving detectably higher readings in the homologous specimen when compared with samples from sheep carrying other gastrointestinal parasites. In comparison to faecal egg counting which has been questioned in terms of the relationship between the worm burden and the faecal egg count, the assay described shows recognition of coproantigens in faecal samples taken from animals with known worm burdens, where the faecal egg count indicates a negatively infected animal. It is suggested that future studies should be aimed at the development of a kit to detect Ostertagiasis by a rapid and cost-effective immunodot test.

a.5.08 DETECTION OF COPROANTIGENS IS MORE SENSITIVE THAN EGG SEDIMENTATION FOR DIAGNOSIS OF CHRONIC BOVINE FASCIOLOSIS

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Background: There is a need for a simple test for rapid diagnosis of bovine fascioloses in the field. A well recognised limitation to accurate diagnosis by coprological examination is the low egg count seen in chronic fascioloses.

Method: We compared the sensitivity of diagnosis by sedimentation versus the detection of coproantigens by gel diffusion in a double-blind test.

Results: When egg count was below 50epg, coproantigens (23,45,80Kda) were detected in 24/30(80%) vs 17/30 (54%) detection by sedimentation ($P<.05$).

However, crossreactions occurred with F.gigantica in faecal samples of 16.7% P.microthobrium and 12% S.bovis cases and 3.3% negative controls.

Conclusion: Coproantigen detection is a more sensitive and rapid means of diagnosis of chronic bovine fascioloses than the egg sedimentation method. However, cross-reactions with sympatric trematodes is a serious limitation to coproantigen assay in the field

b.1.01-04 Parasiticide resistance and drug use

b.1.01 ANTHELMINTIC RESISTANT NEMATODES IN ENGLISH DAIRY CALVES

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Background: Since anthelmintic resistant nematodes have been reported from cattle in New Zealand, their possible presence was investigated on dairy farms in the South West of England.

Methods: Randomly selected dairy farmers, who had first year stock at grass, were asked to return questionnaires and calf faecal samples. Farms with sufficient numbers of nematode eggs in calf faeces were visited, larval development tests run on the eggs and in one case resistance confirmed in a controlled trial of eight calves.

Results: Sixty five percent of farmers turned out calves onto the same paddock each year, and fifty six percent treated second year calves with anthelmintic. Only sixteen out of ninety farms had sufficient eggs in the autumn to justify a farm visit and only eight had sufficient for *in vitro* tests. Two larval development tests were positive for levamisole resistance and one for ivermectin resistance. Numbers of *Cooperia* were not reduced by ivermectin injection in a controlled trial.

Discussion: The low numbers of eggs found on farms suggests over treatment with anthelmintics which might be reducing development of natural immunity. Reduced immunity might explain why farmers perceive the need to treat in the second year. Using the same paddock each year and treating in the second year is likely to enhance the selection for resistance. Two suspect cases of levamisole resistance were found. A controlled test confirmed ivermectin resistant *Cooperia,* the first case in the northern hemisphere.

Supported by the Milk Development Council.

b.1.02 A QUESTIONNAIRE SURVEY ON WORM CONTROL PRACTICES OF SHEEP AND GOAT FARMERS IN THE REGION OF TRIKALA, GREECE

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Background: Information concerning worm control practices of sheep and goat farmers in Greece that may promote development of anthelmintic resistance is lacking. For this reason, a questionnaire survey was carried out on nematode parasite control practices in the region of Trikala, which is an important livestock production area located in central Greece.

Method: Data on worm control practices was collected through a questionnaire survey of 57 sheep and goat farmers residing in 23 rural communities.

Results: Anthelmintics were used by 89% of the farmers. On average lambs, kids and goats were treated once annually, while sheep were treated either once or twice annually. Only 2% of the farmers reported treatment of animals with anthelmintics when moving to new pastures. Farmers who preferred the veterinarian as their information source did not use the anthelmintics correctly, indicating that the veterinarians themselves were not well informed about proper anthelmintic use.

Conclusion: Worm control practices of sheep and goat farmers in the region of Trikala are not in line with the general recommendations for avoidance of anthelmintic resistance. On the other hand, their worm control practices that help avoidance of anthelmintic resistance are their grazing on extensive common pasture lands and the low number of treatments per year.

b.1.03 PREVALENCE OF BENZIMIDAZOLE-RESISTANCE IN EQUINE CYATHOSTOME POPULATIONS IN SLOVAKIA

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The objective of the present study was to provide data on the prevalence of anthelmintic resistance in Slovak horse herds.

The present study included 11 horse studs and total number of 149 horses. The number of horses on the farms varied from 9 to more than 100. Horses of all ages were included. Animals had to be left untreated at least 10 weeks. Three sets of faecal samples were collected, first set at the first visit on the farm, second at the day of treatment and third from the same horses 7-14 days later. Horses with negative egg counts at first visit were excluded. Minimum of 7 horses were used for faecal egg count reduction (FECR) tests. The following anthelmintics were used at the recommended dose rates: fenbendazole (paste formulation), ivermectin (paste formulation) and pyrantel (powder). Egg hatch test were also used to measure the sensitivity of eggs *in vitro* to thiabendazole.

Resistance to benzimidazole were found on 8 farms, with FECR values ranging from 66 % to 80 %. Ivermectin was effective on five farms examined with this drug. Pyrantel was tested on 2 farms and FECR test indicated high efficacy (92-97 %). Results from *in vitro* egg hatch assays indicated that mean concentrations of thiabendazole that inhibited hatching in 50 % of the eggs (ED_{50}) in resistant populations were over 0.1 $\mu g.ml^{-1}$.

The present observations of the prevalence of benzimidazole resistance in Slovakia indicate that the problem is widespread. The results showed a clear need for innovative approaches such as alteration between drugs and using modern anthelmintics.

b.1.04 EXPERIMENTAL INDUCEMENT OF *H. CONTORTUS* RESISTANCE TO MACROCYCLIC LACTONES

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An USDA strain of *Haemonchus contortus*, which was originally sensitive to both moxidectin and ivermectin, was exposed to subtherapeutic levels of these macrocyclic lactone endectocides for 22 generations in lambs. For each passage, the drug dosages were increased or maintained on the basis of previous passage EPG counts to project 80-95% efficacy. After 22 generations, the ivermectin-selected strain became resistant to the recommended dose of 0.2 mg ivermectin/kg b.w. A dose titration study was conducted with the parent sensitive strain (PF22), the moxidectin-selected strain (MOF22) and the ivermectin-selected strain (IVF22) using 5 dosage points 5 lambs per point to determine ED_{90}. Based on necropsy worm counts, the ED_{90} for PF22 was calculated to be 1.3 µg/kg for moxidectin and 7.1 µg/kg for ivermectin. Against MOF22, the ED_{90} was determined to be 19.3 and 170.0 µg/kg for moxidectin and ivermectin, respectively. Against IVF22, moxidectin required only 24.9 µg/kg to achieve ED_{90} whereas ivermectin needed 260.4 µg/kg which is higher than the recommended dose. Moxidectin was consistently more potent than ivermectin against PF22 (5.5X), MOF22 (8.8X) and IVF22 (10.5X). The results indicated that the rates of *H. contortus* resistance development to macrocyclic lactones were relatively slow and that the development of resistance to moxidectin was slower than that to ivermectin.

b.1.05-08 Chemotherapy against horse and cattle parasites

b.1.05 EFFICACY OF PRAZIQUANTEL HORSE PASTE 9 % AGAINST EQUINE TAPEWORM

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Equine tapeworms occur commonly throughout the world. For Europe prevalences of 30-70 % are recorded . In most reports *Anoplocephala perfoliata* was the only tapeworm species found, fewer reports demonstrated *A. magna* and *Paranoplocephala mamillana*. There is growing evidence that heavy tapeworm burden in horses predispose to certain types of colic.

In a dose confirmation trial we treated 12 horses naturally infested with *A. perfoliata* with 1 mg/kg. A modified critical technique for efficacy evaluation with necropsy 24 hours post treatment was employed. Only 5 of 1105 tapeworms exposed to praziquantel survived treatment, representing an efficacy of 99.5 %.

In clinical field trials in New Zealand, Canada, France and Germany we assessed the efficacy of praziquantel against natural tapeworm infections in horses. In total more than 1000 horses were assessed. 264 horses with patent tapeworm infestations (*A. perfoliata, A. magna and P. mamillana*) proven by fecal examination were selected. 219 horses were treated orally with 1 mg/kg, 45 horses served as untreated controls. We took account of the unreliability of fecal examination for demonstrating tapeworm infections in horses by using a special centrifugation/flotation technique. Such technique proved to reach a sensitivity of about 60 %. Additionally we sampled on three consecutive days 1, 3 and 5 weeks post treatment to reach a sensitivity > 90 %.

Thus, 9 post treatment fecal samples were examined from each horse and the observation of just one tapeworm egg on one of these occasions was interpreted as treatment failure. Based on such stringent criterion we found an overall cure rate of 94.1 %. This confirms the efficacy of > 99 % as demonstrated in the dose confirmation study.

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b.1.06 A DOSE TITRATION TRIAL WITH PRAZIQUANTEL ORAL PASTE IN EQUIDS WITH *ANOPLOCEPHALA PERFOLIATA*

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Twenty-two equids naturally infected with *A. perfoliata* were in the trial. Eighteen were given an oral paste containing 0.5, 1.0, or 2.0 mg praziquantel/kg body-weight, euthanised 48 hr later and examined for tapeworms. After treatment, all feces from each equid was examined for tapeworms. Only worms with scolices were counted in assessing efficacy and were deemed abnormal if on microscopic examination they were disintegrating or had a brown discolouration. Four untreated equids were necropsied and the location in and attachment to the intestine were noted. Feces from these equids were also collected for 48 hours prior to euthanasia and examined for tapeworms.

Tapeworms were not found in the feces of the 4 untreated horses. In these horses, 407 *A. perfoliata* were recovered; 24 in the ileum, 58 in the area of the ileocecal valve, 321 in the cecum and 4 in the ventral colon. Twenty-six worms were unattached; 22 in the cecum and 4 in the ventral colon. All worms attached and detached appeared normal.

Treated horses accepted and consumed the praziquantel oral paste readily and adverse reactions were not seen in any horse following treatment. There were 1697 tapeworms recovered and 1171 were in the feces. Normal worms were found in only two equids and these had been treated with 0.5 mg praziquantel/kg body weight. The efficacy of praziquantel was 100% at 1.0 and 2.0 mg/kg and 83.8% at 0.5 mg/kg.

b.1.07 Efficacy of Moxidectin Gel Against Encysted Stages of Equine Cyathostomes

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Moxidectin 2% gel has been shown to be effective against mucosal stages of equine cyathostomes. This study was designed to confirm the effectiveness of the drug against early L_3 and developing larvae in the mucosa at varying time points following treatment and to compare that with the mucosal and lumenal burden of animals receiving no treatment and animals repeatedly treated with pyrantel pamoate, an anthelmintic effective against only lumenal stages.

Three groups of 4 ponies each with natural cyathostome infections were treated with moxidectin at a dose rate of 0.4 mg/kg b.w. Necropsies were performed on days 21, 49, and 84. Three groups of 4 ponies were left untreated and necropsied at similar time points. A third set of animals was treated with pyrantel pamoate at 28 day intervals.

Moxidectin was highly effective against early L_3 (85%) and developing larvae (95%) 21 days following treatment; 53-83% effective against developing larvae at 49 and 84 days; and 88% effective against EL_3 at 49 days and 20% at 84 days. Total parasite burdens in control animals were reduced by 66% over the 84 day period, which probably explains the seemingly lower efficacy of moxidectin at this point. Treatment with pyrantel pamoate effected removal of lumenal parasites and subsequent development and emergence of mucosal larvae into lumenal stages.

b.1.08 EFFECT OF RAIN ON THE EFFICACY OF TOPICAL ABAMECTIN AGAINST PARASITES OF CATTLE

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Background: Grazing cattle treated with a topical formulation may be exposed to unpredictable weather conditions.

Method: A critical trial was undertaken to assess the effect of simulated rain applied in a fine spray, delivering 20 mm of rain over a 30 minute period starting 1 hr after treatment (group 2) or ending 1 hr prior to treatment (group 3). Untreated, unwetted cattle (group 1) and treated, unwetted cattle (group 4) were used as negative and positive controls respectively. Following confirmation of worm-free status, 20 beef calves (92-163 kg, 5-7 month old) were challenged orally with a mixed dose of infective larvae as per WAAVP guidelines (*Ostertagia ostertagi* [Os] : 15000 L_3; *Cooperia spp.* [Co] : 15000 L_3; *Trichostrongylus axei* [Tr] : 15000 L_3; *Haemonchus placei* [Ha] : 12000 L_3). Cattle were randomly assigned to one of the 4 groups and treated 28 days later with 500mcg/kg abamectin (Virbamec® Pour On, Virbac, 1 ml/10kg) applied along the backline of each animal. Cattle were maintained under controlled pen conditions and on wholly supplementary feed sources, to prevent larval reinfection. Animals were slaughtered 13-14 days after treatment for worm burden determininations on abomasum and small intestine content according to WAAVP parasitological procedures.

Results: The geometric mean worm counts and % efficacy (Abbots formula) in groups 1, 2, 3 and 4 were respectively: 738(-), 1(100%), 1(100%) & 0(100%) for *O.ostertagi*; 8072 (-), 287(96%), 720(91%) & 69(99%) for *Cooperia* spp., 507(-), 2(100%), 18(96%) & 2(100%) for *T.axei*, and 421(-), 0(100%), 0(100%) & 0(100%) for *H.placei*. No adverse drug reactions were noted.

Conclusion: Exposure of cattle to precipitation had no effect (α=5%, Kruskall Wallis, one way anova) on anthelmintic efficacy.

b.2.01-08 Immunity against protozoan and helminth infections

b.2.01 MATERNAL IMMUNE RESPONSES TO *NEOSPORA CANINUM* AND FETAL INFECTION

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Background: The protozoal parasite *Neospora caninum* is a significant cause of abortion in dairy cattle. Results from several epidemiological studies suggest that the major natural route of infection is through transplacental transmission of the parasite from an infected dam to the fetus. To understand the mechanisms of vertical transmission, studies were conducted to investigate the immune responses in naturally infected dairy cattle.

Method: Cows with a previous confirmed *Neospora* abortion and/or congenital infection were bred by artificial insemination and evaluated for immune responses throughout gestation until 2 weeks before term, when calves were removed and sera analyzed for *Neospora*-specific titers. Humoral responses of pregnant cows were assessed by measuring serum antibodies to whole parasite and to 2 immunodominant tachyzoite proteins. Cellular immune responses were determined by measuring *Neospora*-specific proliferative responses in peripheral blood mononuclear cells (PBMC).

Results: An increase in serological responses and in PBMC proliferation were observed in all cows following gestation. In cows, whose fetuses were not infected, an increase in both humoral and cellular responses occurred within the first 20 weeks of gestation. Cows which had infected fetuses did not show an increase in immune responses in the first 20 weeks of gestation, but a peak in responsiveness occurred during the last trimester of gestation.

Conclusion: An increase in *Neospora*-specific humoral and/or cellular immune responses within the first 20 weeks of gestation in naturally infected cattle results in a reduced incidence of fetal infection.

b.2.02 *NEOSPORA CANINUM* INFECTIONS IN FETUSES AND DAIRY COWS WITH ABORTIONS IN ARGENTINA

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Background: *Neospora caninum* is a major cause of abortion in dairy cattle in several countries. The purpose of this study was to determine the frequency of transplacental infection by *N. caninum* and its prevalence in dairy cattle from Argentina.

Method: Sera from 104 fetuses (82 from dairy and 22 beef cattle), 5-8 month gestational age, were obtained from a slaughterhouse and examined for *N. caninum* antibodies at a 1:80 dilution using an indirect immunoflorescence test (IFAT). Brains from 8 seropositive fetuses were examined histologically. To attempt to isolate *N. caninum*, brain tissue from 1 seropositive fetus was inoculated into 22 BALB/c mice. Mice were tested for antibodies to *N. caninum* at 1:50 and 1:100 dilution by IFAT. Sera from 177 dairy cows that had aborted from 16 dairy farms were tested serologically at 1:800 dilution by IFAT.

Results: Antibodies to *N. caninum* were found in 20 of 82 (24.4%) dairy cow fetuses, in 1 of 22 (4.5%) beef cow fetuses, and in 70 of 177 (39.5%) dairy cows. Lesions suggestive of neosporosis were found in brains of 7 of 8 fetuses. Antibodies (IFAT, 1:100) to *N. caninum* were found in 1 of 22 mice inoculated with brain tissue from the seropositive fetus.

Conclusion: The results indicate that transplacental *N. caninum* infection is common in Argentina.

b.2.03 *NEOSPORA CANINUM*: IMMUNITY IN WILD TYPE C57BL/6 VERSUS TRANSGENIC B-CELL-DEFICIENT MICE

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The immunology of infections with *Neospora caninum* was studied in an appropriate mouse model. Thus, experimentally infected wild type (wt) C57BL/6 mice and B-cell deficient mice (transgenic mutation in the transmembrane exon of the IgM μ chain gene; μMT) exhibited divergent immune responses and courses of infection. While C57BL/6 mice were resistant to disease, μMT mice died between day 29 and 31 p.i.. PCR-analyses and histology showed that more organs with a significantly higher infection and multifocal necrotic lesion intensity were parasitized in μMT mice than in wt mice. In wt mice, no inducible nitric oxide synthase (iNOS) could be detected in any brain cell. Conversely, in the brain of μMT mice, characterized by multifocal lesions and numerous parasites, often clustered around a central necrotic lesion, numerous macrophage-like cells were iNOS-positive. Splenocytes from infected mice were *in vitro* cultivated. Cells from μMT-mice produced less IFN-γ and IL-10 than wt splenocytes. The phenomenon became more evident for IL-10 with increasing time of infection. Consequently, the susceptibility of μMT mice seemed to be partially related to a decreased production of IL-10 in spleen cells and to an increased iNOS production in the brain. In a vaccination trial, we assessed both recombinant protein rec-p43 and tachyzoite-somatic-antigen (TSA) for their potential to provide protective immunity against challenge *N. caninum* infections. Compared to infected (non-immunized) control animals, infected mice having been immunized with rec-p43 or TSA exhibited a statistically lower PCR-positivity in brain and liver, suggesting that vaccination with either Nc-p43 or *N. caninum* TSA may be partially protective against subsequent *N. caninum* challenge infection.

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b.2.04 T CELL RESPONSES IN CALVES TO A PRIMARY *E. BOVIS* INFECTION: PHENOTYPICAL AND FUNCTIONAL CHANGES

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The study aimed to characterize T cell responses in calves to a primary *E. bovis* infection. For this purpose peripheral blood lymphocytes (PBL) were isolated from six infected calves and three controls during prepatency (day 12 p. i.), patency (day 25 p. i.) and postpatency (day 35 p. i.). In addition, lymphocytes were isolated from various lymphatic organs, peyer's patches and spleen at necropsy (day 35 p. i.). FACS analyses showed significantly increased proportions of $CD4^+$ and $CD8^+$ PBL 12 days p. i. in infected calves. Subsequently these levels tended to normal until postpatency. Levels of PBL $CD2^+$ cells were elevated throughout the observation period when compared to controls, whereas proportions of $\gamma\delta^+$ PBL remained unchanged. In contrast, lymphatic organs of infected animals contained increased proportions of $CD4^+$, $CD2^+$ and $\gamma\delta^+$ cells but not $CD8^+$ cells when compared with uninfected calves (day 35 p. i.). PBL and cells from lymphatic organs showed strong proliferative responses to Con A, without significant differences between the groups. *E. bovis* merozoite I antigen (EbAg) did not stimulate cells of control calves. PBL of infected animals reacted strongly 12 days p. i. but subsequently decreased and reached control values until day 35 p. i. In contrast, strong proliferative responses to EbAg were observed 35 days p. i. by cells from lymphnodes draining *E. bovis* infected gut areas. Increased transcription of the IL-2 gene but not of the IL-4 gene in gut associated lymphnodes of infected calves were detected when compared with controls. In conclusion the data suggest a predominant role of Th1 cells in the immune response to a primary *E. bovis* infection.

b.2.01-08 Immunity against protozoan and helminth infections

b.2.05 IMMUNOPROTECTION OF MICE AGAINST *TRYPANOSOMA BRUCEI* WITH TUBULIN

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Tubulin forms the major protein component of microtubules, which are well distributed pellicularly beneath the surface coat of trypanosomes and are well dispersed in the cytoplasm participating in very vital cellular functions. We report here the immunotherapeutic potential of tubulin against trypanosomosis. Tubulin was purified from *Trypanosoma brucei* and used for immunizing mice. Synthetic peptides with 12 (STP 12) or 14 (STP 14) amino acids from the carboxyl terminal of *Trypanosoma brucei rhodesiense* tubulin cDNA were also synthesized and used in similar immunization studies. The trypanosome tubulin (NTP) was strongly recognised by anti-chicken tubulin monoclonal antibodies in immunoblots. This tubulin fraction induced protection in mice against an otherwise lethal challenge of 10^3 trypanosomes and 87% of the mice did not develop any parasitaemia at all in the 60-day observation period. No protection was observed for the synthetic peptides when compared to the controls. The NTP fraction induced a strong antibody response by ELISA assays which was specific to trypanosome tubulin in western blots. These results suggest that trypanosome tubulin may serve as an immunotherapeutic target against trypanosomosis

b.2.06 ISOTYPE RESPONSES OF RUMINANTS TO *FLUKE* INFECTION TO DEFINED ANTIGEN

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Background: This study investigated the immunoglobulin isotype responses of sheep and cattle chronically infected with *F. hepatica* and *F. gigantica* to adult *F. hepatica* Cathepsin (Fh-cathepsin)
Method: ELISA was used to determine serum antibody (total Ig, IgG1, IgM, IgG2 and IgA) responses to adult Fh-cathepsin.
Results: There was an early (2-3 weeks post infection (wpi) total Ig and IgG_1 responses to Fh-cathepsin in both *F. hepatica* infected sheep and cattle. However, there was a slight delay (7 wpi) noted in the response to Fh-cathepsin by *F. gigantica* infected sheep and cattle. The serum isotype response was predominantly IgG_1. The IgM response was early in both species. The serum IgG_2 and IgA isotype responses to Fh-cathepsin were late and more pronounced (11 wpi and 19 wpi respectively) in cattle, less marked in sheep.
Conclusion: The dominance of the IgG_1 response in *Fasciola spp.* infected sheep and cattle suggest an associated Th_2 response in both species. The late IgG_2 response in cattle may suggest late Th_1 involvement in bovine cellular responses to adult Fh-cathepsin. There is stronger response to Cathepsin by cattle as compared to sheep The detection of serum antibody responses to Fh-cathepsin in *F. gigantica* infected sheep and cattle confirmed antigenic cross-reactivity.
Acknowledgement: We wish to thank Prof. J.P. Dalton of city university Dublin for providing Cathepsin L1 proteases and Dr. Beh of McMaster laboratories in Australia for providing monoclonal antibodies.

b.2.07 ACQUIRED RESISTANCE TO *SCHISTOSOMA JAPONICUM* INFECTION IN PIGS.

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Background: While it has been shown that pigs are resistant to challenge infection with *S. japonicum* when harbouring a primary patent infection, the time-span required for pigs to mount this resistance after a primary infection is unknown.
Method: Different groups of pigs were exposed to a primary infection with one *S. japonicum* isolate and to challenge infection with another isolate at weeks 1, 4, 6, 8 or 12 past primary infection. The isolates could be distinguished by PCR-RFLP after perfusion of worms.
Results: Only the challenge infection at week 1 resulted in a higher worm burden when compared to a single primary infection. PCR-RFLP of perfused worms revealed that challenge schistosomulae were not entirely prevented from establishing. On the contrary, the proportion of worms originating from challenge infection was shown to increase at the later challenge infections, however without an increase in the total worm burden. None of the challenge infections resulted in elevated faecal or tissue egg counts when compared to primary infected groups.
Conclusion: Pigs are able to mount a partial resistance to reinfection already four weeks after a primary infection.
Acknowledgement: This study was supported by the Danish National Research Foundation.

b.2.08 IMMUNOGENICITY OF PRAZIQUANTEL-ELIMINATED *SCHISTOSOMA BOVIS* INFECTION IN GOATS

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Background: West African Dwarf (WAD) goats acquire protective *S. bovis* immunity (anti-fecundity). Praziquantel treatment will eliminate this parasite.
Methods: 42 WAD goats were divided into weight-balanced groups. Variables: primary exposure (P) week 0, praziquantel treatment (T) week 13, challenge exposure (C) week 36. Exposure: 800 cercariae per goat. Groups: P_1, P_2, PT, PTC, PC and F (parasite-free). Necropsy (worm and tissue egg counts): week 36 for group P_1, week 53 for all other groups. Current parametres monitored at two-week intervals: faecal egg counts, body weights, haematology.
Results: Following challenge infection, neither faecal egg excretion nor its consecutive pathogenic effects were significantly increased in goats previously exposed to primary infection (groups PC and PTC). Mean worm counts of groups P_2, C and PC were 173, 287 and 457, respectively, i.e. the challenge worm burdens were established independently of previous exposure.
Conclusion: Under experimental conditions, acquired protection against the pathogenic effects of a homologous challenge infection was not hampered by praziquantel elimination of primary *S. bovis* infection in WAD goats. Thus, praziquantel could be used in the field without impairing the acquisition of protective immunity in goats. If the same applies to cattle, one or few routine treatments in young stock may be useful in schistosomosis bovis control.
Acknowledgement: We wish to thank the Danish National Research Foundation and RVAU for supporting this project directly or indirectly through DCEP.

b.3.01-08 Integrated and biological parasite control

b.3.01 ENTOMOPATHOGENIC NEMATODES AS CONTROL AGENTS OF *IXODES SCAPULARIS*

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Background: Significant human illness and associated economic cost can be attributed to the tick, *Ixodes scapularis*, which transmits the etiological agent of Lyme disease, *Borrelia burgdorferi*, and other human pathogens. Although acaricides are commercially available for the control of *I. scapularis*, concerns about the environmental impact and safety of such chemical compounds has limited their use by the public. Entomopathogenic nematodes (EPNs) are promising biological control agents for a number of economically important insect pests, and may be useful as tick control agents.

Method: Thirteen species or strains of EPNs of the genera *Steinernema* and *Heterorhabditis* were tested in vitro against unfed and replete larvae, nymphs, and adults of *I. scapularis*. We also tested and compared 2 variants of *S. riobravis* (355 and OSCA) for efficacy against replete *I. scapularis* females in field trials using box plots ($1m^2$) placed along the meadow/woodland interface. The plots were seeded with replete female *I. scapularis* and sprayed with $1x10^5$, $5x10^5$, or $1x10^6$ *S. riobravis* 355 or OSCA.

Results: All EPN strains were pathogenic to replete female ticks in vitro, but not to unfed or replete larvae, nymphs, males, and unfed females. *Steinernema riobravis* (355) and *H. megidis* (M145) killed replete female ticks most rapidly in vitro, with a mean day of death of 2.5 and 3.5 days post-infection, respectively. In field trials, *S. riobravis* 355 and OSCA were both highly pathogenic to *I. scapularis* replete females, resulting in killing within 5 days of 93 and 100% of ticks respectively, in plots treated with $1x10^6$ EPNs.

Conclusions: These data suggest that EPNs may be useful as an alternative management method for *I. scapularis* populations, and may be more acceptable than acaricidal chemicals for use in tick infested areas.

b.3.02 COMPARISON OF THREE TRAP DESIGNS FOR THE NON-CHEMICAL CONTROL OF LIVESTOCK FLIES IN THE UNITED KINGDOM.

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Background: Nuisance flies are one of the major groups of ectoparasites attacking cattle in the United Kngdom and are a major source of economic loss to the cattle industry as well as a threat to animal welfare. The control of nuisance flies is heavily reliant upon chemical methods beset by toxicity problems and insecticidal resistance. Alternative, non-chemical methods are sought. A novel panel trap is compared to two existing designs of trap.

Method: 3 types of trap were compared. Nylon-reinforced plastic panel traps ($4000cm^2$ area, coated in non-drying adhesive), Manitoba and Tsetse F3 traps. All traps were sited on pasture with a history of nuisance fly problems. Effectiveness of the traps was compared by the relative numbers of *Stomoxys calcitrans*, (Stable fly), *Musca autumnalis*, (Face fly) and Tabanidae (Horse flies) caught. A comparison of trap design was carried out with carbon dioxide as a simple olfactant.

Results: The number of *Stomoxys calcitrans* and *Musca autumnalis* caught by the panel traps with or without carbon dioxide were significantly higher, $p<0.05$ than numbers caught by Manitoba or Tsetse traps. Manitoba traps caught significantly more ($p<0.05$) Tabanids than panel or Tsetse traps only when carbon dioxide was used. Overall carbon dioxide increased the number of flies and number of species caught by all traps.

Conclusions: The nylon-reinforced plastic panel trap was the most effective trap design for capture of *Stomoxys calcitrans*, *Musca autumnalis* and Tabanids. The design looks promising for the control of these species. The importance of olfactory and visual cues for trapping nuisance flies have been demonstrated.

b.3.03 TRICHOSTRONGYLOSIS IN SEPARATE AND MIX-GRAZED FIRST- AND SECOND-SEASON CALVES

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Background: This study was designed to evaluate whether the grazing of young, susceptible calves together with second season resistant heifers can reduce the levels of infective trichostrongyle larvae on herbage and protect susceptible calves from outbreak of clinical trichostrongylosis.

Method: Four groups of ten animals each were turned out and grazed according to the following scheme: plot A was grazed by first-season calves; plot B was grazed by first-season calves together with second-season heifers; plot C was grazed by second-season heifers alone.

Results: The herbage larval counts were significantly reduced in the second part of the grazing season on mix-grazed plot B compared to that on plot A grazed by first-season calves alone ($P<0.05$). The second-season animals did not suffer parasitologically in mix-grazing with the first-season calves.

Conclusion: The lower pasture contamination with infective larvae on plot B presumably was achieved due to older cattle which played their role through simple dilution of larval density, and hence larval intake of calves. Thus, the mix-grazed first-season calves were protected from clinical outbreak of trichostrongylosis.

Acknowledgement: The investigation was supported financially by the Food and Agriculture Organization of the United Nations.

b.3.04 CLEAN PASTURE STRATEGY SELECTS FOR BENZIMIDAZOLE RESISTANCE IN *HAEMONCHUS*

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A selection study was performed to investigate the effect of the clean pasture strategy on the development of resistance in *Haemonchus contortus*. Three groups of seeder lambs infected with composite *Haemonchus* strains consisting to 1, 10 or 50% of a benzimidazole (BZ) resistant strain were divided into 4 subgroups which were treated with 0, 0.25, 1.25 or 5 mg/kg fenbendazole (FBZ) 4 weeks p. i. The subgroups were then kept on separate clean paddocks for 3 weeks. After a short non-grazing period, each plot was grazed by tracer lambs for 3 weeks. Controlled tests using 5 mg/kg FBZ were conducted with each tracer group. In addition, an allele-specific PCR which allows to determine the genotype of individual *Haemonchus* (Kwa et al., Mol. Biochem. Parasitol. 63, 1994, 299) was performed on DNA from worms collected from untreated tracers.

Even at the "low" proportion of 1% BZ resistance in the initial worm population, already one single treatment mit 5 mg/kg FBZ followed by a turnout onto clean pasture resulted in a total drench failure in the following worm generation as shown by worm counts. The higher the portion of BZ resistance in the initial worm population and the higher the FBZ dose used at turnout the lower the frequency of susceptible allele homozygotes was in the worm populations of untreated tracer lambs.

The present data confirm simulation models which indicate that a drug application - when the source of reinfection from pasture is low - will result in rapid selection for anthelmintic resistance in trichostrongyles.

b.3.01-08 Integrated and biological parasite control

b.3.05 SYSTEMATIC VS SELECTIVE TREATMENT TO CONTROL TRICHOSTRONGYLOSIS IN GOATS

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Background : Resistance to anthelmintics (AH) in population of nematodes, parasite of the digestive tract is of a major concern in ruminant breeding and particularly in goats. One solution to slow down the spread of AH resistance is to apply treatments selectively on the most receptive animals of a flock rather than on a systematic basis. In dairy goats, previous results have shown that the animals in first lactation and with the highest level of milk production are the most susceptible to parasitism. The objective of the present study was to assess the effectiveness of a selective treatment to control trichostrongylosis in goats.

Method : A flock of 120 dairy goats was divided in 2 equal groups according to the age composition and the level of production. In group 1, all the animals were drenched at the start of the study. In group 2, AH was given only to 11 goats in first lactation and 13 high producers. Hence, only 40 % of the animals from group 2 were treated. The 2 groups grazed separately infected pastures for 4 months. Parasitological and production data were collected monthly.

Results : No difference in fecal egg excretion was detected between the 2 groups throughout the whole study as well as no difference in the level of pasture contamination. This lack of difference in parasitological measurements was associated with similar level of milk yield, protein and fat contents.

Conclusion : These results suggest that a selective programme control of nematodes in dairy goats could be as efficient as a systematic one. In addition to financial advantages, this selective use of AH will also avoid further development of resistance in worm populations.

Acknowledgments : This work was supported by the FAIR3CT96-1485 program.

b.3.06 INDUCTION OF IMMUNITY TO *DICTYOCAULUS VIVIPARUS* WHILE UNDER TREATMENT WITH ENDECTOCIDES

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Three groups of five parasite naïve calves were used. The treatments were: Group 1; calves were weighed on Day 0 and injected with doramectin at 200µg/kg. From Day 1 to 19 they were dosed orally with 2000 infective larvae of *D.viviparus* . On Day 28 they were again injected with doramectin, and infected with *D.viviparus* larvae from Days 33 to 41. They were then left untreated until Day 81 when they were infected with 20 infective larvae of *D.viviparus* / kg body weight. They were killed on Day 110 and lungworm counts carried out: Group 2 were immunised with oral lungworm vaccine on Days 0 and 28, and infected and slaughtered as Group 1 on Days 81 and 110 respectively: Group 3 acted as infection controls. Blood samples were taken at Days 0, 21,49, 77 and 110 for antibody tests to *D.viviparus* . At autopsy there were no significant differences between the number of lungworms from Groups 1 and 2 (Means 17.4 and 31.3 respectively); both had significantly less than Group 3 (Mean 228) ($p,0.01, p<0.05$). Increased antibody titres to the larval sheath of the infective larvae were observed from Groups 1 and 2, showing that the larvae in Group 1 had penetrated the intestine before being killed by the circulating anthelmintic. This experiment shows that if calves are exposed to infective larvae while under systemic endectocide cover, an immune reaction is stimulated.

b.3.07 A RECOMBINANT *COOPERIA ONCOPHORA* ELISA FOR HERD HEALTH MONITORING IN CALVES

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Background: An ELISA using a recombinant 14.2 kDa excretory/secretory *Cooperia oncophora* protein has been developed and shown to be highly sensitive to and specific for *C. oncophora* infection in first season grazing calves. The ELISA is evaluated for use in herd health monitoring programs. Here, we present both experimental and field data supporting the use of IgG levels to assess the cumulative exposure to infection during a first grazing season.

Method: Data from one experiment were used to obtain a quantitative relationship between IgG levels and cumulative exposure (*i.e.* cumulative number of L3 calves ingested). That relationship was validated against data from another experimental study and from field trials. The former consisted of 3 groups of calves receiving either 250, 1,250 or 5,000 L3 twice a week for 18 weeks. The latter included different pasture management strategies with or without an anthelmintic treatment. Validation involved 'predicting' cumulative exposure based on observed IgG levels, and subsequently comparing those 'predictions' with observed cumulative exposures. In the field trials, observed exposure was based on calculations from pasture larval counts and tracer worm burdens. A 4 week delay was assumed for an IgG response to infection.

Results: 'Predicted' cumulative exposures correlated well to observed exposures. However, 'predictions' underestimated observed exposures in the field trials. This might have been caused by differences between parasite strains involved in the experimental studies and the field trials. The anthelmintic treatments in some groups of the field trials reduced the accuracy of the 'prediction'.

Conclusion: The results suggest that IgG levels allow evaluating how much exposure to infection calves have experienced in the first grazing season.

b.3.08 EFFECT OF DUNG BEETLES ON THE NEMATODE LARVAE RECOVERED FROM COW FAECES

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The objective of this study is to evaluate the ability of dung beetles (Coleoptera: Scarabaeidae) as the biological control insect by dispersal of faecal masses containing bovine gastrointestinal nematodes. Two species of local dung beetles, *Onitis* spp. and *Onthophagus seniculus* were used for accomplishment of 2 experiments. Experiment I was achieved by the introduction of adults *Onitis* spp. and *O. seniculus* to 100 or 200 g of buffalo faecal mass for overnight. The result showed that the most effective number of these dung beetles for dispersal of the faecal masses (≥ 90%) were 10 and 20, respectively. Experiment II was proceeded by using 10 and 20 adults of *Onitis* spp. and *O.seniculus* for seperately introduction to 100g of cattle faeces which contaminated with gastrointestinal nematodes. The result indicated that *Onitis* spp. and *O.seniculus* were able to reduce the amount of larva at 95.9 and 89%, respectively by using indoor laboratory conditions whereas 96.8 and 91.3% were observed by using the outdoor laboratory conditions.

b.4.01-08 Parasites of wildlife

b.4.01 CRYPTIC *TELADORSAGIA* SP. IN MUSKOXEN: FAUNAL DIVERSITY & HISTORY IN THE ARCTIC

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Background: The polymorphic ostertagiine, *Teladorsagia circumcincta* has a broad host range, extensive geographic distribution and is characterized by substantial morphological variation. Such observations are consistent with a hypothesis that *T. circumcincta* represents a complex of sibling species in wild Holarctic ruminants. We conducted a test of this hypothesis based on collections of abomasal nematodes in muskoxen from the central Canadian Arctic.

Methods: Nematodes in muskoxen and caribou were evaluated by integrating data for comparative morphology, morphometry and molecular sequences.

Results: A new polymorphic *Teladorsagia* sp. was diagnosed based on constant differences in the ND4 region of mtDNA (13% divergence), and a suite of significant morphometric and structural characters. Discovery of a cryptic *Teladorsagia* sp. suggests the necessity to re-evaluate the distribution of *T. circumcincta* in wild artiodactyles.

Conclusions: A new *Teladorsagia* in muskoxen in part corroborates the existence of a cryptic species complex among Caprinae and perhaps Cervidae at high latitudes and indicates the importance of climatological determinants during the late Tertiary and Pleistocene in the diversification of the Arctic fauna. The Arctic and Boreal fauna, particularly in North America, is a mosaic of endemic and introduced species. A new *Teladorsagia* sp. highlights the paucity of knowledge for faunal diversity among nematodes in Holarctic ruminants.

b.4.02 ARCTIC DEVELOPMENT OF *UMINGMAKSTRONGYLUS PALLIKUUKENSIS* IN *DEROCERAS LAEVE*

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Background: *U. pallikuukensis* (*Up*) is a protostrongylid lungworm in muskoxen near Kugluktuk, Canada (67°54'N, 116°38'W). We studied development of larvae in the slug *D. laeve*, and overwinter survival of infected slugs in the field.

Methods: Slugs were experimentally infected with first stage larvae of *Up* and placed in enclosures on the tundra every two weeks from June 19 to August 28, 1997 and weekly from June 10-24, 1998. Slugs from subsets of enclosures were examined biweekly (1997) or weekly (1998). Some 1997 enclosures were left on the tundra overwinter (OW). Vegetation from June and July 1997 enclosures was examined for third stage larvae (L3) in fall, 1997 and spring, 1998.

Results: L3 were present within four to six weeks in slugs infected before or on July 17. Slugs infected on July 31 or later did not contain L3 by winter. In fall 1997 and spring 1998 L3 were recovered from vegetation of June 19 and July 3, 1997 enclosures. In spring 1998 slugs containing larvae were recovered from OW enclosures and these larvae completed development to L3 during summer 1998.

Conclusions: Development and transmission of *Up* may be influenced by change in temperatures. Global warming is anticipated to impact arctic ecosystems. *U. pallikuukensis* in muskoxen may be a model system to study the linkage of climate change to wildlife health and emerging helminthic disease.

Acknowledgments: Supported by: Government of the Northwest Territories, a WCVM Interprovincial Graduate Scholarship, Merial Inc., Northern Scientific Training Program (DIAND Canada) and the WCVM Wildlife Health Fund.

b.4.03 ANTIBODIES AGAINST *TOXOPLASMA GONDII* IN FENNOSCANDIAN SEMI-DOMESTICATED REINDEER

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Background: Reindeer husbandry has been intensified strongly during the last few decades, especially in Finland. It is now common to collect reindeer herds in corrals for winter-feeding. Corral-feeding has been associated with some bacterial and viral diseases transmitted directly between animals. Corralling will also increase contact with cats, the faeces of which is for herbivores the main source of the protozoan *Toxoplasma gondii.* The aim of this study was to identify determinants associated with antibodies against *T. gondii* in reindeer.

Methods: Serum samples (n=2577) collected between 1993 and 1996 from reindeer abattoirs in Finnmark county, Norway, and several locations in northern Finland, were investigated by the Direct Agglutination Test (DAT) for specific antibodies against *T. gondii.* Logistic regression analysis was used to identify determinants significantly associated with antibodies (=infection).

Results: The overall prevalence in this sample was 0.9 %. Seropositivity was positively associated with the age of the animal, and with «*corralling index*», based on the proportion of animals corralled and the length of the corralling period. No association was found with sex, or the frost sum of the pasture area.

Conclusions: Toxoplasmosis in reindeer is a rare infection, but increasing domestication increases also the probability of animals getting infected.

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b.4.04 THE FREE-LIVING STAGES OF GASTROINTESTINAL NEMATODES OF ARCTIC REINDEER.

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Background: To understand the interaction between gastrointestinal nematodes and their reindeer host on the arctic island of Spitsbergen it was crucial to investigate the abundance and distribution of nematode larvae on the pasture. In this context morphology studies and experiments on the development of larvae were important instruments.

Method: Larvae lengths were measured and different microscopical techniques including SEM and laser confocal microscopy were used to obtain images of the larvae. Discrimination between infective larvae of the *Ostertagia gruehneri* morphs and *Marshallagia marshalli* morphs were given particular emphasis. Laboratory cultures at different temperatures with controlled relative air humidity were used to study development. Field experiments to study development and migration of larvae from faeces to the pasture were performed. The density and distribution of infective larvae on pasture was investigated by sampling of herbage.

Results: Culture studies showed that about 200 day °C were needed for development from egg to infective larvae. Development to first and second stage, but not to infective stage was observed within 32 days at 4 °C. In faecal paths eggs develop to infective larvae and moved on to the pasture within 14 days in the first of September. The distribution of larvae on the pasture were highly aggregated with low numbers of larvae per unit area.

b.4.01-08 Parasites of wildlife

b.4.05 HELMINTH DIVERSITY DIFFERENCES IN THE GENERALIZED AND SPECIALIZED MUSTELIDS

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Background: To reveal differences in helminth diversity between generalized and specialized mustelid species, the helminthocenoses of the otter (*Lutra lutra*) and polecat (*Mustela putorius*) populations were compared.

Methods: In Belarus, 49 otters and 73 polecats were helminthologically dissected as well as ecological niches (diets and habitat utilizations) of these predators were investigated in detail.

Results: By comparing the helminthocenoses of the otter and polecat populations as well as the helminthocenoses of other mustelid species (in publications and by own data), the following important regularity of helminthocenosis forming in mustelids was revealed. Taking into account the fairly low level of the morpho-physiological divergence in mustelids and, so, the high similarity of habitat conditions for helminths therein, helminth diversity in mustelid populations mainly depend on their ecological niches which determine different probability to be infested by higher diversity of the mustelid helminths. Thus, in Belarus otters inhabiting water ecosystems and specializing in feeding on fish and crayfish were infested by 11 species (total infestation varied 67-72%), whereas polecats inhabiting all terrestrial ecosystems (including shores and river banks) and having a catholic diet were infested by 22 species (total infestation varied 85-100%).

Conclusion: Width of ecological niche is one of the main factors conducts helminthocenosis forming in mustelid populations.

b.4.06 PHYLOGENY OF HUMAN AND WILDLIFE PIROPLASMS FROM THE WESTERN U.S.A.

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Background: In the western United States, a new species of human *Babesia* has been documented with the occurrence between 1991 and 1995 of seven human cases in the California and Washington. Concomitantly, we have isolated similar babesial parasites from four species of artiodactylids and one species of rodent in California. The purpose of this study was to investigate the hypothesis that the wildlife *Babesia* spp. are the same as the human *Babesia* spp. as evidenced by matching genotypes of the 18S ssu-rRNA gene.

Method: The 18S ssu-rRNA gene of each *Babesia* isolate was sequenced. An alignment file containing the western isolates, piroplasms from the U.S.A., and other wildlife piroplasms was generated. Phylogenies were inferred using maximum parsimony and maximum likelihood methods.

Results: The western artiodactylid wildlife and human isolates though not genetically identical, were more closely related to each other than to *B. microti* or the *Theileria* spp. The isolate from the rodent was more closely related to the *Theileria* spp. than the human *Babesia* spp.

Conclusion: There is evidence that many of the U.S. western species of *Babesia* are closely related. This suggests that the wildlife may maintain potentially zoonotic *Babesia* spp.

b.4.07 The African Buffalo (*Syncerus caffer*): Gastro-intestinal parasites with special reference to *Toxocara* sp. in Kenya.

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Background: *Toxocara vitulorum* is a large roundworm affecting the small intestine of large ruminants mainly cattle and buffaloes. Infections are common among domesticated buffaloes in eastern Asia where they cause significant losses through reduced production and sometimes deaths. In Africa, little is documented on this nematode among cattle. Research in Kenya has established that, cattle grazing in cooler and humid pastoral areas have higher incidences of this nematode infestation. These same areas are forested and harbour the wild African buffalo (*Syncerus caffer*) and preliminary studies have indicated they may serve as hosts of this parasite. Research was therefore undertaken to determine the possible role played by the wild buffalo in the epidemiology of *Toxocara* infections and other gastro-intestinal parasites in cattle. Studies of this nature have not been conducted earlier with this wild animal which sometimes shares common grazing areas with cattle.

Method: Local Masai herdsmen around the forested Ngong Hills of Kajiado District of Kenya were recruited and supplied with labeled faecal bottles. They were asked to collect fresh individual buffalo droppings and indicate on the label the age of the source animal ie. adult or calf. The samples were collected weekly and analysed in the laboratory using the standard parasitological techniques for EPG, OPG, nematode egg and larval identification after culture.

Results: A total of 88 samples were analysed of which 47 were of calf origin, 8 % were positive for *Toxocara* eggs. Strongyloids were also encountered (38.6 %) and were made up mainly of *Haemonchus* and *Oesophagostomum*. Coccidian oocysts (26.1 %) comprising various species were also encountered. In addition, trematode eggs were observed in some limited faecal samples.

Conclusion: African buffaloes are hosts to *Toxocara* nematodes as well as strongyloids, coccidian and trematode parasites which do also occur in cattle. Since cattle and buffaloes sometimes share common grazing grounds especially during the dry season, there is a possibility of cross-transmission of these gastro-intestinal parasites. This is important for the institution of control measures in areas bordering buffalo inhabited pastures.

b.4.08 HELMINTHOSES IN THE REGULATION OF WILDLIFE POPULATIONS IN THE NATIONAL PARKS OF TANZANIA

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Background: Disease used to invade wildlife populations in Tanzania with devastating effects. Recent outbreak of a disease in buffalo and bushbuck in Arusha National Park resulted in the revelation of heavy internal parasite burden in these species. The need for a long term monitoring and assessment of the impact of helminthoses in the wildlife populations of Tanzania therefore becomes of paramount importance.

Method: Routine post-mortem examination of carcasses and fecal sample screening for parastes in national parks is being done by the staff of the Veterinary Department of Tanzania National Parks (TANAPA). **Results:** Deaths of new born as a result of Helminthoses is increasingly becoming a serious problem in highly encroached parks such as Arusha and Rubondo Island National Parks. The problem is less serious in parks with larger ecosystems such as Serengeti, Tarangire and Ruaha.

Conclusion: A need for intervention in affected parks is recommended to safeguard the well being and existence of their biodiversity. Measures should also be taken earlier to protect the less affected parks.

Acknowledgement: The authors wish to thank the organizers of WAAVP 99 for enabling us to attend. The paper is produced with permission of the Director General, Tanzania National Parks.

b.5.01-05 Economic impact of parasitic infections

b.5.01 VALUING TOTAL COSTS OF HEARTWATER IN SOUTHERN AFRICA.

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Background: Heartwater (HW), transmitted by *Amblyomma sp.* ticks, is considered one of the most important tick-borne diseases of cattle, goats and sheep in southern Africa, causing considerable losses through mortality and control costs. In support of a project to develop a new inactivated vaccine, we have carried out, as a first step for an economic impact assessment, an evaluation of the total costs associated with the disease and its control.
Method: A spreadsheet model was developed which incorporated uncertainty by using probability distributions of direct costs associated with HW for different species and production systems. A sensitivity analysis was undertaken to determine the relative importance of estimated parameters used for valuing costs.
Results: Preliminary results show that the commercial farming sector is more affected by HW than the communal sector due to the high susceptibility of animals undergoing intensive tick control. Since control costs are often shared with other vector-borne diseases, mortality and subsequent productivity losses are responsible for the majority of HW costs in both sectors. Sensitivity analysis highlighted the importance of obtaining reliable measures of incidence and mortality.
Conclusion: This study confirms the substantial costs associated with HW. It also provides a first indication of its the relative importance as a constraint across the principal production systems and will serve as the basis for an economic impact assessment study for alternative control methods including vaccination.
Acknowledgement: This study was supported by USAID grant No. LAG-1328-G-00-3030-00 awarded to the University of Florida.

b.5.02 EPIDEMIOLOGY AND ECONOMIC IMPACT OF BOVINE NEOSPOROSIS IN SWITZERLAND

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Neosporosis is a major cause of abortion and loss of young animals in the Swiss bovine industry. Using different diagnostic tools (*in vitro*-cultivation; histology; immunohistochemistry; serology; PCR), a retrospective abortion study has shown that *Neospora*-DNA could be amplified by PCR in 29% of the brains from aborted fetuses, with a simultaneous finding of typical cerebral lesions in most cases. The diagnostic technology was furthermore validated upon investigation of experimentally *Neospora*-infected pregnant cows and their fetuses. Based upon these first findings, a case-control-study was initiated, covering six cantons in three geographically disparate areas of Switzerland. So far, the results obtained in this study confirmed the relative high incidence of neosporosis and its role as a major cause of abortion in cattle. Epidemiological data indicated that vertical transmission occurs much more frequently than horizontal transmission. The duration of the abortion problem keeps on for a longer time in neosporosis-affected dairy farms than in other dairy farms with other abortion herd problems, thus reflecting the importance of the vertical transmission route. Our epidemiological data were compiled to provide an extrapolative basis for the estimation of the putative economic impact of bovine neosporosis in Switzerland: 800'000 cows/heifers with 85% annual birth potential (= 680'000 pregnancies) exhibit an average annual abortion rate of 2%, thus resulting in 13'600 total abortions. From these, 25% (= 3,400 abortions) may be caused by neosporosis, according to our epidemiological estimates. Taking into account the average economic loss of abortion of 3'000 CHF per case, the annual loss due to neosporosis in cattle industry will consequently amount to 10.2 million CHF, equivalent to 6.4 million €.

b.5.03 EFFECTS OF MOXIDECTIN TREATMENT ON MILK YIELD OF EWES AND GROWTH OF LAMBS

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Background: The beneficial effects (increased productivity) of anthelmintic treatment of dairy cows, lambs and wool-producing sheep have been assessed. In this study was attempted to investigate the effects of anthelmintic treatment of ewes on their milk yield and on the birthweight and the growth of their lambs.
Method: Thirty ewes were allocated into one of 3 equal groups: ewes in group A were given moxidectin (0.1% oral drench) and those in group B albendazole (2.5% oral drench) 3 to 5 weeks before lambing; ewes in group C were untreated controls. Faecal nematode epg counts, milk yield measurements and lambs weighing were carried out.
Results: The anthelmintic efficacy of moxidectin and albendazole was 100% and 95%, respectively, for at least 21 days but less than 70 days post treatment. All ewes completed pregnancy and lambed twins normally and without neonatal deaths. Mean milk yield throughout lactation of group A ewes was 27.2 l, that of group B ones 25.3 l and of group C ones 25.4 l. Mean birth weight of lambs born from group A and B ewes was 3.6 kg and that of group C 3.4 kg.
Conclusion: It was concluded that moxidectin treatment of ewes resulted in increased milk production. Furthermore, anthelmintic treated ewes gave birth to heavier lambs.

b.5.04 THE BENEFIT OF TOPICAL EPRINOMECTIN ON MILK PRODUCTION IN DAIRY CATTLE

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Background: Eprinomectin, an endectocide for beef and dairy cattle with a zero milk withholding period permits highly effective anti-parasitic treatment of lactating dairy cows at any stage of lactation.
Method: A study was conducted to evaluate the effect of eprinomectin administered at 500 mcg/kg topically or placebo treatment at calving on milk production of 742 dairy cattle, 560 cows and 182 heifers, in three New Zealand locations. Animals were pasture-fed on predominantly a single pasture at each location, supplemental feed was provided as needed, the same for all animals at each respective location. Cows were allocated to treatments within location based on their parity, expected calving date and production worth or breeding worth to replicates of two animals. Treatments were randomly assigned within replicates. Milk production was measured at approximately monthly intervals using the local dairy recording system. Actual amounts of milk and milk components (fat and protein) were reported.
Results: Daily milk production in liters and daily milk protein were significantly ($p<0.05$) higher for the eprinomectin-treated cows than for the placebo-treated controls (19.28 L milk/day compared to 18.86 L/day and 0.661 kg protein/day vs. 0.650 kg/day). None of the other variables differed significantly ($p>0.05$) between the treatment groups, although total milk production (+79.9 L), total milk protein production (+1.6 kg), total milk solids (+2.0 kg), daily milk fat (+0.007 kg) and daily milk solids (+0.017 kg) were also higher in the medicated group.
Conclusion: These results indicate that the treatment of dairy cows with eprinomectin at calving can improve their milk production.

b.5.01-05 Economic impact of parasitic infections
b.5.06-08 Implementation of research findings to the end user

b.5.05 TOTAL COMMITMENT TO DONKEY AND MULE WELFARE WITH NON-INTRUSIVE RESEARCH

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Background: Smallholder farms, transporters and women are increasingly using donkeys for cultivation, for transport and for income-generation, particularly in areas where draught cattle numbers have declined due to recurrent droughts. Donkeys are exposed to a wide variety of infections, of which parasitism is the major cause of ill health (Saul C; Siefert, L and Opuda-Asibo, Donkey Power Benefits, Workshop Reader, 1997, Volume 2, 58-63) probably causing marked losses in the productivity and activities of the donkey.
Method: The International Donkey Protection Trust runs mobile clinics and clinics in various parts of the world providing anthelmintic treatment and veterinary care to donkeys and mules. The donkeys and mules presented at the clinics were observed.
Results: The economic benefits from the use of anthelmintics has resulted in increased body condition, longer life expectation, reduced sores and in turn improved income-generation. For example, 10 years ago the donkeys of Lamu were in very poor condition. Many presented at the clinic were malnourished and due to beatings and lack of body fat were suffering from bad sores. The donkeys can now carry more weight per load, have more energy to perform their tasks willingly, therefore suffer less beatings and hence less sores.
Conclusion: Non-intrusive research into equine parasites can produce cost-effective improvement in animal health and welfare and therefore their draught ability and longevity. The charity is totally committed to expanding the work to all areas with a high proportion of working equines.

b.5.06 SMART DRENCHING – PHARMACOKINETICALLY BASED "BEST USE" OF ANTHELMINTICS.

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Background: Pharmacokinetic characteristics of anthelmintic compounds can be used to improve efficacy of commercial drench formulations. This information was provided to farmers as a number of on-farm recommendations that encouraged improved drench use.
Methods: Successful worm treatment utilised the term "Smart Drenching" and was described in articles in newspapers, farmer journals and magazines and supported by interviews on regional television and radio. Presentations at regional farmer meetings, agricultural field days and discussions with farmer advisers, with an information pamphlet succinctly describing concepts and major points of successful worm treatment, provided clear "take home" messages. The recommendations were developed to be in accordance with routine farm practices.
Results: Simplicity of presentation and minimal use of scientific jargon without trivialising the content clarified understanding. The advantages of "Smart Drenching" recommendations have been recognised and are now widely accepted by Australian sheep farmers. They are also being promoted in other major sheep producing countries.
Conclusion: Taking scientific information to the sheep farmer as a series of easily understood recommendations facilitated efficient anthelmintic use. The "Smart Drenching" recommendations can contribute to better worm control and extend the useable life of existing drench products.

b.5.07 HEALTH EDUCATION IN CYSTIC ECHINOCOCCOSIS IN MOROCCO

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Background: Cystic echinococcosis is highly prevalent in Morocco both in man and in domestic animals and is a serious public health concern. We have implemented a regional health education programme as part of a larger control system.
Method: Sectors involved in educational processes, including women taking care of pre-school children, were the primary aims. Attention was paid to various socio-cultural customs and religious beliefs. Brochures and posters were designed in order to inform children and women in rural areas about parasite transmission and prevention. Efforts to induce the participation of the community in the programme were made as well.
Results: Preliminary statistical analysis showed that the use of educational aids was of great benefit to the population at risk. People and school-children were very keen to learn about the disease and preventive measures. By its participation in the control programmes, the community gets involved in the development of its own health by improving its living conditions and restoring health and preventing diseases.
Conclusion: The dissemination of information through health education at the local level is an essential element in the prevention and control of diseases.
Acknowledgement: We wish to thank the Thrasher Research Foundation, Utah, for encouraging this work and funding the Compendium on cystic echinicoccosis in Africa and in the Middle Eastern Countries, with special reference to Morocco.

b.5.08 CONSIDERATIONS ON IMPLEMENTATION OF RESEARCH FINDINGS, -A NICARAGUAN CASE, WITH SOME COMPARISONS TO TANZANIAN.

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A socio-cultural community study has been carried out in order to understand indigenous knowledge and practice regarding traditional poultry-production. The fieldwork took place during 1998 in two villages in Tanzania and Nicaragua, and is part of the 3-year running multi-disciplinary research project, "Improvement of traditional poultry management".
Qualitative research methods such as participant observation, ethnographic interviews, focus group discussions and illness records have been used. The material is analysed according to anthropological theories.
Several socio-economic and cultural considerations should be taken into account when implementing research findings to the end user. Peoples management strategies for keeping or not keeping poultry varies according to cultural system of land-use, labour division, ecology, sex- and age-group. Different categories of women have different interests, resources and possibilities regarding poultry keeping. By categorising women according to a number of characteristics, the Nicaraguan case shows that married women in separate households with two or more children and who are capable of some planning and have access to feed for the poultry, are the most apt to use low scale poultry production as a income generating activity. This group find poultry production a reasonable, if not the only, possibility of generating economic resources. The poster concludes that poultry management is not a static practise but complex, dynamic and flexible where the poultry keeper changes practise and strategy according to her stage in life cycle.

c.6.00-10 Pathology of parasite infection

c.6.00 EICOSANOID PRODUCTION BY ADULT *FASCIOLA HEPATICA* AND PLASMA EICOSANOID PATTERNS DURING SHEEP FASCIOLOSIS.

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Background: *Fasciola hepatica* infection in sheep is known to cause anaemia, fever and elevated levels of liver enzymes. It was hypothesized that eicosanoids play a role in *Fasciola* associated pathophysiology.
Methods: The pattern of plasma eicosanoids during the course of acute and chronic fasciolosis was studied in sheep each infected with a single dose of 800 *F. hepatica* metacercariae. Blood plasma was collected weekly until week 17 post infection (w.p.i.) from infected sheep and uninfected controls. Adult *F. hepatica* were recovered from bile ducts and incubated for production of excretory/secretory products (ESP). Eicosanoids were determined by enzyme immuno assay in blood plasma, homogenates and ESP.
Results: Fever and anaemia were seen from 3 w.p.i. until 12 w.p.i. and 8 w.p.i. until 17 w.p.i., respectively. Onset of fever was accompanied by elevated liver enzyme activities (aspartate amino transferase and gamma glutamyl transferase) in the plasma. In general, the plasma levels of prostaglandin E2 (PGE2), prostaglandin I2 (PGI2) and leukotriene B4 (LTB4) were reduced during the acute and chronic stage of the infection, whereas thromboxane B2 (TXB2) was reduced only at 8 w.p.i. The TXB2/PGI2 ratio was increased in favour of TXB2 at 3 and 11 w.p.i. TXB2, PGI2, PGE2 and LTB4 were detected both in ESP and homogenates of *F. hepatica*.
Conclusion: Eicosanoid depletion in the plasma is caused by parasite-induced liver damage. The changes in eicosanoid levels are highly correlated to the clinical signs of the disease. Changes in the pattern of host plasma eicosanoid during fasciolosis as well as parasite derived eicosanoids may reflect or contribute to the pathology of the disease.

C insign assoc with larval cyath

c.6.01 PATHOLOGICAL AND PARASITOLOGICAL STUDIES OF *CYATHOSTOMINAE* INFECTIONS

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Background: Larval cyathostomiasis (LC) is an emerging disease in Argentina, notably in horse farms regularly using anthelmintic drugs. *Cylicocyclus insigne* is seen in these farms. Our aim is to study the pathology of cyathostome infection and the role of different species in LC. Have reported a farm (XX) that has suffered severe losses due LC and are studying it.
Methods: A) More than 3000 cyathostomes from XX and other farms were collected after Ivermectin treatment and classified. B) Standard histological techniques were used in 13 euthanized horses, with no LC signs. C) One horse in XX that died of LC during was also studied.
Results: A) In XX, *C. insigne* had a mean 43 % representation in the population of all resident horses. Horses from other origins had few or no *C. insigne*. B) In all normal horses, larvae of different sizes were seen in the large gut mucosa. They were lodged in cysts surrounded by an inflammatory granulomatous reaction, where lymphocytes predominated; there were many eosinophiles in the periphery; mast cells were scarce. The mucosal granulomae were smaller than the submucosal. The latter were sometimes placed above lymphoid nodules showing germinal centers. Some large larvae were seen in emergency, always surrounded by very strong inflammatory reactions. A marked, diffuse eosinophilic infiltrate was seen in the large gut mucosa. 2/4 horses had no *C. insigne* C) The gut contents of the LC horse were liquid and full of large red larvae. Similar larvae were seen massively emerging from the submucosa; an extensive ulcerative inflammation of caecum and colon was recorded. Only 2/101 collected worms were adults, classified as *C. insigne*. Above 60% of the larvae could be ascribed by size to the same species.
Conclusion: We confirm that acute LC is caused by the inflammation induced by emerging larvae. *C. insigne* may be more pathogenic than other species.

c.6.02 PATHOLOGICAL CHANGES ASSOCIATED WITH *GASTEROPHILUS NASALIS* INFECTIONS IN EQUIDS

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Background: Although the pathological significance of *Gasterophilus* spp is debated, inspection of infected ponies at an abattoir suggested pathological changes were associated with *G.nasalis*.
Methods: Stomachs and upper duodenum from animals infected with *Gasterophilus* spp were placed in ten per cent buffered formal saline at the abattoir. Samples were processed by the paraffin method, sectioned at five µm and stained with haematoxylin and eosin.
Results: Clusters of *G.nasalis* up to six cms in diameter were found in the duodenum adjacent to the pylorus. Elevated craterform lesions were present. The mucosa and submucosa was variably thickened, firm and white. Mononuclear cells, PMNs and eosinophils were observed in the lamina propria and submucosa. Ulceration associated with attached larvae was present. Deep mucosal accumulation of eosinophils representing early abscess formation was seen in two animals.
Discussion: Whilst there was no evidence for interference with function, the pathological changes observed suggest that treatment and removal of *G.nasalis* would be beneficial.
Supported by Merial Animal Health Limited.

c.6.03 ON THE USAGE OF PARASITIC DISEASE NAMES TEN YEARS AFTER THE PUBLICATION OF SNO(A)PAD

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The WAAVP initiated a move for consistency in the usage of parasitic disease names, and appointed an expert committee with the aim of codification of the principles of a standard disease nomenclature. The Standardized Nomenclature of Animal Parasitic Diseases (SNOAPAD, 1988) was based on the principle that the disease name is constructed solely by the suffix -osis, which is added to the stem of the name of the parasite taxon formed from the nominative of the taxa. After its endorsement by WFP in 1990 this guideline was termed SNOPAD and recommended for general use.
Major types of views expressed on SNOPAD are:
1/ welcome and support recognising that SNOPAD gives a consistent approach in contrast to the previous inconsistency of terms;
2/ indifference not recognising the advantageous aspects,
3/ antagonism on principle, based on the opinion that it may corrupt a fundamental tenet of the English language.
While the use of SNOPAD-conform terminology increased in the past decade, more in the field of veterinary than medical parasitology, inconsistency in nomenclatural usage still continues.
SNOPAD is meant to adopt the "one disease, one name" concept and thus to simplify both communication and information-handling in the field of parasitology. SNOPAD presents a standard list of disease names available as a source of reference for all those who wish to use a consistent disease terminology. Evolution makes its course both in parasitism and in the usage of parasitic disease names!

c.6.00-10 Pathology of parasite infection

c.6.04 EXPERIMENTAL CEREBROSPINAL ELAPHOSTRONGYLOSIS (ELAPHOSTRONGYLUS CERVI) IN GOATS

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Elaphostrongylus cervi is a common parasite of intermusclar connective tissue or central nervous system of Euroasiatic deer. The aim of work was to check its patogeny for domestic ruminants pastured on woodland pastures together with deer.

Ten goats 2 - 8 months old were infected orally with doses 300 - 10000 infective larvae of E. cervi. Pathological symptoms were registrated and died animals were autopsied.

Clinical symptoms occured after 7 - 22 dpi in animals infected with 3000, 5000 and 10000 larvae. At first were obserwed weakness, cough and difficult breathing, than paresis of hind legs, "sitting dog" position and paralisis of extremities.

Goats died between 15 and 36 days of invasion. Postmortem examinatin showed oedema and inflammatory changes in lungs, effusions and yellow-gray foci in liver, kidneys and heart muscle. In brain and spinal cord occured numerous extravasations and live larvae of E. cervi.

Nematodes E. cervi can make in goats demages and inflammatory changes in central nervous system causing clinical symptoms and death of animals.

c.6.05 PATHOLOGICAL OBSERVATIONS ON *SCHISTOSOMA BOVIS* INFECTION IN CATTLE IN IRINGA ,TANZANIA.

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Background: Although liver-fluke infection is known to be among the causes of ill-health and lowered productivity in cattle in the southern highlands of Tanzania little has been done on the effect of the co-existing schistosome parasites.

Method: The pathological observations were carried out in six tracer calves kept in an endemic farm at different durations (two for 8 months, another two for 6 months and the last two for 4 months) and in two old cows with chronic illness.

Results:. The pathological lesions were more severe and more prominent in the very old cows than in the tracer calves. Lesions were more severe in those calves kept for 8 months The livers of the old cows were severely affected, being dark-brownish-black in colour and invariably fibrosed. Histopathological lesions were mainly seen in the portal and peri-portal areas. However, in old cows large areas of parenchyma were destroyed and there was an unusually high number of Kupfer cells filled with dark-brown-black granular pigment mixed with iron. In the intestines, there were haemorrhages on the mucosa, cystic dilatation of the crypts, and single as well as clustered egg granulomas on the mucosa and sub-mucosa lesions and these were more dramatic in the old cows. Other organs involved were pancreas and abomasum. However, the finding of the egg granulomas in the gall bladder mucosa of the old cattle has rarely been reported.

Conclusion: In the southern highland of Tanzania Schistosome infection is just as pathogenic to cattle as liver-flukes and the severity and extent of the pathological lesions in cattle under natural conditions depend on duration and intensity of infection.

c.6.06 PATHOLOGY OF *TRYPANOSOMA EVANSI* INFECTION IN TWO SPECIES OF WALLABY

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Movement of livestock from western Indonesia to south-eastern Irian Jaya may pose a risk of introducing *T. evansi* into Papua New Guinea (PNG) via feral deer and native wildlife including wild pigs and wallabies. A pilot experimental study was conducted to observe infection in 2 species of wallaby.

Two agile wallabies (*Macropus agilis*) and 3 pademelons (*Thylogale stigmatica*) were infected by intravenous injection of $2x10^4$ *T. evansi*/kg at the RIVS, Bogor. Animals were observed daily for evidence of clinical disease and blood collected twice weekly to determine the parasitaemia. Moribund animals were euthanased, post mortem examination conducted and gross and histological changes noted.

All wallabies developed a high parasitaemia at day 6 post-infection which persisted until euthanasia at 8, 29, 31, 38 and 64 days after infection. Clinical signs included anorexia, weakness and ataxia. Gross pathology was unremarkable. Histological changes were seen in tissues containing antigen-processing cells and consist of a mononuclear cell infiltrate into the interstitium with little cellular destruction. *T. evansi* could be seen in the tissues using a specific immuno-staining technique.

Agile wallabies and pademelons are highly susceptible to infection with *T. evansi*. Histopathological changes in wallabies infected with *T. evansi* are pathognomic. Wallabies have the potential to spread *T. evansi* into PNG and may suffer high mortality and, thereby acting as an indicator of recent introduction.

This study was supported by the Australian Quarantine and Inspection Service.

c.6.07 THYSANOSOMA ACTINIOIDES AS POSSIBLE PREDISPOSING AGENT OF BLACK DISEASE IN SHEEP

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An outbreak of Black disease in Argentinean Patagonia, where 80 (6.7%) out of 1200 Merino ewes died suddenly, is reported. Congestion of subcutaneous tissue, accumulation of fluid in the thoracic and abdominal cavities, distended gall bladder and a heavy burden of Tysanosoma actinioides in bile ducts were the most relevant findings at post mortem examination. Histological sections showed multiple focal necrotic foci in the liver parenchyma, distended bile ducts and presence of the parasite in bile ducts. Clostridium novyi was early detected in liver smears through immunofluorescence. Lately, from liver cultures, Clostridium novyi type B was isolated. Due to the absence of the commonest predisposing agent Fasciola hepatica and other suspected parasites i.e Cysticercus tenuicollis and Dicrocoelium dendriticum in the liver, ducts and gall bladder, the severe parasitism by T. Actinioides is proposed as the predisposing agent of the present outbreak of Black disease. The mechanism by which the parasite could act as the trigger factor in this case is unknown. However, it was observed that the parasite produces an enlargement of bile ducts compressing the surrounding parenchyma, and also restricts or obstructs the normal bile flow. Both of these conditions could produce anoxia in the surrounding parenchyma, giving the Clostridium the ideal conditions of latent spores to activate and growth.

c.6.00-10 Pathology of parasite infection
c.6.11-24 Immunity against helminth infections

c.6.08 CLINICAL AND HEMATOLOGICAL ALTERATIONS IN CATS INFECTED WITH *Lagochilascaris major*

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Background:.Among the nematodes of the genus *Lagochilascaris, L. major* parasitizes the oro-pharyngeal region of cats. The eventual possibility of its transmission to humans, similarly to *L. minor*, which constitutes an important zoonosis in the Neo-tropical region, including Brazil, justifies a more detailed study of this species related to its biology, clinical and pathological alterations in the feline host.

Method: Six cats were orally infected with *L. major*, receiving 50 cysts each, and another cat remained as a negative control. Clinical signs and faecal egg counts were monitored daily and hemograms were performed weekly.

Results: Two cats showed intermittent mandibular movements, one of them during the 2nd and 3rd and the other on the 4th and 5th weeks post-infection (p.i.). Clinical signs such as hyperthermia, anorexia or apathy were not observed. Intermittent diarrhea was observed on the 2nd, 3rd and 5th weeks p.i. Leukocytosis, neutrophilia, monocytosis and eosinophilia occurred on the 4th week p.i. Higher egg counts were observed during the 3rd and 4th weeks p.i., and infection was eliminated by 4 out of 6 animals 70 days p.i.

Conclusions: Domestic cats may host L. major developing a mild infection when inoculated with 50 cysts, with discrete clinical and pathological alterations, and are able to eliminate a primary infection.

c.6.09 OXIDATION OF BOVINE ERYTHROCYTES INFECTED WITH *THEILERIA SERGENTI*

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Background: Japanese bovine theileriosis ,which is caused by *Theileria sergenti* is one of the most serious diseases of grazing cattle in Japanese pasture. Although the main symptom of this disease is an anemia, the precise mechanism of the anemia is still unknown. We investigated the relationship between oxidative damage of erythrocytes and development of the anemia.

Method: Five splenectomized Holstein calves were infected with *T.sergenti* by infestation with *Haemaphysalis longicornis* and by inoculation of gland homogenates of the tick vector. Blood samples were taken from the calves at constant intervals before and after the infection. We checked hematologic parameters and analyzed methemoglobin(MHB), erythrocyte membrane lipidperoxide(LPO) and oxidized erythrocyte membrane protein(OP), as indicators of erythrocyte oxidations.

Results: It is observed that the increase in the parasitemia was accompanied with the lowering hematocrit. Before the infection, the concentrations of MHB, LPO and OP were about 3%, 5 nmol/gHb and 0.4-0.6 mol carbonyl/mol protein, respectively. However, at the maximum of the anemia, the concentrations of MHB, LPO and OP increased about 20%, 20-30 nmol/gHb and 1.5-1.9 mol carbonyl/mol protein, respectively.

Conclusion: Present study suggest that oxidation of the erythrocyte was related to the development of the anemia in *T.sergenti* infection. This oxidation might play a part of pathogenesis of the anemia of Japanese bovine theileriosis.

c.6.10 STUDIES ON *SARCOPTIS SCABEI* INFESTATION IN DROMEDARY

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Out of some 250 clinical cases of sarcopticosis in dromedary observed during the period of 2 years (1997-1998), 10 camels for either sex, different age groups and breed were selected for detailed clinico-pathological and therapeutic trial for the purpose of the present report. The infested animals showed emaciation and skin lesions showing alopecia alongwith hyperaemia and blood oozing out as a result of itching. The lesions were mainly found on the face, neck and ventral part of abdomen, perineal region, prepuceal sheath and vulva in females. Sarcopticosis was confirmed by skin scrapping examination for presence of mites. Pre and post treatment haematological parameters viz., haemoglobin, packed cell volume, total erythrocyte count, total leucocyte count and differential leukocyte counts were recorded. Some of the pre and post biochemical parameters viz. serum glucose, total protein, calcium and phosphorus were also studied. The animals were treated with deltamethrin plus ivermectin successfully and there were significant improvement in values of haemoglobin, serum glucose and total proteins in the treated camels.

c.6.11 EARLY IMMUNE RESPONSE DURING HYDATID SECONDARY INFECTION IN MICE.

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Hydatid disease, caused by the metacestode of *E.granulosus*, is a world zoonosis which affects a wide range of domestic animals. The innate defences induced by *E.granulosus* metacestode in both natural and experimental infections have been poorly described. In this work, we attempted to characterise the systemic acute phase and the early local inflammatory responses induced by *E.granulosus* metacestode using a murine secondary infection model based on the injection of protoscoleces (PSC) within intraperitoneal diffusion chambers. Normal (N/I group) and decomplemented (D/I group, treated with cobra venom factor) mice were infected with 1000 PSC on day 0. Three mice of each group were killed on days 0, 3, 10, 17 and 43 postinfection and the chamber contents analysed for complement C3 (C3) and serum amyloid P (SAP) proteins, cell types and number of viable parasites (NVP). C3 and SAP were also measured in plasma on the same days. Local and systemic increases of C3 (1.5 fold) and SAP (2 fold) and high PMN infiltration were observed on day 3 in N/I mice. D/I mice showed similar increases in SAP and PMN levels on day 3. On day 43 NVP was comparable between groups, although N/I mice did have higher percent of cyst forms. Also, there was a positive correlation between PMN level and NVP in N/I group, whereas D/I mice showed consistently high PMN infiltration even with low NVP. These results show that the absence of native C3 did not impede the development of the local inflammatory and systemic acute phase responses.

Also, even though NVP was not affected by complement depletion, the low level of cyst forms developed in D/I mice suggests that complement is one of the signals involved in the induction of PSC differentiation to cyst.

c.6.11-24 Immunity against helminth infections

c.6.12 RESPONSES OF *FASCIOLA HEPATICA* INFECTED SHEEP TO VARIOUS INFECTION LEVELS.

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Materials and methods: The response to *Fasciola hepatica* was studied in 15 sheep infected with 5 (5 sheep-Group B), 30 (5 sheep-Group C) or 150 (5 sheep-Group D) metacercariae; 5 non-infected served as control (Group A). The 20 animals were necropsied 12 weeks post-infection (pi) for counting and measuring flukes. Cellular and humoral responses were detected by peripheral eosinophils count, peripheral blood lymphocytes proliferation with excretory-secretory products (FhESP), ELISA and Western blot.
Results: All sheep were infected at necropsy excepted one sheep in Group B. Mean parasitic intensities were 40%, 44% and 27% of infection dose in Groups B, C and D respectively. FhESP-specific lymphocytes responses of the 3 infected groups were significantly enhanced in weeks 3 and 4 pi ($p<5\%$). The kinetic of the specific humoral response and the antigenic recognition profile were similar for the 3 infected group but antibody level was significantly lower in group B than in the 2 other infected group from week 5 pi to week 12 pi ($p<5\%$). Peripheral eosinophils count was significantly enhanced ($p<5\%$) in weeks 6 for Group B and, in weeks 4,5 and 6 for Group C and D. The numbers of peripheral eosinophils were significantly different between the 3 infected groups in week 3,4 and 6 pi and was related to infection level.
Conclusion: These results confirmed that sheep are higly susceptible to *F. hepatica* infection, even when infection pressure is very low. Peripheral eosinophilia seemed dependent of the infection level. The immune response seemed similar in the sheep infected with various number of flukes. A lower antibodies level is observed only in the group infected with 5 metacercariae..

c.6.13 IgA RESPONSE ASSOCIATED WITH REDUCED FECUNDITY OF *OSTERTAGIA OSTERTAGI* IN CATTLE

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In sheep infected with *Teladorsagia circumcincta,* parasite specific IgA responses in the abomasal mucosa have been associated with a reduction in worm fecundity, a feature which is regarded as a sign of acquired immunity. In the present study, the effect of parasite specific IgA responses on worm fitness was investigated in cattle infected with the abomasal nematode *Ostertagia ostertagi.* Fourty one calves were exposed to different levels of natural *O. ostertagi* infections during their first grazing season. The degree of acquired immunity against *O. ostertagi* was evaluated after either a natural challenge infection (exp. 1, n = 18) or an artificial challenge infection (exp. 2, n = 23). In both experiments the worm burdens, the length of the adult worms, the faecal egg counts and the number of eggs per female worm indicated distinctly different levels of acquired immunity, depending on the infection level during the first grazing season. To determine a possible role of IgA antibodies in the protective immune response, *Ostertagia*-specific IgA were measured in the abomasal mucus and the serum of the animals after the challenge infection, using an ELISA. Parasite-specific IgA levels in the abomasal mucus were significantly negatively related with faecal egg counts and the number of eggs per female worm, but not with the worm burden or the size of the adult worms. No significant correlation was observed between serum IgA levels and any of the parasitological parameters. These results suggest that parasite specific IgA responses in the abomasal mucosa of cattle infected with *O. ostertagi* are associated with a reduction in worm fecundity.

c.6.14 RECOGNITION BY W-B OF ANTIBODIES AGAINST *ASCARIS SUUM* ANTIGENS IN IBERIAN PIGS

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Background: To know the immune response produced in naturally and experimentally infected Iberian pigs against *A. suum,* we have carried out a study about the different proteins of five different antigens from adults of this parasite. Sera from the afore mentioned pigs were tested by WB against the five antigens.
Methods: The composition of the different somatic antigens has been carried out by 12 % SDS-PAGE technique and the analysis of the immune response by WB technique. Sera from 9 experimentally infected Iberian Pigs (with single and trickle doses) and 39 naturally Iberian Pigs were analized to carry out this study.
Results: Protein bands were located in all antigens, especially between 33 and 66 Kda. It is possible to find different common bands but also specific proteins in each antigen. By WB, the reactivity of pigs with patent infections was very low for all antigens, while some pigs without worms in the small intestine had strong and easily recognisable bands. A similar response was found in the experimentally infected Iberian Pigs in which intestinal worms were not found at 42 dpi.
Conclusions: The immune response of the Iberian Pigs against *Ascaris suum* is independent of the adults parasitation level.
Acknowledgment: The study was supported by the Comisión Interministerial de Ciencia y Tecnología of the Spanish Goverment (Project AGF96-0557).

c.6.15 IgG1 AND IgG2 HUMORAL RESPONSES IN EXPERIMENTAL CANINE TOXOCARIOSIS

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The humoral-mediated response (IgG1 and IgG2 subclasses) against *Toxocara canis* in dogs infected with embryonated eggs was established by ELISA and crude antigen. One group of dogs was treated with procodazol (G-E), prior and after infection, to modify its response to toxocariosis. Another infected group remained without treatment (G-I), and one group of non-treated and non-infected animals was left as control (G-C). Animals of groups G-E and G-I remained infected throughout 14 weeks, when all infected animals received a single oral dose of piperazine.
All infected dogs passed *T. canis* eggs by faeces, and adult worms were spontaneously recovered during the infection and at the 14^{th} week after infection. The mean number of worms obtained was 17.86 ± 15.60 in G-I and 7.86 ± 6.91 in G-E. The IgG1 response was higher in G-I than in G-E, increasing earlier and peaking at 10^{th}-11^{th} weeks after infection. The values of IgG2 increased at 4^{th}-5^{th} w.a.i. in G-I, and decreased to the end of the study, whereas in G-E a constant level was achieved throughout the experiment.
In toxocariosis the gut acts as a barrier to infection reducing greatly larvae that migrate to the liver and the lungs; furthermore the humoral response seems moderate the intensity of the parasitosis, limiting the number of toxocaras that attain maturity in the intestine. Production of IgG1 and IgG2 is antagonistically regulated.

c.6.11-24 Immunity against helminth infections

c.6.16 SERUM IGG ANTIBODIES AGAINST *HAEMONCHUS CONTORTUS* IN POLISH WRZOSÓWKA HOGGETS NATURALLY INFECTED WITH GASTROINTESTINAL NEMATODES

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The study was carried out on Polish primitive prolific (Romanov type) Wrzosówka hoggets, 11-months-old over one grazing season 1997. Sheep were naturally infected with gastrointestinal nematodes and anthelminthics were not used. The aim of the study was to determine humoral response (IgG) against *H. contortus* crude antigen.

Eggs per gram of faeces (EPG) were performed by a modified McMaster method from April to November 1997. IgG level was detected using method described by Stankiewicz at al., 1996 (Int. J. Parasit., 26: 97-103).

Increase of faecal egg counts was observed from April to September (EPG=2.3 and 129.8, respectively). Mean IgG level peaked in June (OD=0.402), decreased to the lower level in August (OD=0.214) and increased again in the end of grazing season (OD=0.382). Very similar IgG pattern was observed in small groups of hoggets selected for low and higher faecal egg counts. Although observed differences are not statistically significant, IgG level was higher in hoggets passed no more than 10 eggs per gram of faeces.

The results of this trial demonstrate that IgG level corresponded with the percentage of L_3 *H. contortus* larvae cultured from the faeces.

The research was supported by grant No. 6 PO4C 008 10 of State Committee for Scientific Research in Warsaw, Poland.

c.6.18 HORSE *CYATHOSTOMINAE* ANTIGENS: IMMUNOBLOTS USING HORSE AND RABBIT SERA.

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Background: Larval cyathostomiasis (LC) is a widespread emerging horse disease. Although cyathostomes induce immune reactions in their natural host, these reactions may play a role more in LC than in protection. Our aim is to study *Cyathostominae* specific antigens in order to better understand and prevent LC. There are more than 50 species of cyathostomes, and some may be more pathogenic than others. In a breeding farm under study (see accompanying poster), *Cylicocyclus insigne* is the most preponderant species, and was elected as a model.
Methods: Cyathostomes were collected from the feces of horses in this farm, 24 hs after Ivermectin treatment. L4 forms were removed and adult *C. insigne* (CI) were isolated. The 2 resulting adult cyathostome populations were used to prepare polyspecific (PS) and CI extracts, using or not different detergents and centrifugation forces. PS extracts, prepared without detergents, were used to immunize rabbits. All extracts were analyzed by SDS-PAGE and immunoblots, using naturally infected, symptom free horse sera and a cyathostome free control foal serum, as well as normal and immunized rabbit sera.
Results: There were some quantitative differences between protein bands of the PS and CI extracts. In all homogenates, there was an important 30 kD protein band that did not react in immunoblots. Infected horse sera reacted against all extracts, with bands in the 90 to 40 kD range. There was a 40 kD band detected by rabbit sera, not seen with infected horse sera. There were some qualitative differences in the bands detected by horse sera in the CI and PS extracts.
Conclusion: Cyathostomes may have antigens that are not recognized by their natural host's sera, that may be important in prevention and/or pathology. A technique for species determination using monospecific antisera may be developed.

c.6.19 EARLY DIAGNOSIS OF CEREBROSPINAL NEMATODIASIS (SETARIASIS) IN GOATS

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Background: Cerebrospinal Nematodiasis (CSN) is a common paralytic condition of goats in Sri Lanka, caused by migration of larvae of *Setaria spp.* in the central nervous system. Early detection and treatment could reduce diseases incidence in the field. This study was carried out to develop a diagnostic assay for early detection of CSN for effective treatment.
Methods: A total of 12, six month old goats was used in the experiment. Eight were infected with 50 L_3 larvae recovered from infected *Aeds aegypti*. Serum and Cerebrospinal fluid (CSF) were collected twice a week for three months. Antibody levels against a PAGE fractionated, 54kDa adult excretory-secretary antigen (SpES), were elucidated using ELISA. CSF was also examined for its cellular components
Results: Serum antibodies against SpES were detected by day 18 in all the infected animals with titres ranging from 1:20 to 1:80. However, only five of the infected animals developed CSF antibody (21 - 31 days). Animals with CSF antibodies developed neurological symptoms after 42 days, accompanied by elevated levels of eosinophils (13-42%) in the CSF.
Conclusion: Increase in eosinophils levels and appearance of SpES specific CSF antibodies occur during the early stages of CSN infection. Thus, a specific CSF is a useful assay in the early detection of CSN infection.
Acknowledgement: IFS, Sweden for financial support (Grant No.B/2508-1)

c.6.20 SUSCEPTIBILITY OF MICE EXPRESSING SAG-1 PROTEIN TO *TOXOPLASMA GONDII*

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Toxoplasma gondii is a protozoan parasite that infects most species of domestic animals, bird and human in the most parts of the world. One of the major surface antigens, SAG-1 (P30), appears to have an important role in binding to the host cells in the process of invasion, pathogenesis and immunogenicity of the parasite. Aim of this work is to study cell-mediated immune response in the mice expressing the SAG-1 to *Toxoplasma gondii*.

P30 transgenic mice generated were infected parenterally with RH and Beverley strains of *T. gondii*. All of the P30 transgenic mice and non-transgenic mice died on day 8 postinfection when they were infected intraperitoneally with RH strain tachyzoites. However, when the mice were infected intraperitoneally with Beverley strain bradyzoites, about 70 -100 % and 40 % of mortality in the P30 transgenic and the non-transgenic groups, respectively, were observed. These mice succumbed infection on days 10-15 postinfection.

P30 transgenic mice were more susceptible to *T. gondii* infection as compared to non-transgnic mice. This relative susceptibility may be associated with the expression of P30 protein in thymus of the P30 transgenic mice. Analyses of cell population and cytokine profile responsible for the susceptibility are being carried out.

c.6.11-24 Immunity against helminth infections

c.6.21 ANTIGEN OF *FASCIOLA SP.* IN THE EXOGENOUS STAGES

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Background: Identifying antigens of fasciola in the exogenous stages has received little attention. We studied antigens of *Fasciola sp.* at various stages of development by an indirect immunofluorescent antibody (IFA) test.

Methods: The intermediate host, snail *Lymnaea ollula,* was exposed to miracidia of fasciola, and the sporocysts, rediae, cercariae and metacercariae were collected. The juvenile flukes in the liver phase were recovered from mice inoculated with metacercariae. IFA was performed with paraffin-embedded sections of the parasites at each stage. Antiserum to *Fasciola sp.* was collected from rabbits 7 weeks after infection with metacercariae.

Results: IFA test showed specific fluorescence on the surface of all developmental stages. The fluorescence was also detected in the gut cells of cercariae and metacercariae, and the gut caeca of juvenile fluke. After absorption of the serum with antigens of metacercariae or juvenile fluke, no fluorescence was found in metacercariae and juvenile fluke homologusly, however, the fluorescence was observed in the gut cells of metacercariae or in the gut caeca of juvenile fluke heterologusly.

Conclusion: The antigen of *Fasciola sp.* recognized by rabbit exists in the parasites at the exogenous stages as well as in the juvenile fluke of the defenitive host, and the stage-specific antigen also exists in the metacercariae and the juvenile fluke.

c.6.22 RECOGNITION OF *Cooperia punctata* ANTIGENS BY SERUM OF INFECTED AND CHALLENGED CALVES

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Background: Immunity to *C. punctata* in calves is evident after first exposure, however, little is known on the antigen recognition patterns, which might help the development of more accurate serological tests and to design immunoprophylactic studies. The development of infection in prime-infected and challenged calves and recognition of soluble adult and larval *C. punctata* antigens by antibody (Ab) isotypes in the sera of those calves was studied.

Method: Six Holstein male calves aged 5 months were single infected with 130000L_3 per os(G-A) and another 6 calves remained as non-infected controls(G-B). On DAI100 calves were treated with an anthelmintic. Eighty days later, 4 of the infected animals were re-infected with 260000L_3(G-A.1), and the non-infected group was divided into 2 subgroups, one prime-infected with the same dose as G-A.1(G-B.1), and another as control(G-B.2). Daily faecal and weekly serum samples were collected. Sera were tested in immunoblots against adult and larval *C. punctata* soluble antigens, using anti total IgG, IgG1, IgG2, IgA and IgM MAb to detect the response. Blots were revealed with horseradish peroxidase-conjugated MAb and 4-chloro naphtol as substrate.

Results: IgG and IgG1 from sera of G-A and B.1 reacted with a doublet of proteins of apparent MW of 12-14KDa and 17-20KDa from soluble adult proteins. Sera from G-A.1 reacted with an additional 29KDa band after DAI28. IgG from pooled sera of G-A and B.1 reacted with greater intensity from DAI28 until DAI49 with bands of 33 and 43 KDa and with less intense bands of 29-30 and 37KDa from L3 soluble extracts, but after DAI49, reactivity decreased in intensity. IgG and IgG1 from a *Cooperia*-hiperimmunized (HI) calf reacted strongly with a 29KDa band from adult proteins, similarly to re-infected animals, and IgA reacted weakly with a 12-14KDa band, which was recognized by IgM from all infected animals and by IgG2 from sera of G-A.1, B.1 and the HI calf. There was no reactivity of sera from *Haemonchus placei*-infected calves with adult *Cooperia* antigens.

Conclusions: Sera from calves prime-infected at different ages recognized the same adult protein doublets, with the same intensity. Sera from re-infected calves reacted more intensely with this doublet and recognized an additional 29KDa band.

c.6.23 IMMUNE RESPONSE AGAINST *UNCINARIA STENOCEPHALA* INFECTIONS IN DOGS

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Background: *Uncinaria stenocephala* infections are prevalent in dogs of Central and Northern Europe. This parasite can cause anaemia, eosinophilia and other non specific symptoms. However, there have been just a few reports so far on the dog humoral response to antigens of this nematode.

Methods: In the present experiment serum antibody responses to somatic proteins of infective larvae and adults of *U. stenocephala* were investigated in mongrel dogs. Groups of dogs were immunised with two doses of 1000 L3/kg body weight administrated orally or percutaneously in two week intervals and then challenged orally with similar dose of larvae. Another group of naive dogs was infected orally only once with the same dose of larvae and served as a challenge control. Serum samples were collected weekly and adult worms were obtained from the intestines during necropsy.

Results: The vaccinated dogs showed 5.4 and 2.2 times lower worm burdens as compare to challenge controls. ELISA values of all immunoglobulin isotypes measured in sera of immunised dogs were significantly higher then in challenge controls. These differences were more evident when extracts from adult worms were used.

Conclusion: Dogs became resistant to *Uncinaria stenocephala* after the first infection. The route of larvae administration play an important role in the development of immunological response (percutaneous infection is more immunogenic than oral one).

c.6.24 ANTIGEN RECOGNITION BY SERUM OF NELORE AND HOLSTEIN CALVES INFECTED WITH *Haemonchus placei*

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Background: Infection with *Haemonchus placei* on Holstein calves, showed greater establishment and development of the worms compared with Nelore (SATO et al, II Novel Approaches Conf. Procedings, Baton Rouge, 1998, p.50). This experiment aimed at comparing the patterns of antigen recognition of Nelore and Holstein calves sbjected to H. placei infection *H. placei*.

Method: 5 Holstein (group A) and 5 Nelore (group B) 9-month-old calves were infected with a single oral dose of 1.000 *H. placei* L3/Kg, monitored for 9 weeks and then slaughtered. Faeces and blood were collected weekly for counting of eggs per gram (epg), haematocrit and assess the humoral response to the adult *H. placei* total antigens by ELISA and Immunoblotting.

Results: The pre-patent period was 5 weeks for nelores and 4 weeks for the holsteins. Mean peak egg counts of holsteins were higher and appeared earlier than in nelores. Haematocrits of the nelores were always higher than on holstein calves, and they did not show anaemia. On the 3rd week anaemia was observed on holstein calves. Mean group worm burden in nelores was 2.000 ±2.570 and in holsteins was 21.133 ±4254 ($p<0,015$). The ELISA results in nelores and holsteins show an elevation of serum IgG levels (IgG1 levels were the same as these of IgG) against soluble adult antigens after DAI14, with a peak on DAI21 ($p<0,01$). A similar pattern of reactivity was observed on immunoblots. Two bands of ±43kDa were observed on DAI14 and on DAI21 another band of ±18,4kDa was observed, disappearing on DAI35. On DAI56 the ±43kDa band became weak on nelores and disappeared on holsteins (two factor analisys of variancy was the Statistical method utilized).

Conclusions: The worm burden that established on holsteins was greater in number and the sintomatology was more severe than on nelores, although the serological levels of total IgG in ELISA and the recognition of soluble adult antigens of *H placei* on immunoblotting were similar.

c.6.25-30 Immunity against protozoan and ectoparasitic infections

c.6.25 EFFECTS OF *Boophilus microplus* INFESTATIONS ON NAIVE CATTLE

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Background: Due to its large distribution and economic losses caused, the *Boophilus microplus* tick is one of the most important ectoparasite of cattle in tropical and subtropical areas. The objective of this study was to evaluate some tick biological parameters along three successive infestations of naive cattle.
Method: Five animals were infested three times, 4 weeks apart, with 75 mg of *B. microplus* larvae. For each infestation, the number of engorged females detached was recorded. Samples of 20 ticks per animal were weighed, incubated individually for oviposition and the egg masses produced were determined. The packed cell volume (PCV) of infested animals was also determined.
Results: While no significant differences ($P>0.05$) were observed in the mean number of engorged females detached between the first (199a ±69) and the second infestation (90a ±53), there was a significant decrease ($P<0.05$) of detachment of ticks in the third infestation (15b ±13). In addition, the weight of engorged females and egg masses produced after the third infestation decreased significantly ($P<0.05$). No differences were observed in the PCV values at Day 28, in the first, second and third infestation.
Conclusions: Biological parameters of ticks were affected after the third infestation. This could be due to the immune response aquired by cattle after successive infestations.

c.6.26 ROLE OF ACQUIRED IMMUNITY AND NATURAL AGE RESISTANCE ON COURSE OF *ISOSPORA SUIS* COCCIDIOSIS IN PIGLETS

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Background: Coccidiosis in nursing piglets is a clinical disease syndrome caused by the coccidium *Isospora suis* which has been found in all types of farrowing facilities and under all types of management systems.
Method: Thirty-two piglets from three litters were experimentally inoculated with 200,000 sporulated oocysts of *Isospora suis* at 3 days of age and/or rechallenged at 19 days of age or primary inoculated at 19 days of age, to compare the role of acquired immunity and natural age resistance on the course of coccidiosis. Twelve piglets were not inoculated and served as a control.
Results: Following challenge, the signs of coccidiosis characterised by clinical symptoms, oocysts shedding and weekly weights were similar to those occurred in piglets primary inoculated at 19 days of age.
Conclusion: This comparison suggests that the maturation of non-specific components of the immune system plays a more important role in resistance of neonatal piglets to *I. suis* infection than specific immune mechanisms. Prospective vaccination against *I. suis* have to override obstacles of an immature immune system. The key to sufficient means of controlling of *I. suis* coccidiosis in nursing piglets is still improved sanitation and chemotherapy with anticoccidial compounds.

c.6.27 Changes of epithelial ion transport by intracellular coccidia

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An *in vitro* system was established to study the effect of coccidian parasites on ion transport systems in epithial tissues. It used HT29/B6, a human colon carcinoma cell line, which builds a polarized monolayer like an epithelium. *Toxoplasma gondii* served as a model parasite as tachyzoites invade HT29/B6 and replicate rapidly. Mature schizonts are formed within 20 h and merozoites are released after 24-30 h.
Ion transport was measured in a special perfusion chamber (Ussing system). Parameters determined were: short circuit current and conductance to record the electrogenic ion transport, chloride fluxes with ^{36}Cl (mucoserosal, seromucosal) and the paracellular water transport with ^{3}H-mannitol. Measurement was performed 5 h, 10 h and 15 h *post infection* using monolayers in which approximately 30% of the cells were parasitized.
The infection had rapid effects on the conductance of infected monolayers, which was two to three times higher than that of uninfected HT29/B6 monolayers throughout the observation period. Chloride fluxes of both directions were also increased two to three times when compared to uninfected cells independent of the time after infection. However, the chloride netto fluxes and short circuit currents were unaffected by the parasites.
Data on the paracellular water transport will be presented to determine whether the increased chloride fluxes and conductances are due to a higher chloride transport through the cells or through paracellular pathways.

c.6.28 LANGERHANS CELLS HARBOUR *L. INFANTUM* IN SKIN AND LYMPH NODES IN CANINE LEISHMANIASIS.

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Backround: Langerhans cells (LC) are antigen-presenting cells that reside in the epithelium of the skin and mucosal surfaces. Following antigen up-take and elaboration, these dendritic cells migrate to the regional lymph nodes, where they become interdigitating dendritic cells (IDDC) and where they present antigen to T cells in an MHCII-restricted manner. This work reports the presence of intracellular amastigotes of *Leishmania* in LC/IDDC during spontaneous canine leishmaniasis.
Method: Skin lesions and draining lymph nodes were obtained from dogs naturally infected with *Leishmania*. Monoclonal antibodies specific for canine LC (CD1a) and for IDDC (CD1c) were applied to frozen tissue sections and immunopositivity was revealed using an avidin-biotin-peroxidase system.
Results: *Leishmania* amastigotes were observed in the cytoplasm of CD1a CD1c positive cells in both the skin and regional lymph nodes of infected animals. Infected cells usually harboured few parasites. In lymph nodes, infected IDDC were in close proximity to lymphocytes in the paracortex.
Conclusion: This report confirms the presence of *Leishmania* amastigotes in LC during canine leishmaniasis and strongly suggests that LC play an important role in antigen presentation and stimulation of immune response.

c.6.25-30 Immunity against protozoan and ectoparasitic infections
c.6.31-40 Development of vaccines agaisnt parasite infections

c.6.29 EXCRETORY/SECRETORY PRODUCTS (ESP) FROM *OESTRUS OVIS* LARVAE AND NITRIC OXIDE (NO) PRODUCTION BY OVINE AND MURINE MACROPHAGES.

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Oestrus ovis larvae, a common parasite of sheep and goats, live in contact with the nasal and sinusal mucous membranes and feed from plasmatic proteins. ESP of these larvae are highly immunogenic and induce severe inflammatory responses. Nitric oxide, a powerful inflammatory agent, is involved in vasodilatation and capillar permeability. In this study, we report the effects of ESP of *Oestrus ovis* on NO production by macrophages. Ovine macrophages derived from peripheral blood monocytes and tumoral murine macrophages were stimulated with 25 micrograms/ml of ESP from first (L1), second (L2) or third (L3) instar larvae. LPS was used as a positive control of macrophage activation and macrophages without ESP were used as negative controls. NO production reached 15-20 and 30-35 µM/ml with respectively ovine and murine macrophages after L1, L2 or L3 ESP stimulation and remained low (< 2 µM/ml) in control macrophages. Addition of L-N(G) monomethyl -L- arginine (a specific inhibitor of the inducible NO synthase) to stimulated macrophages led to a decrease of 80% in NO production. Moreover, a high expression of iNO synthase was shown by means of immunocytochemistry after stimulation by the ESP. 85-90 KD proteins (present in L1, L2 and L3) obtained in gel filtration (Sephadex G-100) induced high quantities of NO in murine macrophages. The NO concentrations were not deleterious for larvae (*in vitro*, NO kills larvae at non physiological concentrations >10mM/ml) but could improve the passage of plasmatic proteins through the mucous membrane for larval nutrition.

c.6.30 IMMUNITY IN RATS AGAINST *EIMERIA SEPARATA*, A COCCIDIAL PARASITE OF THE LARGE INTESTINE

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The study aimed to characterize the local reactions in the caecum of naive rats and rats immune against *E. separata* to a primary and a challenge infection, respectively. Optimum immunity was induced by two infections with 5000 oocysts per animal each, given in a 10 days interval. The oocyst production of a challenge infection administered after further 10 days was almost completely abolished. Histopathological investigations performed up to 72 h after challenge showed, that predominantly the development of first generation schizonts were affected in immune rats. Challenged animals showed significantly increased infiltrations with lymphocytes, plasma cells, macrophages, eosinophiles and mast cells when compared with rats after primary infection. Immunohistological studies compared local tissue infiltrations in naive and immune rats over a period of 48 h after primary infection and challenge, respectively. There was no difference between the groups concerning $CD45R^+$ cells, whereas significantly more $CD3^+$ cells were found in the caecum wall of the immune rats. $CD4^+$ cells predominated in animals after primary infection, whereas $CD8^+$ cells respresented the predominant T cell subset in challenged rats. The proportion of $\gamma\delta^+$ T cells did not differ between the groups, whereas challenged rats showed significantly increased numbers of $\alpha\beta^+$ T cells in the caecum wall when compared with animals after a primery infection. These findings suggest that predominantly $CD4^+$ cells may be involved in the immune response after primary infection, and that immunity to a challenge infection may be mediated by $CD3^+$, $CD8^+$ and $\alpha\beta^+$ T cells.

c.6.31 ATTEMPTS TO VACCINATE PIGS AGAINST *SCHISTOSOMA JAPONICUM* USING RECOMBINANT ANTIGENS AND NAKED DNA

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Background: The search for a defined vaccine against schistosomiasis japonica continues. Here four formulations were tested in pigs.

Method: Five groups of 8 male pigs were thrice vaccinated with rSj28GST in FCA/FIA, liposome formulated rSj28GST, Sj28GST as naked DNA, liposome formulated rSj26GST or adjuvant alone. These pigs together with 8 unvaccinated controls were challenged intramuscularly with 1500 cercariae each; perfusions and tissue egg counts were carried out 8 weeks later.

Results: The mean worm recoveries in the vaccinated groups were not signicantly different from the controls though interestingly, very few immature worms were found in the vaccinated groups compared with the controls. Although there were no significant differences between liver egg densities in the various groups, the mean tissue egg count in pigs vaccinated with Sj28GST in FCA/FIA was 44% lower than the adjuvant control group (P = 0.078).

Conclusion: Vaccination with the above mentioned formulations did not have a conclusive effect on the establishment of *S. japonicum* in pigs following intramuscular challenge, though there was a 44% reduction in tissue egg count in the group vaccinated with Sj28GST in FCA/FIA. Detailed data will be presented on antibody assays carried out on these pigs to compare the immunogenicity of the various vaccine preparations.

c.6.33 ANTIBODY RESPONSE AFTER DNA IMMUNISATION WITH PARAMYOSIN OR A PROTEIN DISULPHIDE ISOMERASE FROM *ANCYLOSTOMA CANINUM*

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Nucleic acid vaccination by intramuscular or intradermal delivery of plasmid DNA encoding antigenic proteins has been shown to confer protection in experimental animals against viruses and unicellular protozoan parasites. Experiences with metazoan parasites are rare. First trials have been done with the trematode *Schistosoma japonicum* and *Taenia ovis*. This approach, however, has not been tested for induction of immunity to nematodes such as the hookworm *Ancylostoma caninum*. We report here, for the first time, that a protein expression of hookworm proteins can be achieved by intramuscular injection with plasmid DNA encoding a paramyosin homologue or a protein disulphide isomerase. Seroconversion and, therefore, in vivo expression of the hookworm antigens could be demonstrated. A certain degree of protection against challenge infection could be demonstrated compared to control infections and non-immunised animals Still, these results need to be confirmed due to the limited number of animals used in the study. With this immunisation method an alternative way can be used avoiding the usual problems with protein immunisation. These preliminary data show, that DNA can be effectively used for vaccination against nematodes and that full length clones of the antigens used might be promising vaccine candidates.

c.6.31-40 Development of vaccines against parasite infections

c.6.34 INDUCTION OF PROTECTION IN MICE BY *BABESIA RODHAINI* RECOMBINANT ANTIGENS

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Background: *Babesia rodhaini* is an intra-erythrocytic protozoan and causes a lethal infection in mice. Acute infection can be cured by chemotherapy in mice and recovered mice are resistant to reinfection. In present study, recombinant p26 antigens of *B. rodhaini* were produced in *E. coli* or baculovirus-insect cell, And protective effect of the recombinant antigens was examined against *B. rodhaini* Infection in mice.

Method: The p26 gene was amplified by PCR from genomic DNA isolated from the *B. rodhaini* and ligated into *Bam* HI site of bacterial expression vector pGEMEX-2, and the pGEMEX/Br26 was expressed as T7 gene 10 fusion protein in *E. coli* JM109 (DE3). The p26 gene was also ligated into *Bam* HI site of baculovirus transfer vector pBacPAK8 and a recombinant virus expressing p26 gene (AcBr26) was isolated. Sf-9 insect cells were infected with AcBr26 to produce recombinant p26 antigen.

Results: These recombinant proteins were recognized by immune serum from drug-cured *B. rodhaini* immune mice and mouse sera immunized with these recombinant antigens recognized *B. rodhaini* parasites with IFAT. BALB/c mice were immunized with the recombinant antigens using saponin or Freund's adjuvants and challenged with *B. rodhaini*. Mice with immunized with Freund's adjuvants showed 40-100% protection in both recombinant antigens.

c.6.35 *Haemonchus placei* ANTIGEN RECOGNITION BY SERA OF CALVES IMMUNIZED WITH Co^{60}-IRRADIATED LARVAE

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Background: Haemonchosis is an important nematodiosis for cattle in the tropics, so effective immunological control methods are desirable. Calves immunized with *Haemonchus placei* irradiated L_3 show increased IgG levels in ELISA and reductions in egg counts. However, it is not known if the antigens recognized by these calves differ from naturally infected calves, which is the aim of this study.

Method: Twelve 4 month-old Holstein calves were divided in 3 groups: four calves were double immunized with 50000 *H. placei* L_3 irradiated with Co^{60} at a dose of 400Gγ (Group1, G1) on DAI(day after infection) 0 and 37; other 4 calves were double infected with 50000 *H. placei* normal L_3 (Group2, G2) on the same days as G1; the remaining 4 calves were not infected (Control Group). On DAI65 the 3 groups were challenge infected with 100000 *H. placei* normal L_3 and were killed on DAI100. Weekly blood and faecal samples were collected for hematocrit(Ht), egg counts and serum collection, which was used in immunoblots against soluble adult *H. placei* antigens, using HRPO-conjugated anti-bovine IgG to develop the reaction. Worm burdens were assessed upon necropsy.

Results: G1 and Control Group calves shed eggs in faeces 4 weeks after challenge infection, while G2 calves were positive in egg counts since 4 weeks after the first dose, increasing until slaughter. G2 mean PCV values decreased significantly after DAI42, until DAI 77, when the PCV of the other 2 groups also decreased. Upon necropsy, mean worm burdens of the 3 groups did not differ significantly. The immunoblots showed 2 major bands of apparent MW of 44 and 49KDa in G2 calves on DAI35, which persisted until necropsy. The same pattern occurred for G1 calves from DAI63 and for Control calves from DAI77 onwards. Serum from one G1 calf reacted weakly with a 87KDa band and one G2 calf serum recognized a 98KDa band, both on DAI63, when another G2 calf serum reacted with a 36KDa band.

Conclusions: All groups recognized basically the same bands in immunoblots after first exposure to either irradiated or normal larvae. It would be desirable, however, to increase the number of calves tested so that the tendency of lower egg counts in immunized calves could be confirmed.

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c.6.36 THE CONSTRUCTION AND PROTECTIVE IMMUNITY OF Sj23KDa DNA VACCINE

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Purpose: To study the protective immune effect of DNA vaccine against Shistosoma japonicum, Sj23 was chosen as the antigen molecule of DNA vaccine.

Methods: According to the published gene sequence of Sj23, a pair of primers were designed. With the two primers, the Sj23 gene was cloned into eukaryotic expression vector pCD by using RT-PCR. Indentification was carried out by enzyme cleavage analysis, PCR amplification and sequencing. After identification, pCD-Sj23 was injected into the muscle of mice to be oberserved its expression. Then male BALB/c mice were divided into two groups, the experimental group were inoculated by intramuscular injection with 100μg of pCD-Sj23 at weeks 0,3,5. Two groups were challenged with 40 cercarie at week 7.

Results: Sj23 can be expressed in skleletal muscle of mice by IFA. The protective test of Sj23 DNA vaccine on mice showed that the the worm, egg and egg of single female reduction rate are 33.01%, 49.59% and 15.70% respecitively in comparision with the control group.

Conclusion: Sj23 might serve as a potential vaccine and DNA vaccine could provide a new approach to develop immunizition of S.japonicum.

c.6.37 A NEW PROTECTIVE ANTIGEN FROM *HAEMONCHUS CONTORTUS*

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Background: This work is part of a research program to develop vaccines against economically important parasites of livestock.

Methods: A candidate protective antigen was identified from the adult stage of *H. contortus*. The antigen was expressed in recombinant form in insect cells using the baculovirus Bac-to-Bac system and was purified on a nickel column via its N-terminal His-tag. Sheep were given 3 subcutaneous injections of the purified recombinant protein in Freunds adjuvants.

Results: The antigen was expressed at very high levels in the cytoplasm of insect cells infected with recombinant baculovirus. 3/5 vaccinated sheep were highly protected against an *H. contortus* challenge infection. However, protection did not correlate with serum IgG levels, as very uniform antibody responses were induced in immunised sheep, suggesting that the mechanism of protection is not (solely) antibody.

Conclusions: This antigen shows promise as a candidate vaccine for *H. contortus*, but further work is needed to determine the mechanism of immunity and subsequently to optimise immune responses.

Acknowledgments: This work is supported by Novartis Animal Health.

c.6.31-40 Development of vaccines against parasite infections
c.6.41-49 Diagnosis of parasitic infections

c.6.38 H11 DNA VACCINATION AGAINST *HAEMONCHUS CONTORTUS*

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Background: H11 is a highly protective antigen derived from the gut microvilli of adult *H. contortus*. This work aimed to determine whether sheep could be successfully vaccinated with H11 delivered as plasmid DNA, with or without IL-1β as an immunostimulant.

Methods: H11 and ovine IL-1β cDNAs were cloned in a mammalian expression vector and expression confirmed in transient transfection assays. Recombinant IL-1β was expressed in *E. coli* and H11 protein was purified from adult worms. Sheep were given 3 injections, intradermally with plasmid DNAs or subcutaneously with protein antigens.

Results: High antibody responses were obtained to injection with H11 protein in either Freunds adjuvants or in Alum supplemented with IL-1β protein, and the sheep were protected against *H. contortus* challenge infection. However, no antibody response was induced in any of the DNA vaccine groups. A subsequent injection of H11 protein without adjuvant failed to induce a booster response, suggesting that there was no priming effect by the DNA vaccines, and the sheep were not protected against challenge infection.

Conclusions: While DNA vaccines are a promising new technology, work needs to be done to optimise antibody responses, particularly in large animals.

Acknowledgments: This work was funded in part by Novartis Animal Health.

c.6.39 EFFICACY OF A *GIARDIA* VACCINE IN DOGS AND CATS

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Background: *Giardia* is a common intestinal parasite of dogs and cats in most of the world and is the most frequently identified canine intestinal parasite in areas of North America, Europe and Australia. Infected animals may be asymptomatic or have moderate to severe gastrointestinal clinical signs. Giardiasis is recognized as an important zoonotic disease. A vaccine has been recently developed to protect animals from infection and clinical signs.

Method: Male and female 8 week old puppies and kittens were vaccinated and boosted 3 weeks later. Animals challenged with cysts at 21 days, 6 months and 1 year following the last vaccination. Clinical signs, fecal cysts and body weight change were evaluated for 42 days following challenge. Intestinal trophozoites were enumerated at the end of the study.

Results: Following challenge, fewer vaccinated animals developed diarrhea and the diarrhea was of shorter duration than controls. Control animals passed cysts following challenge from day 7 to day 42 while vaccinated animals were not infected or past cysts for a short duration. Vaccinated animals were free of intestinal trophozoites while control animals had large number of trophozoites in the small intestine.

Conclusion: A specific and protective immune response was produced in *Giardia* vaccinated animals. The vaccine may be used to reduced the number infected animals, shorten the duration of *Giardia* infection and minimized or eliminate clinical signs of giardiasis. It is also beneficial in reducing environmental contamination and preventing zoonotic transmission.

Acknowledgement: Natural Sciences and Engineering Research Council and Fort Dodge Animal Health.

c.6.41 Leucocytic changes in guinea pig experimental infestation by T. equinum

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Background: The Trypanosomiasis is an important parasitosis of the horse in Argentina.
For diagnosis is very usefull to inyect laboratory animals and guinea pigs are the most used.

Method: Leucocytic change was studied in guinea pigs by Trypanosoma artificial infection; they were selected by weight (400gr) and sex (female);
12 animals were inoculated subcutaneously as challenge group and other 12 were used as control.
Leucocytic control was carried out for 9 weeks by blood extending samples and colouring them by Giemsa.

Results: Important leucocytic changes were observed, with decreasing lymphocyte and increasing neutrophils during trypanocidal crisis.

Conclusion: This study confirmed the importance of leucocytic changes in experimental animal for the diagnosis.

c.6.42 EVALUATION OF A LATEX AGGLUTINATION TEST IN THE DETECTION OF *CRYPTOSPORIDIUM* OOCYSTS FROM GOAT KIDS FAECES

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Methods : A quantitative latex agglutination test (QLAT) with monoclonal antibodies (Sphervet Crypto[ND], Vétoquinol, France) for the detection of *Cryptosporidium* oocysts in faeces was compared with 3 other conventional techniques : Heine staining on faecal smears (HS) giving semi-quantitative results (scores from 1 to 5), sucrose flotation on diluted faeces (SF) with results expressed in oocysts/g of faeces, direct ELISA (DE) giving qualitative results (Pathasure Crypto[ND], Vétoquinol, France). 234 goat kid unconcentrated faecal samples from 8 farms were processed according to the 4 techniques. Data were analyzed with Win Episcope 1.0 and Testview 1.1 softwares.

Results : The oocyst outputs ranged from 100,000 (detection limit for SF) to 200 millions opg (output mean : 15 millions opg). A very good agreeement was recorded between QLAT and HS, SF, DE : Kappa values ranged between 0.82 and 0.90. When considering as positive (or negative) samples those exhibiting oocysts (or not) with both HS and SF (n=219), the sensitivity and the specificity of QLAT were respectively 95.1 and 96.0 %. The lack of sensitivity was observed in faeces harboring few oocysts (≤ 200,000 opg, scores≤2) whereas the lack of specificity was only observed in 3 samples originating from the same farms. A highly significant correlation was calculated between percentage of agglutination in QLAT and number of oocysts in SF or scores in HS (Spearman's correlation ranging from 0.45 to 0.48, $p<0.001$).

Conclusion : QLAT is a rapid, simple and reliable tool for routine detection of *Cryptosporidium* oocysts in faeces.

c.6.41-49 Diagnosis of parasitic infections

c.6.43 TRYPANOSOME INFECTION STATUS AND TRYPANOTOLERANCE

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Host ability to control the development of trypanosomes is a major component of *trypanotolerance*. However practical indicators of the parasite control capability have still to be identified as they rely on the availability of more precise and practical diagnostic techniques than those available.
Trypanosome antigen detection techniques used in conjunction with parasite detection techniques are being evaluated in a population of postweaner N'Dama cattle raised under trypanosomosis risk. Matching infection, anaemia control, trypanocidal drug requirements and animal growth aspects have been serially recorded at weekly intervals in four herds covering a range of trypanosomosis risk levels.
The analysis of covariance of changes in average packed cell volume (%) and growth (g/day) per unit change in antigenaemia stratified by parasitaemia status demonstrated, for each of these status, significant decreases in PCV and weight gain with each unit increase in antigenaemia.
The results suggest thus that, despite their limitations, antigen test information, serially recorded, can contribute to more accurately characterising infection status and animals trypanosomosis resistance capability. They support the justification for further development of more reliable diagnosis tools for trypanosomosis.

c.6.44 ANTIBODIES LEVELS DETECTED BY INDIRECT FLUORESCENT ANTIBODY TEST, LATEX AGGLUTINATION AND ENZYME-LINKED IMMUNOSORBENT ASSAY IN CAPRINES INFECTED WITH *Toxoplasma gondii*

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Background: Toxoplasmosis is a widespread zoonosis, causing large losses in production animals. In Brazil, only few works on caprine toxoplasmosis were published, using different serological methods. In this work, three methods were tested in experimentally infected animals. **Method:** Antibodies levels against *Toxoplasma gondii* were detected using indirect fluorescent antibody test (IFAT), latex agglutination test (LAT) and indirect enzyme-linked immunosorbent assay (ELISA). Six 6 to 9 months old outbreed goats received orally 10^5 oocysts and two other animals were kept as uninfected controls. Twice a week blood samples were collected for detection of parasitemia and serum samples for serological assays. Eight weeks after infection all animals were slaughtered and different tissues (heart, skeletal muscle, brain, lymph nodes, kidneys and liver) were pepsin digested and inoculated in mice. **Results and Conclusion:** Hiporexya, fever and lethargy were observed from the 3rd to the 7th day after infection (DAI). Parasitemia was detected in 50% of the infected goats from the 7th to the 14th DAI by bioassay in mice. Viable tissue cysts were isolated from all infected animals in muscle samples. In some animals, positive sera were detected on the 10th DAI by the IFAT and LAT and on the 14th DAI using ELISA. The infected goats were seropositive at the 17th DAI. Samples from the uninfected controls remained negative through the experimental period. **Acknowledgement:** We wish to thank FAPESP (São Paulo State Research Assistance Foundation) by the financial support (proc. 97/1177-7)

c.6.45 ASSESSMENT OF A RECOMBINANT HYPODERMIN C FOR THE SERODIAGNOSIS OF HYPODERMOSIS IN CATTLE

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An indirect ELISA test using as antigen a recombinant parasite protein, hypodermin C, was developed to measure *Hypoderma*-specific antibodies in cattle sera and compared with natural hypodermin C. To evaluate the field efficacy of the ELISA test, 334 serum samples were collected from cows raised at farms in Galicia (Spain) and a serological survey performed. The study was completed with 10 Frisian cows naturally infested by *Hypoderma* sp. that were sampled monthly, during a cycle of the parasite, in order to monitor the antibody response against both antigens. Compared with an ELISA based on traditional parasite antigen, the recombinant hypodermin C gave excellent results, with a sensitivity of 95.8% and a specificity of 95.7%. Considering the cut-off point, with the recombinant hypodermin C, 70.9% of the animals had positive levels of antibodies to *Hypoderma* and 73.6% with the natural hypodermin C. In relation to the dynamics of antibody response, both antigens showed very similar profiles. The recombinant hypodermin C appears to be a useful alternative to the traditional parasite antigen for the serodiagnosis of hypodermosis in cattle.

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c.6.46 CLONING AND EXPRESSION OF THE 48 KDA *BABESIA CABALLI* MEROZOITE ANTIGEN AND POTENTIAL USE OF RECOMBINANT ANTIGEN FOR DIAGNOSIS

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Equine babesiosis, caused by *Babesia equi* and *B. caballi*, is widely distributed in tropical and subtropical regions of the world and results in the great economic losses. Since serological methods such as complement fixation test and enzyme-linked immunosorbent assay (ELISA) have some problems in specificity and sensitivity, there is a need to improve these methods to detect antibodies in equine babesiosis.

The monoclonal antibody BC11D against *B. caballi* was produced and used for immunoscreening of a cDNA expression library. Recombinant phage was collected for pBluescript clone by *in vivo* excision. This open reading frame encodes a protein consisting of 458 amino acid residues with a predicted molecular weight of 52 kDa. Recombinant protein was expressed as glutathione S-transferase fusion protein with an apparent molecular weight of approximate 75 kDa by transformation of *E. coli* (BL21 strain). The 96-well plate coating with the fusion protein was used for ELISA. The test was able to distinguish sera of *B. caballi*-infected horses from those of *B. equi*- or non-infected horses. Moreover, the results of the test were consistent with those of a well-established indirect immunofluorescence antibody test (Avarzed *et al.* 1997) using sera from Mongolian field horses. These results suggest that this recombinant protein is a useful antigen for ELISA to diagnose *B. caballi* infection.

c.6.41-49 Diagnosis of parasitic infections
c.6.50-58 Protozoan parasites of wildlife

c.6.47 COPROANTIGENS OF *Fasciola hepatica* IN RATS PRIMO AND REINFECTED

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A polyclonal antibody-based capture ELISA was developed for detection of coproantigens of *F. hepatica* in the faeces of experimentally infected rats. The main objective consisted of comparing the effect of challenge on the dynamics of antigens in faecal samples. Three groups of Sprague-Dawley female rats were used. Group 1 (G-1) was infected with 20 metacercariae of *F. hepatica*/rat; group 2 (G-2) was infected with the same dose and challenged 11 weeks after primary infection (w.a.p.i.). Another group remained uninfected as control group (G-3). Anti-IgG values were estimated by indirect-ELISA and excretory/secretory products. Coproantigens were detected by using a capture ELISA. In the two infected groups, an early IgG response to *F. hepatica* was observed, and the titres remained significantly higher than in controls from the 3rd w.a.p.i. to the end of the study. All rats passed eggs and harboured flukes in the liver. Coproantigens were detected at 6 w.a.p.i., and preinfection faecal supernatants also were tested. All infected animals exhibited antigens in their faecal contents. These results were corroborated by the appearance of *F. hepatica*-eggs in the faeces at the 7th w.a.p.i. Optical densities were significantly greater in G-1 and G-2 than in G-3 from the 6th w.a.p.i. to the end of the trial. No statistically differences were noted between the two infected groups, nor in the detection of coproantigens, egg-output neither in the number of flukes. The detection of antigens of *F. hepatica* in the faeces of rats infected is not influenced by a challenge infection.

c.6.48 OBSERVATIONS ON FLOTATION OF *Balantidium coli* CYSTS IN MCMASTER COUNTING CHAMBERS

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Background: There have been reports of investigations using saturated NaCl as a flotation media for detecting cysts of the ciliate *Balantidium coli* in pig faeces. We have not found this method suitable and have therefore performed further experiments with $ZnSO_4$ as recommended in the literature for flotation of protozoan cysts.

Method: The number of cysts in faeces was determined by a standard sedimentation method of washing, sieving, centrifuging and counting of the sediment in Sedgewick-Rafter chambers (S-R count). Flotation solution was added to sediment or faeces 14:1 (v/w) and the counting was performed in a McMaster counting chamber (McM).

Results: Results are expressed as % of S-R count. Within the 1 minute (1min.) after adding the flotation solution $ZnSO_4$ ($1 H_2O$) 331 g/l, 94% of the cysts floated but after 5 minutes this was reduced to 7%. In saturated NaCl only a maximum of 3% cysts floated in 1min. sample . If the 1min. McM sample was left undisturbed under the microscope, the cysts remained attached to the coverglass of the counting chamber and thus extended the period for optimal cyst counting. It was possible to float fresh faecal samples directly but only a maximum of 42% of the cysts were found floating.

Conclusion: It is possible to float and count cysts of *B. coli* adequately in $ZnSO_4$ from washed preparations of faeces but many cyst are lost when using fresh faeces.

c.6.49 AN IMPROVED, ROBUST MINI-ANION-EXCHANGE METHOD FOR DETECTING *T. EVANSI*

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The miniature anion-exchange centrifugation technique (MAECT) is considered the most sensitive method for detecting *T. brucei*, however, the technique has not been widely used in other trypanosome species and is not sufficiently robust for field surveys. Improvements to the method have been made to both improve the sensitivity of the test and its robustness.

Modifications to the MAECT include the use of 10ml centrifuge tubes containing hypodermic needle caps to protect the pasteur pipettes during centrifugation, the addition of buffy coat instead of whole blood to the column and the use of 10ml plastic disposable chromatography columns (Poly-prep columns, Bio-Rad).

Blood from a *T. evansi* infected rat was diluted into bovine blood, shown to be free of *T. evansi*, to give samples with levels from 250 organisms per ml to 1.25 organisms per 4ml. At least 5 replicates of each sample were tested using the haematocrit centrifugation technique and the MAECT with whole blood and the MAECT with buffy coat.

The MAECT was the most sensitive test; able to detect 1.25 *T. evansi* per 2ml of blood if buffy coat is used in place of whole blood.

This modified MAECT is the most sensitive test to detect parasitaemia yet devised. The test has the advantage over rodent inoculation that it is not affected by biological variation in the infectivity of trypanosomes for mice. The method now enables use of a small portable centrifuge in true field conditions.

This study was supported by the Australian Quarantine and Inspection Service.

c.6.50 COCCIDIA OF ROE DEER AND RED DEER IN NORTH WEST POLAND

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Material and methods: In the second half 1998 faeces' samples from 58 roe deers and 35 red deers were collected in the districts Koszalin and Piła. The samples were examined by Willis-Schlaafa flotation method.

Results: Oocyst of the genus *Eimeria* were found in 37,9% of examined roe deer and 22,85% of red deers. There were found 4 *Eimeria species* in roe deers and 2 species in red deer (table 1).

Table 1. The infection rate of roe deers and red deers with *Eimeria* in North West Poland

Animal Species	Number of Animal	*Eimeria species*	Number of infection animal	%
Roe deer	58	*E.capreali*	17	29.31
		E. panda	7	12.06
		E.rotunda	6	10.54
		E. ponderosa	3	5.17
Red deer	35	*E. sordida*	5	14.28
		E. elaphi	2	5.71

Conclusion: Finding the oocyst of coccidia in feaces of roe dear and red deer is the first registration of these protozoa in North Poland.

c.6.50-58 Protozoan parasites of wildlife

c.6.51 DIURNAL OOCYST OUTPUT OF *ISOSPORA* (EIMERIIDAE) FROM COMMON STARLING

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Background: Diurnal periodicity of *Isospora* oocysts appearance is known for *Isospora* of a few species of passerine birds in captivity. We checked the existence of such rhythm for *Isospora* oocysts from common starling in nature, and compared the results with data from caged starlings.Using the diurnal model of oocyst output allowed us also to get data to compare intensity and prevalence of *Isospora* infection in young starlings of different moult stage.
Method: The work was made on the Courish spit of the Baltic sea. 110 young starlings (*Sturnus vulgaris*) were trapped at different time of day during June and July 1997. Fresh droppings were collected and after sporulation were checked by standard method of Fülleborn. Feces samples from 7 caged starlings were collected every 2 hours during 4 days and were checked by the same method.
Results: The peak of oocyst output was between 4 and 7 p.m. both in nature and captivity. The number of oocysts at this period of time increased with developing of moult, though the prevalence of infected birds was constant.
Conclusions: The diurnal rhythm of oocyst appearance exists in nature as well as in caged starlings and may be an adaptation of parasite to host behaviour. There is a constant part of starlings in population which seems to be, by some reasons, resistant to *Isospora* infection.

c.6.52 PREVALENCE OF *TOXOPLASMA GONDII* ANTIBODIES IN SERA OF DOMESTIC PIGS AND SOME WILD GAME SPECIES FROM ZIMBABWE

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ABSTRACT: Serum samples of domestic pigs (*Sus scrofa*), kudus (*Tragelaphus strepsiceros*), elands (*Taurotragus oryx*), giraffes (*Giraffa camelopardis*), bushbucks (*Tragelaphus scriptus*), sable antelopes (*Hippotragus niger*), nyalas (*Tragelaphus angali*), warthogs (*Phacochoerus aethiopicus*), bushpigs (*Koiropotamus* [*Potamochoerus*] *koiropotamus*), white rhinos (*Ceratotherium simus*), black rhinos (*Diceros bicornis bicornis*), African buffalos (*Syncerus caffer*), wildebeest (*Connochaetas taurinus*), African elephants (*Loxodonta africana*) and lions (*Panthera leo*) from Zimbabwe were tested for *Toxoplasma gondii* IgG antibodies by the modified agglutination test (MAT) incorporating whole formalized tachyzoites and mercaptoethanol in the antigen. Sera were diluted at 1:25, 1:50 and 1:500 for MAT testing. *Toxoplasma gondii* antibodies were found in 8.3% of 144 domestic pigs, 20% of 10 kudus, 36.8% of 19 elands, 10% of 10 giraffes, 57.1% of 14 bushbucks 11.9% of 67 sables, 90% of 10 nyalas, 0% of 21 warthogs, 0% of 5 bushpigs, 50% of 2 white rhinos, 27.3% of 11 black rhinos, 5.6% of 18 buffalos, 14.5% of 69 wildebeest, 10% of 20 elephants and 92.3% of 26% lions examined. Sera with antibodies in a 1:25 dilution were considered to have *T. gondii* infection.

c.6.53 AGE - RELATED SEROPREVALENCE TO NEOSPORA CANINUM AND TOXOPLASMA GONDII IN RED FOXES (VULPES VULPES) IN BELGIUM

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Background : *N. caninum* and *T. gondii* are 2 cysts forming sporozoa. The cat acts as final host for *T. gondiii* whereas the dog has been recently identified as the final host of *N. caninum*. This study was conducted in order to assess the age - related seroprevalence to *N. caninum* and *T. gondii* in the red fox (*Vulpes vulpes*) a widely distributed carnivore in Belgium and elsewhere.
Method : 95 foxes killed in the Province of Luxembourg between August 96 and July 98 were available ; age was determined by radiographical or histological examination of the canine. Animals were classsified as juveniles (less than 1 year) or adults aged over 1, 2, 3 or 4 years. Sera were collected and specific antibodies to *N. caninum* and *T. gondii* were detected using a home-made IFAT (starting dilution and cut-off : 1/100).
Results : 1 (1.05 %) and 73 (76.8 %) animals were seropositive for *N. caninum* and *T. gondii* respectively. The animal seropositive to *N. caninum* had a high titre (1/3.200).There was no difference in seropositivity to *T.gondii* between the different age groups.
Conclusion : Seroprevalence to *N. caninum* and *T. gondii* in red foxes is highly different which suggests that there was no cross reactivity between the two parasites in the IFAT test and that the level of exposure to these 2 protozoa differs markedly.
Acknowledgment : This work was supported by a grant (grant 5774 A) from the Ministry of Agriculture, Belgium.

c.6.54 GIARDIA AND CRYPTOSPORIDIUM IN CANADIAN ARCTIC AND LAND AND MARINE MAMMALS

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Background: Giardiasis is frequently observed in arctic communities in Canada and cryptosporidiosis occasionally occurs. Animal to human transmission may occur and these diseases may also have detrimental effect on the overall health of arctic mammals.
Methods: The presence of *Giardia intestinalis* cysts, *Cryptosporidium parvum* and *Cryptosporidium muris* oocysts in wild arctic land animals (eg. muskox, caribou, wolf, grizzly bear) were determined. Samples were collected from animals in the Yukon and Western Northwest Territories. The prevalence of *Giardia intestinalis* cysts, *Cryptosporidium parvum* and *Cryptosporidium muris* oocysts in pinnipeds from the western arctic and Canadian east coast (including St. Lawrence estuary) was also determined. Fecal samples were collected from hunted or dead seals (ringed, grey, harp and harbour) and whales (beluga, northern bottle-nosed). Fecal samples were processed by sucrose gradient centrifugation and staining with FITC monoclonal antibody.
Results: *Giardia intestinalis* was detected in most carnivorous mammals but Cryptosporidium was not detected. Muskox and caribou harboured Giardia intestinalis, *Cryptosporidium parvum* and *Cryptosporidium muris*. Giardia intestinalis was detected in harp seals, grey seals, harbour seals and ringed seals. Neither *Giardia* nor *Cryptosporidium* were detected in whales sampled.
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c.6.50-58 Protozoan parasites of wildlife

c.6.55 " STRATIGIES FOR RESEARCH AND CONTROL OF TRYPANOSOMIASIS INFECTION AMONG FREE-RANGING INDIAN WILD LIFE IN 21st CENTURY " - A CASE STUDY.

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Background: Trypanosomiasis Caused by *Trypanosoma evansi* have been reported in the form of morbidity and mortality at alarming rate among free living Wild Animals in Ranthambhore National Park INDIA.

Method and Media: Blood & lymph samples were collected from tranquillized, Ailing & carcass of felidae like 9 TIGER (*Panthera tigris tigris*), 26 Panther (*Panthera padus*) as well as Herbivores like 23 black buck (*Antilope Cervicapra*) 83 spotted deer (*Axis axis*), & 57 Sambhar (*Cervus unicolar*). All the samples were anaylized by wet Blood and lymph smear examination (W B L E) and by Micro haematocrits centrifugation technique(M H C T). Blood Samples were also send to various labs. for serological Diagnosis.

Results: Trypanosoma evansi infaction were found positive in felids in 8.57% to 14.28%, 11.45% by WBLE & MHCT and in 12.27% to 18.52%, 15.32% by WBLE & MHCT in wild herbivores. All the labs. also confirmed the presence of parasits in the Blood samples.

Conclusion: This is the first report of the existence of trypanosoma evansi infaction among free living wild animals in the study area and Suggest a high techno-diagnosis-research at the cellular, molicular, genetic and eco-development level in the scientific co-operation with devloped nations for the bright future of indian wild life in 21 st century.

Acknowledgement: I am gratefull to prof. K.M.L. Pathak & Dr. R.M. Bhatnagar for there excellent technical support and advice.

Finally Thanks to Dr. D.S. Raghav for his willing supervision

c.6.56 ATTEMPTED INFECTION OF COMMON WATERBUCK (*KOBUS ELLIPSIPRYMNUS*) WITH BUFFALO-DERIVED *THEILERIA PARVA*.

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Background: Following reports that defassa waterbuck (*Kobus defassa*) may play a role in the epidemiology of *T. parva*-group infections in cattle in East Africa, common waterbuck (*Kobus ellipsiprymnus*) in Kruger National Park (KNP) were investigated as potential carriers of *T. parva*-group infections.

Method: Seven adult and sub-adult waterbuck were captured and screened by conventional and molecular diagnostic techniques for *Theileria* spp infections. Laboratory-reared *Rhipicephalus zambeziensis* nymphs were fed in ear bags on 4 captive buffalo (*Syncerus caffer*) confirmed to be naturally infected with *T. parva*. The ensuing adult ticks were fed on 4 captive sub-adult waterbuck and 2 cattle.

Results: All waterbuck were found to carry microscopically detectable *Theileria* sp. piroplasm infections, found by PCR diagnosis to belong to a hitherto uncharacterised *Theileria* species. *R. zambeziensis* adults which fed as nymphs on the buffalo transmitted fatal *T. parva* infections to cattle. However, no transmission of *T. parva* to the waterbuck could be demonstrated clinically or by PCR diagnosis. Also, *R. zambeziensis* nymphs which were subsequently fed on the waterbuck failed to transmit *T. parva* to cattle in the ensuing adult stage, confirming the absence of *T. parva*-group infections in the waterbuck.

Conclusion: The results suggest that buffalo in KNP probably do not carry *T. parva*-group parasites which are readily transmissible to waterbuck and that waterbuck are thus unlikely to play an important role in the epidemiology of *T. parva*-group infections in cattle in South Africa.

c.6.57 DETECTION OF *TRYPANOSOMA EVANSI* IN BRAINS OF HOG DEER BY STREPTAVIDINE-BIOTIN IMMUNOHISTOCHEMISTRY

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Twenty-two percent of free-ranging hog deer (*Cervus porcinus*) on a farm in Samuthprakarn, Thailand, died between September 1997 and February 1998. Most of them presented with nervous signs including ataxia, paresis of the hind limbs, lateral recumbency, excitation, convulsion and finally death. Several pregnant deer aborted. Six animals showing clinical signs of disease and one carcass were submitted for diagnosis at the National Institute of Animal Health, Bangkok.

Trypanosoma evansi was detected in the blood and cerebrospinal fluid of four and five deer, respectively. Antibodies to *T. evansi* were found in all six hog deer as well as in the dead animal by indirect enzyme-linked immunosorbent assay. Histopathological observation showed that there was a generalized nonsuppurative meningoencephalitis affecting white and gray matter at all levels of the brain. Typically, there were broad perivascular cuffs of mononuclear inflammatory cells including lymphocytes, and some Mott cells. No trypanosomes were found in any tissue examined by conventional histopathology. However, numerous *T. evansi* were demonstrated by streptavidin-biotin immunohistochemistry in neuropil and Virchow-Robin spaces of brain in three of animals examined.

c.6.58 ISOLATION OF *SARCOCYSTIS FALCATULA* FROM *DIDELPHIS ALBIVENTRIS*

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Background: *Sarcocystis falcatula* uses avian as intermediate hosts and the North American opossum (*Didelphis virginiana*) as the definitive host. To extend knowledge on its biology, *S. falcatula* infection in the South America opossum (*Didelphis virginiana*) was investigated.

Method: *Sarcocystis* sporocysts from the intestines of 2 opossums (*Didelphis albiventris*) from Argentina were inoculated into three captive-reared budgerigars (*Melopsitiacus undulatus*). Tissue samples from budgerigars were examined after staining with hematoxylin and eosin and by immunohistochemical methods. Lungs of 1 budgerigar were inoculated in bovine monocyte cell cultures.

Results: Budgerigars died of acute sarcocystosis 8, 9, and 14 days after feeding sporocysts. Schizonts and merozoites, found in the lungs and other organs of the budgerigars, were identified as *S. falcatula* based on structure and immunoreactivity with anti-*S. falcatula*-specific antibody. *Sarcocystis falcatula* was also isolated in bovine monocyte cell cultures.

Conclusion: The results of this investigation indicate that *Didelphis albiventris* is another definitive host for *S. falcatula*. This is the first report of *S. falcatula* nfection in South America.

c.6.59-69 Helminth parasites of wildlife

c.6.59 LUNG NEMATODES IN RED DEER IN BIAŁOWIEŻA FOREST

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Lung nematodes are widespread and the most pathogenic parasites in red deer. To examine the level of invasion of red deer with lung nematodes in Białowieża Forest, animals were tracked on newly-fallen snow, and from each of them the sample of feaces were collected. 86 collected samples were examined with Baermann method to estimate extensity and number of larvae in 5g of feaces.

In red deer were found 3 species of lung nematodes: Elaphostrongylus cervi, Varestrongylus sagittatus and Dictyocaulus noerneri. In 100% of examined in Białowieża Forest red deer were infected with E. cervi. However V. sagittatus occured in 51% and D. noerneri in 21% of red deer. The greater part of red deer were infected with mixed invasion. In 43% of deer were found mixed E. cervi and V. sagittatus invasion, in 13% E. cervi and D. noerneri and in 8% occured all 3 found species of nematodes. Intensity of invasion was measured as number of larvae in 5g of feaces. For E. cervi it was 4 - 1186, V. sagittatus 1 - 1062 and D. noerneri 1 - 17 larvae. During last 15 years extensity of invasion of E. cervi increased 9% and V. sagittatus 16%.

Lung nematodes are very common parasites of red deer in Białowieża Forest and the level of invasion requires continous inspection.

c.6.60 *UMINGMAKSTRONGYLUS PALLIKUUKENSIS*, A LUNGWORM IN CANADIAN ARCTIC MUSKOXEN

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Background: *U. pallikuukensis* (*Up*) is a protostrongylid lungworm of muskoxen in NT, Canada (67°54'N, 116°38'N). Field and laboratory experiments provided information on the biology of this parasite in its hosts and natural environment.

Methods: Experimental infections of captive muskoxen were used to define prepatent and patent periods, development and distribution of lung lesions and intrapulmonary parasite populations. The habitat of infected muskoxen was surveyed for gastropods. Laboratory infections were used to assess the ability of these gastropods to support *Up* larval development and to investigate development rates and the emergence of third stage larvae (L3).

Results: The prepatent period of *Up* was approximately 3 months. The patent period was at least 23 months. Lung cysts were found in all lobes and were primarily associated with major airways and vessels. Two to six adult parasites were found in each cyst. Of eight gastropod genera found in field surveys, five snail genera and the slug *Deroceras laeve* supported larval development. In *D. laeve* at 20°C, larvae required 12 days and, at 15°C 21 days, to develop to L3. Emergence of L3 was a consistent feature of gastropod infections.

Conclusion: Aspects of the development of *U. pallikuukensis* in its mammalian and gastropod hosts have been described.

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c.6.61 PREVALENCE OF THE TREMATODE *COLLYRICLUM FABA* IN SLOVAKIA

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Background: The skin trematode *Collyriclum faba* (Bremser in Schmalz 1831) has been recorded only sporadically in some 20 species of wild passerines in various European countries. There is no enough information about the prevalence of this parasite in indigenous bird populations.

Method: During post-breeding period (August) in years 1996 – 1998, wild birds were caught in mist-nets in the Bukovské vrchy hills in the Poloniny National Park on the boundary between the western and eastern Carpatians in the nord-eastern part of Slovakia. Lesions diagnosed as the subcutaneous cysts of *C. faba* were found in these birds.

Results: *C. faba* was found in 30 (30 %) of 99 robins (*Erithacus rubecula*) and in 1 of 3 redstart (*Phoenicurus phoenicurus*). Other 500 birds of 43 species were not affected.

Conclusion: It is likely that the site in the hills is an endemic focus for *C. faba* where at least one of stage of its still unknown life cycle is probably attached to the food of robins and redstarts.

Acknowledgement: The author would like to thank to Š. Pčola and M. Bural (NP Poloniny) for their cooperation.

c.6.62 *IXODES RICINUS* ON GREEN LIZARDS IN A NATURAL AREA FROM NORTH ITALY

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Background: Lizards have been considered incompetent reservoir hosts for *B. burgodorferi* but able to maintain the younger stages of *Ixodes ricinus*. The importance of green lizard (*Lacerta bilineata)* in the life cycle of *I. ricinus* was studied in an area where roe deers have been recently reintroduced.

Method: 165 green lizards were sampled from June to October 1997 and from April to September 1998 in the Lombard Park of Ticino Valley (North Italy). All lizards were captured by hand, marked individually and released after noting host and habitat informations. Ticks from each host were identified and their life stage was determined.

Results: All the ticks (n= 1940) were larvae (31%) and nymphs (69%) of *I. ricinus*. The prevalence and the abundance respectively were 70.4% and 11.76. Highest abundances have been observed in adults and particularly in males captured in April and July. All collected tick stages were found fully engorged.

Conclusion: The green lizard resulted a suitable host for the life cycle of *I. ricinus* in the study area showing the ability to host mainly nymphs and confirming their zooprophylactic role in the epizootiology of European Lyme borreliosis.

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c.6.59-69 Helminth parasites of wildlife

c.6.63 ON THE STRUCTURE OF HELMINTH COMMUNITY OF BANK VOLE *CLETHRIONOMYS GLAREOLUS*

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Background: It is discussed whether parasites produce predictable community structures or community structure is a fortuitous result of independently evolved species. The aim of the present work was to evaluate interactions among helminth species of bank vole and to ascertain the reasons predetermining the structure and dynamics of parasitic community.
Method: The material was collected in South West Lithuania (Šakiai district). The biotope is the wood-cutting areas of mixed coniferous-deciduous forest. Rodents were caught in seasonal assays in 1983-1986 and 1998. Total helminthological autopsies of about 400 bank voles were carried out. The interaction among helminth species was evaluated using correlation coefficient r. A significant difference was declared if the P value was < 0.05.
Results: 17 helminth species were found in bank voles. There exists a relation among bank voles infection with different species of helminths. Numbers of parasites correlate reliably in 9.2% of cases out of all the pairs of helminth species possible in seasonal assays. Since all the reliable correlations among helminth numbers are positive, it can be assumed that these correlations are determined by a higher probability of infection of certain groups within host population with all the helminths. Distinct changes in the structure of helminth community were observed during the period of investigation.
Conclusion: The dynamics of separate species and changes in the structure of helminth community show that the structure of bank vole helminth community is predetermined by characteristics of separate species and their dynamics. Interaction between separate species of parasites and the host is of greater significance than interaction among parasites.

c.6.64 AN ELISA FOR DETECTING ANTIBODIES AGAINST OPISTHORCHIID FLUKES IN SILVER FOXES

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Background: The red fox (*Vulpes vulpes*) has proven to be a good indicator for the occurrence of opisthorchiid flukes and can be used for mapping the distribution of these parasites in larger territories. Since blood samples for detecting the vaccination status against rabies are taken at necropsy the idea was borne to develop a serological test to detect specific antibodies against liver flukes with an ELISA.
Method: An E/S antigen was produced by in vitro cultivation of *Opisthorchis felineus* and *Metorchis bilis*. 2 and 6 silver foxes (*V. vulpes fulva*) were experimentally infected with either *O. felineus* or *M. bilis* respectively. Serum samples were taken prior to infection, then weekly up to the 7th week post infection (p.i.) and after that at monthly intervals and were tested for homologous and heterologous antigens (AG).
Results: All infected animals excreted opisthorchiid eggs starting between 2 and 4 weeks p.i. Specific antibody titres against homologous AG seroconverted in the 2nd week p.i. and remained positive up to the end of the trial at week 41 p.i. while antibody titres against heterologous AG were significantly lower and stayed near the cut-off. At necropsy, the number of flukes was low, compared to findings in free living foxes. 2 infected foxes were negative for flukes at necropsy.
Conclusions: The E/S-ELISA is specific and sensitive enough to detect liver fluke infections and can be used as a tool for epidemiological investigations.

c.6.65 CRUZIA TENTACULATA ISOLATED FROM DIDELPHIS ALBIVENTRIS IN ARGENTINA.

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In this study the A.A. describe some external morphological features of *Cruzia tentaculata* Rudolphi, 1819 (Nematoda: Cosmocercoidea) using images obtained with conventional optic microscope (COM) and scanning electronic microscope (SEM).
The parasites were collected during necropsy from the caecum and large intestine of *Didelphis albiventris* (white-spotted wild weasel) hunted in its native environment near Corrientes City, Argentina (59° W longitude, 27° S latitude).
Collected specimens of *C. tentaculata* were washed into physiological solution and then observed by COM in order to be classified. Later these specimens were put into a 3% glutaraldehyde solution, dehydrated, dried by critical point, assembled, metalized,observed and photographed with a SEM.
This is the first description of some external morphologic features of this species carried out by SEM in Argentina.

c.6.66 PREVALENCE OF *ALARIA ALATA* IN RED FOXES IN WEST POLAND

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Material and methods: The studies were carried out in 15 provinces in the west part of Poland. During the years
1994-1997 (till April) under a regime of strict laboratory safety regulation the intestines of 1909 red foxes were examined for *Alaria alata*. The section has been done according to the method of Eckert et al. (1991). Additionally faecal samples were examined by using a coprological method (Willis-Schlaaf).
Results: Foxes infection with *Alaria alata* were detected in 15 provinces (tab. 1). Of special interest is the finding in north – west provinces where about 33.8 per cent of foxes were infected with *Alaria alata*. In the central – west 15,4% and in the south – west provinces 2.2% foxes were infection with *Alaria alata* only.

Tab. 1. The infection rate of *Alaria alata* in red foxes in part of Poland.

Part of Poland	Number of the examined foxes	Number of the infected foxes	
		Number	per cent
North-west	1080	365	33.8
Central-west	188	29	15.4
South-west	641	14	2.2

Conclusion: The studies indicate that geoclimatic conditions are on important factor, which influence the infection rate of *Alaria alata*.

c.6.59-69 Helminth parasites of wildlife
c.6.70-76 Parasites of poultry

c.6.67 PARASITIC NEMATODES OF THE FAMILY CAPILLARIIDAE (NEMATODA, TRICHOCEPHALIDA): ECOLOGICAL CONNECTIONS AND INFLUENCE ON HOSTS

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Nematodes of the family Capillariidae are a widely distributed group of helminths. We have found three polyspecific species of Capillariidae *(Capillaria hepatica, C. soricicola, Eucoleus aerophilus)* in natural populations of mammals (Voronezh Biosphere Reserve). These species, which are highly pathogenic parasites, have been found in liver parenchyma *(C. hepatica, C. soricicola)* and in the bronchioles of the lungs *(E. aerophilus)*. Red mouse is a dominant for the prevalence (47.5%) and the abundance (0.7%) of the parasite ($P<0.01$). Thus, red mouse plays a major part in the accumulation and spread of *C. hepatica* in the environment. European beaver has become a member of the parasitic system due to high-level concentrations of infected *C. hepatica* eggs in wetlands. It has been found that the pathology caused by *C. hepatica* reduces the biological productivity of the beaver populations (micropopulations) by 10-12%. *C. soricicola* is a parasite of insectivores, mainly from the family Soricidae. Due to coincidence of the ecological niches of the Soricidae species, *C. soricicola* may be one of the main biotic factors influencing the population dynamics of insectivores. *E. aerophilus* is found mainly in carnivores. Red fox is a dominant for the prevalence (75.0%) and abundance (5.0%) of *E. aerophilus*.

c.6.68 CHRONICAL PARASITIC GRANULOMATOUS TYPHLITIS IN DIDELPHIS ALBIVENTRIS.

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Histopathological lesions of the caecum in *Didelphis albiventris* by *Cruzia tentaculata (Nematoda: Cosmocercoidea)* are described. For this study, the material was fixed in a 10%-formol solution according to the classical technique for parafined blocks and colored with haematoxylin and eosin. On the analysis of the caecum mucous membrane affected by this parasite were observed necrosis of superficial epithelium and a severe chronical granulomatous inflammatory reaction, with giant cells at the levels of submucous and serous membranes. This study is the first report of the histopathological lesions caused by *Cruzia tentaculata* in the caecum of *Didelphis albiventris* in Corrientes, Argentina.

c.6.69 MEDICAL AND VETERINARY MEANING OF WILD ANIMALS HELMINTHS OF POLESIE

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Background: One of the main lines is the determination of the degree of infection of wild animals by helminths dangerous for man and domestic animals. The substance of this report is the results of investigations (1981-1995) of animals of canine family (Canidae)in Belorussian Polesie (southern part of Belarus).
Method: 218 carcasses of 3 species (Canis lupus, Vulpes vulpes, Nyctereutes procyonoides) were subjected by helminthological revelation according to Skrjabin K.I.
Results: The high infection of wild animals of canine family by helminths (83.0±2.5%) was tested. These animals are owners of 35 species of helminths. The 34 species are known as parasites of man and domestic animals (dogs, cats and pigs). The most frequently Uncinaria stenocephala (37.6±3.3%), Alaria alata (36.7±3.3%), Toxocara canis (22.0±2.8%), Isthmiophora melis (17.4±2.6%), Capillaria plica and Trichinella spiralis larvae (16.5±2.5% for each) are registered. Also Echinococcus granulosus and E.multilocularis are very important for medicine and veterinary science. The 1st parasite was discover in C.lupus (11.5±4.4%), 2nd - in V.vulpes (6.8±2.7%). Intensity of infection – 1-3 individuals of helminths in infectious animals.
Conclusion: Wild animals of canine family are the spreaders in Belorussian Polesie of 34 species of helminths, having medical and veterinary meaning. These animals promote to support of natural helminthoses centers which in turn may be dangerous for the human and domestic animals organisms.

c.6.70 Lipeurus caponis control by Fipronil

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Background: The Fipronil is a drug used in dogs and cats to control ticks and fleas infestation with excellent results.
Method: 30 birds with a similar parasitic infestation (12 parasites for feather) were selected in chicken farm.
20 birds were treated by 2 drops of Fipronil 0,25% as spot-on on the back, and 10 birds without used as control group.
Results:48 hours after the spot-on treatement and for 60 days not Lipeurus caponis were found on treated hens. The treaties birds increased their weight significantly, and not toxicity signs were observed.
Conclusion: Fipronil showed an excelent insecticide action and residual effect to control L. Caponis in hens.

c.6.70-76 Parasites of poultry

c.6.71 *Eimeria colchici* in pheasant – endogenous development and immune responses

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Background: Coccidiosis is an important disease of farm-breeding pheasants, where the risk of the occurrence of clinical form increases in parallel with bird concentration.
Method: Coccidia-free 2-week-old pheasant chicks were experimentally infected with a pure suspension of *Eimeria colchici* oocysts (5,000 oocysts per chick). Blood samples for immunological tests, and tissue samples (intestinum) for histological processing were obtained at 24 h intervals, i.e. 24, 48, 72, 96, 120 and 146 h after infection.
Results: Numerous first-generation schizonts were found in the intestinal crypts of the caeca 24 h post-infection, second-generation schizonts were observed 72 h post-infection, and 120 h post-infection numerous macro- and micro gamonts could be seen. Lymphocytes response of pheasant chicks to *Eimeria colchici* antigen in blasttranformation test significantly increased from the 6th day, with maximum on 14th day. Metabolic activity of phagocytes increased from 14th day post infection, with increasing of T-lymphocyte subpopulation on 2nd day post infection.
Conclusion: the patent period began on the 4th day and ended on 11th day post-infection with the largest production of oocysts on days 7-9, with positive changes in immune response.
Acknowledgement: This work was supported by a research grant from the Scientific Grant Agency of The ministry of Education of The Slovak Republic and Slovak Academy of Sciences (Grant 1/4254/97).

c.6.73 EFFECTS OF THE CHICKEN MITE, *DERMANYSSUS GALLINAE*, ON EGG LAYERS.

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Background: The chicken mite, *Dermanyssus gallinae*, is a well known blood sucking ectoparasite in Danish egg production units. However, there has been conflicting reports about the effects of mite infestations on the host birds.
Method: 2 groups of each 15 hens were infested with *D. gallinae* and 2 similar groups were used as uninfected control. Changes in the population size of the mites were observed as well as differences in the weight gain and blood parameters of the hens.
Results: In the groups infested with *D. gallinae* there was a significant decrease in the packed cell volume and the mean cell haemoglobin at a time of rapid growth in the mite population. On the same time, the mean cell volume was significantly higher in the infested groups. At the peak of the mite population, 6 birds in the infested groups died within 1 week. The infested hens also showed a reduced weight gain compared with the control hens. But no significant differences were observed in the immunological parameters of the blood.
Conclusions: During periods of rapid population growth of *D. gallinae*, severe physiological problems or even deaths may occur in infested birds. Changes in blood parameters indicate that the infested hens were suffering from a regenerative anaemia, due to the blood intake by *D. gallinae*.

c.6.74 DIFFERENCES IN PARASITE BURDENS BETWEEN DANISH LANDRACE CHICKENS AND LOHMAN BROWN CHICKENS INFECTED WITH *ASCARIDIA GALLI*.

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Background: Due to consumer demands organic farming systems have developed in Denmark. More than 10% of the total number of table eggs are now produced in such systems. However, mortality has increased compared to traditional cage or deep-litter systems. Part of this mortality is presumably due to the high prevalence of *Ascaridia galli*.
Method: Two groups (Lohman Brown and Danish Landrace, respectively) of each 90 animals were infected as follows: Thirty animals were trickle infected on a weekly basis with 50 embryonated *A. galli* eggs, thirty animals were infected once with a single dose of 500 eggs and thirty animals were kept as controls. After 8 weeks 15 animals from each group were killed and examined for the presence of parasites. Two weeks later all animals were challenged with a single dose of 500 eggs. Eight weeks later the the remaining animals were killed and examined for the presence of parasites.
Results: There were no differences in worm burdens between trickle and single infected animals in either groups. However, significantly lower different worm burdens were seen in the Lohman Brown chickens at the first examination, whereas significantly lower worm burdens were seen in the Danish Landrace chickens after the challenge infection.
Conclusion: A genetic component might explain the differences in worm burdens and additionally that the Danish Landrace might have a capability of "self-cure" when challenge infected.
Acknowledgement: The Danish Council for Development Research (Danida) and the Danish National Research Foundation is acknowledged for the financial support of the study.

c.6.75 SOME MORPHOLOGICAL ASPECTS OF THE FOWL NEMATODE *ASCARIDIA GALLI*

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Background: Despite its global occurrence and significant pathogenicity, morphological characteristics of the various stages of *Ascaridia galli* seem limited. There are to date no studies describing the entire life cycle using scanning electron microscope methods.
Method: Naturally infected hens were purchased in order to obtain adult female *A. galli* worms. After isolation and identification, the worms were dissected and the eggs were incubated in 0.1N H_2SO_4 at 28 °C. The eggs were followed on a weekly basis and light microscope and scanning electron microscope pictures were taken. Once embryonated, the eggs were orally administered to parasites naive chickens. Chickens were slaughtered with weekly intervals and different stages of worms were obtained for further documentation using scanning electron microscopy, transmission electron microscopy and light microscopy.
Results: The life cycle of *A. galli* has been described in pictures. The number of male papillae differs compared to those in the existing literature. Furthermore, sensoric areas close to the anal opening have been identified for the first time.
Conclusion: The morphology of *A. galli* in this study differs compared to the existing literature. This suggests that there might be geographical differences between *A. galli* populations. DNA studies are needed to study whether these differences are only morphological in nature or whether they are also genetic in origin.
Acknowledgement: The Danish Council for Development Research (Danida) and the Danish National Research Foundation is acknowledged for the financial support of the study.

c.6.70-76 Parasites of poultry
c.6.77-87 Parasites of fish

c.6.76 SEASONAL WORM FLUCTUATIONS IN NATIVE CHICKEN IN WEST JAVA INDONESIA

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Background: Native chicken or kampong chicken plays important role as protein source for people in Indonesia. The present study was carried out to investigate seasonal fluctuation of gastrointestinal (GI) helminths in native chicken in wet and dry regions of West Java Indonesia.

Methods: Every month from July until December 1997, fifty-six worm-free native chicken tracers were turned out in four chicken farms in West Java Indonesia situated in wet (type A) and dry regions (type C). After the tracers had been exposed to infection for one month, they were reared under worm-free condition for 21 days until before post-mortem worm counting.

Results: GI helminth populations of chicken in both climatic regions were highest in July. The helminth population in dry area (C) suddenly dropped in August and remains low until the end of the study. In wet area the population remain high until September. GI nematode predominated worm burdens of animals originated from type C climatic region, whereas cestode population was higher in farms of the type A climatic region. Despite total worm counts were higher in animals from type A climatic region compared with those of the type C, the difference is not statistically significant.

Conclusion: Climatic regions affected seasonal fluctuation of helminth parasites in native chicken in West Java.

c.6.77 DIPLECTANID MONOGENEANS OF THE GENUS *PSEUDORHABDOSYNOCHUS* FROM BRAZILIAN GROUPERS WITH POTENTIAL FOR MARICULTURE

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Background: The direct life cycle of monogeneans is the main reason for their rapid population growth observed when appropriate abiotic conditions are provided. Thus, when infected fish are kept in confined environments with suitable temperatures epidemic outbreaks will often result. The groupers *Epinephelus niveatus* (Valenciennes) and *E. marginatus* (Lowe) (Serranidae) are currently being evaluated in mariculture facilities near Rio de Janeiro as potential species for future fish farming. It was therefore considered important to examine these fish for monogeneans at an early phase in their domestication.

Method: The fish hosts were captured close to Ilhas Cagarras off the Brazilian coast. The worms, collected from the gills, were fixed in ethanol and several staining procedures were used (Gomori's trichrome, Mayer's paracarmine).

Results: *Pseudorhabdosynochus* n. sp. from *Epinephelus niveatus* is characterized by having a vagina with a non-sclerotised ampulla, its tegument armed with scale-like spines and the squamodisc with 15-16 open concentric rows of elements. Characters as ventral and dorsal bars, cirrus-bulb and vagina and geographical distribution serve to differentiate it from other species of the genus. *P. beverleyburtonae* (Oliver, 1984) is recorded from *E. marginatus* and redescribed.

Conclusion: Infections of serranid fishes with diplectanids are known to be a source of stress and a major contributing factor to disease outbreaks. The species of Pseudorhabdosynochus, recorded for the first time in Brazil, must therefore represent a potential threat to farming of groupers in the region.

c.6.78 PARASITE FAUNA OF ROACH RUTILUS RUTILUS (L.) FROM THE RUDA WODA LAKE (POLAND)

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From May 1997 until Januar 1998 the seasonal occurrence of parasites of roach *Rutilus rutilus (L.)* originating from the lake Ruda Woda were investigated. The examined fish (74 specimens) were of 14 - 26cm of total length, of 44,3 - 210g of weight and age of 2+ to 4+. Totally 17 species were recorded belonging to: 2 Protozoa (*Myxobolus muelleri, Trichodina sp.*), 8 Monogenea (*Dactylogyrus crucifer, D. namus, D. sphyrna, D. similis, D. fallax, D. suecicus, Dactylogyrus sp., Paradiplozoon homoion homoion),* 4 Digenea *(Tylodelphys clavata met., Diplostomum sp.met., Sphaerostomum globiporum, Bucephalus polymorphus met.),* 1 Cestoda *(Paradilepis scolecina), 1* Nematoda (*Raphidascaris acus larv.),* 1 Crustacea (*Ergasilus sieboldi*). The parasite fauna of roach was dominated by *Tylodelphys clavata, Diplostomum sp., Dactylogyrus crucifer* with an overall prevalence and intensity (86,4%, 2-300; 85,1%, 2-121; 58%, 1-23 worms)fish) respectively. Less abundant occurred *Trichodina sp.* (32,2%, 1-6), *D. namus* (25,7%, 1-23), *S. globiporum* (25,6%, 1-4), *P. scolecina* (25,6%, 1-8), *Paradiplozoon h. homoion* (17,6%, 1-4), *D. similis* (12,2%, 3-22), *D. fallax* (12,2%, 1-6). The other species were found rarely with low intensity and lower than 10% prevalence. The majority of these species was most abundant in spring-summer period and less abundant in autumn-winter period; only prevalence of *Diplostomum sp.* and *Tylodelpys clavata* were steadily high during the whole period of the study.

c.6.79 Prevalence of intestinal nematodes in aquarium fishes

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Background: There is a problem with therapy and control of some intestinal nematodes with direct life cycle in aquarium fishes.

Method: 532 complete parasitological dissections of 35 fish species were performed. In small species a compresoric method digestive tract was used.

Results: Intestinal nematodes were found out in 15 species of fish. The highest prevalence was observed in 4 species *(Ancistus sp., Symphysodon sp., Poecilia reticulata, Pterophyllum scalare)* with typical clinical symptoms as anorexy, kachexy. Typical sign was white trailing faeces. The clinical symptoms depend on an amount of nematodes in intestine. The numbers of parasites ranged from 1 till 145 with an average of 5 to 8 species.

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c.6.77-87 Parasites of fish

c.6.80 GYRODACTYLIDS ON SALMONIDS FROM DANISH STREAMS – A PRELIMINARY REPORT

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Background: The first country-wide survey of *Gyrodactylus* species of wild salmonids in Denmark is presented. The survey has been carried out as part of an EU-Fair project on improved diagnosis of *Gyrodactylus* species of aquacultured species. The project includes participants from Scotland, Norway and Denmark.
Methods: Fish sampling included brooks and major streams from 10 of the 14 counties in Denmark. A total of 61 localities from 45 major streams were sampled by electro-fishing. Dissected fins and body proper were preserved separately in 80 % ethanol. At the laboratory, gyrodactylids were enumerated and isolated from the fins and specimens mounted in ammonium-picrate-glycerin.
Results: A total of 599 fish were recovered. Five different salmonid species were caught: Atlantic salmon *Salmo salar* (n = 13), brown trout *S. trutta* (n = 537), houting *Coregonus oxyrhynchus* (n = 3), rainbow trout *Oncorhynchus mykiss* (n= 38) and grayling *Thymallus thymallus* (n = 8). *Gyrodactylus derjavini*, *G. salaris* and *G. truttae* have thus far been identified. Another, not yet identified, morphotype has also been recovered. Infection prevalence of 100 seems to be the general pattern in the majority of the streams. Intensities of gyrodactylids on brown trout vary considerably ranging from 1 to 1421 parasites, as well as mean intensities and abundances varies between the investigated streams and regions. The prevalence of *Gyrodactylus* spp. on all salmonids were 82 % with an abundance of 71.5 and a mean intensity of 86.7. Concerning each salmonid species, the figures are as follows (prevalence – abundance – mean intensity): Brown trout: 83.9 % - 76.8 - 91.5; salmon: 30.8 % - 0.9 – 3.0; rainbow trout: 92.1 % - 38.0 - 41.3; grayling: 50 % - 1.0 – 2.0. None of the three houting were infected.

c.6.81 NON-SYSTEMIC RESPONSE IN RAINBOW TROUT AGAINST *Gyrodactylus derjavini*.

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Background: Epidermal changes of host skin during infection and development of host response were studied. Furthermore it was studied whether the aquired protective immunity could be transferred by passive immunisation of naive hosts.
Methods: Two groups of rainbow trout (1.4 g) were infected with *G. derjavini* and infections followed for 70 days. Additional uninfected fish were transferred to each experimental group at 34 days p.i. Infections on these subgroups were likewise followed. Mucous cell densities were determined on Alcian Blue stained fins including uninfected controls. The carbohydrate composition of these cells was determined by using a lectin assay. A group of 15 fish (5 cm) were passively immunized with native immune sera whereas another 15 fish received heat-inactivated sera. Finally 15 fish received decomplemented non-immune sera. The fish were then infected and the infections monitored for a total of 32 days.
Results: Parasite populations increased on all naive host groups and peaked 5-6 weeks p.i. after which infection levels decreased. Introduction of naive fish into responding host populations resulted in heavy infections of naive fish, whereas immune fish remained infected at a very low level. Mucous cell hyperplasia was seen in one group 34 days p.i., but at the termination of the study a significant depletion was evident. Small changes in lectin binding patterns were seen during infection. Thus, a stronger expression of mannosyl, acetyllactosamine and glucosyl residues was evident in the infected fish. No differences in abundance between the groups were detected in the passive immunisation trial at any time.
Conclusion: The observed immune response was not caused by a systemic humoral component. The epidermal changes observed indicate a role of these components in the response against *G. derjavini*.

c.6.82 BRANCHIOBDELLIDS ON THE NOBLE CRAYFISH ASTACUS ASTACUS L. IN AQUACULTURE

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Background: The class of the Branchiobdellida belongs to the phylum Annelida, subphylum Clitellata. Branchiobdellids have been found on crayfish throughout Northern hemisphere. The majority of branchiobdellid species are commensal, but some species are parasites. They feed on organisms growing on crayfish body, however, parasitic species feed on gill tissues or eggs of the host. The purpose of our study was to identify branchiobdellid species found in aquaculture and to estimate effectiveness of prophylaxis measures against them.
Method: The study was carried out in the First Noble Crayfish Aquaculture Centre at Simnas Fishery Farm (Alytus district, Lithuania). Branchiobdellids were removed from the carapace and gills of crayfish and fixed in 70% ethanol. Later they were placed in glycerol solution to become transparent. The shape of the jaws was considered to be the main systematic characteristic. Treatment with 5% NaCl for 20 minutes was used as prophylaxis means against branchiobdellids.
Results: In spring and early summer of 1996-1998 up to 30 adult branchiobdellids per host were found on the crayfish maintained in a pond. A lot of white branchiobdellid cocoons on egg walls on egg-bearing females were observed. Treatment with 5% NaCl before transferring of the crayfish into the pond was not sufficient for prophylaxis.
Branchiobdella parasita (Braun 1805) has been found on crayfish exoskeleton, while *B. astaci* Odier 1823 - in the gill chambers. *B. parasita* has not been found in Lithuania before this study.
Conclusion: *Branchiobdella parasita* and *B. astaci* have been found in crayfish aquaculture. Treatment with 5% NaCl for 20 min. was not sufficient as prophylaxis means against branchiobdellids.

c.6.83 THE PARASITOSES OF CULTURED FISH IN SOME FISH FARMS FROM ROMANIA

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Background. In the last few years, when the stress factors multiplied, an increasing of parasitological diseases was observed at the cultured fish, with impact on fish production. In order to diminish the loses by applying efficient prophilactical and therapeutical measures it was necessary a good knowledge of parasitological diseases, their extensive and intensity degree and also the influence on fish population. In those circumstances, during the period 1996-1998, we made parasitological researches on the cultured fish species in some fish farms from the Eastern part of Romania
Method. The researches were carried in the following fish farms: Brates, Lozova, Vladesti and Oltina. For every fish farm a number of 100 samples (0+-2+) from each fish species: common carp (Hypophthalmychthys molitrix Val.), big head (Aristichtys nobilis Rich.) and grass carp (Ctenopharingodon idella Val) were analyzed. All parasitological investigations were carried on live stock and fresh samples by classic method. The obtained results were analysed using the extensive (E%) and intensity (I- feeble, average, severe) degree. The sporadic cases were not taken into account.
Results. As a results of the researches were identified 12 parasitoses: myxosporidiosis,trichodiniosis,ichthyobodosis,icthyophthiriosis, dactilogyrosis,diplozoonosis, diplostomiosis, bothriocephalosis, hepaticolosis, argulosis, lerneosis and sinergasilosis.
Conclusion. The most extensive and with severe intensity degree were parasitosis: trichodiniosis, icthyophthiriosis, dactilogyrosis, hepaticolosis and argulosis. The most affected species being the common carp and silver carp from farm Lozova, Vladesti and Oltina at which numerical loses and decreased growing rate were registered.
Acknowledgements. We wish to thank to the staff of fish farms.

c.6.77-87 Parasites of fish

c.6.84 *IN VITRO* CULTURE OF THE FISH PARASITIC CILIATE *ICHTHYOPHTHIRIUS MULTIFILIIS*: PRELIMINARY OBSERVATIONS

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Background: The pathogenic protist *Ichthyophthirius multifiliis* parasitizing the skin of various freshwater fishes is relatively immunogenic. However, development of vaccines against this ciliate is hampered by the lack of techniques for *in vitro* propagation of the parasite from the theront to the trophont stage. Experiments with various substrates including cell cultures for in vitro propagation of the parasite are described in the present work.
Method: Theronts released from tomocysts originating from a Danish rainbow trout farm were used to infect monolayers of EPC (Epithelioma Papulosum Cyprini) cells in Multidish plates (Nunc) supplied with medium (E-MEM or L-15) or cell cultures produced from trout epithelia.
Results: Parasites developed into trophonts in cell cultures. They survived for a significantly longer time compared to parasites in water or culture medium alone. Attachment of theronts were induced by adding host factors as skin mucus or serum from rainbow trout.
Conclusion: *In vitro* propagation of *I. multifiliis* could be one way to produce sufficient antigen for production of vaccines against the skin parasitic ciliate. However, the technique still needs some improvement before the total life cycle of the parasite can be conducted in the laboratory.

c.6.85 ON PARASITISM OF MICROSPORIDIANS IN LARVAE OF CESTODE KHAWIA ARMENIACA

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Background: The data on some intracellular microsporidia in helminths are available. Microsporidia of the genus *Nosema* from larvae of *Khawia armeniaca* - a parasite of *Varicorhinus capoeta sevangi* is for the first time described.
Method: Procercoids of caryophyllidean cestodes removed from the body cave of oligochaeta *Pothamothrix hammoniensis* were fixed in 4% glutaraldehyde in cacodylate buffer, post-fixed in OsO_4, dehydrated and processed for TEM examination.
Results: It has been schown that microsporidians reproduce intensively and develop normally in the epidermis cells. The cytoplasm of this cells contains invasive, vegetative and sporogony microsporidians. The destruction of endoplasmatic reticulum. Golgy apparatus and secretory granules have been revealed in the cytons of tegument. The distal cytoplasm of the tegument do not contain the secretory granules also. Secretory functions of the tegument are disturbed. The secretion protects worms from the host's immune response. The invasive procercoids has no such means of protection.
Conclusion: The microsporidians able to reduce a number of procercoids are the most promising for biological control of cestodes.

c.6.86 PATOGENIC EFFECT OF CESTODES OF THE FAMILY LIGULIDAE IN SMALL LAKES

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Background: Nekrasov flood plain of the river Volga has numerous group of small water bodies (8 - 323 hectares) using for sport fishing by members of the Yaroslavl Amateurish Society of fishermen. This flood plain is protected by a dam with pumping stations from the Gorky reservoir.
Method: The level of fish infecting by plerocercoids of *Digramma interrupta* (Rudolphi) has been estimated. A total of 515 sp. *Carassius carassius* (Linne) and 253 sp. *Carassius auratus gibelio* (Bloch) were captured from 2 slow-flowing rivers, 2 deep lakes and 4 shallow lakes.
Results: Crucian carps (*C.carassius*) play the leading part in the maintenance of *D.interrupta* populations (prevalence - 4.5±0.9%). Only 1 of 253 giebel carps were infected. Parasites were absent in lakes with depth 7 - 9 m.
A positive correlation was found between the ratio of second intermediate hosts and the prevalence of *D.interrupta* in the populations of *C.carassius* from shallow lakes (0.1:1-0%; 2.5:1-2.6±1.5%; 4.3:1-3.6±1.3%; 10.0:1-10.0±3.9%) and slow-flowing rivers (0.9:1-3.1±3.1%; 2.1:1-7.2±2.8%).
Conclusion: The anthropogenic succession of this group of water bodies is a result of two simultaneous processes (eutrofication and agricultural pollution). The optimum ratio between hosts of *D.interrupta* is 1:1.
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c.6.87 MORPHOLOGY OF PARADIPLOZOON FROM FISHES IN SOUTH AFRICA

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Only a few species from the family Diplozoidae have been described in Africa, namely: *Diplozoon aegyptensis* from Egypt, Kenya and Uganda, collected from *Labeo* spp. and *Barbus* spp.; *D. ghanense* on *Alestes macrolepidotus*; *Neodiplozoon polycotyleus* on *Labeo* spp. and *Barbus* spp. in Kenya and Tanzania and on *Barbus marequensis* and *Barbus trimaculatus* in the Olifants River in South Africa whereas larvae where present on *Barbus nefi* from the same river.
Possible host fish species were collected at the Vaal Dam in the Gauteng province, with the aid of gill nets. The gills of the fish were removed and the parasites collected using a dissection microscope. They were fixed between a cover slip and glass slide in Aseto-formaldehyde alcohol. Whole mounts were prepared and serial sections made.
The parasites are relatively large (±4mm). The anterior section possesses two muscular buccal suckers and a pharynx, which extends into a branched intestine. The posterior section is occupied by the reproductive system of this hermaphroditic worm, and opisthaptor with 4 pairs of clamps.
On grounds of the number of hooks, the presence of an egg without a filament, and the fact that the reproductive system is situated posteriorly, the conclusion is made that the organism is a representative of the *Paradiplozoon* genus. This genus has at present not been described from Africa.
Prevalence, incidence and intensity is high but infestation is limited *to Barbus aeneus* and *Barbus kimerleyensis* in the Vaal River.

c.7.00-01 Economic impact of parasitic infections
c.7.02-11 Epidemiology and control of protozoan and ectoparasitic infections

c.7.00 THE EFFECT OF GASTROINTESTINAL NEMATODES INFECTION ON THE METABOLIC PROFILE AND MILK YIELD OF COWS

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Backgraund: The aim of the study was to evaluate the influence of parasitc infection in treated and non-treated cows on metabolic profile and milk yield of dairy cows.
Method: Thestudies were carried out on 194 experimental cows, treated with 1.5% Levamisol and 49 control non-treated cows. The infection rates were estabilised on the basis of coprological examination (Willis-Schlaff and Mc Master methods). Profiles of 14 constituens of blood were analised.
Results: Observation on the dynamics of gastrointestinal nematodes infection in cows during the yearling cycle exhibited one vernal peak in April (infection rate 100%, PGF 285). After treatment the infection rate decreased to 30% and the intensity to 5 EPG. In cows naturally infected with gastrointestinal significant decreases in Ht, Hb, total serum proteins and serum albumin and significant increase in total eosinophils were the noticeable changes.
The milk yield and percent of fat content in milk of treated cows were higher by 500 kg and 0,24 % comparing with the control cows.
Conclusion: The result idicasted that the the subclinical infection of gastro-intestinal namatodes influence on the metabolic profile. Worthly of notice is the influence of nematodes' control on increase of milk production.

c.7.01 PREVALENCE AND ECONOMIC IMPORTANCE OF LIVER FLUKES AND OTHER HELMINTHS IN CATTLE AND SHEEP IN NYANDARUA DISTRICT OF KENYA

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Information on parasitic and other conditions causing whole or part condemnation of cattle and sheep carcasses was recorded daily for a period of one year from slaughter slabs in Nyandarua District. A total of 5259 cattle and 5967 sheep were slaughtered in the slabs during that period. This represented approximately 58% of all cattle and sheep slaughtered in the entire district. Only 3 cattle carcasses out of the total killed (0.06%) were condemned entirely due to septic peritonitis. The annual condemnation for organs in cattle due to helminth parasites was 20.2% of the total kill. The causes of condemnation were Fasciola (liver flukes) 15.5%, Oesophagostomum (pimply gut) 2%, Cysticercus bovis 1.5% and Paramphistomum (stomach flukes) 1.2%. The highest number of livers condemned in cattle due to liver flukes was in October and February during the dry season. There was no seasonality in the number of other organs condemned due to the other parasites. In sheep, the annual rate of organ condemnation was 42.4 % of the total kill. This was due to Fasciola (liver flukes) 35.4%, Stilesia hepatica 5.3%, Oesophagostomum (pimply gut) 1.2% and Cysticercus tenucolis 0.5%. The highest number of livers condemned due to liver flukes in sheep was in September, October and February in the dry season. It was estimated that annually there was a loss of approximately US$ 14,805 and US$ 25,917 in cattle and sheep production respectively, as a result of condemnation of organs due to helminth infections. Most of the losses were due to Fasciola in cattle (94%) and sheep (84%) and Stilesia hepatica (13%) in sheep. This does not include production losses in milk, meat, disease susceptibility, cost of treatment and mortality due to Fasciolosis. Intensification of control strategies for Fasciolosis is advocated.

c.7.02 *CRYPTOSPORIDIUM MURIS* IN CATTLE: FIRST REPORT IN SCANDINAVIA

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Background: *Cryptosporidium parvum* is widespread in Danish cattle herds but prevalence of *Cryptosporidium muris* has not been well studied. In November 1997 *C. muris* was diagnosed in a heifer with no previous history of diarrhoea. A prevalence study was performed in the herd of origin (beef suckler herd).
Material & method: Faecal samples obtained during two separate visits were examined using the modified Ziehl Neelsen (MZN) technique and immunofluorescens. Oocysts were purified by Sheater's sugar flotation technique and $5x10^6$ administered to a 4 d old calf. Neonatal mice (n=12; BALB/c and outbreed) were inoculated with $5x10^3$ oocysts (11 mice served as uninoculated controls). Faecal samples were screened for cryptosporidia using MZN. The calf was euthanized 23 weeks PI. Mice were euthanized 12, 17 and 31 d PI. Tissue samples from stomach, duodenum, jejunum and ileum were stained with haematoxylin and eosin.
Results: At the first visit 19% (27/142) were infected with *C. muris* while 4.2% (6/142) excreted *C. parvum*. Of these only one mixed infection was found. At the following visit 28% (42/150) of the samples contained *C. muris*, 16.7% (25/150) *C. parvum*; 8 of these were mixed infections. In wet preparation a mean size of 7.3x5.7µm (x1000) was determined for the oocysts considered to be *C. muris*. Oocysts were excreted intermittently by the infected calf from 25 d PI. Clinical signs were absent and the number of discharged oocysts was low: 1-5 per slide ~ 100.000 per g. The mice remained uninfected and histological examination revealed no signs of cryptosporidia.
Conclusion: This is to our knowledge the first report of *C. muris* in Scandinavia. Further studies concerning epidemiology, economic significance and interaction with other enteropathogens are needed.

c.7.03 *HAEMATOBIA IRRITANS*: CURRENT CONTROL AND PROSPECTS IN ARGENTINA

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Haematobia irritans **entered Argentina in 1992, when treatment with cypermethrin allowed cattlemen and field veterinarians alike, to significantly reduce infestations over extended periods. Since then incorrect management of treatment times and the use of off-label formulations has increased fly resistance to cypermethrin 10-fold, creating the need to change the current concept of control from "fly-free" cattle to "infestation levels compatible with productivity". In 1997/98 the overall perception of resistance and lower efficacy of all commercial products was considerably higher. New parametres now include treating only those approximately 20% higher infested cattle in each herd, when infestation surpasses 250/300 flies/animal, with selective treatment of categories where horn-flies cause most economical damage. Acceptance by cattle owners of these rules aimed at checking the advance of resistance, is proving difficult and requires careful planning. Current treatment options include PO monodrug OP's, synergic pyrethroid/OP mixtures in PO and spray formulations, and 4 months-active OP ear-tags. An injectable ivermectin 1% long-acting endo-ecto formulation has been approved as an adulticide (28 days), whilst research on future biological control with Pteromalid Hymenoptera has commenced, based mainly on experience in the USA.**

c.7.02-11 Epidemiology and control of protozoan and ectoparasitic infections

c.7.04 SEASONAL VARIATIONS OF CULICOIDES SPP (DIPTER: CERATOPOGONIDAE) COLLECTED FROM SAUDI ARABIA

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There are four principal viral diseases known to infect domestic animals in Saudi Arabia (African horse sickness, Bovine ephemoral fever, Blue tongue and Akabani). Since these diseases are vectored by Culicoides spp this investigation was initiated to identify and study the seasonal variations of the different species which prevail around animal operations.
NewJersy light traps were used for collecting Culicoides spp at two different sites of El-Ahsa locality (Eastern province) of Saudi Arabia during a period of 13 month.
The mean monthly number of Culicoides spp per trap reached its minimum value during January then it increased gradually from February to reach its maximum in September.During the study period the following species were reported+ C.schultzei group (September), non-spotted group of Culicoides (September), C. sahariensis (September), C.imicola (May) and C.newsteadi (March). It was concluded that C.schultzei group and C.imicola (The principal vectors of viral diseases) were present during June to October and March to June respectively.

+ Peak numbers between brackets.

c.7.05 SEASONAL DYNAMIC OF THE PARASITIC STAGES OF *Amblyomma cajennense* IN HORSES

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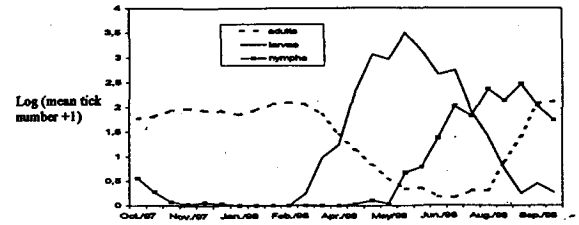
The Cayenne tick, *Amblyomma cajennense*, is a common parasite of horses and others hosts, including man, among Brazil. Since field control of *A. cajennense* has had few success, studies of its seasonal dynamic is essential to formulations of strategic control techniques. From October/97 to September/98 ten mares were kept in a paddock without acaricide treatment, in Pirassununga County, São Paulo State, Brazil. Each 14 days, tick assessment was performed on the left side of the body of all mares. Attached adults were counted individually. For assessment of larvae and nymphs, the left side was entirely brushed with a horse brusher and only engorged immatures were dislodged into a plastic bag, which was taken to laboratory to be counted. Mean number of adults and engorged larvae and nymphs are presented on figure. *A. cajennense* showed an one-year generation pattern, with larvae predominating from April to July, nymphs from July to September and adults from October to March.



Financial support by FAPESP-Brasil (Processo 95/6938-0)

c.7.06 BOVINE NEOSPOROSIS IN ITALY: SEROLOGICAL EXAMINATIONS, PCR AND ISOLATION IN CELL CULTURE

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Background: *Neospora caninum*, a protozoan parasite related to *Toxoplasma gondii*, is considered a major cause of abortion in cattle and of neurological disease in calves all over the world. In the last two years, we investigated its prevalence in dairy herds reared in northern Italy.
Method: We employed an indirect fluorescent antibody test (IFAT) and a PCR assay targeted to the ssu-rRNA gene of cyst-forming coccidia. A few tissue samples were also inoculated onto cell culture for isolation of the parasite. We are currently sequencing the ssu-rRNA gene and the internal transcribed spacer (ITS) regions from positive samples, in order to investigate the relationships among them and the strains recovered in other countries.
Results: We found antibodies to *N.caninum* in 24.3 % of 10,684 blood samples of aborting cows and in 24.8 % of 525 bovine fetal pleural fluids. We detected *Neospora* by PCR test in 103 out of 349 samples and we isolated a *Neospora* strain in cell culture. The sequence of fragments of ITS and ssu-rRNA gene has been compared to the ones deposited in the databases and found to be identical (ssu-rRNA) or closely related (ITS) to them.
Conclusion: *N. caninum* is widespread in the Italian dairy herds. The IFAT is an useful tool for the presumptive identification of the infected herds, while the PCR test is easy to perform and provides a faster diagnosis for the infection than the isolation in cell culture. Sequencing of the amplified PCR fragments is useful to confirm PCR identification and to investigate the relationships of the different strains.

c.7.07 PRODUCTIVE PERFOMANCE IN SHEEP CURED FOR PSOROPTIC MANGE IN PATAGONIA

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The impact of sheep scab was evaluated during 35 months in 92 female Merino lambs artificially infected by placing 15 to 20 mites on the skin and treated conventionally, according to 3 types of management used in extensive production systems. The animals were allocated in similar paddocks during 150 days; hereafter, all of them grazed on the same pasture. Group 1 (G1) and 2 (G2) were treated with two dips with diazinon: G1 (careless management) at shearing (150 days post infection) and G2 (traditional management) after obvious clinical signs of mange were observed and according to weather conditions (90 days p.i.), Group 3 (G3) (modern management) was dosed twice with ivermectin, as soon as mange was diagnosed (15 days p.i.). Complete control of Psoroptes ovis was achieved after treatment. The evolution of body weight between groups was different ($p < 0,05$), but at the end of the trial (420 days), the mean body weight of all groups were similar.
At first shearing (150 days p.i.), wool weight between groups was different ($p < 0,05$), but 9 month later, at second shearing G2 and G3 were similar and superior to G1.
Lambing percentage was G1= 13 %, G2= 74 %, and G3= 85 %. This finding was in line with the poor condition at mating time.
This study shows that despite the deteriorated production induced by psoroptic mange, in patagonian conditions young sheep which had been cured and realimentated achieve during the second year live weights similar to those of almost continuously grown animals.

c.7.02-11 Epidemiology and control of protozoan and ectoparasitic infections

c.7.08 SEROLOGICAL SURVEY AND FIRST FINDING OF *NEOSPORA CANINUM* IN TAIWAN

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Background: *Neospora caninum* has not yet been reported in Taiwan. We carried out a serological survey for antibodies against *N. caninum* in cattle, goats and farm dogs, and also tried to isolate the parasite to prove its presence in Taiwan.

Methods: Sera of 488 cattle from 17 dairy farms throughout Taiwan, 22 goats and 13 farm dogs were tested for antibodies against *N. caninum* using indirect fluorescence antibody test (IFAT). In conjunction to the IFAT test, we also tested the same sera for antibodies against *T. gondii* using latex agglutination test (LAT).

Results: Of the 488 cattle sera, 32.0% (156/488) were found to have antibodies against *N. caninum*. Among these 156 positive cattle, 48 also possessed antibodies against *T. gondii*. Most cattle with *N. caninum* antibodies had a history of abortion or stillbirth. Nevertheless, 89 cattle which were negative for *N. caninum* showed antibodies against *T. gondii*. Of the 22 goat sera tested, none was found to be positive for *N. caninum* but 45% (10/22) were positive for *T. gondii*. Of the 13 farm dogs tested, 3 were found to possess antibodies against *N. caninum*, 2 of which were tested negative for *T. gondii* antibodies. We sacrificed 1 cattle which was serologically positive for *N. caninum* but negative for *T. gondii* for confirming the presence of the parasite. The brain of that cattle was homogenized and then inoculated intraperitoneally into 13 prenisolone-treated SPF ICR mice. About 4 months later, we detected a 50μm cyst with thick cyst wall in the brain of one of the inoculated mice.

Conclusion: Thus, we have confirmed for the first time the presence of *N. caninum* in Taiwan and also observed that it is widespread among dairy cattle and farm dogs.

c.7.09 THE PREVALENCE OF ANTIBODIES TO *THEILERIA PARVA* INFECTION IN EXOTIC AND CROSS-BRED CATTLE IN UGANDA.

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Background

East Coast Fever (ECF), a disease of cattle caused by *Theileria parva* and transmitted by *Rhipicephalus Appendiculatus*, is the most important tick-borne disease in Uganda. In some farms ECF is controlled by immunisation using infection and treatment method. The main target cattle for immunisation are the exotic and cross-bred cattle of 3-12 months on fenced farms practising intensive tick control using either Organophosphate or Pyrethroid. The paper reports on the prevalence of exposure to *T. parva* of cattle prior to immunisation.

Methods

Blood for serology was collected from calves on the day of immunisation. Antibodies to *T. parva* were detected using indirect antibody Enzyme-Linked Immunosorbent Assay. Altogether, 2947 sera from 16 districts were tested.

Results

Of the calves tested, 67.1% carried antibodies against *T. parva*. Farms using Pyrethroid had the highest sero-prevalence of 75.8% while those using Organophosphate had 61.7%. The lowest prevalence was recorded in zero-grazing animals.

Conclusion

The high sero-prevalence of *T.parva* in all these farms suggests that tick control using acaricide alone is not sufficient. It is therefore justified to immunise susceptible cattle against ECF as an additional control measure.

Acknowledgements

We wish to thank DANIDA and NARO for financial and material support.

c.7.10 PREVALENCE OF *B. burgdorferi* IN *Ixodes ricinus* (ACARINAE, IXODIDAE) IN NORTHERN ITALY

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Background: Previous data demonstrated the presence of *Borrelia burgdorferi* sensu latu (s.l.) in ticks populations in the North of Italy. Ticks are well known vectors of *B. burgdorferi* s.l. and this represents an important problem for public health in relation to the risk of Lyme desease. The aim of this study was to assess the prevalence of infection in ticks from North-Eastern Alpine area (Trento province).

Method: Ticks were collected by dragging in different areas of Trento province from March to October 1997 and 1998. DNA from each tick sample was amplified by PCR using a couple of primers specific for the internal transcribed region of *B. burgdorferi* rRNA genes. Amplification products were visualized by gel electrophoresis.

Results: Two thousands and 267 ticks were examined (29 larvae, 2152 nymphae, 52 adult males and 34 adult females). We found a high prevalence of the infection (362 positive samples, corresponding to 16% of the ticks). Specimens collected during the month of June showed the highest prevalence.

Conclusion: Our data confirm the widespread diffusion of the infection in tick populations and the high risk for human health.

Acknowledgements: This work was partially supported by a grant from Centro di Ecologia Alpina and by MURST "Progetti di Rilevante Interesse Nazionale 1997".

c.7.11 FRECUENCY OF *Hypoderma lineatum* (de Villiers 1789) IN CALVES FROM CHIHUAHUA, MEXICO

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Background: The aim of the present study is to comunicate the frequency of *H. Lineatum* in calves from Chihuahua, Mexico as part of a project related to the presence of this miasis in adult cattle.

Method: Two visits to the coupling place of Chihuahua were made in november 1997 and december 1998 to carry out visual observations of young cattle , looking for suspicious animals, using the oxigenated water method for the larvae extraction.

Results: In november 1977, from 160 animals observed, 31 (19.4%) were positive to the presence of larvae. In december 1998, from 145 calves, 32 (22.0%) were positive. The age of animals ranked from 5 to 22 months,. The isolated larvae were LIII of *H. Lineatum*.

Conclusion: LIII of H. Lineatum were found in animals aged between 2 to 22 months. This data is relevant for the knowledge of the biological cicle of *H. Lineatum* in Chihuahua, Mexico for future establismenth of serologic diagnosis and control of this parasitic disease.

Acknowdledgements: To the Comision México-Estados Unidos para la prevencion de fiebre aftosa y otras Enfermedades exóticas de los animales for finacial support..

c.7.12-19 Epidemiology and control of horse parasites

c.7.12 Horse flies (Tabanidae) - the parasites of live-stock in Low Volga region

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Background: The high abundance of horse flies in some areas of Low Volga region brings considerable economic damage to cattle-breeding. The absence of detailed recent data about the horse flies fauna of this area became the reason for investigation of species composition and ecology characteristics of this parasites.

Method: The collections of insects were made in Astrahan, Volgograd and Saratov regions, Russia during the whole period of horse flies activity (from the end of May to September).

Results: 42 horse flies species belonged to 7 genera were recorded. 2 genera and 9 species were found in this area for the first time. Diurnal and seasonal activity of horse flies, influence of climatic and meteorological factors on change of periods of their activity were studied. It was established, that at high day's temperatures ($+30^0$ C and higher) and dry winds the diurnal activity of Haematopota genus beginning et 4.00 (40 minutes before sunrise) and have two peaks of biting activity (et 7.00 and 19.00). As a result of our research it became obvious that big animals - cattle and horses - serve as preferable hosts for all Tabanidae.

Conclusion: On the base received data the optimum periods for stay of live-stock on the pastures were recommended.

c.7.13 AN ABATTOIR SURVEY OF HELMINTHS IN HORSES

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Background: It is not known how effectively anthelmintics are being used by horse owners in the UK. Therefore a five month survey of the incidence of tapeworms, bots and faecal nematode egg was made on one hundred and sixty four horses at a local abattoir.

Methods: Numbers, weights and species of *Gasterophilus*, numbers and total weights of *Anoplocephala perfoliata* and nematode faecal egg counts were recorded from October to February.

Results: Fifty five percent of horses were infected with a mean burden of forty *G.intestinalis*, five percent with *G.nasalis* and forty eight percent with *A.perfoliata*. Sixty two percent had fifty or more nematode eggs per gram of faeces. The main growth of the third stage bots was confined to December and January. Only horses with one tapeworm showed no mucosal damage. Those with more than one hundred worms showed extensive damage. The mean burden of *A.perfoliata* was eighty five worms, wet weight two hundred and fifty mgs.

Discussion: Gastrointestinal parasites are still common in horses in Southern England and half the horses were infected with bots despite widespread use of ivermectin. *G.nasalis* was confined to wild ponies from Dartmoor or the New Forest. Tapeworms were common suggesting insufficient use of double dose pyrantel by owners. Nematode egg counts were maintained during the winter but the epidemiological significance of those reaching pasture is not known.

Supported by Pfizer Animal Health.

c.7.14 PREVALENCE OF EQUINE TAPEWORMS IN ARGENTINA.

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*** Rural Practice

Background: Recent reports suggested that tapeworms are potentially dangerous in horses. There were no previous data of the prevalence of tapeworms eggs in fecal samples in Argentina. The objective of the present trial, was to evaluate the prevalence of tapeworms in equine feces collected in the Buenos Aires Province, Argentina.

Methods: Fecal samples taken directly from the rectum of one hundred sixteen horses raised on pasture in the Buenos Aires Province, were examined using a simple saturated sodium chloride flotation technique (A) and by two sedimentation methods, one, using saturated sucrose solution (B) and the other using a saturated zinc sulfate solution (C).

Results: Using method A, 14% of the samples were positive; using method B, 45% of the samples were positive, while in method C, 33% of the fecal samples were positive to tapeworm eggs. The prevalence reached 45% using the most sensitive test.

Conclusions: In the present trial, a high prevalence of tapeworm eggs in equine fecal samples was observed. Both techniques using a sedimentation/flotation method, were more sensitive than a technique based only in a flotation procedure.

c.7.15 PREVALENCE OF STRONGYLOÏDES WESTERI IN NORMANDIE STUDFARMS FROM 1993 TO 1997

Hamet Nicole, Lamidey Corinne, Collobert Claire

From May 1993 to 10 September 1997, fecal samples were collected from the rectum of 242 foals less than 6 months of age during necropsy. These feces were examined for Strongyloïdes westeri eggs by the Mac Master method.

The prevalences obtained according to breed, sex and age are presented in the following table :

	Number examined	Number infected (%)
Breed		
Thoroughbred	93	5 (5.4)
Standardbred	117	14 (12)
French saddlebred	32	5 (15.6)
Sex		
Female	104	9 (8.6)
Male	138	15 (10.9)
Age		
0 to 1 months	139	5 (3.6)
1 to 6 months	103	21 (20.4)

The most abundant parasite burdens (90 to 600 eggs per g of feces) were observed after one month of age ; no eggs were detected from the fecal samples of younger foals.

c.7.12-19 Epidemiology and control of horse parasites

c.7.16 AN ESTIMATION OF TAPEWORMS (ANOPLOCEPHALA SPP.) PREVALENCE IN BRAZILIAN HORSES

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Background: Tapeworms (Anoplocephala *spp.*) commonly found in horses and ponies are a potential risk factor for colic or unthriftiness. Treatments that provide >90% control of infection by tapeworms are not many in equids, and this situation may explain the tapeworm prevalence.
Method: A field trial was conducted in Brazil, from March to April (wet season). Cull equids from the Province of Rio de Janeiro were purchased and housed in trial facilities, split in 7 replicates. A faecal egg count was done on day of arrival with a routine technique. The total number of equids included was 38, both males and females, weighing 143 to 378 kg, aged between 1 and 16 years. Tapeworms or proglottids expulsion in total volume of faeces was assessed during the 4 days period between arrival and sacrifice. They were necropsied and tapeworms were looked for in the digestive tract, identified and counted.
Results: Among the 38 horses included in trial, a total of 26 horses had tapeworms and 12 horses were tapeworm free. Only 2 had_cestode eggs observed in their faeces on arrival. The number of adult tapeworms recovered varied between 1 and 183 (mean=14). 22 horses had both nematode and cestode infection, 10 had nematodes only, 4 had cestodes only, and 2 were free of cestodes and nematodes. All cestodes recovered were identified as Anoplocephala perfoliata.
Conclusion: A sample of horses taken at random around trial facilities showed a high prevalence of tapeworm infection (68 %). A common faecal egg counting method is not a sensitive tool to detect cestode infection. Infection with Anoplocephala perfoliata is most of the time coincidental with nematode infection (22 out of 26 i.e. 84%) and a treatment active on nematodes and cestodes is then most of the time needed.

c.7.17 SEASONAL POPULATION DYNAMICS OF HELMINTH PARASITES OF DONKEYS IN KENYA.

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Background
The main objective of the study was to study the seasonal population dynamics of Helminth parasite of Donkeys in Kiambu district , Kenya.
Method
Faecal samples were collected from the rectum of the donkeys (once a fortnight) for a period of 12 months. 353 Donkeys were considered . The faecal samples were taken to the laboratory for faecal egg counts and culture for subsequent larva identification.
Results
It was observed that there was a statistical significance in the difference in egg per gram counts observed between the short dry season and the long rain one, the long rain season and the short rain one, the long dry season and the short dry one. However, there were no differences in egg counts between the short dry season and the short rain one.
Conclusion.
The infestation with the internal parasites showed a seasonal prevalence and therefore, apart from the use of antihelmintics, the epidemiological considerations are critical in successful control designs.
Acknowledgment
I'm grateful to the DANIDA funded Livestock Helminth project at the University of Nairobi, Kenya.

c.7.18 ANOPLOCEPHALA PERFOLIATA IN HORSES IN THE CZECH REPUBLIC – TAPEWORM INFECTION, MORPHOLOGY, CLINIC AND THERAPY.

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Anoplocephala perfoliata is a significant risk factor for the onset of the equine spasmodic colic and ileal impaction colic. The parasite has a predeliction for the ileo-caecal junction, which results in a high infection rates at that site.
The present study of eight clinical cases of *A.perfoliata* was based on an intensive 3-year collaboration with veterinary surgeons. Two of the eight horses died and their necropsy was performed. Their intestines were split longitudinally and the contents examined for *A.perfoliata* tapeworm adults. Ileocaecal pathological finding was proportional to the infection intensity. Mature tapeworm proglotids were collected, prepared for SEM (scanning electron microscopy) and their morphological details were studied. All faecal samples were assayed for the presence of *A.perfoliata* eggs by the method described and validated by Proudman and Edwards (1992). Strongyle eggs were counted using the McMaster counting method (EPG).
Long-term administration of anti-cestode drugs (fenbendazole and pyrantel tartrate) was responsible for some of the observed decrease in colic incidence.

c.7.19 FACTORS EXTENDING THE EGG REAPPEARANCE PERIOD IN DONKEYS ON SANCTUARY FARMS

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Background: The egg reappearance period (ERP) has been defined as the period after an anthelmintic treatment in which mean egg counts do not exceed 100epg (eggs per gram), however, it has also been defined as the number of weeks elapsing until 25% of the herd had patent infections of >200epg. Studies have shown that two herds consistently achieve ERP greater than those expected by the efficacy of anthelmintic treatment.
Method: This trial was conducted at three Sanctuary farms in Devon. The herds varied in age, population densities, pasture management and parasitological burdens. The donkeys were treated with ivermectin prior to spring turnout. The faecal egg count of 212 donkeys was monitored using the Modified McMaster technique. Sampling commenced eight weeks post worming and then fortnightly until the ERP was reached.
Results: All 4 herds exceeded the expected ERP of 9 weeks, by 3, 5 and 9 weeks and one herd which had an ERP of 22 weeks was returned to winter housing but was still below the defined ERP. In each herd sex differences were also recorded and donkeys over the age of 25 show higher mean epg.
Conclusion: Since two herds were situated on one farm (ERP 14 and ERP 18) and were turned out within seven days of each other it was unlikely that weather conditions or delayed turnout accounted for the 4 week difference in ERP. Other factors include the affects of soil type, microclimate, dung beetles and nematode-trapping fungus to reduce pasture larvae contamination.
Acknowledgements: The Donkey Sanctuary, especially the Veterinary Department and Farm staff.

c.7.20-27 Chemotherapy against horse parasites

c.7.20 INSECT BITE HYPERSDENSITIVITY IN 25 HORSES AND TREATMENT WITH A PERMETHRIN + PYRIPROXYFEN CONTAINING SPRAY.

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Background : Insect bites hypersensitivity (Sweet itch) is a major skin disease in horses in the mid-north of France. The control is very difficult and the efficacy rarely of treatments rarely demonstrated. The modern use of insecticides underlines the importance of actions like contact-repellent, knock down and antifeeding effects. The aim of this study was to evaluate precisely the efficacy of a Permethrin containing spray and as the only treatment of established clinical sweet itch in horses.
Method : 25 horses were included. The only treatment was the application of 250 ml of a spray (permethrin 18.8mg/ml. + Pyriproxyfen 0.2 mg./ml DUOWIN Spray®) on days O, 15, 30 and 45. On days 0, 30 and 60 (or 120) pruritus: (0 to 3), lesional index (LI), index distribution (DI) were evaluated A clinical evaluation was performed on d.15: O = no change to 3 excellent to cure. **Results :** . The tolerance to treatment was excellent in all. On day 15 the clinical evaluation on 22 horses was : 0 in 2, 1 in 10 and 3 in 10. Pruritus was decreased in 69,5% (16 of 23) of horses on day 30. It was respectively absent, decreased , identical or worsened in 4, 4, 5 and 3 on day 60 and on 2, 2, 1 and 1 horse on day 120. On day 30 reduction or disappearance of the scored pruritus, lesional index and distribution index in 69.5%
Conclusions : These results suggest that the application of an insecticide with important contact repellent, knockdown and killing effects may rapidly decrease the allergenic challenge conducive to a clinical benefit obtained in one month and that such a method could be very useful as a treatment

c.7.21 ENGLISH EVENTER HORSE OWNERS OVER USE ANTHELMINTICS

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Background: Benzimidazole resistant cyathostomes are more common in intensively managed horse enterprises. To investigate this further, anthelmintic use was determined by means of a questionnaire and faecal egg counts made on samples submitted by owners of eventer horses.
Methods: Completed questionnaires and faecal samples were submitted by owners at the time of anthelmintic treatment. Further samples were submitted fourteen days later. Faecal egg counts and egg hatch test (EHT) were performed by standard WAAVP methods.
Results: Eighty one percent of owners treated horses every six to ten weeks and seventy five percent used multidose fenbendazole. Only twelve percent removed faeces from pasture at least twice per week. Eighty two percent of horses had fifty or less epg at the time of treatment. Three horses were treated unsuccessfully by their owners with single dose fenbendazole. Further samples were only sent from one in which an EHT failed to indicate resistance.
Discussion: Eventer owners worm control policy is largely based on reliance on chemicals rather than pasture management. Anthelmintics are being over used as indicated by very few eggs in faecal samples at the time of treatment. This made it impossible to determine the extent of benzimidazole resistance. The reasons for apparent failure in three horses could not be determined.
Supported by Pfizer Animal Health.

c.7.22 PARASITE CONTROL USED BY PONY CLUB MEMBERS

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Background: There is little information on parasite control practices adopted by horse owners in the UK. The way chemicals are used will affect the development of drug resistance in endo- and ectoparasites.
Methods: Three hundred and eighty five questionnaires were sent to members on ectoparasite control and six hundred on nematode control.
Results: Thirty percent replied on ectoparasite control. Fly worry was the most commonly perceived problem (eighty six percent) followed by sweet-itch (forty four percent) and lice (fourteen percent). Pyrethroids dominated in sweet-itch and lice control but repellents were important in fly control. Only ten percent replied on nematode control. Thirty one percent were worming horses seven or more times per year. Ivermectin was the most commonly used anthelmintic, but sixty percent of owners used pyrantel for tapeworm control. Only fifty three percent removed faeces from pasture at least twice per week. There was relatively little involvement of veterinary surgeons in planning of parasite control.
Discussion: It would be of considerable interest to determine how effective are the methods used for ectoparasite control and whether there is resistance to pyrethroids in lice. Anthelmintics were used very frequently. Whilst this will control nematodes it poses the risk of selecting for anthelmintic resistance.
Supported by Pfizer Animal Health.

c.7.23 EFFECT OF GASTROINTESTINAL HELMINTHS AND ANTHELMINTIC TREATMENT ON BODY WEIGHT OF PONIES

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Background: The concept of sub-clinical weight loss or poor weight gain associated with helminth infection in young cattle is well established and has been dubbed "less than optimum productivity". The relationship between helminths and weight maintenance and gain in horses has been subject to less scrutiny, for a number of possible reasons. This study assessed the impact of helminth infection on weight gain and maintenance in a group of twelve ponies, compared to that of a second group of ponies treated at monthly intervals with pyrantel embonate (Strongid™ paste, Pfizer Ltd).
Method: Animals were set-stocked by group on separate, but comparable paddocks, for the grazing season from May to November. All ponies were treated on day –3 with pyrantel embonate at a target dose of 19mg/kg. Ponies in Group One remained untreated throughout the grazing season except for a single treatment in mid-July when their geometric mean faecal egg-count exceeded 500 eggs per gram of faeces (epg). Group Two ponies were treated with pyrantel embonate at monthly intervals.
Results: Group One mean faecal egg counts were significantly higher ($p<0.05$) than those of Group Two on eight out of the twelve fortnightly sampling occasions. At the end of the grazing season, animals in Group One had gained 3kg on average, whilst those in Group Two had gained a mean of 21.2kg. This difference in gain was significantly different ($p<0.05$).

c.7.20-27 Chemotherapy against horse parasites
c.7.28-33 Surveys of parasiticide resistance and drug use

c.7.25 PERSISTENT EFFICACY OF TWO ENDECTOCIDES IN HEAVILY CONTAMINATED HORSES (FECRT)

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Background: Recommended treatment intervals vary between regions and parasite pressure conditions. A faecal egg count reduction test was run to assess the remanent activity of two endectocide-based oral pastes for equids with natural heavy infection.
Method: A field trial was conducted in the State of Sao Paulo (Brazil), from December to April. 34 adult Arab mares were selected from one pasture based on positive (minimum 150) medium to high EPG counts (natural infection) and kept on this same pasture for the entire study duration. They were allocated to 3 groups from D-7 individual EPG counts: a Moxidectin* (0.4 mg/kg) (n=11), an Ivermectin/Praziquantel combination** (0.2mg/kg)(2.5 mg/kg) (n=11), and a control group (n=12). All treatments were administered orally on D0. All animals were taken faecal samples on D14, D28, D42, D56, D63 and D70.
Results: Arithmetic mean of egg counts was 963 in all groups on D-7. A mixed infection was present (predominance of small strongyle larvae, less than 4% of large strongyles). The control group remained at very high levels of egg output until the end of the trial period (AM= 1087.5 on D70, peak at 1362.5 on D63). The two treated groups had mean EPG less than 200 and significantly lower than untreated group from D14 until D42 included. On D42, Moxidectin and Ivermectin/Praziquantel groups had 159.1 and 140.9 EPG respectively. The threshold of 200 EPG was attained on D56, D63 and D70 for both treated groups.
Conclusion: When less than 200 EPG is considered as a bearable nematode egg output in horses, both endectocide oral pastes remained efficient for maximum 8 weeks after treatment. Knowing this persistent efficacy, a treatment interval of 2 months is validated even for horses reared on heavily contaminated pastures.
*EQUEST®, **EQUIMAX®

c.7.26 FIELD EFFICACY OF IVERMECTIN INJECTION IN HORSES IN ICELAND

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Objective: To evaluate the level and duration of suppression of faecal strongyle egg output and the effect on genera/species composition by a single ivermectin injection in horses in Iceland.
Material and methods: Thirty adult horses were included in the study. They had not been treated with anthelmintics in the 6-24 months preceding the study. The horses grazed from July to September 1995 in six experimental paddocks with varying stocking rates and grazing conditions. The anthelmintic was injected 9-10 days after the horses entered the paddocks. Faecal egg counts were carried out once before the treatment and on six occasions after the treatment. Third stage larval (L3) cultures were prepared from the faecal samples before and ten weeks after the treatment. Pasture larval (L3) counts were carried out on grass samples taken from July to October.
Results: Strongyle eggs per g (EPG) counts were high before the treatment (mean=2322). One week after the treatment 98.9% reduction in EPG was observed. More than half of the faecal samples had positive EPG counts after seven weeks. Both large and small strongyle species were found before the treatment but only small strongyles after the treatment, *Cyathostomum, sensu latu* accounting for 99.9% of L3 found, with a prevalence of 97%. No L3 were recovered from the paddocks until August and the density onwards varied greatly depending on both stocking rates and grazing conditions.
Conclusion: The study indicates that ivermectin injection produces a slightly less suppression on faecal strongyle egg output than treatment with oral ivermectin paste, as shown in previous studies in Iceland and abroad.

c.7.28 ANTHELMINTIC RESISTANCE IN STRONGYLID NEMATODES IN CZECH REPUBLIC

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In selected farms of horses, sheep, goats and cattle with naturally acquired strongylid infections levamisole, mebendazole, fenbendazole, albendazole, pyrantel, ivermectin and doramectin were tested for the prevalence of anthelmintic resitance. *In vivo* fecal egg count reduction test (FECRT) was used and in the case of the benzimidazole anthelmintics also *in vitro* egg hatch assay (EHA). Considerable resistance was found in equine small strongylides against mebendazole (10 $mg.kg^{-1}$) with the FECRT values 61.5% and 70.4% and against fenbendazole (7.5 $mg.kg^{-1}$) with 76% and 84.1%. The FECRT results in sheep proved the occurence of resistant strains of *Ostertagia* spp. and *Trichostrongylus* spp. to levamisole (7.5 $mg.kg^{-1}$) 67.8% and to fenbendazole (5$mg.kg^{-1}$) 83.7% and 85.4%. The resistance to benzimidazoles was confirmed by EHA in horses and sheep, as well. Resistance against albendazole, pyrantel, ivermectin and doramectin was not found.

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c.7.29 THE SPREAD OF RESISTANCE TO LEVAMISOLE IN OVINE NEMATODES.

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Background: The most likely cause for the spread of anthelmintic resistant nematodes is the movement of stock. Two recent cases suggest this could explain levamisole resistance on two farms.
Methods: Faecal egg counts (FECRT), egg hatch (EHT) and larval development (MALDT) tests and post-mortems followed standard procedures.
Results: Case one. Levamisole resistance had been diagnosed when the flock was grazed on rented pasture. On moving to a new cattle farm during the winter, sheep were dosed with ivermectin in the morning and turned out in the afternoon. In July FECRT at day seven was thirty four for fenbendazole and sixty three for levamisole.
Case two. Eggs counts were not eliminated in lambs from Wales which were maintained on concrete following treatment with levamisole. An EHT was negative but the MALDT for levamisole positive. Adult worms were *Trichostrongylus* sp. The farmer treated purchased stock with moxidectin and placed animals immediately on 'clean' pasture. Ewes, but not lambs, were treated twice per year with levamisole.
Discussion: On neither farm were animals treated with a group 3 anthelmintic and then yarded for one day before turning out to pasture. Animal movement rather than treatment regimes probably account for the levamisole resistance on both farms. Our data suggests levamisole resistant nematodes are more common than generally realised in the UK. This is the first case of levamisole resistant nematodes from Wales.

c.7.28-33 Surveys of parasiticide resistance and drug use

c.7.30 DETECTING ANTHELMINTIC RESISTANCE IN HORSE STRONGYLES

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Background: Faecal egg count reduction tests (FECRT) are widely used to detect anthelmintic resistance. *In vitro* tests, such as the larval development assay (LDA) and egg hatch assay (EHA), avoid large inter-animal variation. This study compares FECRT, LDA and EHA results on 52 Danish horse farms.
Method: Faecal samples were collected from horses on the day of treatment (day 0) and 14 days later. Drugs used were the oral paste formulations Panacur vet. (18.75% fenbendazole, Hoechst), Banminth vet. (43.9% pyrantel pamoate, Pfizer) and Eqvalan vet (1.9% ivermectin, MSD Agvet). An untreated control group was included on each farm. LDAs and EHAs were performed on pooled day 0 samples from 32 and 52 farms, respectively. FECRTs were performed on 42 farms. Relationships between the 3 tests were examined with the aim of using one test as a predictor for another of the tests.
Results: Benzimidazole (BZ) resistance was indicated on 79% and 62% of farms tested by FECRT and EHA, respectively. It was not possible to determine BZ resistance by LDA due to suspected BZ resistance of the reference strain. Pyrantel (PYR) resistance was indicated on 3 of 15 farms by FECRT and on 1 of these farms plus an additional 2 farms by LDA. It was not possible for one test to predict the outcome of another.
Conclusion: The 3 tests measure different attributes of the parasite's response to anthelmintics. The best test to use is likely to depend on the drug against which resistance is suspected, the purpose of the testing and the available resources.

c.7.31 RE-EMERGENCE OF IVERMECTIN-RESISTANT *OSTERTAGIA CIRCUMCINCTA* IN SHEEP GRAZED ON PASTURE CONTAMINATED WITH GOAT DERIVED IVERMECTIN-RESISTANT *OSTERTAGIA* SPP.

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Background: Ivermectin-resistant isolate of *O. circumcincta* rapidly re-emerged in goats but not in similarly treated sheep after an interval of 5 years with no ivermectin (IVM) usage. A follow up cross-over study was conducted to investigate if sheep are at risk from grazing pasture contaminated with goat-derived IVM-resistant *O. circumcincta.*
Method: The same trial area was divided into 8 paddocks, half previously grazed by goats and half previously grazed by sheep. Each paddock was setstocked with either a group of weaned goats (n=6) or weaned lambs (n=4). After 1 year, the animals were replaced by recently weaned animals of the same species. Each host species was drenched with ivermectin (0.2 mg/kg) when any paddock mean faecal egg count for that host species exceeded 500 epg. Anthelmintic resistance was monitored with faecal egg count reduction tests (FECRT) and larval development assay (LDA).
Results: IVM resistance re-emerged rapidly in all goat groups. It was never identified in sheep on paddocks previously grazed by sheep but appeared after 5 months in sheep on paddocks previously grazed by goats and remained apparent up to the end of the experiment. The level of IVM resistance as assessed by FECRT was higher in goats compared with sheep. These trends were also reflected in LD_{50} values from LDAs.
Conclusions: Sheep are at risk from grazing pasture contaminated with goat-derived ivermectin-resistant *O. circumcincta.* Ivermectin was more effective in sheep than goats.

c.7.32 RESULTS FROM A QUESTIONNAIRE SURVEY ON ANTHELMINTIC USE IN DAIRY GOATS IN FRANCE

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Background : Since the spread of anthelmintic (AH) resistance in nematode populations of the digestive tract, recommandations have been rised to improve the use of antiparasite substances in ruminants. To evaluate the diffusion of these recommandations to farmers, a questionnaire survey was undertaken in France in dairy goat flocks where AH resistance is particularly widespread.
Method : Information were collected from 54 farms in the 3 main regions of dairy goat production. The data refered to 3 years (1995-1997).
Results : AH were used to control nematodes in 52 out of 54 farms and the mean number of treatment per year was 2,7. In 12 farms, a single treatment was applied only. In the 42 remaining farms, a change in the use of different class of AH within or between year was practiced in 43 per cent of the investigated flocks. Avermectines, pyrantel and levamisole were used respectively in 27 ; 11,5 and 6 per cent of the farms. Benzimidazoles (BZ) and probenzimidazoles were given in all, except 2, of the farms. They represented more than 80 % of the treatments applied. BZs were applied at the recommanded double ovine dose in only one half of the farms. In addition, in the vast majority of the farms, the substances were given on the basis of the estimated mean weight of goat and not using the heaviest animal as reference.
Conclusions : The present results show that errors in the use of AH are still frequent in goat breeding (underdosing of BZ, lack of alternance of molecules,..) which could further the spread of AH resistance. This underline the need to improve the diffusion of recommandations for control of nematodes to farmers.
Acknowledgments : This work received the financial support from the contract FAIR3CT96-1485.

c.7.33 FIELD STUDIES ON ANTHELMINTHIC RESISTANCE IN VILLAGE GOATS IN UGANDA

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A survey was conducted to assess the prevalence of anthelminthic resistance in nematode populations in village goats on 36 farms in two parishes in Mbale district, Uganda. Two commonly used anthelminthics, representing two major groups of broad spectrum anthelminthics with different modes of action [Levamisole, (LVM) and Albendazole, (ALB)] were applied at the manufacturer's recommended dose rates. Using the faecal egg count reduction test (FECRET), percent efficacy rates of 98% and 100% were obtained for ALB in the two parishes, respectively. While, LVM gave percent efficacy rates of 90% and 48% in the same parishes, respectively. Larval cultures identified *Oesophagostomum spp* (36%), *Nematodirus spp.* (29%), *Haemonchus contortus* (21%) and *Bunostomum spp.* (14%) to be the most predominant nematode species in the goats after treatment. According to these preliminary results, existence of resistance against LVM in nematode populations in village goats in Mbale district is highly suspected, since LVM percentage efficacy rates were well below 95%.

c.7.34-37 Molecular studies of parasiticide resistance

c.7.34 GENETIC DIAGNOSTIC OF BZ-RESISTANCE IN SMALL RUMINANT NEMATODE COMMUNITIES.

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Background : The intensive use of benzimidazoles (BZ) to prevent losses from helminth infections, soon resulted in emergence of resistance. Sensitive essays in order to detect early stage of resistance are also highly needed. Egg hatch essay is a widely *in vitro* test used to monitor resistance to BZ in nematodes but it is an "all species" diagnostic. It is not always reliable to detect "one species resistance", which is the most frequent case. The less frequent species, of one particular sampling period, might be overlooked for resistance when the egg hatch essay technique is used.

Method : Genetic mechanisms of BZ-resistance have been studied. A recessive point mutation on the β-tubulin gene has been shown to be responsible for BZ-resistance. Consequently, genetic essays have been investigated. Using allele specific polymerase-chain-reaction (PCR), the resistance status of adults *Haemonchus contortus* and *Teladorsagia circumcincta* has been determined. These tests permit detection of resistance at individual level, but are time consuming (being based on a two step-PCR) and can be applied only on adult worms. In the present work, we describe a new method which allows to determine, in a first step, the generic composition of a community of nematodes and, in a second step, the BZ-resistance status of each species. The major advantage is the identification on larvae which can be easily recovered from culture of faeces. In addition, this technique is quick, very sensitive, and suitable for field conditions of multispecific infections. It would be useful to monitor the emergence of resistant allele in a community of nematodes.

Acknowledgement : This work was supported by a PhD grant from INRA and "Bureau des Ressources Génétiques".

c.7.35 A PCR TEST FOR MACROCYCLIC LACTONE RESISTANCE IN *Haemonchus contortus*

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Background: Anthelminthic resistance in nematode parasites is a serious problem worldwide. The proposed mode of action of the avermectins and possibly the milbemycins involves the binding of the drug to the alpha subunit of a glutamate-gated chloride channel, which opens the channel and leads to the hyperpolarization of the target neuromuscular cell. The mechanism of resistance is not yet known but one possibility is that the glutamate-gated chloride channel is involved.

Method: We have analyzed by SSCP the genetic variation of a gene encoding a glutamate-gated chloride channel subunit from two sets of unselected and three anthelmintic-selected strains of the parasitic nematode *Haemonchus contortus*. The resulting alleles were then sequenced in an attempt to find mutations that could be used to design primers for a PCR based test for selected alleles.

Results: One allele (allele A) increased in frequency in the three selected compared to the unselected strains of *H. contortus*. Sequence analysis revealed a unique mutation in this allele that was used to design primers for a PCR test. The PCR method was further tested and found to be accurate in detecting the frequency of allele A in both laboratory and field strains of *H. contortus*.

Conclusion: Our results provide evidence that the glutamate-gated chloride channel in involved in macrocyclic lactone resistance. We have further developed a PCR test that can detect a potential resistance allele that may be useful as a genetic marker for the detection of macrocyclic lactone resistance in the field.

c.7.36 GLUTAMATE GATED CHLORIDE CHANNELS IN IVERMECTIN RESISTANT *Haemonchus contortus*

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The development of resistance to ivermectin (IVM) has been well reported. One of the major target sites of IVM is the inhibitory glutamate-gated chloride channels (Glu-Cl). Resistance may therefore be mediated by an alteration in these target sites. We examined [^{3}H]-glutamate binding to membrane preparations from L3 larvae of an IVM-sensitive and a South African IVM-resistant field isolate of *H. contortus*. Highest binding was observed in the P2 membrane fraction. Equilibrium binding studies showed the presence of large numbers of low-affinity specific glutamate binding sites. These sites were more numerous in the resistant (B_{max}^{res}=659±92.7 pmol/mg protein) than the sensitive isolate (B_{max}^{sen}=119±11.7 pmol/mg) and may have altered affinity (K_d^{res}=8.3±0.3 μM; K_d^{sen}=1.4±0.8 μM) though the nature of the site makes accurate estimation of binding parameters difficult. The rate of dissociation of 1 μM [^{3}H]-glutamate binding from *H. contortus* membranes at 4°C showed 2 components (K_{offa}=0.84 min^{-1};K_{offb}=0.004 min^{-1}). The rate of association of was 78.23 μM^{-1} min^{-1}. It was difficult to determine the nature of this binding site due to the lack of suitable ligands. A similar low-affinity binding site was observed in membranes from *Ostertagia circumcincta*, but in this case no significant change in this site was observed in an IVM-resistant field isolate. Full-length cDNAs of Glu-Cl subunits (HG2-HG5) were compared in the IVM-resistant *H. contortus* isolate. The amino-acid sequences of the HG2, HG3 & HG4, plus the N-terminal domain of HG5, were identical in the IVM-resistant and -sensitive isolates. The expression of the HG2/3 & HG4 subunits was examined using specific antibodies; again, no differences were observed between the two isolates.

c.7.37 CHARACTERISATION OF THE BETA TUBULIN GENE OF *CYLICOCYCLUS NASSATUS*

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Beta tubulin is the target of the anthelmintic efficacy of benzimidazoles (BZ). The characterisation of the ß-tubulin gene of *C. nassatus* is necessary for the understanding of the interaction of benzimidazoles with nematode microtubules. Adult worms of small strongyles were morphologically differentiated. cDNA and genomic DNA was isolated from adult worms of *C. nassatus*. The cDNA was amplified by PCR using degenerate primers which were designed from comparison of ß-tubulin cDNA sequences of other nematodes such as *Haemonchus contortus* and *Trichostrongylus colubriformis*. To complete the coding sequence the 3`end was amplified by 3`RACE, for the 5`end the SL1-primer was used. The cDNA of the ß-tubulin gene of *C. nassatus* comprises 1362 bp and codes for a protein of 454 amino acids. cDNA sequence identities with *H. contortus* are 84%. Specific primers are developed from the cDNA sequence to amplify the genomic DNA sequence and to analyse the genomic organisation of the ß-tubulin gene. To examine the molecular basis for the often reported BZ-resistance, the sequences of the ß-tubulin gene from BZ-susceptible and -resistant strains of *C. nassatus* will be compared.

c.7.38-56 Chemotherapy against livestock parasites

c.7.38 COMPARISON OF EPRINOMECTIN WITH OTHER MACROCYCLIC-LACTONES IN CATTLE

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Seventy-two Belgian Blue crosssbred cattle aged 9-12 months were allocated by restricted randomization based on faecal egg counts among 4 treatment groups: (1) eprinomectin 0.5% w/v solution; (2) ivermectin 0.5% w/v solution; (3) moxidectin 0.5% w/v solution; and (4) doramectin 0.5% w/v solution. All doses were administered topically, at 500 µg/kg body weight according to label recommendation on Day 0. The calves were grazed together as a single group for the duration of the 64-day study, and faecal egg counts were monitored regularly. Geometric mean counts reached zero only in the eprinomectin group on Days 3 and 4. Significantly ($p<0.05$) lower mean counts in the eprinomectin group were obtained on Days 2, 3 and 4 compared to the doramectin and ivermectin treated cattle. From Day 10 to 63, there were occasional significant differences in mean faecal egg counts between eprinomectin and other groups. However, the number of cattle shedding eggs was not significantly different between the same groups. From Day 29 until the end of the study, mean egg counts counts in the moxidectin group were numerically higher than those in the other groups. The overall pattern of faecal egg output indicated equivalent duration of persistent activity for all treatments, with reductions in egg counts in the eprinomectin group occurring significantly faster than in the ivermectin or doramectin groups.

c.7.39 EFFICACY OF AN IVERMECTIN CR BOLUS AGAINST RARER SHEEP NEMATODES

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To evaluate the therapeutic and prophylactic efficacy of the ivermectin controlled-release bolus (IVM-CRB) which delivers ivermectin intraruminally at 0.9 mg per day for 100 days, against some rarer gastrointestinal nematode species, twenty-one male and female Schwarzkopf lambs weighing 21.5-35.5 kg were used. The animals were allocated by restricted randomisation based on body weight within sex to one of three treatments: Untreated control; IVM-CRB on Day 0; and IVM-CRB on Day 70. The animals were challenged with infective third stage larvae of *Bunostomum trigonocephalum* (Days 10-14), *Cooperia oncophora/surnabada, C. punctata, Nematodirus helvetianus, N. roscidus, Ostertagia leptospicularis* and *O. ostertagi* (Days 38-42), and *Strongyloides papillosus* (Days 45-49). On Day 84 the lambs were slaughtered for nematode recovery. Prophylactic treatment with the IVM-CRB on Day 0 prevented the establishment of incoming larvae of all parasite species tested by >99% ($p<0.01$). Efficacy against adult nematodes (IVM-CRB on Day 70) was 96% or greater. The results show that the efficacy of the IVM-CRB against the tested nematode parasites was as high as that reported against the most common species.

c.7.40 EFFICACY OF TRICLABENDAZOLE, ITS METABOLITES & OTHER COMPOUNDS IN SHEEP

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Background: Triclabendazole (tcbz) has been on the market for 15 years. We report here on 5 controlled efficacy trials previously unpublished.
Methods: Sheep were infected with 100-200 Fasciola hepatica metacercariae. After 3-42 days groups of 3-8 sheep (see brackets in table) were given 10mg/kg tcbz, its sulfoxide (tcbzSO) or sulfone ($tcbzSO_2$) metabolite using drenches of different strengths, or 7.5mg rafoxanide/kg orally, or subcu-taneously 10mg nitroxynil/kg. Groups of 5-10 sheep were not treated. After 8-17 weeks all Fasciola stages found in bile ducts and liver tissue were counted.
Results: The following worm reductions (%) were calculated and compared in Mann-Whitney Tests (comparable data with different letter differ at $p \leq 0.05$).

Treatment	3days	7days	14days	21days	28days	35days	42days
tcbz 5%	94(8)				99(6)		
tcbz 5%		97a(5)	97a(5)	100a(5)	100a(5)	100(5)	100(5)
tcbz 10%		98a(5)	93a(5)	95a(5)	100a(5)	100(5)	100(5)
rafoxanide					36b(5)		
tcbz 2.5%		82(5)	99(5)		95(5)		99a(5)
rafoxanide							90b(5)
tcbz 5%			92a(5)				100a(5)
rafoxanide			34b(5)				
nitroxynil			18b(5)				42b(5)
tcbz 5%					99a(5)		
tcbzSO					100a(3)		
$tcbzSO_2$					41b(3)		

Conclusions: Tcbz kills Fasciola aged ≥3 days. Dosing tcbz or sulfoxide entails similar efficacy, sulfone is inactive. Drench strength does not influence efficacy.

c.7.41 PERSISTENT EFFICACY OF 3 ENDECTOCIDES AFTER EXPERIMENTAL NEMATODE INFECTION IN CALVES.

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Background: This study compared the persistent efficacy of 3 endectocides based on long acting Ivermectin*, long acting Abamectin** or Doramectin*** against artificial infection in calves.
Methods: 80 calves were randomly allocated into a Control (n = 5) and 5 groups (n = 15) treated at 42, 35, 28, 21 and 14 days prior a single mixed inoculum of *O. ostertagi, T. axei, H. placei* and *C. oncophora*. Sacrifice occurred 22 days after infection.
Results: The percentage efficacy (GM worm count) was determined by comparing parasite populations at necropsy, with a cut off point established at 90 %, and a significant reduction compared with Control. L.A Ivermectin protected for 35 days against *H. placei* and *T. axei*; 21 days against *O. ostertagi* and *C. oncophora*. L.A Abamectin protected for 35 days against *O. ostertagi, H. placei*, and *T. axei*; 21 days against *C. oncophora*. Doramectin protected for 35 days against *O. ostertagi* and *T. axei*, 28 days against *H. placei* and 21 days against *C. oncophora*.
Conclusion: The study results indicate similar persistent efficacy (no significant difference) between groups according to the fixed cut off point and significant difference with Control. The L.A. formulation extends the persistent efficacy compared to a classical Ivermectin or Abamectin formulation. The results also indicate that duration of efficacy is nonetheless variable, depending on parasite species.
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c.7.38-56 Chemotherapy against livestock parasites

c.7.42 IMPACT OF AN IVERMECTIN LAI FORMULATION ON THE PRODUCTIVITY OF BEEF CATTLE

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A study was carried out to compare the impact of a year-long treatment program using an ivermectin long-acting injectable (IVM-LAI) formulation with a similar program using a doramectin (DOR) injection and untreated controls (UC) on parasite control and weight gain of grazing beef steers in southern Brazil. Seventy-two Angus steers and 12 similar paddocks were used. The trial had a partially hierarchical design with Day -1 weight, paddock location and forage as blocking factors. Within blocks, one paddock was randomly allocated to each treatment group. The following treatments were administered to steers on Days 0 and 140: UC; IVM-LAI at 630 mcg/kg *sc*; or DOR at 200 mcg/kg *sc*. Several salvage treatments against ticks (*Boophilus microplus*) and gastrointestinal worms were performed on some UC and DOR treated steers. No IVM-LAI treated animal needed salvage treatment against these parasites. Compared to the UC group, the IVM-LAI treated cattle had significantly ($p<0.05$) lower mean nematode fecal egg counts (FEC) from Days 14 to 209 and from Days 325 to 363; and lower ($p<0.05$) mean tick counts on Days 14, 28, 69, 83, 139, 167, 195, and 349. Compared to the DOR treated cattle, IVM-LAI treated cattle had significantly ($p<0.05$) lower mean FEC on Days 69 and 97 and lower ($p<0.05$) tick counts on Days 111, 195, 335, and 349. The mean weight gain of the IVM-LAI treated cattle was significantly ($p<0.05$) higher than UC group and DOR treated cattle from Days -1 to 140 and from Days -1 to 364. By the end of the year-long trial, mean weight gains per group were 156.4 kg for the IVM-LAI treated cattle, 111.1 kg for the UC group, and 137.4 kg for the DOR treated cattle. These results indicate that a program using two ivermectin LAI treatments administered to cattle provides significantly better nematode and tick control resulting in a better weight gain than doramectin injection administered according to the same schedule.

c.7.43 EFFICACY OF AN IVERMECTIN CONTROLLED RELEASE BOLUS AGAINST ENDOPARASITES OF SHEEP

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Background: A trial was conducted to confirm that a controlled-release (CR) bolus formulation of ivermectin was effective against endoparasites throughout its release period.

Method: Twenty-two, 4 to 6 month-old Döhne Merino rams weighing 25.5 to 36 kg on Day 0 and 28 to 39.5 kg on Day 90 were ranked on body weight. Within replicates of 7 sheep, 3 were randomly allocated as unmedicated controls and 4 were allocated to treatment once on Day 0 with a CR bolus releasing a minimum of 20 µg ivermectin/kg/day for 100 days. All sheep were treated with levamisole on Day –12 and dosed with approximately 1500 *Haemonchus contortus*, 2000 *Trichostrongylus colubriformis* (culture contained low levels of *Teladorsagia circumcincta*) and 400 *Oesophagostomum columbianum* on Days 89 and 90, and were necropsied for worm counts on Days 125 or 126.

Results: The group treated with the ivermectin CR bolus had reductions in worm counts from control of >99.9% for *H. contortus*, *O. columbianum*, *T. colubriformis*, *T. circumcincta* and *Trichuris* spp. The presence of *Trichuris* spp. in the controls is considered to be due to the relatively poor efficacy of levamisole against this parasite.

Conclusion: These results confirm the sustained efficacy of the ivermectin CR bolus against major endoparasites of sheep.

c.7.44 EFFICACY OF MOXIDECTIN AND FENBENDAZOLE AGAINST A RESISTANT STRAIN OF HAEMONCHUS.

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Background: To evaluate efficacy of Moxidectin (MOX) and Fenbendazole (FZ) against an ivermectin resistant strain of *Haemonchus contortus* in sheep, in Argentina, using experimental infections.

Methods: Sixteen worm free Pampinta lambs, 3-6 months of age and weighing 25-38 kg were orally infected with 12000 infective larvae composed of a known ivermectin resistant strain of *Haemonchus*, isolated from the field and kept under laboratory conditions as reference. The percentage composition of the inoculum was 75 % *Haemonchus* spp, 20 % *Teladorsagia* spp. and 5 % *Trichostrongylus* spp. After 21 days post-infection, the animals were sampled and ranked by epg in decreasing order and then allocated into four homogeneous experimental groups: Group 1 (FZ 5 mg/kg bw, orally), Group 2 (MOX 200 mg/kg bw, SC), Group 3 (ivermectin 200 mg/kg bw, SC) and Group 4 (non treated control). Treatments to groups were assigned at random.

Results: Using the FERT test, the efficacy of fenbendazole and moxidectin was 99 and 100 % respectively. The strain inoculated was resistant to ivermectin (76 % of efficacy).

Conclusions: The inoculated strain of *Haemonchus contortus*, resistant to ivermectin was highly susceptible to Moxidectin and Fenbendazole treatment.

c.7.45 PERSISTENT EFFICACY OF DORAMECTIN AGAINST *TRICHOSTRONGYLUS AXEI* IN CATTLE.

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Two separate studies were conducted to evaluate the persistent efficacy of doramectin injectable solution (200 µg/kg, subcutaneously) and doramectin pour-on solution (500 µg/kg, topically), respectively, against experimentally induced infections of *Trichostrongylus axei* in cattle. On day 0, 40 parasite-free calves were selected and randomly assigned to 1 of the 4 treatment groups (n=10) in each study. The 4 groups for each study consisted of 1 control group and 3 treatment groups, receiving treatment on day 0, 7 or 14 respectively. Beginning on day 21 (injectable study) or day 22 (pour-on study), 2,000 infective *T. axei* larvae were administered orally each day to all calves in all groups up to day 35. This experimental design allowed to for the evaluation of the efficacy of doramectin for 35, 28, and 21 days after treatment. All calves were slaughtered for worm burden determination on days 55 and 56. *T. axei* worm burdens in the 3 doramectin-treated groups for each formulation were significantly ($P \leq 0.05$) lower than those in their respective control groups. For each study, percent efficacies were calculated for the reduction in worm burden geometric means in the doramectin-treated groups compared to the worm burden geometric mean in the control group (see table below). No abnormal clinical signs or adverse reactions were observed in any calf following administration of the injectable or pour-on formulations of doramectin.

	21 days	28 days	35 days
Doramectin injectable	98.9 %	97.4 %	92.8 %
Doramectin pour-on	99.5 %	98.5 %	97.3 %

c.7.38-56 Chemotherapy against livestock parasites

c.7.46 EFFICACY OF DORAMECTIN AGAINST OESTRUS OVIS AND NEMATODES IN SHEEP.

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In order to investigate the efficacy of doramectin in sheep, 24 animals with naturally acquired infections of *Oestrus ovis* and gastrointestinal nematodes were used. Sheep were randomly assigned to a non-medicated group (n=12) or to a treated group (n=12) that received a single intramuscular injection of doramectin at a dose rate of 200 µg/kg. Faecal egg counts were performed daily from days 0 to 7 and on day 14. On day 14, all sheep were slaughtered and *O. ovis* larvae and nematode worm burdens were determined. Efficacy was assessed by comparing faecal egg counts, *O. ovis* larvae counts and counts of nematodes (geometric means) between non-medicated control sheep and doramectin-treated sheep. In doramectin-treated animals, faecal egg counts decreased to zero by day 4 for all recovered types of nematode eggs, including strongyles, *Nematodirus* spp, *Trichuris* sp, and Rhabditidae. On day 14, percentage reductions were 100% for *Nematodirus* spp and Rhabditidae, and 99.8% and 99.1% for strongyles and *Trichuris* sp, respectively.

At necropsy, only *O. ovis* L1 larvae were found in control animals, whereas in doramectin-treated animals the efficacy was 100%.

Doramectin was highly efficacious against the adult stages of the following species (no larvae were present in control and treated groups) : *Teladorsagia circumcincta,* 100%; *Nematodirus battus,* 100%; *Nematodirus filicollis,* 99.9%; *Oesophagostomum venulosum,* 99.8%; and *Trichuris* sp, 93.3%.

No abnormal clinical signs or adverse reactions were observed in any animal treated with doramectin.

c.7.47 A NEW IN VITRO ASSAY OF ANTHELMINTIC ACTIVITY AGAINST LARVAE OF *ASCARIS SUUM*

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Background: Few studies have reported the anthelmintic resistance (AR) of *Ascaris suum* (As) and the methods to evaluate AR of As in vitro. In this study we tried to develop an in vitro method to detect AR of As.

Method: In a 24 well culture tray (Nunc) an agar gel culture (AGC) system was developed for newly hatched AS larvae. Larval viability was tested in different concentrations of anthelmintics. Xtree-gold program was used to analyze data.

Results: There were significantly different percentages of larvae penetrating to the surface of the agar gel using AGC with different agar thickness, 17.78%, 5.97%, 1.50% and 0.41% were recovered from the agar surface using 0.5ml, 1ml, 2ml and 3ml agar gel per well, respectively. After 3 days of exposure to different concentrations of fenbendazole (FBZ), mebendazole (MBZ), thiabendazole (TBZ), levamisole (LEV), pyrantel (PYT) and moxidectin (MXD), the number of larvae recovered from the agar surface was different, when the AGC system was used. There were significant relationship between the number of larvae moving to the agar surface and the concentrations of FMZ, MBZ, TBZ, LEV and PYT. The EC50 (concentration of drug giving a 50% reduction of migrating larvae compared to controls) was for FBZ: 3.842nM, MBZ: 3.684nM, LEV: 1.363 M and PYT: 3.330 M, respectively. No relationship in AGC found with different concentrations of moxidectin.

Conclusion: AGC method for As larvae could be used to evaluate the effects of anthelmintics against As in vitro, thus, AGC could be used as an in vitro test of possible anthelmintic resistance of As.

Acknowledgments to The Danish National Research Foundation.

c.7.48 THE SAFETY AND EFFICACY OF ABAMECTIN FORMULATIONS FOR CATTLE, HORSES AND SHEEP

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Background: Abamectin, the natural fermentation product from *Streptomyces avermitilis*, has high level endo- and ecto-parasiticidal activity. Abamectin was developed as injectable and pour-on formulations for cattle; paste and tubing liquid for horses and an oral presentation for sheep. Combination formulations of abamectin and praziquantel were developed for horses and sheep.

Results: Safety. Cattle were treated at multiple doses of abamectin formulation by injection and pour-on application without signs of toxicity.

Horses were dosed at rates of abamectin up to 800 µg/kg (4x recommended) without toxic signs. Weanlings were treated safely at 600 µg/kg and foals, pregnant mares and stallions were treated at 400 µg/kg without effect on the unborn foal or sperm production or quality. Sheep were treated at doses up to 1000 µg/kg (5x recommended) without signs of toxicity. Lambs as young as 4 weeks of age, pregnant ewes and rams were treated at 400 µg/kg. No toxic signs were observed and no effect was seen on the foetus or spermatogenesis. Food residue studies were conducted in cattle and sheep to support withholding periods of 30 days (cattle injection), 35 days (cattle pour-on) or 14 days (sheep oral).

Efficacy was demonstrated for an extensive range of cattle internal parasites as well as cattle tick and buffalo fly. Greater than 99% efficacy was demonstrated against all major horse parasites including arterial and lumen stages of *Strongylus* spp, large and small strongyles, pinworms, ascarids, lungworms and bots.

High levels of efficacy were demonstrated for all the major genera of sheep nematodes as well as *Oestrus ovis and Psorergates ovis*. The addition of praziquantel to some of these formulations did not compromise efficacy against nematodes or arthropods and demonstrated up to 100% efficacy against heads and scoleces of *Moniezia* spp. in sheep and *Anoplocephala* spp and *Paranoplocephala* in horses.

Conclusion: Abamectin administered alone or in combination with praziquantel was a safe and highly effective endectocide for cattle, horses and sheep.

c.7.49 WEIGHT GAINS OVER 6 MONTHS IN 100 CATTLE TREATED WITH 4 ENDECTOCIDES

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Background: A weight gain and EPG test was run in Brazil to assess the economic value over a 180 days period of different endectocide compounds and formulations used three times at 2-month interval.

Method: A field trial was conducted from April to October. 100 cattle (*Bos indicus*) were selected from one pasture based on positive EPG counts (natural infection) and kept on this same pasture for the entire study duration. They were allocated to 5 groups of 20 according to D0 bodyweights: an Ivermectin*group **A**, a Doramectin**group **B**, a Control (untreated) group **C**, a long acting (L.A.) Ivermectin***group **D** and (LA.) Abamectin****group **E**. All treatments were administered S.C. on D0, D60 and D120 at 0.2 mg/kg. All animals were weighed and sampled on D0, D30, D60, D90, D120, D150 and D180.

Results: Geometric mean (GM) of egg burdens varied from the lowest GM=302.3 in group A to the highest GM=372.4 in group D on D0 ($p>0.05$). A mixed strongyle infection was present (Cooperia, Haemonchus, Oesophagostomum). Since randomisation was based on bodyweights, all groups had similar bodyweights on D0 (AM=218.7 kg) ($p>0.05$). On D180, AM bodyweights were respectively 234.2 kg, 241.9 kg, 221.1 kg, 244.1 kg, 242.1 kg in groups A, B, C, D, E with groups B, D, E statistically different from control group ($p<0.0001$). The maximum average 180 days weight gain was observed in group D (25.5 kg) and the minimum in group C (2.7 kg). All groups had GM of EPG statistically different from control group on D180: the lowest GM was observed in group E (GM=1.4) and the highest in group C (GM=254.4).

Conclusion: All products tested but one (group A) improved economic performance of cattle at the end of a 6 months period, when applied three times at 2-month interval.

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c.7.38-56 Chemotherapy against livestock parasites

c.7.50 EVALUATION OF EFFICACY OF MOXIDECTIN AGAINST ASCARIS SUUM IN SWINE

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Background: Preliminary studies showed good activity of moxidectin (MOX) administered as a 0.5% pour-on (P/O) and as a 0.1% oral drench (O/D) against a range of GI nematodes in swine. A study was conducted to confirm efficacy in pigs naturally infected with Ascaris suum.
Method: Thirty six pigs were divided into the following groups of 12 according to pretreatment Ascaris faecal egg counts (FEC's): (Group 1): Untreated control (UC), (Group 2): MOX P/O at 1.0 mg/kg applied along the midline of the back, and (Group 3): MOX O/D at 0.6 mg/kg administered by stomach tube. Pigs were slaughtered 14 days posttreatment and their FEC's and worm burdens determined.
Results: Both MOX formulations reduced the geometric mean FEC by more than 99.8% compared to the UC value of 1,785 epg (range 120 7,280 epg). MOX P/O and MOX O/D reduced the G. mean worm burden by 97% and 99% respectively, compared to the UC value of 10.3 (range 0 - 93). No adverse effects were observed.
Conclusion: The results confirm that MOX P/O (1.0 mg/kg) and MOX O/D (0.6 mg/kg) are highly effective against A. suum in swine.

c.7.51 EFFICACY OF CLORSULON AGAINST FASCIOLA HEPATICA INTEGRATED AS ECTOPARASITE CONTROL

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Psoroptes ovis and Melophagus ovinus are commonly found in Fasciola hepatica affected sheep in Patagonia. The objective of this study was to evaluate the therapeutic efficacy of clorsulon when dosed with ivermectin as an ectoparasiticide. 34 Merino sheep naturally infected with F. hepatica were randomly allotted to 3 groups: Group 1, 11sheep were subcutaneusly dosed on Days 0 and 7, with 1% ivermectin/10 % clorsulon, 1ml/50 kg; Group 2, 11 sheep, same dose, but Days 0 and 21; Group 3, 12 untreated control sheep. Seven days after the second dose, all animals from Group 1 and 6 of Group 3 (Day 21) and all of Group 2 and 6 remaining of Group 3 (Day 28) were slaughtered. Parasitological observations were carried out in fecal samples by sedimentation-filtration technique and post mortem examination for parasites was performed in liver parenchyma, bile ducts and gall bladder. The drug efficiency was monitored comparing egg and worm recovery in treated groups with that in untreated control group.
The results showed only a few eggs of liver fluke in fecal samples of 18 % of the treated groups, and at necropsy revealed a 99,9 to 100% efficacy against mature F. hepatica and 82,8 to 98,7 % efficacy against immature stages.
The current trial clearly showed that in treatments against keds or scabies concurrently affected by liver fluke, the compound ivermectin-clorsulon is highly recommended.

c.7.52 A COMPARISON OF THE LD_{50} VALUES IN A LARVAL DEVELOPMENT ASSAY BETWEEN SINGLE AND TRICKLE INFECTIONS WITH *TRICHOSTRONGYLUS COLUBRIFORMIS*

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Background: LD_{50} values in a Larval Development Assay (LDA) have been observed to change with time after a single infection with trichostrongyloid nematodes, increasing from a baseline to peak about 50-70 DPI and then returning to baseline levels. The hypothesis of this experiment was that in a trickle infection LD_{50} values would increase slightly and then stabilise at a level slightly above baseline.
Method: Three sheep (Group 1) were trickle-infected with 2000 *T. colubriformis* /week for 14 weeks. Another 3 sheep (Group 2) were given a single infection of 22000 *T. colubriformis*. LDAs were performed weekly with ivermectin, avermectin B_2 and levamisole as test products.
Results: For each anthelmintic LD_{50} values for all sheep were initially similar until 35 DPI. Group 1 values then increased for each drug to a peak about 50 DPI (ivermectin 3.5X, avermectin B_2 2.5X and levamisole 4-7X) before returning to baseline levels on about 80 DPI. LD_{50} values for Group 2 increased slightly from about 35 DPI, then remained at this slight elevation before also returning to baseline levels about 80 DPI.
Conclusion: The change in LD_{50} was reduced by the trickle infection but the trend remained the same. This implies that the change in LD_{50} value with time may have less influence on the interpretation of LDA results under field conditions

c.7.53 PERSISTENT EFFICACY OF DORAMECTIN AND MOXIDECTIN VERSUS *C. ONCOPHORA*

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Background: Two studies were conducted to the same protocol in France and the UK to investigate the persistence of activity of pour-on formulations of doramectin and moxidectin against naturally acquired infestations of *Cooperia oncophora.*
Method: Pastures were seeded in early spring by grazing with previously parasite-naive calves that had each been infested with *C. oncophora* larvae of a strain known to be susceptible to anthelmintics. The seeder animals were replaced by study animals that, in equal numbers on day 0, had been treated with either doramectin or moxidectin administered topically down the mid-line of the back at a dose rate of 500 mcg/kg of bodyweight. From day 21 to 56 in each study, samples were obtained weekly from the 30 animals and the pastures for coproculture and determination of faecal egg counts.
Results: In both studies, the moxidectin-treated group showed an increase in faecal egg output on day 28, whereas in the doramectin -treated group this did not occur until day 42. The predominant nematode cultured from the faeces or isolated from the herbage was *C. oncophora.*
Conclusion: Doramectin pour-on administered to grazing cattle delayed faecal egg output longer than moxidectin pour-on under field conditions, confirming the superior persistency profile of doramectin pour-on against *Cooperia oncophora* compared to moxidectin pour-on.

c.7.38-56 Chemotherapy against livestock parasites
c.7.57-63 Integrated and biological parasite control

c.7.54 PERSISTENT ACTIVITY OF ORAL MOXIDECTIN AGAINST GI NEMATODES IN GOATS

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Background: There is a need for an anthelmintic for goats with an extended persistent effect against GI nematodes. A controlled artificial infection trial was conducted to determine the period of persistency of oral moxidectin in goats.

Method: 24 parasite free kids, weighing 14 – 25 kg, were randomly allocated to 4 groups. Three groups were treated with moxidectin (0.1% oral drench for sheep; 0.2 mg/kg body-weight): one on day 29, another on day 22 and the third on day 15 before infection. A further group was kept as untreated control. Artificial infection with *Haemonchus contortus* (2500 L_3), *Teladorsagia circumcincta* (5000 L_3) and *Trichostrongylus colubriformis* (3000 L_3) was performed on day 0. Goats were sacrificed on days 21-23 post-infection and worm counts performed.

Results: Geometric means for *H. contortus* and *T. circumcincta* in the control group were higher than corresponding values in other groups ($P<0.0001$). Efficacy values for groups treated 29, 22 and 15 days before infection with *H. contortus* were 99.7, 100 and 100 per cent, respectively. Corresponding values for *T. circumcincta* were 94.9, 99.9 and 95.7 per cent. *T. colubriformis* counts in control and treatment groups were not different ($P>0.05$).

Conclusion: Moxidectin 0.1% oral drench at 0.2 mg/kg in goats provides a high degree of protection against re-infection with the abomasal nematodes *H. contortus* and *T. circumcincta* for at least 29 days. No useful effect was evident at 15 days post-treatment against the small intestinal species *T. colubriformis*.

c.7.55 EVALUATION OF THE PERSISTENT EFFICACY OF DORAMECTIN AND IVERMECTIN INJECTABLE AGAINST *OSTERTAGIA OSTERTAGI* AND *COOPERIA ONCOPHORA* IN CATTLE.

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The persistent efficacy of doramectin and ivermectin injectable against a moderate and high infection level of *Ostertagia ostertagi* and *Cooperia oncophora* was evaluated. Helminth free calves were allocated to 6 groups of six animals. On day 0, animals of groups I1/I2 and D1/D2 were treated with 0.2 mg/kg ivermectin and doramectin injectable, respectively, while the animals of group C1/C2 remained untreated and served as controls. Animals of the C1, I1 and D1 groups received a daily infection of 1,000 L3 of *O. ostertagi* and *C. oncophora*, and animals of the C2, I2 and D2 groups received a daily infection of 10,000 L3 of each species. The animals were infected for 21 days with both species, the infections with *C. oncophora* and *O. ostertagi* started from day 8 and 15 post treatment, respectively. Animals were necropsied on day 40. The calculation of the persistent activity was based on the efficacy against the different developmental and adult stages of both parasites. The present study confirmed that infection levels can affect the duration of persistent efficacy of an anthelmintic. Doramectin had at the moderate infection level a persistent efficacy of at least 35 days against *O. ostertagi* and at least 28 days against *C. oncophora*; at the high infection dose persistent efficacy was somewhat shorter i.e. at least 33 days and approximately 28 days, respectively. The duration of persistent efficacy of ivermectin against *O. ostertagi* infection was at the moderate infection level less than 25 days (but more than 14 days), at the high dose level up to 25 days. Persistent efficacy of ivermectin against *Cooperia* could, at both infection doses, not be observed in the present experimental conditions.

c.7.56 THE EFFICACY OF LG IVERMECTIN FOR PIG PARASITES IN KOREA

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Background: The pig industry is very important, and some parasites are important for that in Korea. LG Chemical Ltd. synthesized ivermectin, and we investigated its efficacy against *Ascaris suum*, *Trichuris suis* and ectoparasites. To evaluate the efficacy of LG-ivermectin on piglets, growing pigs and sows, a serial experiment was carried out in field pig farms and animal houses.

Methods: We investigated the efficacy of LG-ivermectin on pig parasites in the field trials and artificially infected pigs.

Results: In the artificial infection with *A. suum* and *T. suis*, egg production rates were 0% in treated groups compared to 80-100% and 0-60%, respectively, in control groups at 2 weeks after infection. At 5 weeks after infection, there were no parasites in the intestinal lumen of treated pigs, but 100% parasites in control pigs. Field trials: egg reduction rates were 100% in treated pigs, but no egg reduction in control pigs. Egg production and excretion of adult worms in pigs treated with LG-ivermectin were faster than in pigs treated with another ivermectin. Ectoparasites of piglets: bodyweight gains were 6.5-7.4 kg in groups treated with LG-ivermectin and 6.2 kg in the group treated with another ivermectin, but 5.9 kg in the control group. Lesion scores of LG-ivermectin treated groups were 0.07-0.36, and 4.20 in the control group.

Conclusion: The efficacy of LG-ivermectin was more rapid and better than that of another ivermectin. LG-ivermectin had no side effects. If LG-ivermectin is supplied to domestic pig farms, endo- and ectoparasites will be controlled rapidly and effectively. Thus, the income of pig farms and the competitive potentials on the international market will be rapidly increased.

c.7.57 PRODUCTION AND PARASITES OF SHEEP IN ALTERNATE GRAZING WITH CATTLE IN THE CARIBBEAN

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Background: It has been shown that mixed sheep and cattle grazing decreases infection rate of sheep by gastro-intestinal nematodes (GIN) and also promotes sheep and cattle production in Caribbean. However, no data exist on alternate grazing in the humid tropics. Therefore, alternate grazing of «Martinik» sheep with Brahman cattle in intensively and rotational managed irrigated *Digitaria decumbens* pastures was studied during 2 years in Martinique (F.W.I.).

Methods: Performances and internal parasitism of two flocks of hair sheep conducted in 3 matings in 2 years were compared: ewes grazing alone (GA: stocking rate 579 kg metabolic body weight (MBW)/ha ; rotation: 4 weeks (w) out, 1 w in) *vs.* ewes alternatively grazing with heifers (AGH: 631 kg MBW/ha ; rotation: 3 w out, 1 w heifers in, 3 w out, 1 w ewes in).

Results: Annual production was 1616 and 1241 kg weaned lambs /y/ha, for AGH and GA ewes. Milk production was 1531 and 1313 g/d ($P<0.01$) for AGH and GA ewes, respectively. Ewes and lambs were mainly infected with *Haemonchus contortus* in the 3 lambing seasons but *Cooperia* sp. were more frequently recorded in fecal culture and worm population of AGH than of GA lambs ($P<0.05$). Alternate grazing markedly decreased Fecal Egg Counts and enhanced Packed Cell Volume ($P<0.01$) in lambs until weaning and in pregnant and lactating ewes, comparing with grazing alone animals.

Conclusion: Alternate grazing might be a reliable method to control GIN in sheep in the humid tropics.

c.7.57-63 Integrated and biological parasite control

c.7.58 SIGNIFICANCE OF ENDOPARASITC INFECTIONS IN SUCKLING CALVES IN SWITZERLAND

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A follow-up study was carried out in 20 cow/calf husbandries in Switzerland. 1355 faecal samples from 369 calves were examined qualitatively and quantitatively for the presence of parasitic stages. The sampling period of the calves extended from the birth to the age of ten months (slaughter). The cumulative incidences of endoparasitic infections were as follows:
Protozoa: *Cryptosporidium parvum* 56%; *Eimeria bovis* 92%; *E. ellipsoidalis* 79%; *E. alabamensis* 73%; *E. zuernii* 69%; *E. auburnensis* 67%; *E. cylindrica* 42%; *E. bukidnonensis* 24%; *E. subspherica* 22%; *E. brasiliensis* 18%.
Helminths: Trichostrongyles 94%; *Trichuris sp.* 44%; *Nematodirus sp.* 32%; *Strongyloides papillosus* 21%; *Bunostomum phlebotomum* 18%; *Moniezia sp.* 18%; *Dictyocaulus viviparus* 8%; *Capillaria bovis* 7%; *Dicrocoelium dendriticum* 2%. During the stabling period relatively high shedding of *Eimeria* oocysts was observed, whereas only few calves shed high numbers of *Cryptosporidium* oocysts. No clinical disease related to parasitism was observed during the entire study. The husbandry conditions of cow/calf herds especially during the stabling period must be regarded as favourable concerning development and transmission of *coccidia, Strongyloides, Trichuris* and *Bunostomum*. The intensity of trichostrongyle infections was very low during the entire grazing period in nearly all farms, which is mainly due to the low stocking rate with susceptible animals, the low egg output of the cows and the milk intake of the calves. Anthelmintic prophylaxis against trichostrongyles did not induce better liveweight development compared with untreated calves at a stocking rate below 1.3 livestock units per hectar. Due to the low intensity of endoparasitic infections during the grazing period cow/calf husbandry systems are beneficial from the parasitological view.

c.7.59 BIONOMICS OF *ASCARIS SUUM* AND *TRICHURIS SUIS* EGGS ON PASTURES

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Background: To improve pasture management in the growing number of outdoor swine herds in western Europe, it is important to know the developmental and survival rates of *Ascaris suum* and *Trichuris suis* eggs on pastures.
Method: Pig faeces, containing known numbers of *A.suum* and *T.suis* eggs, were deposited on a Danish pasture on 4 occassions: spring, summer, autumn and winter. Faeces were placed either in short grass or 2 cm below the surface of well cultivated bare soil, imitating pastures that were either grazed or rooted by pigs. The number and developmental stage of the eggs were recorded in faeces and soil samples for up to 50 weeks post deposition.
Results: Embryonation took place only during the summer months and was seemingly independent of the microclimate. While *A.suum* eggs developed within 1-2 summer months, the majority of *T.suis* eggs did not develop until the following summer. Most eggs of both species disappeared before becoming fully embryonated. They also disappeared significantly faster in the summer than in the winter and when deposited in short grass versus buried in soil (less exposed).
Conclusion: The fast disappearance rates are in striking contrast with the general belief that eggs of these two helminths survive for years regardless of environmental conditions and suggest that pasture rotation may have a controlling effect.
Acknowledgement: The study was supported by The Danish Veterinary and Agricultural Research Council and The Danish National Research Foundation.

c.7.60 STRATEGIC ANTHELMINTIC TREATMENTS OF DAIRY CALVES IN FLORESTAL COUNTY, MINAS GERAIS STATE, BRAZIL.

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Epidemiological studies of gastrointestinal helminthiasis were previously performed in dairy cattle in Florestal County Minas Gerais-Brazil. Based on that data, 80 swiss and crossbreed zebu x holstein 8-10 months old calves were selected for testing the efficacy of three different protocols of Ivermectin treatment for gastrointestinal helminth control. The cattle was raised grazed in *Brachiaria* grass paddocks and were naturally infected with *Haemonchus, Cooperia, Oesophagostomum, Trichostrongylus and Bunostomum* species . The calves were divided in 4 groups of 20 animals each: Group 1-Treated with 200µg/kgbw in April(end of rainy season) and October(beginning of rainy season); Group 2-Treated in April, August(middle of dry season) and October; Group 3-Treated in April, August, October and December(middle of rainy season) and Group 4-control. The treatments were effective in eliminating the worm burden only in Groups 2 and 3 ($P>0.05$) . However, after each treatment with Ivermectin the calves remained eliminating *Cooperia* eggs.

c.7.61 THE ESTABLISHMENT OF *Ostertagia circumcincta* AND *Trichostrongylus colubriformis* IN LAMBS FED LUCERNE OR SULLA

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Background: Previous grazing studies have shown that lambs which grazed sulla (*Hedysarum coronarium*) had lower faecal egg counts and adult worm burdens than those which grazed lucerne (*Medicago sativa*). This study aimed to determine whether this effect was due to direct plant activity or mediated through the immune response of lambs.
Method: Lambs were trickle dosed with 15000 *O. circumcincta* and *T. colubriformis* larvae weekly for 5 weeks, drenched and challenged with 15000 *O. circumcincta* and *T. colubriformis* two weeks later. A subgroup in each herbage treatment was either treated with anabolic steroids prior to the final larval challenge or was not trickle dosed for 5 weeks (control).
Results: Control lambs fed sulla had lower *O. circumcincta* burdens than those fed lucerne. Steroid treated lambs fed sulla had higher *T. colubriformis* burden than those fed lucerne while antibody titre to *T. colubriformis* larvae and numbers of L_4 larvae was higher from lambs fed sulla than lucerne.
Conclusion: This study suggests that the effects of sulla in reducing faecal egg count and adult worm burdens is primarily due to an enhanced immune response rather than a direct plant effect.
Acknowledgements: This study was funded by the New Zealand Foundation of Research, Science, and Technology (FRST).

c.7.57-63 Integrated and biological parasite control
c.7.64-75 Biological parasite control using nematophagous fungi

c.7.62 INTEGRATED CONTROL OF NEMATODES OF SHEEP UNDER RUBBER PLANTATIONS IN INDONESIA

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Gastro-intestinal nematodes dominated by *Haemonchus contortus* and *Trichostrongylus* spp. are a major constraint in sheep production in humid tropics. Sole reliance on intensive chemotherapy is neither practical nor sustainable. An integrated approach incorporating pasture management, minimal anthelmintic treatment and breeding of sheep with higher genetic resistance was evaluated in rubber plantations in North Sumatra.
Epidemiological studies through regular monitoring of sheep on pasture and tracer lambs showed that very few larvae survive on pasture over 12 weeks, there is no well demarcated seasonal pattern of infection and sheep attain peak counts of upto 2000 eggs/g of faeces by 3rd month following anthelmintic treatment. Therefore, pastures were spelled for 3 months. All grazing animals were treated with anthelmintic once every 3 months and then moved to clean spelled pastures. As the local Sumatra wool sheep is small in size, two hair sheep breeds - St.Croix and Barbados Blackbelly - have been used to produce a synthetic breed to incorporate the high prolificity of Sumatra and higher body weight and higher resistance to nematodes of two hair sheep breeds. Dams productivity index of synthetic crossbreds is 35-51% higher than that of the local breeds. No anthelmintic resistance has developed. It is concluded that three-monthly anthelmintic treatment followed by movement to clean pastures and use of better and resistant hair sheep for crossbreeding provide optimal conditions for sheep production under rubber plantations in the humid tropics of Southeast Asia.

c.7.63 THE EFFECT OF IRON SUPLEMENT ON THE PARASITIC BURDEN AND GROWTH OF MUSCLE LARVAE IN MICE INFECTED WITH *Trichinella spiralis*

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Background: Previous studies have shown that iron is important for the survival of *Trichinella spiralis* muscle larvae *in vitro* and the pathogenesis of trichinellosis in swine. The objective of the present study was to determine whether iron supplement in the host increases establishment and growth of *T. spiralis* muscle larvae.
Method: Sixteen mice received Ringer's solution as placebo and were infected either with 100, 200, or 400 *T. spiralis* larvae. Another seventeen mice received iron supplement and were inoculated with similar doses. Thirty days after inoculation muscle larvae intensity (lpg) was measured for each mouse. The growth of larvae was assessed by measuring the width, length, perimeter and surface area of individual larvae by using an image analysis system.
Results: The intensity of infection was lower in the low infection dose than the other two levels of infection doses ($p = 0.05$). The iron supplement had positive effect on establishment of muscle larvae ($p=0.03$). The difference in lpg between iron supplemented and control mice was highest in mice inoculated with 100 larvae, less expressed in mice inoculated with 200 larvae, and not significant in the mice inoculated with 400 larvae. Larvae from mice given iron supplement were wider than larvae in mice not given iron ($p=0.002$).
Conclusion: The present observations show that iron plays a role in the establishment and growth of trichinous larvae in the muscle tissues of the host.

79
=2370

c.7.64 PERSPECTIVES OF APPLICATION OF THE BIOLOGICAL CONTROL OF STRONGYLIDS OF HORSES IN LARGE HORSE FARMS IN UKRAINE AND RUSSIA

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In the former Soviet Union studying of predator fungi for creation of method of biological control of strongylids of horses was start by Soprunov (1958) and continued by Priadko and others. (1972, 1980). Cultures of predator fungi with high activity against nematodes was distinguished. However, its were not used because of using of high-effective benzimidazoles.
Resistant to anthelmintics races of strongylids of horses appeared in Ukraine (Borgsteede et al., Veterin. Parasitol., 1997, 68, 113-117). So, because of it and taking into consideration exotoxicity of avermectines and proceeding from the conception of saving biodiversity we worked out the technique of extraction and cultivation of predator fungi, local populations were founded, their activity against nematodes was tested. On the large horse farms the most important actions for control of nematodoses are: monitoring of pastures, change of paddocks and using of high active predator micro fungi. It allows to decrease using of avermectines.

c.7.65 EFFECT OF *DUDDINGTONIA FLAGRANS* AGAINST *OSTERTAGIA OSTERTAGI* IN GRAZING CATTLE

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Background: The nematode-trapping fungus *Duddingtonia flagrans* has been shown to reduce the number of *Ostertagia ostertagi* L_3 on herbage in plot trials. Two field trials were conducted to test this under grazing conditions with a high and low risk of parasitic exposure, respectively.
Method: In each of the two trials, thirty calves, experimentally infected with *O. ostertagi*, were divided into three comparable groups and allocated to three similar paddocks. Two of the three groups received fungal material (high or low dose) once per day during the initial two months. The third group remained as an untreated control group. Faecal, blood, and herbage samples were collected and animals were weighed every month from May to September.
Results: Under the high risk management, the number of L_3 on pasture was reduced and calves were protected from clinical disease using *D. flagrans* at the high dose-level. In the low risk situation, the parasitism of the animals seemed not to be severe, and no conclusive effect of the fungus could be detected even at the high dose-level.
Conclusion: The present study showed that different factors affect the efficacy of the applied biological control agent, such as stocking rate and number of overwintering infective larvae. Future studies should be conducted to elucidate the optimum dose-level of the fungal material to be used under different grazing conditions.

c.7.64-75 Biological parasite control using nematophagous fungi

c.7.66 EFFICACY OF *DUDDINGTONIA FLAGRANS* AGAINST TRICHOSTRONGYLIDS IARVAE IN GOATS

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A number of performed studies have shown that the nematophagous fungus *Duddingtonia flagrans* can be an effective biological agent against free living stages of trichostrongylids in cattle and sheep.
The experiment was conducted for testing the ability of *D.flagrans* to reduce the number of infective larvae of trichostrongylids in the faecal cultures after feeding goats with fungal spores. 8 adult goats naturally infected with trichostrongylids were used. During 5 consecutive days they were fed with four doses of *D. flagrans* spores: $5x10^4$, 10^5, $2.5x10^5$ and $5x10^5$ per 1 kg of body weight. Each goat was served as its own control.
The larval reduction capacity of the fungus was evaluated by faecal cultures set up untill tenth day after feeding. Significant differences were found between the number of fungal spores and the level of reduction caused by fungus, with 98.8% efficacy of the highest spore dose. Good reduction capability was observed untill second day after fungal spores feeding.
An important observation is positive correlation between number of eggs in faeces and fungus efficacy.
The study has shown the nematode-trapping fungus *D.flagrans* is highly effective against free living stages of nematodes in goats.

c.7.67 THE POSSIBLE IMPACT OF THE NEMATODE-TRAPPING FUNGUS *DUDDINGTONIA FLAGRANS* ON SURVIVAL AND GROWTH OF THE EARTHWORM *APORRECTODEA LONGA*

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The nematode-trapping fungus Duddingtonia flagrans may be used in biological control of parasitic nematode larvae in faeces of various domestic animals after feeding the hosts with fungal chlamydospores. In this experiment the potential fungal impact on earthworms, of the species Aporrectodea longa, was investigated since any adverse effect would be undesirable. As earthworms ingest animal faeces, D. flagrans may come into contact with both the earthworm alimentary tracts and their body surfaces. However during a study period of 20 days, when earthworms were living in soil and eating cattle faeces that were experimentally inoculated with high numbers of viable chlamydospores of D. flagrans, and also afterwards, there were no indications of internal or external mycosis, or any detrimental effects of the earthworms.

c.7.68 SELECTION OF POTENTIAL EGG-PARASITIC FUNGI AGAINST EGGS OF *NEMATODIRUS* SPP. - AN IN VITRO STUDY.

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Background: A variety of micro fungi are known to infect eggs of plant-parasitic cyst-nematodes, but no detailed information exist with regard to possible fungal antagonist of long lasting egg stages of animal parasitic nematodes. Infective larvae of *Nematodirus fillicolis* and *N. battus* develop inside the egg, which remain in the environment for an extended period. This experiment was set up to test the infective capacity of a range of micro fungi against eggs of primarily *N. fillicolis*.
Method: Three isolates of *Verticillium suchlasporium*, 2 *V. chlamydosporium*, 1 *V. leptobactrum*, 2 *Paecilomyces lilacinus* and 1 *Gliocladium roseum* were each inoculated onto water agar with antibiotic in 9 cm petri dishes. When the surface was covered by mycelium, 150-200 unembryonated eggs of *Nematodirus* spp. were added to the plates, which were then sealed and incubated at room temperature. Plates were inspected weekly.
Results: After 3 weeks distinct differences could be observed with respect to infection of the eggs. One isolate of *V. chlamydosporium* and *G. roseum* were unable to penetrate the eggs. Two *V. suchlasporium* isolates and one *V. chlamysdosporium* isolate had infected >75% of all the eggs while the *P. lilacinus* isolates infected approx. 50% of eggs. The last *V. suchlasporium* isolate and *V. leptobactrum* only infected a small proportion of eggs, approx. 25%.
Conclusion: There are marked differences both between and within fungal species with respect to ability to infect *Nematodirus* eggs when tested in vitro. Further testing on agar as well as in faecal cultures should be undertaken.

c.7.69 NEMATOPHAGOUS ACTIVITY OF PREDACIOUS FUNGI *DUDDINGTONIA FLAGRANS* AND *ARTHROBOTRYS OLIGOSPORA* IN THE FLUID PREPARATION

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In the investigations on the predacious fungi as agents of biological control of zooparasitis nematodes the dry fungal preparations were mainly used. The aims of our investigation were to estimate the viability of the fluid preparations of predacious fungi *Duddingtonia flagrans* and *Arthrobotrys oligospora* after feeding them to horses and to examine the nematode-trapping activity in fecal cultures *in vitro* against strongylid infections larvae.
Fluid preparations of two strains of *Duddingtonia flagrans* and one strain of *Arthrobotrys oligospora* were used in experiment. They were obtained by the deep cultivation method. Each of three horses naturally infected by strongylid nematodes were fed on 700 ml of the preparation mixed with chopped corn. Daily the fecal samples were collected from each horse and then cultivated in the thermostat during 22 days. The estimation of decrease of number of strongylid infective larvae was carried out on the 6th, 10th, 14th, 18th and 22nd days of culturing. Fecal samples collected from the same horses on a day before experiment were used as the control.
The predacious fungi have been revealed in fecal cultures *in vitro* on the 2nd day of experiment. The nematophagous activity against infective larvae of horse strongylids in fecal cultures was maximum on the 4th-6th day. On the 14 day of cultivation up to 86-87% of larvae were eliminated by the fungi comparing with 13-29% of natural larval mortality in the control cultures.
The results obtained confirm the ability of fluid preparations of predacious fungi *Duddingtonia flagrans* and *Arthrobotrys oligospora* to save viability and nematophagous efficiency after passage through the digestive tract of horses. These preparations thus may be recommend for industrial use for biological control of zooparasitic nematodes.

c.7.64-75 Biological parasite control using nematophagous fungi

c.7.70 THE DEMONSTRATION OF NEMATOPHAGOUS ACTIVITY OF DRIED *DUDDINGTONIA FLAGRANS* PREPARATION AFTER ITS PROLONGED STORAGE

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In the studies on the schemes of predacious fungi usage for biological control of zooparasitic nematodes the fungal preparations were used immediately after growing, not after prolonged storage. The objective of our investigation was to reveal the ability of the predacious fungus *Duddingtonia flagrans* to retain the nematode-trapping activity in fecal cultures *in vitro* after the prolonged storage of dry grain preparation.

Dry preparation of two strains of *Duddingtonia flagrans* was used in experiment. It has been grown on barley grains and was stored during 6 months at room temperature and humidity. Two horses naturally infected by strongylid nematodes were fed on 500 g of the grain preparation. Daily the fecal samples were collected from each horse, the number of eggs was counted and the samples were cultivated in the thermostat during two weeks. The estimation of the decrease of the number of strongylid infective larvae was carried out on the 7th and 14th days of culturing. Fecal samples collected from the same horses on a day before experiment were used as the control.

The predacious fungus was revealed in fecal cultures in vitro on the 2nd day of experiment. It has demonstrated high nematophagous activity against infective larvae of horse strongylids: up to 98.6% of larvae eliminated on the 7th day and 99.9% - on the 14th day of cultivation (comparing with 20.5 and 25.5% of died larvae in control cultures respectively).

The results obtained confirm the ability the dry barley grain preparation of predacious fungus Duddingtonia flagrans to retain its nematophagoous efficacy after prolonged storage (during at least 6 months) and thus it may be recommend for industrial usage for biological control of zooparasitic nematodes.

c.7.71 A FIELD TRIAL WITH *Duddingtonia flagrans* TO CONTROL STRONGYLE INFECTIONS IN FOALS IN PORTUGAL*

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Background: A field trial was performed to evaluate the capacity of the nematode trapping fungus *Duddingtonia flagrans* to control strongyle infection in a group of yearlings on a horse farm near Lisbon.

Method: The research was carried out since November 1997 until March 1998 and it involved 2 groups of 9 male horses with mixed infections of strongyles maintained on 2 similar size pastures. All horses were dewormed with pyrantel pamoate, at the beginning of the trial. One of the groups (D) received a daily dose of *D. flagrans* ($5X10^5$spores/Kg body weight) mixed with a feed supplement, while the other (C) received a similar amount of supplement without fungus. During the trial period, faecal and pasture samples were collected every fortnight and EPG and the number of L3/kg dry herbage determined. In addition, faecal cultures were made to assess larval mortality in the faeces. Blood samples were collected at the beginning, in the middle and at the end of the research.

Results: The number of larvae in cultures from group D was significantely reduced compared to numbers in faeces from the control group (ratio N°L3 C/D: min.0 and max.17) and the herbage infectivity of the pasture used by the horses fed fungi was reduced to zero at the end of the trial. EPG counts were very similar at the start of the trial, but in the last 2 months of the work the group D had lower EPG counts than group C. Blood samples from the group D more closely followed the normal values than samples from group C.

Conclusion: *D. flagrans* is effective on reducing the number of infective larvae of horse strongyle transmited to herbage in a Mediterranean ecossystem. Research will be continued during other periods to obtain a better knowledge of *D. flagrans* ecology in the field.

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c.7.72 SURVIVAL OF *DUDDINGTONIA FLAGRANS* AFTER *IN VIVO* AND *IN VITRO* PASSAGE

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Background: Passage of *D. flagrans* spores through the alimentary canal of animals is considered to be detrimental to spore survival, but this effect has never been quantified. The effect of rumen fluid on chlamydospore spore germination was first studied *in vitro* and the studies extended to assessing the survival of spores after passage through calves *(in vivo)*.

Method: The *in vitro* experiments were carried out using chlamydospores produced on both SDA and millet. Chlamydospores were incubated in bovine ruminal fluid and samples taken periodically. These samples were incubated on PDA for 24 hrs. at 26°C and germination determined. In the *in vivo* experiments animals were fed a single dose of chlamydospores and faeces collected during the following 48 hours. Chlamydospores were extracted from the faeces and germination determined.

Results: Incubation in ruminal fluid for 24 hours reduced spore germination from 92% to 3%. Spores harvested from millet survived longer and 48 hrs. incubation was necessary to markedly reduce spore germination. In calves, spore germination of chlamydospores extracted from faeces, 42 hours after ingestion, had fallen from 35% to 6%.

Conclusion: The results clearly indicate that many spores fail to germinate after incubation in ruminal fluid or after passage through animals. It is suggested the isolation of more resilient fungal isolates could result in improved efficacy of nematode trapping fungi for nematode control in livestock.

c.7.73 BIOLOGICAL CONTROL OF PRE-PARASITIC STAGES OF HAEMONCHUS CONTORTUS IN SHEEP WITH DUDDINGTONIA FLAGRANS

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Background : This experiment is designed to determine whether chlamydospores of Indian isolate of Duddingtonia flagrans can survive gut passage of sheep, climatic conditions on pasture and can reduce the number of infective larvae of Haemonchus contortus in faeces and on pasture.

Method : Chlamydospores of D. flagrans were fed to a group of H. contortus infected sheep (n=5), @ 1.0 million spores twice daily for 30 days. Individual faecal egg counts, faecal culture, pasture larval burden of the plot grazed by these sheep were enumerated and compared with those of infected fungus unfed controls. Nematode establishment was measured by tracer lamb technique.

Results : The fungus could be isolated from the faeces of sheep following 36 hours of feeding of chlamydospores. There was significant reduction ($p<0.001$) in the larval harvest of spore-fed sheep compared to controls and the pasture larval burden per kg dry matter was significantly decreased ($p<0.01$) in fungal contaminated plot. Adult worms recovered from the abomasum of tracer lambs were significant lower compared to controls.

Conclusion : This experiment shows that D. flagrans is able to survive passage through sheep gut and reduce the number of infective larvae on pasture.

c.7.64-75 Biological parasite control using nematophagous fungi
c.7.76-92 Basic parasite biology

c.7.74 THE NEMATOPHAGOUS FUNGI IN FRESH FAECES OF LIVESTOCK IN THAILAND

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Nematophagous fungi are considered to be the useful candidates for biological control agents of nematode parasites of livestock. During 3 months of investigation, 65 fresh faecal samples from grazing livestock, cattle, sheep and goats from various provinces of Thailand were collected and examined using faecal cultures on water agar plate for the presence of nematophagous fungi. Twelve isolates of endoparasitic fungi, *Harposporium anguillulae*, and 3 isolates of nematode-trapping fungi, *Arthrobotrys* spp., were obtained from goat and sheep faecal specimens, respectively. The discovery of the gut-resistant and local isolated nematophagous fungi are benefit for studies and development of the biological control of nematode parasites in the livestock of Thailand.

c.7.75 DUNG COLONISATION BY NEMATOPHAGOUS FUNGI

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Background: Sheep dung on pasture is colonised by nematophagous fungi (NF) which can kill larvae of gastro-intestinal nematode parasites thus reducing populations on herbage. Our study reports on (a) the identity and rate of colonisation of dung by NF, (b) the role of soil-living nematodes in transporting NF into dung, (c), the nematode-killing ability of dung NF.
Method: (a) Samples of sheep dung, placed on pasture plots in summer (February) and autumn (April), were retrieved at intervals for isolation and identification of NF. (b) Colonisation of dung from soil inoculated with individual NF was compared in laboratory trials using filters to differentially exclude nematodes and NF from dung. (c) Numbers of larvae migrating from dung containing eggs of *Trichostrongylus colubriformis*, were compared following incubation with/without NF isolates from dung.
Results: (a) *Monacrosporium haptotylum*, several *Harposporium* spp. and a *Nematoctonus* sp. were the most common NF. Invasion was rapid; only 3 days after dung deposition in February and April, NF were found in 35% and 54% of samples respectively. (b) Presence of a nematode–excluding filter significantly reduced colonisation of dung by the endoparasite *H. helicoides* but not trapping species of *Arthrobotrys* and *Monoacrosporium*, suggesting that colonisation of dung by endoparasites is assisted by soil-living nematodes (c) Many isolates of *Harposporium*, *Monoacrosporium*, and *Arthrobotrys* spp. reduced numbers of *T. colubriformis* larvae by >50%.
Conclusion: Enhancement of colonisation of dung by NF naturally present in pasture soil should be considered further in relation to biological and managerial control of nematode parasites of livestock.

c.7.76 INFECTION OF LAMBS WITH TWO ISOLATES OF *HAEMONCHUS CONTORTUS* FROM THE CARIBBEAN

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Background: Between strains differences in biology of gastro-intestinal nematodes are rarely experimentally estimated. These experiments require cryopreservation, and thus exsheathment of infective larvae (L3). Therefore, the objective of this study was to assess the effects of exsheathment and cryopreservation on the compared development in lambs of two isolates of *Haemonchus contortus* from Guadeloupe (GUA) and Cuba (CU).
Methods: The stocks of L3 were prepared by *in vitro* hatching and development of eggs collected from immuno-suppressed infected lambs by isolate larvae from female worms. Exsheathment was performed by checking L3 during 4 hours in Earle buffer with CO2 bulling and rinsed in distilled water. Cryopreservation of exsheathed L3 during 1 week, was carried out at a freezing rate of 1°C/min during 2 hours prior plunging into liquid nitrogen. 44 6-months Black Belly naive rams were allocated in a 2 x 3 experimental design : (a) lambs infected with L3 from Guadeloupe *vs.* from Cuba ; (b) lambs infected with 5000 L3 of fresh L3 *vs.* 5000 freshly exsheasthed L3 *vs.* 5000 thawed surviving L3.
Results: The exsheathment rate was 91 and 90 % for GUA and CU, respectively. The geometric means of establishment of living L3 and the development rate in adult did not differ between treatment : 11.7, 13.5 and 9.1 %, respectively for fresh, exsheathed and cryogenized L3. The establishment of CU was slightly but not significantly higher than GUA: 13.0 *vs.* 9.4. No interaction between treatment and isolates was recorded.
Conclusion: Cryopreservation and exsheathment can be used for comparison of infection of lambs with different *Haemonchus contortus* isolates.

c.7.77 EFFECTS OF LARVAL CUTURE ON INFECTION OF LAMBS WITH *HAEMONCHUS CONTORTUS*

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Background: Fecal culture is the most commonly method used to prepare infective larvae (L3) of gastro-intestinal nematodes prior experimental infection. However, in the humid tropics, the development rate of eggs in L3 in fecal culture is highly variable. Furthermore, no data exist on the effect of this variability on the development rate of *Haemonchus contortus* in lambs. Therefore, the objective of this study was to study the effects of fecal or *in vitro* L3 culture on the development of *Haemonchus contortus* in lambs.
Methods: The stock of L3 were prepared either by fecal culture during 10 d and Baermann extraction or by *in vitro* culture as followed: extraction of eggs from fecal material by filtration and centrifugation in dense solution, culture in sterile Earle medium (pH 7.2) during 10 d. 40 6-months Black Belly naive rams were allocated in a 2 x 2 experimental design plus a control group: (a) lambs infected with L3 prepared from fecal culture *vs.* from *in vitro* culture ; (b) lambs infected with 10500 L3 *vs.* 2300 L3/d during 14 d.
Results: Fecal egg counts (FEC), packed cell volume (PCV), circulating eosinophil response to infection (EOS), establishment of worms (EW) and adult proportion (AP) were not significantly affected by the type of L3 culture. In contrast, these parameters were significantly affected by the type of infection, trickle infected lambs having lower FEC, higher PCV, higher EOS and a lower AP comparing to single infected lambs.
Conclusion: *In vitro* culture can be used for experimental infection of lambs with *Haemonchus contortus* but a great attention must be paid to the daily rate of infection rather than the total dose of L3.

c.7.76-92 Basic parasite biology

c.7.78 *IN VITRO* EXCYSTATION OF *Eimeria tenella* WITH RUMINANT BILES FROM SLAUGHTER HOUSE

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Background: This research used 3 kinds of ruminant biles (sheep, goat and buffalo) obtained from slaughter house for *in vitro* excystation of *E. tenella* to produce sporozoites.

Method : Excystation of oocysts proceeded in 2 distinct steps. The first step consisting of the mechanical rupture of cell wall by using glass beads to release the sporozoites from oocysts. The second step is to release the sporozoit from the sporocyst under the combined action of trypsin (0.125 % and 0.250 %) and biles (sheep, goat and buffalo) in shaker water bath at 41° C (time on incubation were 15, 30, 45, 60, 75 and 90 minutes). Every kinds of biles had 4 concentrations: 6.25 %, 1.25 %, 25 % and 50 % (v/v).

Results : ***In vitro*** excystation on *E. tenella* produced the highest percentage of sporozoites : 78.26 %, 74.25 % and 66.44 % by using trypsin 0.25 % and ruminant biles : sheep, goat and buffalo 50 %, respectively, after incubation for 90 minutes.

Conclusion : Ruminant biles obtained from slaughter house can be used for *in vitro* excystation of *Eimeria tenella* to produce sporozoites.

c.7.79 MODELLING CHANGES IN THE AGGREGATION OF WORMS OVER THE HOST POPULATION : *ASCARIS SUUM* - PIG INTERACTIONS

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Background: The distribution of macroparasites over the host population is typically highly aggregated and empirically best described by the negative binomial distribution. The degree of aggregation is represented by the inverse of the negative binomial parameter k.

Method: Maximum-likelihood methods were used to obtain an estimate of k for several distributions of *Ascaris suum*. The changes in aggregation across different exposures to *Ascaris* were analysed.

Results: The distribution of worms across piglets was less aggregated in those born to parasite-exposed sows than those born to parasite-naive sows. Past experience of infection in pigs on pasture also corresponded to a significant reduction in aggregation. Moreover, continuous infections were considerably less aggregated than single experimental infections.

Conclusion: An increase in exposure to *Ascaris suum* may result in a less aggregated distribution of worms across the host population.

Acknowledgement: We wish to thank researchers at DCEP for allowing their data to be used in the models. The Danish National Research Foundation is acknowledged for financial support.

c.7.80 Pathogenesis of bovine otoacariasis by *Raillietia* (Acari:Gamasida)

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Background: The bovine ear mite *Raillietia flechtmanni* (Faccini, Leite & Costa, 1992) has been described only in Brazil. *R. flechtmanni* and *R. auris* are the most important cause of otites in cattle in our country and, probably, elsewhere. The correct identification of the species is essential, considering that both parasite the same host.

Method: In this work, female of *R. flechtmanni* was collected from ear canal of cattle, fixed in glutaraldeyde 2,5%, pH 7.2, 0,1M, processed and prepared for scanning electron microscopy (S.E.M.) according to Ferry et al. 1994 (Rev. Bras. Parasitol. Vet. 3:65-68). Additionally, external auditory meatus from naturally infected bovine was collected, fixed in formaldheyde 10%, sectioned about 5 µm and stained with hematoxilin-eosin.

Results and conclusion: The histological sections of the external meatus of the hosts and S.E.M studies of *Raillietia* mites show two important piercing structures: the tarsi of the legs (that has two nails, each like a hook) and the chelicerae (that has two horn-like structures distally in its body: the fixed digit and movable digit, that makes contact like a tweezer); both contribute to make damage in the external ear canal of the hosts, compressing, cutting and penetrating the epithelia. The action of the normal bacterial flora follow up to a suppurative inflammatory process.

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c.7.81 THE MORPHOGENESIS OF *ASCARIS SUUM* TO THE INFECTIVE THIRD-STAGE LARVAE WITHIN THE EGG

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Background: According to most textbooks in parasitology it is generally accepted that the infective stage of *Ascaris suum* that develops within the egg is the second stage larvae. In spite of this, severel authors have reported that two molts occur within the egg.

Method: Development of *A. suum* eggs was followed in vitro by cultivating the eggs at 18-22 C in 0.1 N H_2SO_4. Artificial hatching and microscopical examination of the eggs were performed 3 times weekly. Every second week from week 4 to 12 the infectivity of the eggs was tested by mouse inoculation.

Results: The start of first molt occurred after 22 days of cultivation. After artificial hatching the larvae were fragile, deformed and died promptly. From day 27 and onwards the second molt started, and double loose cuticula could be demonstrated surrounding the larvae indicating a second molt. From day 38, all hatched larvae survived and were motile. Egg should be cultivated for 6 weeks or more to be infective for mice. Transmission electron microscopy clearly showed two loose larval sheaths surrounding the larvae within the egg after 31 days of cultivation.

Conclusion: The present study brings strong support to previous rather neglected investigations in that, it clearly documents that 2 molts occur in *A. suum* eggs, before the eggs are infective for mice. We therefore suggest that a final evidence has been provided to confirm, that the infective stage is the egg containing the third stage larva, opposite what is written in most textbooks in parasitology, where the second stage larva is claimed to be the infective one.

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c.7.76-92 Basic parasite biology

c.7.82 OCCURRENCE OF ORAL PROTOZOA OF THE GENUS *TRICHOMONAS* IN DOGS

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The aim of the study was to characterize the frequency of infection with canine trichomonas in animals and the trial of identification of protozoan species.

The investigations involved 81 dogs of various breeds aged 1 to16 years. Trichomonads were isolated from smears from the gums and periodontal areas of 21 dogs. All had clinical symptoms associated with the presence of tartar and gingivitis.

The trichomonads, studied in native preparations and smears from culture stained with Giemsa, had: oval, round or spindle shapes, a length of 6 – 10 μm, width 4 – 6 μm, 4 free flagella 8 – 12 μm long, a fifth flagellum linked with a long undulating membrane extending for more than half the length of the organism, an axostyle standing out beyond the cell for a length of 3 – 5 μm, a circular or oval nucleus and cytoplasm with granulation and absorbed bacteria.

The biological and morphological features of the canine trichomonads indicate membership of the protozoa to the family *Trichomonadidae* and the genus *Trichomonas*. Comparative morphological analysis under a light microscope revealed considerable similarity between the trichomonads obtained from dogs and *Trichomonas tenax* from humans. This species differs from earlier described *Tetratrichomonas canistomae* mainly with the lack of free posterior flagellum connected with undulating membrane, but the final identification of species will require additional research methods such as electron microscopy and molecular analysis.

c.7.83 CRYOPRESERVATION OF FIRST AND THIRD STAGE LARVAE OF *Haemonchus placei* AND *Cooperia punctata*

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Background: Cryopreservation of L_3 is a well established process for sheep nematodes, but little is known on cattle thrichostrongylids, especially from tropical climates. It also requires exsheathment, reducing larval infectivity. Preservation of L_1 may overcome this problem, however, these larvae are more sensitive to freezing. Data on cryopreservation of L_1 and L_3 of the most common cattle nematodes *H. placei* and *C. punctata*, and on infectivity of the cryopreserved L_3, are presented.
Method: Eggs obtained from monospecifically infected donor calves were separated by gradient centrifugation and incubated on 2% agar gels overnight at RT. Hatched larvae were frozen in DMSO in a polystyrene insert at -80°C for 48h, then transferred to liquid N_2. Thawing was performed on air or in water at RT or 37°C and live larvae were counted and incubated for 5d at 28°C, to assess viability. L_3 were exsheathed and frozen over liquid N_2 in saline. Thawing occurred at 37°C in water and live larvae were counted. One Holstein calf was injected with 50.000 cryopreserved L_3 of both *H. placei* and *C. punctata* into the abomasum and duodenum, respectively, and another calf was given 100.000 *H. placei* L_3 per os. Infection was monitored by egg counts and worm burden was assessed after necropsy of both calves.
Results: Survival of L_3 was high for both nematodes and infectivity of *H. placei* L_3 after surgical injection was comparable to normal larvae (13,76% of the initial dose). The *C. punctata* worm burden was lower (3,7% of the initial dose) than obtained with normal larvae, despite high egg counts. Infectivity of frozen L_3 by the oral route was low (0,03% of the initial dose), but eggs were detected and cultured, allowing for maintaining the strain. Survival of L_1 was low and did not allow for testing of infectivity.
Conclusions: L_1 seem to be very sensitive to the freezing period, so freezing rates should be carefully established. L_3 survived very well to freezing and retained their infectivity, however, oral infectivity may have been low due to calf age.
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c.7.84 PIG'S EAR AS A HIDDEN HABITAT FOR ECTOPARASITES

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A total of 1637 gilts of 8-11 weeks, originating from 214 breeding farms distributed in the area of 17 Regional Animal Breeding Offices (RABO) and tested at 4 Pig Testing Stations (PTS), were examined for the presence of ectoparasites during 1996-1997. The samples of skin scrapings from the inside part of the ear pinna together with earwax were collected for microscopical investigation twice: at the beginning of the control test and after 3 months of fattening, when pigs gained a 100 kg of body weight. The weaners were treated routinally with ivermectin on the 7th day following delivery to PTS.
In 15 out of 17 RABO'es the gilts were found to be infested with *Sarcoptes scabiei* var. *suis*, and in 9 RABO'es with *Haematopinus suis*. The mean incidence rate of inapparent sarcoptic mange infestation was 10,6%, ranging from 1,9% to 25%, whereas louse infestation ranged from 0,7% to 8,9%, with the mean of 3,4%. Usually, several mites or lice were detected and the intensity of infestation varied from 2 to 70 specimens or from 1 to 15, respectively.
During the fattening at PTS, a decrease in the occurrence of mites infestations to the level of 2,6-6,9% was observed, whilst for lice it was noticed either an increase to 3,1-17,6% or a reduction to 0,0-0,4%, depending on particular PTS. These results indicate that the common control regimen do not supress the ear-hidden ectoparasite infestation and that the regular treatment is less efficient in the case of lice. Studies on the occurence of mites conducted over 20 years ago in the area of Opole RABO (Bogatko W., Med. Wet. 1974, 30: 38-39) detected very similar incidence rate of hidden scabies infections. No such data are attainable regarding louse infestation.

c.7.85 COMPARISON OF EXSHEATHMENT AND CULTURE METHODS FOR TWO ABOMASAL NEMATODES

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Background: To provide materials for molecular studies from early developmental stages of the nematodes *Haemonchus contortus* and *Teladorsagia circumcincta*, infective larvae (L3) were cultured *in vitro* to early fourth stage (eL4). While much is known about exstheathment and culture of *H. contortus*, very little has been published about *T. circumcincta*.
Method: Storage of L3, as well as various exsheathment methods, axenisation solutions, culture media and techniques to obtain clean exsheathed larvae (xL3) were examined. Best procedures were adapted to large scale production of *H. contortus* and modified to produce eL4 of *T. circumcincta*.
Results: Optimal exsheathment with CO_2 gas was achieved in EBSS in a shaking waterbath at 39°C. With *H. contortus*, > 95% exsheathment occurred within 90 min of CO_2 stimulation with cultures stored at 10 °C for 2-8 weeks. For *T. circumcincta*, > 85% xL3 were obtained after 24 h with cultures stored at 4 °C for 10-20 weeks. Migration of xL3 over a series of sieves (37, 20 and 20 μm) was used to separate them from L2 cuticles and debris. Improved culture success was achieved when RPMI/PIPES (pH 6.6) with 20% sheep serum was used and incubation conditions increased to 40°C and 20% CO_2. Moulting commenced within 3 days and > 80% had attained eL4 by days 5-6.
Conclusion: Optimal storage conditions for *H. contortus*, adapted for subtropical conditions, were markedly different to those required for *T. circumcincta* (temperate) and these greatly affected the rate and percentage of exsheathment.
Acknowledgment: This work is supported by Novartis Animal Health.

c.7.76-92 Basic parasite biology

c.7.86 INTERBREEDING BETWEEN TWO GEOGRAPHICALLY DISTINCT ISOLATES OF SCHISTOSOMA JAPONICUM FROM MAINLAND CHINA

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Background:Previous studies have indicated that a species complex may exist among isolates of Schistosoma japonicum from mainland china.The present study aimed at a further investigation of this hypothesis by performing an interbreeding experiment between geographically distinct isolates from the Sichuan and the Anhui provinces.

Method:S.japonicum cercariae were obtained from single miracidium infected Oncomelania hupensis snails collected from the Sichuan Province and Anhui Province.Fourty cercariae of the Sichuan isolate and 40 cercariae of Anhui isolate were used to percutaneously infect mice using the coverslip method.The F1 generation was used to infect rabbits.The interbreeding experiment was terminated at the F2 miracidial stage.

Results:S.japonicum eggs were obtained from 32 of 55 livers from the experimental mice after 35 days of infection.These F1 eggs were hatched and used to infect 150 negative snails to obtain F1 miracidia.Twelve of 115 (10.4%) snails were cercariae positive after 70 days of infection.These cercariae were used to infect two rabbits.The rabbits were killed 42 days after infection.No gross morphological difference was observed between the F1 adult worms.F2 egg,deposited in the rabbit livers,were hatched and host finding activity was observed.

Conclusion: Despite previously observed morphologic, enzymatic and genetic differences between the S.japonicum Sichuan isolate and the Anhui isolate from mainland China these isolates were capable of interbreeding.

c.7.87 ESTABLISHMENT AND DEVELOPMENT OF *Lagochilascaris major* CYSTS IN MICE

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Background: It is known that small rodents make part of the life cycle of the nematodes of the genus Lagochilascaris. The fact that infection could not be established in cats using larvated eggs of *L. major* (personal communication) led our laboratory to investigate the degree of participation of mice in the life cycle of *L. major*. Parameters that were observed included hematological alterations, location, establishment and development of the cysts with L3 in mice infected with various doses of *L. major* eggs.

Method: Three groups of 10 Balb/c mice were each orally infected with either 50, 100 or 200 *L. major* eggs, and another 10 mice remained as non-infected controls. Complete hemograms were carried out weekly. On day after infection (DAI) 180, the mice were sacrificed, cysts were counted and their location recorded.

Results: The hemogram revealed eosinophilia and monocytosis during infection. On DAI 180, independent of infecting dose, all cysts contained L3 and were located preferentially in the subcutaneous tissue of the armpits, but also being found all through the skeletal muscle, liver, diaphragm, spleen, lungs, brain, tongue and heart. There was no significant difference in the number of established of cysts among the different doses used.

Conclusions: The minor hematological alterations, together with the evident establishment and development of *L. major* cysts in mice and the long-term survival after infection with various doses of eggs indicates that mice are adequate hosts for this ascarid in natural environments.

c.7.88 SCREENING OF IN VITRO DEVELOPMENT OF CO60 IRRADIATED *COOPERIA PUNCTATA* LARVAE

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Background: Up to date no experiments with irradiated *Cooperia punctata* have been carried out. The aim of this work was to study the development of in vitro cultivated *C. punctata* larvae exposed to different levels of Co^{60} irradiation.

Method: Infective *C. punctata* larvae were exsheathed and divided in 6 groups of 20.000L3, and exposed to levels of Co^{60} irradiation of 100Gy (Group A); 200Gy (Group B), 300Gy (Group C), 400Gy (Group D), 500Gy (Group E) and a non irradiated Control Group (Control). The larvae were cultivated for 26 days, and development was measured by length, motility, mortality and morphology. Statistical analysis was made using one-way ANOVA.

Results: At day 0 the larvae, 100% L3, presented good motility, 0% mortality and mean length of 542 ±46,86µ. On the 3rd day of culture, Control larvae moulted to L4 with total exsheathment of the L3, on irradiated groups the exsheathment occurred but some sheaths were attached to the larvae. At 12th day of culture, the number of larvae attached to the L3 sheath and the mortality were proportional to the radiation level. From the 16th day onwards there were no more sheaths attached to larvae. Higher mortality was observed in Groups D and E. At the end of the experiment the percentage L5 in Control was 17,25% and in Groups A, B C D e E were 14,3%, 9,53%, 7,42%, 16,14% e 16,14%, respectively. The mean length of Groups A, C and D on 3rd day of culture were 686,205 ±51,558µ, 698,614 ±32,37µ and 707,469 ±42,111µ, significantly different of the Control, 686,205 ±51,558µ ($p<0,05$). On 12th day of culture the Groups C e E, 701,533 ±46,336µ e 696,886 ±48,034µ respectively were significantly different of the Control, 727,239 ±37,268µ ($p<0,05$). On 26th day the values of irradiated Groups A, 775,979 ±50,611µ; B, 757,119 ±67,18µ; C, 759,066 ±52,545µ; D, 743,778 ±42,71µ and E, 740,32 ±49,762µ were significantly different of the Control 810,112 ±55,246µ ($p<0,05$).

Conclusions: Co^{60} gamma radiation compromises *C. punctata* larval development, increases sheath retention proportional to the intensity of irradiation and delays growth and development in irradiated groups.

c.7.89 ENDOGENOUS DEVELOPMENT OF *EIMERIA MINASENSIS* IN THE DOMESTIC GOAT

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Background: Few life cycle studies have been carried out with *Eimeria* species in experimentally infected goats. Endogenous development of *E. minasensis*, a recently described species in goats from Brazil (Lima & Silva. Mem. Inst. Oswaldo Cruz, 1998, 93:741-744), was studied in coccidia-free kids.

Method: Nine kids inoculated with 10^5 sporulated oocysts/ kg b.w. of *E. minasensis* were killed 4, 7 (2 animals), 10, 13, 16, 18, 19 and 22 days after inoculation (DAI). Tissues samples from lungs, heart, liver, spleen, kidneys, gall bladder, mesenteric lymph nodes, abomasum, small intestine, cecum and colon were collected, fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 3-5 µm, stained with HE and examined using light microscopy.

Results: Two generations of meronts, gamonts, gametes and oocysts were found in the intestines. The first generation meronts developed deeply in cells of the lamina propria close to *muscularis mucosae* of the jejunum and ileum. Giant mature meronts (299.4 x 243.8 µm) with canoe shaped merozoites were found 16 DAI. Trophozoites, immature and mature second generation meronts were found 16 DAI in the epithelial cells of the crypts of the ileum, located above the host cell nuclei. Mature meronts measured 11.5 x 10.1 µm and contained 18-28 comma shaped merozoites. Gamonts were found in the epithelial cells of the crypts of the ileum, cecum and colon 18 DAI and lay below the host cell nuclei; and macrogametes (27.8 x 17.6 µm), mature microgamonts (21.3 x 17.0 µm) with microgametes and oocysts (30.5 x 19.4 µm) were first seen 19 DAI.

Conclusion: *E. minasensis* asexual and sexual development occur in the small intestine and in the small and large intestines of the domestic goat, respectively.

Acknowledgment: This work was supported by CNPq and FAPEMIG.

c.7.76-92 Basic parasite biology
c.7.93-99 Parasite morphology

c.7.90 SEROTONIN AND NITRIC OXIDE IN THE NERVOUS SYSTEM OF AN ELK PARASITE

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Background: Little is known about the different categories of neuronal substances in helminths. Hope lies in finding chemotherapeutic agents that act specifically on neuronal signal substance of the worm, with minimal side-effects on the host.

Method: In this study the nervous system of the elk parasite *Parafasciolopsis fasciolaemorpha* was investigated by immunocytochemical and histochemical methods. The pattern of neurons immunoreactive (IR) to 5-HT and neurons positive for NADPH-diaphorase was clarified. NADPH-d indicates the presence of nitric oxides synthase, i.e. the enzyme involved in the synthesis of the transmitter gas nitric oxide (NO). The neuromuscular relationship was studied by application of TRITC-conjugated phalloidin, that binds to F-actin.

Results: In the longitudinal nerve cords, the oral sucker and in association with the reproductive organs 5-HT-IR nerves were found. NADPH-d staining was observed in neuronal elements close to the ventral sucker. Details in the musculature of *P. fasciolaemorpha* was revealed by staining the F-actin.

Conclusions: NO is regarded as one of the first neuronal signal substances. One of the central functions of NO in higher animals is to act as a general muscle relaxer by diffusing through the cell membrane of the target cells, there activating the soluble guanylate cyclase and the second messenger cGMP. This is the first report on NADPH-d stained neurons in a mammalian trematode.

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c.7.91 NEUROBIOLOGICAL ASPECTS OF HOST-PARASITE RELATIONS

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Little is known about neurobiological aspects of host parasite relations. Analyses of the results of our experiments showed that functional activity of serotonin (5-hydroxytryptamine, 5-HT) neurotransmitter system of helminths depends on those in infected host. On the one hand study of 5-HT level in cestodes *Hymenolepis diminuta* and in the intestine of cestode-infected rats showed that there was a positive correlation between 5 -HT content in cestodes and that in the place of their localization. Further, results of experiments on fish cestodes have demonstrated the low concentration of 5-HT in helminths localized in body cavity or liver of fishes comparing with that in helminths from fish intestine, where 5-HT concentration is fairly high. In addition, it has been shown that the 5-HT level in cestodes from pike intestine (where 5-HT concentration is considerably high) is significantly higher than that in cestodes from fish intestine with low 5-HT level. On the other hand, helminthoses cause changes in host neurotransmitter systems. So changes of 5-HT level in different regions of head brain and in other tissues has been observed in experimentally helminth-(Trichinella spiralis, T.pseudospiralis, H. diminuta)-infected rats. These investigations are of importance in terms of rational approach to helminthoses chemotherapy and corrections of neurotransmitter function by means of pharmacological compounds upon serious helminthoses. This work was supported by RFBR grant 98-04-49025.

c.7.93 GNATHOSOMA OF PSOROPTES OVIS: GROWTH PROGRESSION AND FEEDING MECHANISM.

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The authors studied the three major gnathosoma components of the polyxenic species *Psoroptes ovis*: palpi, subcapitular swellings (pseudorutella) and chelicerae, determining their growth progression as well as their percentage of relative growth in comparison with the previous stage, using a combination of techniques including in situ observations of living, frozen and fixed specimens, optical dissections and Scanning Electronic Microscope (SEM) dissections and sections. A discussion is added regarding the relationship among these gnathosomal structures, different depths of hosts integument and the quality and availability of food. The pseudorutella, because of its thin conformation and lack of injurious external structures, its improbable to play some role in abrading skin of the host, but probably participates in maceration or destruction of cellular membrane of erythrocytes. Chelicerae are capable to develop different roles such as scrapping the superficial skin layers, piercing, cutting and tearing the subjacent epidermal stratum, depending on the food resource available which in turn depends of the clinical status of the host as well as the host species involved. Taking into account the different qualities of food resources possible to obtain by direct and indirect action of gnathosomal components, *P. ovis* seems to be more opportunistic than previously suspected, feeding on a material via the epidermis such as cellular debris, lipids, sera, lymph and blood, depending on the species of colonized host and the availability and distribution of these resources in each one.

c.7.93-99 Parasite morphology

c.7.94 THE FINAL STAGE OF THE INFECTIVE EGG FORMATION IN SOME CESTODES WITH AQUATIC AND TERRESTRIAL LIFE CYCLES.

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The aim of the present work was to compare functional ultrastructure of the infective eggs of cyclophyllideans from hosts living in different external environments.
Our TEM and SEM studies on 8 cestode species from aquatic hosts, of the 3 genera: *Diorchis, Fimbriaria, Wardium*, and 3 species: *Dipylidium caninum, Taenia saginata, Hymenolepis diminuta*, with terrestrial life cycles show clear differences in the final arrangement of their oncospheral envelopes. There are differences in ultrastructural details of the outer and inner envelopes, as well as the number, shape,thickness, spatial relation of their derivatives.The differences were observed among: 1) various aquatic species of *Hymenolepididae*, 2) aquatic and terrestrial species of cestodes, and 3) between different terrestrial cyclophyllideans.Clear differences were also visible in the functional ultrastructure of the outermost persisting envelopes. The above- mentioned differences depend on the arrangement of infective eggs "in utero", i.e if they develope singly (*H. diminuta, Fimbriaria czaplinskii*), or if they are grouped into egg capsules (*Fimbriaria fasciolaris, D. caninum*). In the cestodes examined,we distinguished four types of differentiation of the outer envelope, and two types of morphogenesis of the inner envelope derivatives.
This high degree of ultrastructural diversity, observed in oncospheral envelopes of examined cyclophyllideans, certainly reflects different ways of infective egg adaptation to the cestode life cycles, namely: to their intermediate hosts and environmental conditions in which they live.

c.7.95 GNATHOSOMA OF PSOROPTES OVIS: MORPHOLOGY AND FUNCTION.

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Feeding mechanisms of the polyxenic species *Psoroptes ovis* (Gervais, 1841) were determined after a detailed morphology & functioning study of the three major gnathosoma components involved in obtention & intake of food: palpi, subcapitular swellings and chelicerae, correlated with hosts epidermal thickness and eventual availability of food. The three structures have been studied in a comparative form through all developmental stages, using a combination of techniques including in situ observations of living, frozen and fixed specimens, optical dissections, scanning electronic microscope dissections and sections. Internal & external pseudorutellar processes have been determined as highly improbable that they abrade host tissue, but the chelicerae appear to be responsible for that action.
The authors determined that the maximum depth possible to reach by a fully protruded chelicera from the gnathosomal tip is 54 µm in some large female individuals. Subepidermal capillaries of the host, which are located below the epidermis (which ranges from 27 to 50 µm in sheep) make the direct piercing of the chelicera very difficult to reach.

c.7.96 INTRA-SPECIFIC VARIABILITY OF THE MORPHOLOGY IN POPULATIONS OF THE CATTLE PARASITE *HAEMONCHUS PLACEI*, PLACE 1893.

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Background: *Haemonchus placei* is a parasite of cattle abomasum. The females present three main variants of vulval process: Linguiform, Knoblike and Smooth. Different frequency of vulval morphopype seemed to correspond to ecotypes adapted to geographic sites and/or climate in *Haemonchus contortus,* but no information is available on the existence of *H. placei* ecotypes. This is the first aim of the work. The other objective is to study differences between African and American isolates (introduced from Africa?)
Methods: Fifty females per origin (18 isolates) were differentiated into one of the three female morph Linear regression analysis and distance weighted least-square smoothing (DWLS) were used to relate the proportions of each morphotype to rainfall and temperature. Eight measures in 10 males and 10 females of 4 American and 3 African isolates were performed and analysed using discriminant analysis.
Results: The following significant least-square linear regression were found after transformation into natural logarithm of morph percentages and rainfall in mm on 18 isolates: Log (Linguiform)= 0.058 + 0.53 Log (Rainfall) P= 0.05. Log (Knobbed)= 8.76 – 0.77 Log (Rainfall) P=0.04. Two groups were clearly delineated (94 % of males and 89% of females were well classified) in the analysis: *H. placei* found in New World (North and South America) and Old Word (Africa) and were different when their morphometrics was compared.
Conclusion: Ecotypes with hight proportion of knobbed females would correspond to drier climates whereas those with a majority of linguiform females would de found in larger proportions in rainy areas. The different origin *H. placei* evoluated for several century due to massive Indian *Bos indicus* importation the beginning of this century or introduction from Africa to America.

c.7.97 *CYATHOSTOMUM MONTGOMERYI* AND ITS PLACE IN CYATHOSTOMINAE (NEMATODA: STRONGYLIDAE)

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The redescription *C. montgomeryi* (Boulenger, 1920), was one of the tasks of the International Workshop on the Systematics of Cyathostomes in Horses (WAAVP, 1997). This common parasite of zebras has not been redescribed since its original description. It is still almost unknown outside of Africa. This species was placed into Murshidiinea (Hartwich, G., Mitt. Zool. Mus. Berl., 1986, 62, 61-102).
We have studied 210 specimens from *E.burchelli* from Etosha and Kruger National Parks (Republic of South Africa) and made measurements of 15 males and 15 females. Nematodes were fixed in 4% formalin and studied in temporary mounts cleared in 80% phenol in glycerol with the aid of interference contrast light microscopy.
This species has two *corona radiata* or leaf crowns surrounding the mouth. The internal leaf crown (ILC) is inserted near the middle of the buccal capsule. The external leaf crown (ELC) has fewer elements than the ILC. The extra-chitinous supports are similar to those of *C. catinatum*. The distal end of the spicules are identical to others in the Cyathostominea but differ from those in the Murshidiinea. The bursa is typical for *Cyathostomum.* Thus we retain this species in the Cyathostominea. Specimens we have measured were larger than those described by Boulenger. The bases of the ILC elements are inserted in a curved line on the wall of the buccal capsule and those of the ELC are recessed from the tips of the elements of the ILC on which they rest; all typical of other species of *Cyathostomum*. The walls of the buccal capsule of this species are similar to those of *Coronocyclus labratum.* However, the extra-chitinous supports of ELC adjoin the anterior edge of buccal capsule and that is why we place *C. montgomeryi* in the genus *Cyathostomum.*

c.7.93-99 Parasite morphology

c.7.98 FINE STRUCTURE OF THE INFECTIVE ONCOSPHERES OF FIVE SPECIES OF TAENIID CESTODES OF MEDICAL AND VETERINARY IMPORTANCE: TEM & SEM STUDY

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The purpose of the present study was to describe the functional ultrastructure of taeniid eggs. More information on their morphology may prove useful for better understanding of the mechanism of their remarkable resistance to destructive physico-chemical factors and their impermeability to the anthelmintic ovicides. The eggs of five species of taeniid cestodes: *Echinococcus granulosus, E. multilocularis, Taenia hydatigena, T. saginata* and *T. solium* were studied by means of TEM and SEM. The infective oncospheres are surrounded by 3 egg envelopes: the embryophore composed of keratinous blocks, underlying cytoplasmic layer of the inner envelope containing residual nuclei, and the oncospheral membrane. Size of embryophore blocks, namely the measurements of their surface and length by means of SEM and TEM, show significant differences in five species examined. The hook- muscle system and cellular organization of hexacanths of five examined species show great similarity. Five major types of oncospheral cells have been identified: (1) somatic cells [=perikarya of somatic and hook musculature; (2) bi-nucleate subtegument perikaryon; (3) oncospheral penetration glands; (4) nerve cells; and (5) about 10 germinative cells, located in the posterior pole of the larvae, forming the postembryonic stage of the life cycle.

Acknowledgement: Financial support from KBN (Grant No.6 P04C 013 14) is gratefully acknowledged.

c.7.99 A DIGITAL IMAGE ANALYSIS AND NEURAL NETWORK BASED SYSTEM FOR IDENTIFICATION OF THIRD STAGE PARASITIC STRONGYLE LARVAE FROM DOMESTIC ANIMALS

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Background: The identification of third stage parasitic strongyle larvae is based on a combination of quantitative and qualitative morphological features but the qualitative features are subject to inter- and intraobserver variability. For this reason, an Artificial Neural network (ANN) was trained to identify third stage parasitic strongyle larvae from domestic animals on the basis of quantitative data obtained from processed digital images of larvae.

Method: An ANN was trained to identify third stage parasitic strongyle larvae on the basis of quantitative data obtained from processed digital images. A total of 132 images of 26 individual larvae in various shapes belonging to 5 genuses were recorded. Following image processing 16 novel features were measured of which 7 were selected as invariant to larva shape. Two of those novel features "area" and "perimeter" along with the quantitative features used in conventional identification, "overall body length", "width" and "tail of sheath" were used as an effective training data set for the ANN. This ANN became the basis for developing a computer-based larva identification system.

Results: The overall identification performance of the system was 89.3%.

Conclusion: The advantages of this system are its speed and objectivity. The limitations of the system are that it can not handle raw images but only data extracted from images, and its performance depends on the reliability of the input vectors used as training data for the ANN.

d.1.01-04 Chemotherapy against livestock parasites

d.1.01 VERAPAMIL MODIFIES THE PHARMACOKINETICS OF IVERMECTIN IN SHEEP

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Background: P-glycoprotein (P-gp) is a transport protein that participates in the mechanism of active secretion of different molecules from the bloodstream to the gastrointestinal tract. P-gp also acts as an efflux pump reducing intracellular drug concentrations. This protein seems to be involved in the mechanism of resistance in nematodes. Concurrent use of verapamil (VRP) enhanced the efficacy of ivermectin (IVM) against resistant *Haemonchus contortus*. The aim of the current work was to evaluate the effect of VRP on the plasma kinetic behavior of IVM given to sheep.
Methods: Sixteen adult sheep were divided into 2 groups and treated with either IVM alone (oral, 200 µg/kg) or co-administered with VRP (subcutaneous, 3 mg/kg in three treatments, 12 h interval). Blood samples were collected for 30 days post-treatment. Plasma was analyzed to determine IVM concentrations by HPLC. Kinetic analysis was carried out using the PK Solutions software.
Results: The IVM+VRP treatment resulted in higher IVM plasma concentrations over the whole drug detection period (12-15 days). IVM concentration at 12 days post-treatment was 57% higher following the IVM+VRP treatment. The IVM peak concentration (Cmax) was significantly higher (83%) after the IVM+VRP compared to the IVM alone treatment. The total IVM plasma availability measured as area under the curve (AUC) was also significantly higher (54%) following the co-administration. The IVM elimination half-life was longer in the presence of VRP.
Conclusion: The results demonstrate that VRP induced a significant alteration in the plasma disposition and availability of IVM in sheep, probably due to an interference with a P-gp-mediated drug elimination mechanism, which could have an important impact on the efficacy and persistence of its antiparasitic activity.

d.1.02 FASCIOLACIDAL EFFICACY OF TRICLABENDAZOLE & CLOSANTEL (GLDH, GGT, EGGS)

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Background: Anthelmintic efficacy is normally based on helminthological parameters (e.g., egg counts). Clinical impact can be gauged by measuring clinical parameters (e.g., glutamate dehydrogenase [GLDH] for fasciolosis).
Methods: 30 of 40 sheep were infected weekly with Fasciola hepatica 9x starting on Day 0, leaving 10 sheep uninfected. On Day 70, 10 infected sheep each were given orally 10mg closantel/kg or 10mg triclabendazole/kg or left untreated. Faecal samples were examined after sedimentation for Fasciola eggs on Days 91, 105, 119, 133 140 & 147. Sheep without eggs in any of their faecal sample were declared "cured". GLDH and γ-Glutamyl-transpeptidase (GGT) activities of all 40 sheep were determined weekly from Day 0 to Day 147. The mean Area Under the enzyme activity Curve (AUC) was calculated for all 4 groups from Day 91 to Day 147. The clinical efficacy (CE %) was calculated for both treatments as reduction of elevated values:

$CE\ \% = 1-([AUC_{treated}-AUC_{uninfected}]/[AUC_{untreated}-AUC_{uninfected}])x100$

Cure rates were compared by Fisher's Exact Test and enzyme activity log AUCs by analysis of variance.
Results: Parasitological cure rates, clinical efficacy (GLDH, GGT) and differences between treatments are shown in the following table:

		cure	GLDH	GGT
triclabendazole		90%	95%	80%
closantel		0%	59%	14%
difference	p	<0.05	<0.05	<0.05

Conclusions: The significant difference in parasitological efficacy resulted in a significant difference in clinical efficacy underlining the clinical importance of eliminating also the early immature and immature stages.

d.1.03 UPTAKE AND METABOLISM OF ALBENDAZOLE AND ALBENDAZOLE SULPHOXIDE ENANTIOMERS BY *Fasciola hepatica*

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Background: Albendazole (ABZ) and its sulphoxide (ABZSO) metabolite are anthelmintically active molecules. ABZSO exhibits chiral behaviour and its (+) and (-) enantiomers have been detected in plasma. Further understanding of their tissue distribution and capability to reach target parasites is required. The drug/metabolite concentration profiles obtained in plasma, bile and liver in ABZ-treated sheep and the pattern of uptake of ABZ and both ABZSO enantiomers by F. *hepatica* recovered from the same treated sheep, were investigated. Complementary *ex vivo/in vitro* studies characterise the patterns of ABZ/ABZSO uptake and biotransformation by the trematode.
Method: Twelve (12) *F. hepatica* infected lambs received ABZ (7.5 mg/kg) intraruminally. Two animals were sacrificed at either 1, 4, 8, 12, 18 or 24 h post-treatment. Specimens of adult *F. hepatica* and samples of blood, bile and liver tissue were collected, processed and analysed by HPLC using reverse and chiral stationary phases. Flukes collected from untreated sheep were incubated with either ABZ or ABZSO (*ex vivo* studies) to characterise their comparative uptake patterns. ABZ biotransformation in *F. hepatica* was investigated by incubation of the drug with homogenated/ultracentrifugated parasite material.
Results: While only low ABZ concentrations were detected in the parasite (4-24 h post-treatment) and bile (8-18 h), a high availability of the parent compound in liver tissue was observed. ABZSO was the main molecule measured in *F. hepatica* and bile samples collected from ABZ-treated sheep. The (+)ABZSO was the enantiomeric form recovered at the highest proportion in plasma (75.3 %), bile (78 %) and *F. hepatica* (74 %). A significantly greater uptake of ABZ compared to ABZSO was observed after *ex vivo* incubations. ABZ was oxidised under *in vitro* conditions; equivalent proportions of both ABZSO enantiomers were obtained as a product of this parasite-mediated ABZ oxidation.
Conclusion: The high lipophilicity of ABZ accounts for its greater parasite uptake; however, ABZSO (largely the (+) enantiomer) is the main active molecule recovered in *F. hepatica* collected from ABZ-treated sheep. The low ABZ concentrations detected in bile and the oxidative capacity of the adult parasite may account for those results.

d.1.04 NEMATOCIDAL ACTIVITY OF OXYTETRACYCLINE AND ENDOSYMBIOTIC *WOLBACHIA*

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Background: Filarial nematodes are significant global pathogens in humans and domestic animals. We are utilising the bovine parasite *Onchocerca ochengi* to discover compounds lethal to adult *O. volvulus*, the causal agent of "River Blindness" for which there is no safe macrofilaricidal drug for mass application.
Method: A controlled trial was done in Cameroon, using cattle naturally infected with *O. ochengi* and groups treated either with oxytetracycline, moxidectin or untreated. The viability of macrofilariae was determined by motility score, tetrazolium reduction and embryogenesis and worms were also fixed for transmission electron microscopy (TEM), and preserved for DNA extraction.
Results: By 9 months post-treatment (mpt) all worms in the oxytetracycline group were dead. No adult worms in either moxidectin treated or untreated groups were killed up to 12 mpt. TEM demonstrated abundant micro-organisms in the hypodermal tissues which disappeared as treatment progressed. Sequence analysis of the 16S rRNA gene from worms' bacteria showed them to be *Wolbachia*-like organisms of the order *Rickettsiales*.
Conclusion: Oxytetracycline is macrofilaricidal against *O. ochengi*. Its action is hypothesised to be due to activity against endosymbiotic micro-organisms. Since similar organisms occur in other filarial nematodes these results suggest a new target for anti-filarial therapy and the possibility of a safe, macrofilaricidal drug for human use. Thanks to the MACROFIL Programme of WHO TDR/APOC/OCP for funding.

d.2.01-04 Development of vaccines against parasites

d.2.01 STUDIES OF THE IMMUNOLOGICAL CONTROL OF THE SHEEP SCAB MITE, *PSOROPTES OVIS*

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Background: Sheep scab is a severe welfare condition characterised by irritation and wool loss caused by the mite, *Psoroptes ovis*. Although a range of acaricide dips and injectable endectocides are now available for treatment and control there are a number of concerns relating to the use of some of these products. Vaccines offer a possible alternative to sheep scab control.
Method: Evidence was obtained that *P. ovis* ingests sheep immunoglobulin. Mites obtained from the field were cultured on parasite-naive sheep, and subsequently harvested and stored at -70°C. Extracts were prepared from mites by methods designed to enrich for integral gut membrane proteins. Two groups of sheep were hyperimmunised with different fractions of the extract, then artificially challenged with live mites and monitored clinically and parasitologically for a period of 6 weeks post infection.
Results: No clinical or parasitological protection was observed despite good antibody response. Further trials with different fractions are in progress.
Conclusions: It seems likely that the gut antigen approach to vaccination is not appropriate for *P. ovis*, probably because the mites do not ingest adequate concentrations of immunoglobulin. However, on-going research has identified certain mite enzymes and allergens, a greater understanding of which and their role in modulating the response to infection may ultimately lead to a vaccine against this disease.

d.2.02 Expression of P30, the major surface antigen of *Toxoplasma gondii* in baculovirus--insect system and the evaluation of the immune response in mice induced by recombinant P30

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Background: The major surface antigen of *Toxoplasma gondii* (p30) has been known to be highly immunogenic in human and animal. We have cloned the coding sequence of p30 from a Chinese toxoplasma ZS1 strain genomic DNA and have expressed it in a baculoviral expression system.
Method: The purified recombinant p30 was incorporated into the immunostimulating complexes (ISCOM). The mice were immunized by subcutaneous injection of ISCOM containing 0.1, 1, 1.0 μg p30 (per individual), respectively. Control mice were injected with matrix, 10 μg of Quil A per injection. After 6 weeks, all mice were boosted.
Results: One week after the secondary immunization, the specific antibody titers in mice immunized with ISCOM ranged from 1:6400 to 1:25600, the response was related to the immunized dose. The lymphocyte transformation test show that the ISCOM immunized mice displayed p30-specific proliferative responses following in vitro stimulation. Eight mice in each immunized and control group received $1x10^4$ tachyzoites of ZS1 strain administered intraperitoneally 17 days after the second immunization. Immunized mice survived longer than the controls, and the mean survival time was dose dependent.
Conclusion: The present results indicated that the recombinant p30 purified from baculoviral expression system can stimulate mice to produce effective protection to *Toxoplasma gondii* infection.

d.2.03 IMMUNISATION OF RATS AGAINST FASCIOLOSIS WITH A CYSTEINE PROTEINASE cDNA

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Background: Fasciolosis causes great economic losses in cattle and sheep industry. No protective vaccine against the disease exists. A new paradigm of DNA vaccination have been utilised to solve the problem. Rat is a good laboratory model for cattle fasciolosis. Cysteine proteinase cDNA was chosen basing of the relevance of the enzyme in the life cycle of parasite and host - parasite interactions.
Methods: cDNA of a cysteine proteinase of *Fasciola hepatica* was amplified by PCR and cloned into pcDNA 3.1 vector (Invitrogen). Five male and five female rats were immunised with 50μg of recombinant vector diluted in saline with 0.05% bupivacaine by intramuscular injection. Four weeks later rats were challenged orally with 40 metacercariae each. Control groups: injected with empty vector and non-injected were included into experiment. Seven weeks after challenge rats were euthanised and their livers examined for the parasite burden.
Results: The vaccinated rats demonstrated a high level of protection. The mean number of flukes in vaccinated females was 1.0±0.2 and in males 0.0±0.0, 74% and 100% worm burden reduction, respectively, in comparison with controls. The results were statistically significant ($p<0.001$). The livers of vaccinated animals, even containing parasites, appeared to be much less damaged than that of control animals.
Conclusion: DNA vaccination with cysteine proteinase cDNA containing vector appeared to be an efficient method to achieve protection against fasciolosis in rats.This new kind of vaccination seems to be much more promising than traditional protein one.

d.2.04 EPITOPE SPECIFICITIES AND ANTIBODY RESPONSES TO SYNTHETIC PEPTIDE ANTIGENS IN SHEEP

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Background: EG95 is a protective recombinant vaccine against hydatid disease in sheep. The mechanism by which EG95 elicits protection is believed to be antibody dependent, complement mediated lysis.
Method: Linear, antibody-binding epitope specificities of EG95 were mapped by reacting sera raised against the recombinant antigen with a set of 25 overlapping EG95 14mer synthetic peptides. Synthetic peptides of these regions were prepared as either unconjugated or DT conjugated forms and these were used in immunisation trials in sheep.
Results: Four immunodominant regions of EG95 were identified. These regions corresponded to the EG95-derived sequences TETPLRKHFNLTPV (peptide 1), SLKAVNPSDPLVYKRQTAKF (peptide 2), DIETPRAGKKESTVMTSGSA (peptide 3) and SALTSAIAGFVFSC (peptide 4). Immunisation with both conjugated and unconjugated peptides produced specific IgG1 and IgG2 antibodies in all sheep. Mean antibody titres for conjugated and unconjugated peptide were as high as 460,000 and 65,000 respectively. Anti-sera raised against both conjugated and unconjugated forms of all four peptides were reactive with the recombinant antigen.
Conclusion: The identification of these immunogenic peptides of EG95 maybe useful in the development of a synthetic peptide vaccine as a derivative of the EG95 recombinant.

d.3.01-04 Biological parasite control using nematophagous fungi

d.3.01 BIOLOGICAL CONTROL OF SMALL RUMINANT NEMATODE PARASITES IN THE TROPICS

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Background: Nematode parasites are a major problem for sheep and goat farmers in Malaysia. Control has relied on the intensive use of anthelmintics and as a consequence, high levels of resistance to these drugs have now been reported. Alternative methods of parasite control are now an urgent priority.

Method: During 1997-1998, approximately 1,000 faecal samples from a range of livestock species were screened for the presence of nematode-trapping fungi. *Arthrobotrys* spp. was commonly found and has been tested for its ability to control *Strongyloides papillosus* which is an important parasite problem of pen reared young ruminants. Recently an isolate of *Duddingtonia flagrans* has been successfully made. This is the first report of an Asian isolate of *D. flagrans*, which is being extensively evaluated as a potential biological control agent of nematode parasites of livestock, in the temperate regions of the world.

Results: Studies are underway to determine the effectiveness of these fungi to control the range of important nematode parasites of sheep and goats. Investigations are also in progress to determine optimal methods for the production of fungal material and for practical means of deployment.

Conclusion: Studies to date show that exploiting the nematode trapping properties of certain micro-fungi offers great promise in the control of the free-living stages of nematode parasites of small ruminants in Malaysia. Also, the means by which these fungi can be used is well suited to the way in which sheep and goats are reared in this country, whereby they are housed at night and offered feed supplementation.

d.3.02 EFFECT OF DELAYED DEPOSITION OF *DUDDINGTONIA FLAGRANS* ON SHEEP PARASITIC LARVAE ON PASTURE - A PLOT STUDY.

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Background: Field trials conducted in Denmark have demonstrated the successful application of *Duddingtonia flagrans* to control parasitic nematode larvae of livestock. This trial was designed to examine the effect of *D. flagrans* spores on free-living larvae of sheep parasitic nematodes on pasture.

Method: The trial was devided into 2 deposition times, July and August, in which 3 treatments were investigated: A- faeces collected from naturally infected sheep; B - faeces from infected sheep fed *D. flagrans* chlamydospores; C - faeces collected from infected sheep followed by faeces from "worm-free" sheep fed *D. flagrans* chlamydospores deposited 2 weeks after the initial deposition. Pasture was sampled every 2 weeks after deposition, for a total of four sampling times per deposition period. An additional sample was collected 1 month after the 4th sampling. The number and species of infective larvae present on pasture was determined.

Results: Only very few trichostrongylid larvae were found on pasture plots of treatment B compared to treatment A. Treatment C showed no difference from treatment A, although there seemed to be a delayed effect, indicated by differences observed 3 months after deposition.

Conclusion: *D. flagrans* spores germinate in faeces and trap hatching parasite larvae but do not appear to grow into the surrounding soil. This suggests that *D. flagrans* will not effect hatching parasite larvae in faeces deposited prior to administration of the biocontrol agent.

d.3.03 EFFICACY OF *DUDDINGTONIA FLAGRANS* ON INFECTIVE LARVAE IN SHEEP FECES

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Nematode infection, specifically *Haemonchus contortus* is a serious constraint for the sheep industry worldwide. Development of anthelmintic resistance and increasing concern about the impact of anthelmintic use, dictate the need for alternative control. Using biological products such as the nematode trapping fungus *Duddingtonia flagrans* to reduce pasture contamination is such an alternative. Two trials were conducted to determine the efficacy of *D. flagrans* in reducing infective larvae in feces. The 1st trial was to determine the dose effect of *D. flagrans* in reducing infective larvae in feces with a relatively consistent fecal egg count (FEC). Eighteen ewes were dewormed to remove existing infections and randomly assigned to 6 treatment groups. Ewes in the 6 treatment groups were given 5 x 10^4, 10^5, 2.5 x 10^5, 5 x 10^5, 10^6, and no (control) spores of *D. flagrans* per kg of body weight mixed in feed for 7 days. Fecal samples were collected daily from these and from donor ewes (FECs ranged from 5,900-20,150 EPG). Feces from individual treated ewes was mixed with an equal amount of donor ewe feces and cultured. Across dosages and days, percent reduction of infective larvae (predominantly *H. contortus*) ranged from 81.6%-99.5%. The 2nd trial was to determine the efficacy of *D. flagrans* at the dose of 10^5 spores per kg body weight on infective larvae from lambs with variable and very high FECs. Twenty lambs were paired from lowest to highest FEC and 1 of each pair was randomly assigned to either treatment or control. Treatment lambs were fed spores mixed in feed for 7 days. Feces were collected daily for FECs and cultures. FECs ranged from 17,000-135,000 EPG during the trial. Across days, the percent reduction of infective larvae (predominantly *H. contortus*) ranged from 65.9%-99.7%. Results of these trials show that the nematode trapping fungus *D. flagrans* is highly effective in reducing infective larvae in sheep feces. The use of such biological products is a promising aid in nematode control especially when anthelmintic resistance is present.

d.3.04 EFFICACY OF TWO ISOLATES OF THE NEMATODE-TRAPPING FUNGUS *DUDDINGTONIA FLAGRANS* AGAINST *DICTYOCAULUS VIVIPARUS* LARVAE ON PASTURE

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Background: A laboratory dose-titration assay demonstrated that the isolates CI3 and Troll A of *Duddingtonia flagrans* significantly reduced the number of *Dictyocaulus viviparus* and *Cooperia oncophora* L_3 in faecal cultures. Two plot trials were carried out to confirm this effect under natural conditions.

Method: Artificial faecal pats containing *D. viviparus* L_1 and *C. oncophora* eggs plus one of the two fungal isolates were deposited twice on pasture plots and the herbage around the pats was sampled periodically. The pats were recovered after one month to determine the faecal degradation.

Results: The herbage sampling showed a reduced transmission of gastrointestinal as well as lungworm larvae (77 to 100% and 34 to 80%, respectively) around pats containing fungus. The transmission of *C. oncophora* larvae, but not *D. viviparus*, was clearly affected by the climatic conditions during both trials. Determinations of wet and dry weight plus organic matter content of the pats showed no differences between the fungus-treated and non-treated control pats, indicating that the addition of the fungus did not affect the faecal degradation.

Conclusion: The present study is the first report of the activity of nematode-trapping fungi against *D. viviparus* under natural conditions. These results call for further research in this area.

d.4.01-04 Parasites of fish

d.4.01 EXPERIMENTS ON THE CONTROL OF COSTIA NECATRIX

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Background: Costia nexatrix is a host-non specific protozoan parasite (Cl. Flagellata), economically important in trout- and carp culture.
Method: Experiments were performed with fingerlings of rainbow trout (Oncorhynchus mykiss). Tested for efficiency were sodiumchloride (NaCl), formalin (37% formaldehyd), calcium hypochlorite (CaOCl2) and potassium permanganat (KMNO4). After treatment fish were kept for several weeks in running water and mortalities were compared.
Results: Optimal results were obtained by use of formalin 20 ppm 24h, but even treatment with only 2 ppm 24h showed a distinct reduction of mortalities. Use of NaCl gave optimal results with 1,5% for 6h. Use of CaOCl2 2 ppm 2h gave psitive results, while treatment with KMNO4 proofed to be ineffective.
Conclusion: Treatment of Costiasis in tanks and small ponds is probably performed optimal by use of formalin, In large ponds (carp ponds) the optimal alternative is calziumhypochlorite.

d.4.02 THE EVALUATION OF A PARASITE INDEX IN THE OLIFANTS RIVER SYSTEM

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At the previous WAAVP conference held in the Republic of South Africa, an oral presentation was given regarding the use of fish parasites as indicators of aquatic heavy metal pollution. This parasite index was developed from a fish health assessment index, which provides a means of statistically comparing the health of various fish species in different water bodies. Preliminary results indicated a distinction could be made between polluted and less polluted locations using this newly developed parasite index. To indicate the accuracy of these results a follow up study was carried out at the same two locations in the lower Olifants River catchment, South Africa, with an additional two locations in the upper Olifants River catchment being analysed.
As was found with the pilot study, parasites were good indicators of environmental health. Ectoparasites and endoparasites were evaluated separately and it was found that at highly polluted sites, ectoparasite numbers decreased and endoparasite numbers increased.
Various fish species were used during these comparisons, namely, *Clarias gariepinus*, *Oreochromis mossambicus* and *Labeo rose*, representing different trophic niches. During the pilot study it was determined that hardy fish provided the better indication of environmental health, which lead to the use of *C. gariepinus*, *O. mossambicus* and *Barbus marequensis* during the follow up study. On the whole the omnivorous *C. gariepinus* provided the best results. Analysis of heavy metal concentrations in water, sediment and fish tissue, using atomic absorption spectrometry, substantiated results obtained using the parasite index. There was a good correlation between parasite loads and heavy metal values at all four sample sites.

d.4.03 PHAGOCYTOSIS OF PANCEARS RODLET CELLS BY IMMUNE CELLS OF INFECTED *PHOXINUS PHOXINUS*

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Background: Rodlet cells have been described in different organs of freshwater and marine teleosts and were thought to be protozoan parasites by some authors. On the other hand, the view that rodlet cells are integral constituents of fish epithelia is supported by many researchers. The structure and distribution of rodlet cells has lead to speculation regarding their function.
Methods: This investigation was carried out on cestode - parasitized and uninfected specimens of minnow, *Phoxinus phoxinus* (L.) collected by electrofishing from a stream in province of Padua (North Italy). Digestive tract and associated organs were isolated from the rest of the body and processed for light and electron microscopy.
Results: Observations with light and electron microscopy showed that the rodlet cells occurred in the intestine, liver and pancreas of minnows. In this fish a high number of rodlet cell was encountered in pancreas of fish infected with a cestode, mainly in its periphery. In many instances rodlet cells were detached from the pancreas and were observed near the visceral peritoneum. Likely, all the rodlet cells found in the peritoneum were phagocytized by minnow immune cells (e.g. phagocytes) and appeared to have damaged capsules, altered nuclei and no organelles.
Conclusion: The rodlet cells undergo a turn-over involving the phagocytes. In reference to the structure and function of the pancreas, this mode of discharging the "old rodlet cells" most likely developed to protect the organ itself from the autolysis.

d.4.04 ACTUAL AND POTENTIAL PARASITE PROBLEMS IN THE MARICULTURE OF COD

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This paper reviews the information published to date on parasite problems in cod mariculture and anticipates problems which are likely to arise in the future. For parasites with direct single-host life cycles, such as protozoans and monogeneans, the rate of transmission is greater the denser the population, so they are likely to be the most troublesome. Parasites with indirect life cycles, such as most helminths and some crustaceans, can persist in a farmed population only if the other hosts necessary for the completion of their life cycles are present in the immediate vicinity of the farm site and are available for the cod to feed on.
Parasites which are already known as pathogens of cod held in captivity are the monogeneans *Gyrodactylus* spp. and the copepod *Lernaeocera branchialis*. Potentially problematic species are the protozoans *Loma morhua*, *Trichodina* spp. and *Spironucleus torosa*, the myxosporean *Kudoa thyrsites* and the copepod *Caligus elongatus*. Metacercariae of the digenean *Cryptocotyle lingua* and larval ascaridoid nematodes can cause spoilage and render infected fish unsaleable.
We recognise three main routes through which parasites can gain access to farmed cod: 1) with previously infected wild cod caught for stocking purposes; 2) from local wild populations via free-living transmission stages; and 3) by eating infected food items, either provided or as prey organisms entering the cages.

d.5.01-04 Parasites of poultry

d.5.01 PATHOLOGICAL LESIONS ASSOCIATED WITH COMMON HELMINTHS IN FREE RANGE CHICKENS

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Background: Pathological lesions caused by common helminth parasites of poultry and their implications were studied in free range chickens.

Methods: Body conditions of 200 carcasses were assessed. All gastrointestinal tracts from 100 growing and 100 adult free range chickens of both sexes were collected and examined for helminth infections and their associated lesions.

Results: Most of the carcasses were emaciated. Proventriculi that were infected by *Tetrameres americana* appeared anaemic and slightly swollen while those infected by *Dispharynx nasuta* were pale, hardened, and swollen to almost twice their normal size. Moreover, proventriculi infected by both *T. americana* and *D. nasuta* were tremendously swollen and macerated. In addition, 9.1% of small intestines infected by *Raillietina echinobothrida* had nodules while the caeca that were heavily infected by *Heterakis gallinarum* had thickened walls and submucosal nodules. Histological examinations of tissues infected by *D. nasuta, R. echinobothrida* and *H. gallinarum* showed cellular infiltration. Proventriculi that were infected by both *T. americana* and *D. nasuta* showed formation of cellular girdles, hydropic degeneration of the glandular epithelial cells, necrosis of the glands, widened lobules and eosinophilic inclusion bodies in the glandular epithelial cells, whereas the submucosal glands were thrombosed, hyperplastic and with microminiature damage.

Conclusion: In general, the knowledge on the pathophysiology and deleterious effects caused by these helminth parasites is scanty, it is therefore recommended that further studies should be conducted.

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d.5.02 MALE BEHAVIOUR AND MALE HORMONES IN *ASCARIDIA GALLI* INFECTED HENS

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Background: Infections with *Ascaridia galli* are common in hens in deep-litter, free-range and organic production systems and observations have indicated that the infections may increase cannibalism and perhaps male behaviour.

Method: Two groups of 15 young Lohman Brown hens were weekly inoculated orally with 500 *A.galli* eggs per animal for 12 weeks, while 2 comparable groups were uninfected controls.Behavioural observations were carried out by focal animal observations of 6 hens per group and male behaviour was recorded for all individuals. Individual sera were analysed for testosterone at slaughter.

Results: The inoculated hens had 9.8±8.4 worms ($\bar{x}$±SD) at slaughter, while no worms were found in the controls. Cannibalism was not observed, however, the *A.galli* infected hens had increased frequency of male behaviour, including mating and aggression, and a significantly increased level of testosterone (0.117±0.120 ng/ml) compared to the controls (0.036±0.037 ng/ml) (p=0.0014).

Conclusion: The influence of *A.galli* on male hormones and thus egg production should be considered.

Acknowledgement: The experiment was supported by the Danish Environmental Research Programme, Project I-5 in the programme on Strategic and basic research into organic farming systems.

d.5.03 THE EFFECT OF HELMINTHOSIS ON WEIGHT GAIN IN TRADITIONAL CHICKENS.

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Background: A study on the effects of helminthosis on the weight gain of traditionally reared chickens was conducted in Shibuyunji village, Mumbwa District, Zambia.

Method: A total of 100 chickens were selected from around the village with two groups having 25 birds each serving as the treatment and control groups. On day zero (0), Levamisole (25% m/v) was administered to the treatment group at a single dose of 6ml/5Kg. The groups were then weighed weekly for 15 weeks.

Results: There was a mean weight gain (grams) of 594.3 ± 67.4 in the control group and 706.2 ± 45.5 in the treatment group. The mean worm burden from the control group and the treatment group was 101.4 ± 6.8 and 23.2 ± 3.4 respectively. There was no significant difference in weight gain between the two groups (p = 0.1727) however there was a negative correlation between the live weight and the worm burden in the individual chickens in the control and treatment groups (-0.874, R-Squared=0.764 and -0.808, R-Squared = 0.653 respectively).

Conclusion: Although there was no significant difference in the mean weight gain between the two groups, the chickens in the treatment group had a higher mean weight gain than the controls. There is evidence from the negative correlation that the higher the worm burden in individual chickens, the less there weight gain capacity.

Acknowledgement: We would like to thank the ENRECA-Livestock Helminthology Research Project (DANIDA) for the financing the study.

d.5.04 *DERMANYSSUS GALLINAE*: SEASONAL DYNAMICS AND SPATIAL DISTRIBUTION

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Background: The haematophagous poultry mite, *Dermanyssus gallinae,* is known to be more common in alternative floor housing systems than in conventional battery cages, and since the Swedish animal welfare legislation implies a ban of the latter, problems due to mites are expected to increase.

Method: Traps made of corrugated cardboard were used to follow population levels of *D. gallinae* in an aviary system (Oli-Voletage), along with meteorological data, during 6 flock cycles at 2 commercial egg-producing farms between 1994 and 1997.

Results: After their first appearence in the poultry house, equilibrium level of mites was reached in 4-5 months. Increased mite populations during summer and decreased populations during winter was observed. In an analysis of spatial distribution of the mites in the poultry house a difference was noted vertically. There was a correlation between mite numbers and resting sites for the hens. This differed between hybrids.

Conclusion: Populations of *D. gallinae* fluctuate depending on season, and the mites seem to gather on tiers where most of the hens are resting at night.

Acknowledgement: This study was financially supported by the Swedish Farmers' Foundation for Agricultural Research and by the Swedish National Board of Agriculture.

e.1.01-04 Epidemiology and control of swine parasites

e.1.01 PARASITES IN PIGS IN THE UPPER EAST REGION OF GHANA

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Background: The pig population in Ghana consists of almost 400.000 pigs, reared in small semi-commercial systems in peri-urban and rural areas. Among diseases, parasitic infections are, apart from African Swine Fever, estimated to be the most important in domestic pigs in tropical and sub-tropical areas. No studies have been carried out in Ghana to determine the parasite burdens of scavenging pigs.
Method: In total 259 faecal samples and 60 live pigs were examined for the presence of ecto-, endo- and haemoparasites.
Results: All live pigs had endoparasites and 91 % excreted parasite eggs; The clinical examinations revealed ectoparasites on 78,3% of the animals and 23,3 % had haemoparasites. Among a range of parasites also *Cysticercus cellulosae*, *Sarcocystis* spp. and possible hydatid cysts were recovered.
Conclusion: The results show that parasite prevalences in local cross-bred pigs in the Upper East Region of Ghana are high and there is a high risk of zoonotic infections.
Acknowledgement: His Royal Highness Prince Frederik of Denmark is acknowledged for the financial support of the study as well as The Royal Veterinary and Agricultural University and the Danish National Research Foundation

e.1.02 *ASCARIS SUUM:* SEASONAL EGG DEVELOPMENT RATES IN A SASKATCHEWAN PIG BARN.

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Background: *Ascaris suum* eggs are not infective when passed in pig feces but must undergo a period of development in the external environment. This development, to the infective second larval stage (L2), is affected by a number of factors including temperature and humidity. This study investigated the effects of seasonal temperature variations on the rate of development to infectivity of *A. suum* eggs under western Canadian climatic conditions.
Method: Flasks containing a suspension of *A. suum* eggs were placed inside a pig barn located near Saskatoon, Saskatchewan, Canada at monthly intervals from July 1997 to July 1998. Eggs from each flask were monitored weekly for development to the infective larval stage. Infectivity of eggs was confirmed using a mouse bioassay.
Results: Development to the infective stage took three to four weeks in the summer when in-barn temperatures were similar to the external ambient temperature. Fall, winter and early spring egg development took longer as in-barn temperatures were cooler. Mid-winter egg development took as long as 11-12 weeks. The in-barn mean temperature during this period was approximately 17°C, close to the development threshold of *A. suum* eggs.
Conclusion: Results from these experiments may assist in developing more efficient control measures for *A. suum*, combining both properly timed anthelmintic treatments and properly timed barn cleaning procedures.
Acknowledgements: The assistance of Hoechst Roussel Vet, the Saskatchewan Agriculture Development Fund and Saskatchewan Pork International is gratefully acknowledged.

e.1.03 EPIDEMIOLOGICAL STUDIES ABOUT ASCARIOSIS IN IBERIAN PIGS

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Background: *Ascaris suum* is the most common and frequent parasite helminth in outdoor production systems of Iberian Pigs in the southwest of Spain. For this reason, we planned an epidemiological study to know the reality of this parasitation.
Methods: In different slaughterhouses of Extremadura, eigthy Iberian Pigs were studied to compare the presence of worms in the small intestine, faecal egg counts and IgG antibodies. Different organs and sera of this Iberian Pigs were analized. First ones by necropsy while sera were tested by ELISA test against somatic antigens (oesophagus, cuticle, uterus, ovary and body fluid) and excretory-secretory antigen from adult worms.
Results: Evidence of Ascaris presence in the small intestine was found in 48,75% of all Iberian Pigs. The 43,9 % of them were coprologically false-positive and the 28,2 % were coprologically false-negative.The percentage of positivity by ELISA test was very high in all analized Iberian Pigs for all antigens; 100 % antigens from oesophagus, cuticle and ovary; 92,3 % for body fluid antigen and 89,74 % for uterine and excretory-secretory antigens. A high correlation was observed among ELISA results obtained with the different antigens. However, OD was not correlated with the presence and number of intestinal worms or *Ascaris suum* eggs. In fact, the mean OD for all antigens was lower and some times negative for the Iberian Pigs with intestinal worms than in the remaining pigs.
Conclusions: Most of analized swine sera were positive by ELISA demonstrating the extremelly high contact beetwen *Ascaris suum* and Iberian Pigs in the outdoor production system in Extremadura region (Spain).
Acknowledgment: The study was supported by the Comisión Interministerial de Ciencia y Tecnología of the Spanish Goverment (Project AGF96-0557).

e.1.04 HELMINTH TRANSMISSION IN PIGS ON PASTURE IN RELATION TO PIG BEHAVIOUR

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Background: The increased interest in outdoor pig production calls for better understanding of the relationship between helminth transmission and the behaviour of grazing/rooting pigs.
Method: The influence of individual pig behaviour and rank in the flock hierarchy on the uptake of *Ascaris suum*, *Trichuris suis* and *Oesophagostomum dentatum* from 5 contaminated pastures were studied in 75 pigs in '96. Faecal deposition on the pastures was estimated. The level of geophagia was estimated in a total of 125 pigs in '96 and '97 using the same pastures.
Results: The high level of geophagia showed a highly significant variation between pigs ($p=0.0001$), and a positive correlation was found between the level of geophagia and intestinal worm burdens of *T. suis* ($p<0.05$), but not for *A. suum* or *O. dentatum*. Neither rank nor frequency of suspected risk behaviour (snout in contact with soil, grass, mud or latrine) were significantly correlated with egg excretion, worm burdens at slaughter, or antibodies against *A. suum*. Selective defecation resulted in an unevenly distribution of eggs/larvae on the pasture.
Conclusion: The individual level of geophagia was correlated with *T. suis* worm burden, while frequency of risk behaviour and social rank did not influence infection levels significantly, which may possibly be explained by the complex host-parasite relationships.
Acknowledgement: The study was funded by The Danish National Research Foundation and The Danish Environmental Research Programme.

e.1.05-08 Epidemiology and control of ruminant helminths I

e.1.05 DICROCOELIOSIS IN SHEEP & GOATS IN N. GREECE

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Background: *Dicrocoelium dendriticum* is found in the bile ducts mainly of sheep and goats causing a chronic disease. The prevalence of infection may be significant but clinical signs in most instances are absent. Condemnation of livers at slaughter as well as declined performance and growth rate of the infected animals may cause important economic losses. The aim of this study was to estimate the prevalence of dicrocoeliosis in a sample of sheep and goats in N. Greece. **Methods:** Samples were collected from 6 different abattoirs in N. Greece and from 938 animals (486 sheep and 452 goats). The liver of each animal was inspected and a faecal sample as well as a sample from the bile was obtained. The adult trematodes from each liver found infected were collected, by a perfusion technique, and counted. The faecal samples were examined using the mod. McMaster technique and the bile samples using simple sedimentation. **Results:** The prevalence of infection based on liver inspection was 59.9% (95%CI: 55-64%) in sheep and 63.7% (59-68%) in goats. Based on faecal examinations the prevalences were 49.1% (44.7-53.5%) and 18.2% (14.6-21.7%) for sheep and goats, respectively. The prevalences estimated after bile examination were 80.6% (77-84%) for sheep and 76.9% (73-81%) for goats. The mean parasitic burden of sheep was 1506.6 liver flukes (range: 10-15540 per liver). In contrast the mean burden in goats was 532.2 liver flukes (range: 10-4400 per liver). The average number of adult parasites per liver is higher ($P<0.0001$) in sheep than in goats. The estimated prevalences of infection after bile examination were higher ($P<0.0001$) than those after faecal examination in both species. **Conclusions:** Sampled sheep and goats were highly infected with *D.dendriticum*. The prevalences of infection at meat inspection were similar in both species. The sheep had heavier parasitic burden than goats. The most sensitive method for diagnosing the disease is bile examination. Faecal examination for *D. dendriticum* eggs has been proved to be unreliable.

e.1.06 ORIGIN AND OVERWINTERING SURVIVAL OF CATTLE PARASITES IN SWEDEN

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Background: The origin of over wintered bovine infective larvae in Scandinavia is open to some speculation. The only investigation in Sweden was conducted in 1974 and showed prolonged survival. In our study, estimates of the relative contribution of infective larvae on pasture at turn-out were made, derived from faeces of grazing cattle deposited at different times during the previous year.
Method: Faeces from parasitized grazing cattle were deposited on a bird proof pasture area on 7 occasions with 3 weeks interval throughout the grazing season as 12 artificial 400g dung pats. Half the plot was protected from snow cover. The following spring, pasture and soil samples were taken from a circular area (diam. 28cm) centred immediately over the deposited dung pats. Larval recovery was conducted by sedimentation from which 2 subsamples were examined to identify and differentiate between parasitic and saprophytic nematodes.
Results: Larvae from pasture and soil were recorded from all periods of dung pat deposition. The highest number, relative to egg count, was recovered from the mid third of the grazing season. Less larvae was found in soil than on herbage.
Conclusion: Faecal contamination throughout the entire grazing season contributes to available infective larvae at the time of turn-out the following year. However, mid-third season contamination appears to be relatively more important.The upper layer of soil was also found to be a substantial reservoir for infective larvae. Snow was only transient during the winter, therefore the effects of extended snow cover could not be assessed.
Acknowledgement: This work was financially supported by the Swedish Council for Forestry and Agricultural Research (SJFR).

e.1.07 RESILIENCE OF SECOND GRAZING SEASON CATTLE TO PARASITIC GASTROENTERITIS.

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Background: Epidemiological studies on 100 farms in the Netherlands indicated poor resilience of yearling dairy cattle to parasitic gastroenteritis when they had been exposed to low infections in the first grazing season. However, field studies on the subject yielded variable results. Therefore, the effect of different exposure to nematodes in the first grazing season on resilience to parasitic gastroenteritis in the second grazing season was evaluated in a grazing experiment at Utrecht University.
Method: Four groups of 6 yearling cattle were grazed together as one herd from April to October 1998 and parasitic infections and weight gain were monitored. Exposure to nematode infections in the first year had been moderate (G1), low (G2), very low (G3) and negligable (G4).
Results: Pasture infectivity, as measured by pasture larval counts and tracer worm counts, remained low to very low until the end of August but in September and October high pasture infectivity developed. Faecal egg counts remained low in all 4 groups throughout the grazing season, though the increased pasture infectivity in autumn resulted in slight increases in EPG. In G1 and G2 virtually only *O. ostertagi* larvae were found in faecal cultures but in G3 and G4 *C. oncophora* was also present. Weight gain in the second grazing season was highest in G1 and G2.
Conclusions: Despite low faecal egg counts high pasture infectivity can build up on pastures grazed by yearling dairy cattle. Furthermore, resilience to parasitic gastroenteritis of yearling cattle indeed is influenced by nematode exposure in the first year.

e.1.08 NEMATODE PARASITES OF ADULT DAIRY CATTLE IN THE NETHERLANDS

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Over a period of one year, from November 1997 to October 1998, abomasa, blood and faecal samples were collected from 126 dairy cows for examination of nematode infections. Of these, 113 had a grazing history and were suitable for the study. Most faecal cultures were positive (88.5%). *Ostertagia* larvae were most frequent (97%), followed by *Trichostrongylus* (29%), *Oesophagostomum* (23%), *Cooperia punctata* (20%), *C. oncophora* (4%), *Haemonchus* (2%) and *Bunostomum* (1%). The geometric mean of the EPG was 2.4. Worms were found in 108 abomasa (96%). In all 108, *Ostertagia* was found (100%). *T. axei* was seen in 47 abomasa (42%). The geometric mean of the total abomasal worm counts was 1743 and of *Ostertagia* 1615. Almost all male worms were *O. ostertagi*. Occasionally, a few *Skrjabinagia lyrata* were seen. Inhibited stages (EL-4) were particularly present during the winter months (max. 91.5% in December) and lowest in October (3.3%). Worm burdens were highest in old cows (>10 years) and young cows (< 3 years). The highest number of worms was 99,800 with 86% EL-4. Eighty-six cows (76%) had a low to moderate (100-10,000) and 22 cows (20%) a high total worm burden. *Ostertagia*-specific antibodies were highest in late summer and autumn and lowest in spring and early summer. The same pattern, although less pronounced, was observed for serum pepsinogen values.
Two calves excreted larvae of *Dictyocaulus viviparus*, while 7 (6%) had a positive titre for specific antibodies directed against lungworm.

e.2.01-04 Pharmacokinetics of parasiticides

e.2.01 PHARMACOKINETICS OF MACROCYCLIC LACTONES IN FAT AND LEAN PIGS

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Background: Macrocyclic lactones are widely used anthelmintics in swine. Although moxidectin (MOX) is about 100 times more lipophilic than ivermectin (IVM) both drugs are distributed in bodyfat, this fat-drug complex then acts as a reservoir from which the drug is progressively released. This study examined if the body condition of the pig could influence the disposition of MOX or IVM.
Method: 'Fat' and 'lean' lines were established using 2 different diets. Animal liveweight and backfat thickness were determined weekly to monitor the difference in body condition. Plasma samples were taken at regular intervals until day 40 following intravenous or subcutaneous (s.c.) injection of IVM or MOX at the recommended dose (300ug/kg). Fat samples were taken 3 days and 3 weeks after s.c. injection.
Results: Liveweight and backfat measurements confirmed that the two groups of animals had different body conditions. For MOX the 'lean' group had higher rates of exchange between plasma and fat than the 'fat' group, but there were no differences between groups for IVM. Despite similar concentrations of IVM in fat at 3 days the concentration was about 10 times lower in the 'lean' group after 3 weeks for both backfat and omental fat. The concentration of MOX in backfat and omental fat in the 'lean' group was about half the level of the 'fat' group.
Conclusion: The quantity of lipid stored and/or the rate of lipid turnover could effect the availability, and therefore the efficacy of macrocyclic lactones.

e.2.02 THE KINETIC DISPOSITION OF DORAMECTIN IN SHEEP

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Background: The pharmacokinetic behaviour of Doramectin (DOR) has been reported in the peripheral plasma of sheep but there is no information describing the kinetic disposition of DOR in fluid and particulate digesta throughout the gastrointestinal tract nor of the secretion of DOR in bile.
Method: Sheep were surgically fitted with a re-entrant bile cannula and sampling cannulae in the rumen, abomasum and terminal ileum. DOR, containing [^{3}H]-DOR, was administered (150µg/kg) intraruminally (IR) and intravenously (IV) and the behaviour of radiolabelled products determined in the cannulated compartments as well as plasma, urine and faeces.
Results: IR administered DOR extensively associated with rumen particulate digesta, the flow rate from the rumen extending DOR presence in the abomasum. Virtually no IV administered DOR was secreted into the rumen or abomasum. After absorption into the bloodstream, about one quarter of the IR administered dose was secreted in bile with approximately 20% of biliary metabolites being enterohepatically recycled. The proportion of DOR associated with particulate digesta decreased at the ileum. The majority of DOR was excreted in faeces with less than 3% dose excreted in urine.
Conclusion: The rate of passage of particulate digesta-associated DOR from the rumen significantly influenced the duration of DOR in the upper gastrointestinal tract. Thereafter, absorbed DOR was almost completely returned to the intestine in bile, this process increasing DOR presence in digesta fluid and availability to intestinal parasites.

e.2.03 PHARMACOKINETICS OF RICOBENDAZOLE AFTER ITS INTRAVENOUS, SUBCUTANEOUS, INTRAABOMASAL AND INTRARRUMINAL ADMINISTRATION IN CALVES.

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Background: Injectable ricobendazole (RBZ) has been introduced into the argentine market a few years ago. This experience represents the first time that a benzoimidazolic agent is injected. Some RBZ characteristics are presented here.
Method: RBZ solution was administered to six Holando Argentino calves by the intraveous (IV), subcutaneous (SC), intraabomasal (IA) and intrarruminal (IR) routes at 7,5 mg/kg b/w. Plasma samples were obtained. And RBZ and albendazole sulfone (ABZSO2) were determined chromatographically. Pharmacokinetic analysis was performed.
Results: Areas under the curves (AUC) after IV and IA administrations were larger than after SC and IR administrations. Half life of elimination (T ½ ß) and mean residence time, resulted shorter after IV and IA administrations respect SC and IR. ABZSO2 AUCs did not show any statistically significant difference between the different routes of administration.
Conclusion: After IV and IA administrations high and transient concentrations were obtained. The IR administration prolongs the persistence of RBZ and ABZSO2 in the body, probaly because of some "reservoir effect". The SC administration gives rise to a plasma profile statistically similar to that obtained after IR administration. This is attributed to slow liberation from the administration site.

e.2.04 *IN VITRO* DRUG TREATMENT OF *ECHINOCOCCUS MULTILOCULARIS* METACESTODES

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The metacestode stage of *Echinococcus multilocularis* is the causative agent of alveolar echinococcosis (AE), a parasitic disease occurring in rodents and accidentally in humans, affecting the liver, with occasional metastasis formation into other organs. Benzimidazole carbamate derivatives such as mebendazole and albendazole are currently used for chemotherapeutic treatment of AE. However, albendazole is poorly resorbed, and is metabolically converted to its main metabolite albendazole sulphoxide, which is believed to be the active component, and further to albendazole sulphone. Unfortunately, chemotherapy using albendazole has been shown to exhibit mostly a parasitostatic rather than a parasitocidal effect, is not effective in all cases, and the recurrence rate is rather high once chemotherapy is stopped. Thus, development of new means of chemotherapy of AE are needed. Such developments are only possible if more detailed information can be generated on the actual uptake mechanisms, biochemical pathways, and effects of drug treatment. To assess the suitability of an *in vitro* cultivations system of *E. multilocularis* metacestodes to obtain such information we performed an *in vitro* drug treatment study using albendazole sulphoxide and albendazole sulphone. HPLC- analysis of vesicle fluids showed that the drugs were taken up rapidly by the parasite. Electron microscopical investigation of parasite tissues and nuclear magnetic resonance spectroscopy of vesicle fluids demonstrated that both, albendazole sulphoxide and albendazole sulphone, exhibited similar effects with respect to parasite ultrastructure and changes in metabolites in vesicle fluids. This study shows that the *in vitro* cultivation model presented here provides an ideal first-round test system for screening of anti-parasitic drugs, and is a valuable alternative to the animal experimentation practiced to date.

e.2.05-08 Biochemical parasitology

e.2.05 IMMUNE SERA TO TUBULIN INHIBITS PROLIFERATION OF *Trypanosoma brucei*

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Tubulin, a heterodimer of α- and ß-subunits, is the main constituent of microtubules which forms the cytoskeleton and is involved in many functions in all eukaryotic cells. Under appropriate conditions tubulin and other molecules copolymerize into microtubules or vice versa until a tubulin-microtubule equilibrium is established. Compounds which bind and remove free tubulin will promote depolymerization and fatally disrupt microtubule functions. We reasoned that antibodies directed against tubulin can similarly depolymerize microtubules and be cytotoxic. We investigated this possibility using antibodies generated in rabbit against tubulin of *T.b.brucei*. We used a natured tubulin enriched protein (NTP) and a SDS-PAGE denatured tubulin protein (dNTP) prepared from blood stream forms. We also used synthetic peptides (STP) to the c-terminus of the cDNA of *T.b.rhodesiense*. Antisera from each of these immunizations reacted strongly with NTP but failed to react with mammalian and chicken brain tubulins. The immune sera to NTP and dNTP caused over 80% and 30% inhibition of trypanosome growth respectively, while the anti-STP sera had only about 10% inhibition during 24hr incubation with trypanosomes in culture. A sustained level of antibody in the culture medium caused higher levels of inhibition, where even the anti-STP sera had about 30% inhibition by day 4 and up to 50% by day 8. Tubulin is therefore a possible immunotherapeutic target against trypanosomiasis.

Acknowledgment: This investigation received financial support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR).

e.2.06 A NOVEL SECRETORY SERINE PROTEASE INHIBITOR FROM *TRICHURIS SUIS*

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Background: *Trichuris suis*, the swine intestinal whipworm, can produce severe clinical symptoms apparently associated with worm-induced suppression of mucosal immunity. Modulation of host immune processes by parasite protease inhibitors may represent critical mechanisms for parasite survival.
Method: Extracts of adult *Trichuris suis* and culture fluids from 24-hr in vitro cultures of adult parasites were assayed for protease inhibition activity.
Results: A trypsin/chymotrypsin inhibitor, isolated by acid precipitation, affinity chromatography (trypsin-agarose) and reverse phase HPLC, was identified as a single polypeptide. The molecular weight of the inhibitor, termed TsTCI, was estimated at 6.6 kDa by both SDS-PAGE and laser desorption mass spectrometry. TsTCI associated strongly with trypsin (equilibrium dissociation inhibitory constant (K_i) of 3.1 nM) and chymotrypsin (K_i = 24.5 nM); elastase, thrombin, factor Xa, plasmin and kallikrein were not inhibited. The NH_2-terminal sequence and sequences of 4 peptides obtained following Lys C protease digestion of TsTCI showed no significant homology to any previously identified serine protease inhibitors. However, sequence similarity with the reactive site regions of *Ascaris suum* trypsin inhibitor (ATI) and *Ancylostoma caninum* factor Xa inhibitor (AcAP) was apparent.
Conclusion: This novel peptide inhibitor may be involved in protecting the parasite by interfering with protease-mediated host immune cell responses.

e.2.07 PROTEOLYTIC ENZYMES IN METABOLITES OF *UNCINARIA STENOCEPHALA*

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Background: *U. stenocephala* is a hookworm which is prevalent in dogs throughout Europe. However, very little is known about immunological aspects of this host-parasite relationship, including the antigens secreted by the parasite.
Methods: Exsheathing fluid (EF) as well as excretory-secretory products (ES) of the exsheathed L3 and adult nematodes were analysed for proteolytic activity using SDS-PAGE gelatine substrate gels. For characterisation of the detected proteinases, 8 specific proteinase inhibitors were used at concentration of 10 mM. In addition, estimation of pH optimum for proteinases activity was conducted for pH range from 3 to 11.
Results: Four proteolytic polypeptides (186, 155, 112 & 56 kDa) have been detected in the EF, 3 in the ES of the exsheathed L3 (33, 27 & 23.5 kDa) and 6 (182, 159, 98, 50, 39 & 26 kDa) in the ES of adult nematodes. Serine proteinase activity was dominant in both EF and ES of the infective larvae. Also, in the ES of adult *U. stenocephala* the serine proteinase of 26 kDa showed a very high activity. Preliminary vaccination experiments suggest that ES of exsheathed L3 may be a source of protective antigens.
Conclusions: The present results show that serine proteinases account for the most pronounced activity in the ES of *U. stenocephala*. Whether these enzymes are good candidates for vaccine antigens is to be elucidated.
Acknowledgement: This study was supported by the State Committee for Scientific Research project No. 5 PO6K 021 13.

e.2.08 PROTEINS OF THE BACTERIAL ENDOSYMBIONTS (*Wolbachia*) OF FILARIAL WORMS ARE RECOGNIZED BY SERA OF DOGS INFECTED WITH *Dirofilaria* spp.

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Background: Intracellular bacteria are widespread among filarial nematodes. These bacteria have been identified as *Wolbachia*. The presence of *Wolbachia* appears as a stable characteristic of filarial species, with bacteria from different isolates of the same species showing identical or nearly identical DNA sequences. *Wolbachia* thus represent a package of prokaryotic proteins harboured by the body of the nematode. Do these proteins have any antigenic role?
Method: Protein coding genes of the wolbachiae of *Dirofilaria immitis* and *D. repens* were cloned and overexpressed. These proteins were purified and tested in Western blot assays using sera from control dogs and from dogs infected with *D. immitis* and *D. repens*.
Results: Some of the proteins were recognized by the sera of the dogs infected with *D. immitis* and *D. repens*, showing both specific and aspecific patterns.
Conclusion: The *Wolbachia* endosymbionts of filarial worms can be regarded as a stable package of prokaryotic antigens associated with filarial worms. Compared to eukaryotic antigens, prokaryotic antigens are easy to produce in *Escherichia coli*. This should allow a rapid testing of their potential applications.
Acknowlwdgment: Support from EU Actual Cost Contract BMH4-CT98-9578 (DG 12-55 MI)

e.3.01-08 Chemotherapy against protozoan and ectoparasitic infection

e.3.01 PERSISTENT ACTIVITY OF DORAMECTIN POUR-ON AGAINST *DAMALINIA BOVIS*

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Background : A challenge model was developed to investigate the persistent activity of doramectin 0.5% pour-on against *Damalinia bovis* in cattle. Two studies were carried out, each involving 16 treated animals.

Method : On Day 0 in each study the animals were treated with doramectin (500 mcg/kg, pour-on). The animals were then divided into sub-groups of eight animals. Animals in the first sub-group were challenged on Days 14, 28, 42 and 56 and animals in the second sub-group on Days 21, 35 and 49. For the challenge each animal was penned with a naturally infested seeder animal and a louse-naive control animal for six days. The treated and control animals were examined for the number of dead and living lice and the presence of lice eggs seven and fourteen days after the initiation of challenge. Although the animals were divided into subgroups, the intent of the design was to interprete the data as a continuum of time allowing for consideration of a challenge at 7 day intervals.

Results : In both studies the control animals confirmed the infectivity of the challenge. In the first study, live lice were not detected until day 56 for one subgroup and day 63 for the second subgroup following day 49 and day 56 challenge respectively. In the second study, animals challenged on days 14, 21, 28 and 35 did not become infested. Following day 42 challenge, two treated animals of one sub-group developed a transient infestation, which failed to establish, whereas the challenge on day 49 became established.

Conclusion : Doramectin 0.5% pour-on gave protection against challenge with *D. bovis* for 42 days.

e.3.02 STANDARDIZATION OF THE *Boophilus microplus* LARVAL TEST USING FIELD AND LABORATORY STRAINS

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Background: Tick resistance to acaricides is a serious problem for the cattle industry, several efforts have been developed to control it. So, the acaricide resistance tests are the most important tool for acaricide resistance monitoring in control programs. The larval test is the FAO choice for chemical resistance determination. The standardization of the different doses for each chemical using a sensitive strain as reference are presented, and also the comparison with the results from field strains as well as the Resistance Factor (RF) determination.

Method: The filter paper was impregnated according to STONE & HAYDOCK (Bulletin of Entomological Research, v.53,p.563-578,1962.). Firstly, the dilutions were defined for each chemical using the Mozo sensitive strain as standard. It was isolate in 1973, from Montevideo, Uruguay and maintained at our laboratory since 1994. The field engorged *B. microplus* females were obtained from 7 different farms, then taken to the laboratory and after 7 days of oviposition all eggs were weighed and placed in vials. Using a small brush, samples of 70-100 larvae were removed from the vial and placed in the center of packets made of the impregnate paper and incubated for 24 hours, afterwards the packets were opened and live and dead larvae counted. The values were transformed to probits and the LD_{50} and LD_{99} were calculate, then the RF was calculated for the field strains.

Results: The RF ranged from 2,04 to 195,45 for pyrethroid chemicals and from 5,44 to 791,58 for amitraz, however for the organophosphates the Mozo strain LD_{50} was similar or bigger than for field strains. The packed larvae in the filter paper packet impregnated with amitraz needed to stand for 72 hours in the B.O.D. incubator to achieve good results.

Conclusions: The test standardization makes possible to quantify the RF. This fact is important to elaborate an acaricides use strategy, if one chemical can or can't be used in a given situation. No conclusions could be made for organophosphates, since there was no evidence of sensitivity of the Mozo to these compounds. Due the high values found for RF it was possible to identify an established resistance situation among the field strains.

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e.3.03 *IN VITRO* AND *IN VIVO* SYSTEMIC EFFICACY OF NITENPYRAM AGAINST ADULT CAT FLEAS (*CTENOCEPHALIDES FELIS*)

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Nitenpyram is a neonicotinoid acting at the nicotinic acetylcholine receptors of insects. It possesses excellent systemic activity against fleas and has a very low mammalian toxicity. Nitenpyram has been developed by Novartis Animal Health Inc. as an oral product for cats and dogs that rapidly removes adult fleas from the treated animals.

The *in vitro* efficacy of nitenpyram against fleas was discovered and studied in an artificial membrane feeding system. In this system, the fleas were fed cattle blood containing defined concentrations of nitenpyram. The estimated lethal concentrations in ppm of nitenpyram - LC50 and LC95 after 24 and 48 hours feeding - for female and male fleas are given in the following table:

SEX	LC_{50}		LC_{95}	
	24 h	48 h	24 h	48 h
MALE FLEA	0.2 ppm	0.04 ppm	0.8 ppm	0.1 ppm
FEMALE FLEA	0.07 ppm	0.04 ppm	0.2 ppm	0.1 ppm

The efficacy of nitenpyram against fleas was confirmed *in vivo*. Nitenpyram was continuously released by an osmotic pump in the subcutaneous tissue of cats, maintaining constant blood levels for several days. The treated cats were sporadically infested with fleas to assess the efficacy within a short period. The minimum effective concentrations (MEC) of nitenpyram in blood to kill fleas on cats within 6 hours and 24 hours were estimated to be 0.5 - 0.9 ppm and 0.06 - 0.1 ppm respectively.

e.3.04 NATURAL ACQUIRED RESISTANCE IN SHEEP TO THE SHEEP SCAB MITE (*PSOROPTES OVIS*). A PILOT STUDY

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Background: Little information is available on the effects of re-infesting sheep previously exposed to psoroptic mange (sheep scab). These studies investigated the effects of reinfesting sheep one year after eradication of a primary infestation.

Method: In March 1996 3 yearling, full fleeced, close wool breed sheep were artificially infested with 25 adult female ("virulent isolate") *P ovis*. Lesion areas and mite burdens were monitored weekly up to 6 weeks post infestation. The sheep were then treated with ivermectin (s/c) and moved to mite free accommodation, where they received a second s/c injection of ivermectin. Scab was completely cured and five weeks after the last injection the sheep were shorn and introduced to a grass paddock. After 1 year the sheep were reinfested with the same isolate of *P ovis* and the lesion areas and mite burdens monitored as before.

Results: Primary lesions progressed normally with a 14 to 21 day lag period followed by a rapid growth phase, where the mean lesion area after 42 days was 3822.3 cm^2 and the mite burden 240.6 mites per sheep. The progression of disease after the secondary challenge followed a different pattern with extremely low numbers of *P ovis* (range 5 to 16 mites per sheep) 49 days after secondary infestation. The mean lesion area 42 after the second challeng was only 187.6 cm^2. The scab lesions continued to progress up to 56 days post secondary challenge, but the mite burdens continued to remain extremely low.

Conclusions: Sheep can develop a partial natural resistance to *P ovis* infestations, considerably reducing the progress of secondary infestations. This is of great importance, not only on the epidemiology of the disease, but also on potential for the the development of vaccines against sheep scab.

e.3.01-08 Chemotherapy against protozoan and ectoparasitic infection

e.3.05 TREATMENT OF *PNEUMONYSSOIDES CANINUM* - THE NASAL MITE OF DOGS

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Background: The canine nasal mite, *Pneumonyssoides canimum*, is common in Swedish dogs. Reversed sneezing and other upper respiratory symptoms are the most obvious clinical symptoms observed. Loss of scent is a major problem in hunter's dogs. Sometimes the mites are seen crawling on the nostril of a sleeping dog.
Method: Seventy dogs with symptoms associated to *N. canimum* comprised the study group. In 25 of these dogs mites had been observed by the owner. Fortyseven of the dogs were treated at their first visit to the clinic and the remaining 23 dogs were left untreated for a month. The dogs were treated orally with 0.5-1.0 mg/kg bw. milbemycin oxime once a week for 3 consecutive weeks. The owners were interviewed about the symptoms before treatment and one month thereafter.
Results: One month after treatment none of the 25 dogs with verified *P. canimum* infection showed any signs of infection. Fortythree out of 45 dogs with symptoms suggestive of *P. caninum* infection showed no more signs of upper respiratory infection.
Conclusion. Milbemycin oxime at 0.5-1.0 mg/kg orally once a week for 3 consecutive weeks is an effective treatment of *P. caninum* infection in dogs.

e.3.06 EXPERIENCES MONITORING CLINICAL STUDIES TESTING PARASITICIDES IN PETS

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Background: Since 1995 the EU-guideline for „Good Clinical Practice for the conduct of Clinical trials for veterinary medicinal products" (GCPV) has been in place. The veterinary committee for international harmonisation (VICH) is currently reviewing the VICH/GCPV. The Monitor, the link between the Investigator and the Sponsor, has a huge responsibility within clinical development. Little has been published concerning the conduct of studies with parasiticides run to GCPV from a Monitors viewpoint.
Methods/Results: 9 multicentre studies in dogs and/or cats were performed in Germany and France between 1995 and 1999. Both endo- and ectoparasiticide infestations were monitored. Investigator selection, investigator motivation as well as investigator and owner compliance, drug logistics, the selection of laboratory methods and the planning and managing of human resources within standard operating procedures were major factors in the conduct of successful studies. A variety of endo- and ectoparasites were identified (Roundworms, Hookworms, Trichuris, Tapeworms, Fleas, Ticks). Patients with seasonal ectoparasites were easily selected while owner compliance with these patients caused more problems. In studies to test endoparasiticides the selection of positive animals was a major limitation. Of 2629 faeces samples screened for endoparasites, only 7.1 % were positive for at least one worm species. Of 1990 samples screened, 4.6 % were positive for more than one worm species.
Conclusion: It was challenging to conduct field studies according to GCPV. Workload and costs for the conduct of clinical studies increased significantly. An overall completion rate of 95.5 % of 641 enrolled animals was obtained by intensive monitoring. This significantly reduces product development time. Therefore intensive monitoring according to established SOP's is an investment that shortens development programs and allows early launches.

e.3.07 EFFICACY OF MOXIDECTIN AGAINST SARCOPTIC MANGE OF SHEEP AND THE EFFECTS ON MILK YIELD AND LAMB GROWTH

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Background: Sarcoptic mange often causes severe suffering to sheep. The efficacy of moxidectin has not been documented against ovine sarcoptic mange. The objectives of this study, therefore, were to investigate the efficacy of moxidectin against sarcoptic mange of sheep and to evaluate the benefits of the treatment on the milk yield of ewes and the growth of lambs.
Method: Forty-eight dairy ewes naturally infected with sarcoptic mange were divided into 3 equal groups (n=16): ewes in group E1 were treated with moxidectin (0.2 mg/kg), twice 10 days apart; those in group E2 were treated once (same dose rate) and those in group E3 were untreated controls. Furthermore, 45 lambs also naturally infested with sarcoptic mange, were divided into 3 equal groups (n=15): lambs in group L1 were treated with moxidectin as above, twice 10 days apart; those in group L2 were treated once only and those in group L3 were untreated controls. During the study the skin lesions were scored and the number of mites recovered in facial skin scrapings were recorded. Milk yield measurement and lambs weighing were carried out.
Results: The efficacy of moxidectin was found to be 100% in sheep treated twice. Skin lesions disappeared completely 123 days after the first treatment. In sheep treated once only, the efficacy was found to vary from 75 to 92%. Sarcoptic mange caused a decrease in milk production of ewes by 16% and in the growth rate of lambs by 19%.
Conclusion: Two injections of moxidectin, ten days apart, were effective against ovine sarcoptic mange. Furthermore, sarcoptic mange significantly lowers the milk production of the infested ewes and the growth rate of the infested lambs.

e.3.08 CONTROL OF SP RESISTANT BUFFALO FLY (*HAEMATOBIA IRRITANS EXIGUA*) WITH CHLORFENAPYR

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Background: Chlorfenapyr is a novel pyrrole insecticide developed by American Cyanamid Company. It is used in insecticide products and acts by contact and ingestion. Its unique mechanism of action involves uncoupling oxidative phosphorylation at the cellular level. It is highly toxic to insects and slightly toxic to mammals. This study was undertaken to evaluate 4 different prototype eartags against buffalo flies (*Haematobia irritans exigua)* under field conditions in Australia.
Method: A pen trial was set up in South-East Queensland, Australia. 30 Brahman/Angus cross heifers were ranked by weight and allocated randomly to 6 groups. 4 groups were treated with different prototypes of 30% chlorfenapyr impregnated eartags. One group received commercially available diazinon eartags and the last groups remained untreated as a control. The trial was initiated when the average fly burden was 217 per animal. Fly counts were carried out on day 1 and day 3 post-application and then weekly for 16 weeks. A resistance test showed the trial fly population to be resistant to cyhalothrin, deltamethrin, flumethrin and cypermethrin.
Results: All eartags showed good initial knock-down effect and a high level of efficacy (>90%) was achieved through 13 weeks, beyond which the fly numbers on the control animals fell below a useful testing level.
Conclusion: Chlorfenapyr eartags are an effective means of controlling *Haematobia irritans exigua* (including SP resistant strains) in cattle.

e.4.01-08 Parasitic zoonoses

e.4.01 VISCERAL LARVA MIGRANS: MIGRATORY PATTERN OF *TOXOCARA CANIS* IN PIGS

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Background It is well known that *Toxocara canis* larvae migrates in paratenic hosts including humans. Therefore, a suitable non-primate model is needed to use in human research. It was decided to investigate the migratory behaviour of *T. canis* larvae in the early phase of the infection in pigs.
Method 18 pigs was inoculated with 60,000 infective *T. canis* eggs. Groups of 6 pigs were slaughtered 7, 14 and 28 days after infection (p.i.), and the number of larvae in selected tissue samples was determined by digestion.
Results *T. canis* migrated well in the pig, although the relative number of larvae recovered decreased significantly during the experiment. On day 7 p.i., high numbers of larvae were recovered from the lymph nodes around the small intestine and to some extent also from the lymph nodes around the large intestine, and from the lungs and the liver. On day 14 p.i., the majority of larvae were recovered from the lungs and the lymph nodes around the small intestine and by day 28 p.i. most larvae were found in the lungs. Larvae were recovered from the brain on days 14 and 21 p.i. Severe pathological changes were observed in the liver and lungs, especially on day 14 p.i. Finally, a strong specific Ig antibody response was observed from day 14 p.i.
Conclusion The pig is an useful non-primate model for human visceral larva migrans, since *T. canis* migrate well and induce a strong immunological response in the pig. However, the importance of the pig as an paratenic host is probably minor, because of the relatively early death of most of the larvae.
Acknowledgement: The work was funded by The Danish National Research Foundation.

e.4.02 HUMAN TRICHINELLOSIS DUE TO HORSE MEAT CONSUMPTION : A CURRENT PROBLEM OF VETERINARY PUBLIC HEALTH

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Background: Within 25 years (1975-1999) several outbreaks of human Trichinellosis occurred in Italy and in France following consumption of insufficiently cooked horse meat. More than 3,000 cases were recorded with a high morbidity and a low mortality, only during the 1985/86 outbreaks in France (5 fatal cases).

Origin of infection: For every outbreak the origin of infected horses or refrigerated infected horse meat could be evidenced outside of the country of consumption (either in North America: USA, Canada, Mexico or in Eastern Europa: Yugoslavia, Poland). Natural mode of infection remained unknown.- According to each out break Trichinella species or type were determined by the International Reference Centre for Trichinellosis in Rome, Italy as : T. britovi, T 5 and T. spiralis.

The problem of horse meat/carcases inspection: Due to the scarcity of Trichinella larvae in horse muscles the detection of their cysts remains difficult.- It is now recommended to increase the size of the meat samples to be examined (to 5g or 10g and even 20g) by peptic digestion then microscopic examination. PCR is also considered for further evaluation.

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e.4.03 *TRICHINELLA* SP. FROM PAPUA NEW GUINEA

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Background: Since 1988, non-encapsulated *Trichinella* larvae have been detected in domestic and sylvatic swine in one remote locality of Papua New Guinea (PNG) on several occasions. Here, we report preliminary data on the morphological, biological, and molecular characters of this parasite.
Method: Morphological characters (size of adults and muscle larvae, ML; presence/absence of nurse cell at different time after infection) were studied by light microscopy. Cross-breeding infections were carried out in CD1 mice with single larvae of the PNG isolate with single larvae of the 8 genotypes of *Trichinella*. The reproductive capacity index (RCI) in laboratory mice and the infectivity for 2-week-old chickens were evaluated. A portion of ribosomal DNA containing the expansion segment IV was amplified by PCR and sequenced.
Results: Adults and ML of the PNG isolate show a size similar to that of encapsulated *Trichinella* genotypes. PNG muscle larvae did not induce the modification of the muscle cell into a nurse cell over a period of at least 3 months. The RCI was 23.8 in CD1 female mice. PNG larvae were unable to infect chickens. Single PNG larvae did not cross with single larvae of the other 8 genotypes described so far. The size of amplified ribosomal DNA fragment as well as the sequence of the expansion segment IV were different from those of the other 8 genotypes.
Conclusion: These results suggest that the PNG isolate belongs to a new *Trichinella* genotype. Henceforward, the absence of the nurse cell around muscle larvae can no longer be considered as a marker of *T. pseudospiralis*. The size of muscle larvae and the lack of a nurse cell around them can be considered morphological hallmarks of this genotype and allow its identification. This is the first time that *Trichinella* parasites from PNG are isolated and identified.

e.4.04 SERO-EPIDEMIOLOGICAL SURVEY OF *TAENIA SAGINATA* CYSTICERCOSIS IN BELGIAN SLAUGHTER CATTLE

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A sero-epidemiological survey of *Taenia saginata* cysticercosis was carried out to determine the prevalence of the infection in slaughter cattle in Belgium. Between November 97 and June 98, a total of 1164 serum samples were collected in 20 export abattoirs. Meat inspection was routinely carried out by veterinary inspectors. Serum samples were examined for circulating parasite antigen using a monoclonal antibody-based sandwich enzyme-linked immunosorbent assay (Ag-ELISA). Thirty six samples (3.1 per cent) were found positive in the Ag-ELISA, while by meat inspection on the same animals, cysticerci were detected in only two carcasses. Sero-prevalence was positively correlated with the age of the animals. The sero-prevalence found in this study was more than 10 times higher than the annual prevalence (0.26 %) reported by the Institute for Veterinary Inspection. This study clearly indicates that the classical meat inspection techniques detect only a minor fraction of the carcasses infected with cysticercosis.

e.4.01-08 Parasitic zoonoses

e.4.05 SERODIAGNOSIS OF CYSTICERCOSIS IN HUMANS AND PIGS IN IRIAN JAYA, INDONESIA

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Background: Cysticercosis is one of the most serious parasitic zoonoses spread worldwide. The establishment of serodiagnosis for cysticercosis in both human and swine is expected to be highly useful for public health.

Method: Serum samples from humans [suspected neurocysticercosis (anamnesis revealed epileptic seizures or headache), suspected subcutaneous cysticercosis (detection of nodules) and healthy people from the endemic area or non-endemic area] and pigs (grown in the endemic and non-endemic areas) were prepared from Irian Jaya, Indonesia , for serological analysis by both ELISA and immunoblot (Ito *et al.* Am. J. Trop. Med. Hyg. 1998, 59, 291-294).

Results: Approximately 26 %(12/47) of healthy people at risk as well as approximately more than 60 % of either neurocysticercosis (12/18) or subcutaneous cysticercosis (20/31) suspected cases showed strong antibody responses against specific antigens of *Taenia solium*. Ten samples of pig sera from endemic area as well as five parasitologically confirmed pig sera also showed typical responses.

Conclusion: Serology using the antigens for both ELISA and immunoblot revealed that the majority of the local people in some areas in Irian Jaya at least had been infected with ***T. solium***. Based on detection of infected pigs by serology, we are planning to establish the basic methodology for detection of the worm carriers in the local areas.

e.4.06 ON LIVER FLUKES (OPISTHORCHIIDAE) IN THE CITY OF BERLIN, GERMANY

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Background: Apart from single findings of opisthorchiid flukes in carnivorous mammals little was known about the occurrence of these flukes in Germany before 1990. A first larger survey on liver parasites in red foxes (n = 1412) made in the Federal State Brandenburg (Germany) showed a prevalence of 33%.

Method: Current investigations included 409 red foxes originating from the German capital Berlin send for necropsy in 1997. Gall bladders and livers of each animal were examined separately. Flukes isolated were counted and determined.

Results: 240 (= 58.7%) out of 409 were positive for liver trematodes. The fluke prevalence in adult foxes at different seasons of the year was statistically not different and varied between 55.5% and 66.6%. In young foxes it rose from 32.5% in spring to 70.6% in autumn. *Metorchis bilis, Opisthorchis felineus* and *Pseudamphistomum truncatum* were found in 128, 73 and 8 animals respectively. While most animals showed low numbers of flukes one fox harboured 1204 specimens. The preferred site of location of *M. bilis* and *O. felineus* were the gall bladder and the bile ducts of the liver respectively.

Conclusions: Foxes in Berlin showed a much higher liver fluke prevalence and fluke burdens in comparison to foxes from the bordering Brandenburg State. Although voles are considered to be the main diet of free living red foxes under conditions of large cities foxes have to search for alternative food sources which include fish. Due to the growing population density the red fox seems to be the main natural reservoir for opisthorchiid flukes in Germany.

e.4.07 HUMAN, FARM ANIMAL AND WILDLIFE CONTRIBUTIONS TO WATERBORNE PARASITES IN THE NORTH SASKATCHEWAN RIVER

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Background: *Cryptosporidium* and *Giardia* are highly prevalent in Alberta watersheds. The influence of cow-calf operations, wildlife, and sewage effluent on the presence of these parasites in the environment is unknown. The purpose of this study was to determine parasite prevalence within each of these sources and to evaluate their impact on the North Saskatchewan River (NSR) Basin in Alberta, Canada.

Method: Fecal samples from calves aged 3-5 weeks, adult cows, and various wildlife species were collected from the NSR watershed area. Raw sewage was collected from municipal treatment facilites on a bi-montly basis. All samples were analyzed for *C. parvum, C. muris, and G. duodenalis* using sucrose flotation and immunofluorescence (IFA). Cyst and oocyst viability testing was performed on all positive sewage samples.

Results: Both *Cryptosporidium* and *Giardia* were isolated from cattle feces and human sewage, although the prevalence of both parasites in calf feces was the highest. *Giardia* was found in wildlife scat in extremely low incidence.

Conclusion: The results suggest that agricultural waste, wildlife scat, and human sewage all contribute to the presence of *Cryptosporidium* and *Giardia* in the NSR basin. However, the contribution from each source is different. Cow-calf operations potentially have the highest impact on parasite presence within the NSR basin.

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e.4.08 SEROLOGICAL DETECTION OF *TOXOPLASMA GONDII* IN PIGS USING RECOMBINANT ANTIGENS

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Background: Toxoplasmosis is a world-wide zoonosis. Transmission to humans usually occurs by ingesting *Toxoplasma gondii* oocysts shed into the environment by cats or by eating meat of infected animals. Pigs are among the food animal species known to harbor *T. gondii*. For control of *T. gondii* at the farm or in the slaughter plant, it is necessary to have available a simple test.

Method: Serum samples were collected from pigs inoculated with various doses of *T. gondii* and from pigs with naturally-acquired infections. Serum and tissue samples were run in an enzyme immunoassay (EIA), using a native tachyzoite antigen and recombinant antigens P65 (MAG1), P28.5 (GRA2), P22 (SAG1), P30, and an uncharacterized *T. gondii* protein. Tissues from naturally-infected animals were bioassayed in mice.

Results: Native tachyzoite antigen was effective in detecting antibodies to *T. gondii* in pigs for up to 50 weeks PI. Most recombinants detected infection in early stages, but EIA values declined from 5-15 weeks after inoculation. A P30 recombinant detected infection in manner similar to native protein. The P30 recombinant, native P30 and crude tachyzoite antigen were used in an EIA and compared with the modified agglutination test and bioassay for ability to detect pigs with naturally-acquired infection of *T. gondii*.

Conclusion: EIA, using several native and recombinant antigens, is effective in detecting *T. gondii* infection in pigs.

e.5.01-08 Use of PCR in molecular parasitology

e.5.01 COMPARISON OF PCR WITH PARASITOLOGY AND SEROLOGY IN THE DIAGNOSIS OF A LOW VIRULENT STRAIN OF *TRYPANOSOMA BRUCEI GAMBIENSE* IN MICE

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BALB/c mice infected with a low virulent strain of *Trypanosoma brucei gambiense* IL3253 were treated intraperitoneally (ip) with either Mel-B or PSG(+) buffer as controls. The mice were subsequently monitored regularly for parasites by direct microscopic examination of their tail blood or buffy coat and by PCR. Suratex® an assay that detects circulating trypanosome parasite antigen was also used. Mel-B was found to be an effective drug for treatment against *T.b. gambiense* and at the end of the first treatment schedule of 3 series of 3 injections at 7 days post infection, all treated mice were negative for parasites even by PCR, while all the control animals were positive. Suratex® was found to be inappropriate in the serodiagnosis of trypanosomosis due to *T.b. gambiense* in mice as it gave high levels of false positives. At thirty four days post infection, all the BALB/c mice were sacrificed and 0.5 ml of their blood injected ip into individual SCID mice. The SCID mice were likewise monitored for parasites or for evidence of infection by PCR for up to 115 days post infection. Again, an excellent correlation was shown between tail blood and buffy coat techniques. Non of the SCID mice injected with blood from BALB/c previously treated with Mel-B showed any parasite either in their tail blood or buffy coat over the entire sampling period, though some of these mice were shown to harbor an infection by PCR at some time over the sampling interval. This experiment affirms the importance of the various techniques used in the diagnosis of trypanosomosis. It is recommended that a repeated negative PCR test in combination with clinical and conventional microscopical examination, over a one month interval may be used as a gold standard to declare an experimentally infected animal, and by extension, a patient, cured of infection.

e.5.02 MICROSATELLITE SEQUENCE VARIATION AMONG *CRYPTOSPORIDIUM PARVUM* ISOLATES

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Background: Molecular and biochemical methods have recently allowed the identification of 2 genetically distinct genotypes among isolates of the parasite *Cryptosporidium parvum*, one which is exclusively found in humans ("human" genotype), and the other one in both animals and humans ("bovine" genotype). So far, these 2 groups are considered genetically homogeneous, because no recombinant genotypes have been observed even by multilocus analyses.
Method: PCR amplification of a locus containing a GAG microsatellite was performed on 7 human and 30 animal (from calf, goat, and lamb) isolates of *C. parvum* collected from Northern, Central, and Southern Italy. PCR products were directly sequenced on both strands and the sequences aligned for comparison.
Results: Sequence analysis (250 bp) revealed the presence of at least 3 alleles at the investigated locus. One allele is present in some isolates from calves, lambs, kids and humans, and it is likely to be the ancestral one. The other 2 alleles, which are characterised by deletions of different length involving the GAG trinucleotide repeats, are found either in kid and human isolates or in lamb and calf isolates.
Conclusion: The analysis of a microsatellite-containing locus revealed an amount of genetic variation (at least 3 alleles) which is larger than that previously observed using other molecular markers (only 1 allele). Therefore, the "bovine" genotype is not homogeneous. This study suggests that human infections in Italy are of zoonotic origin. This informative marker will therefore be important for a refined analysis of the transmission of the disease and for outbreak investigations. Moreover, it will be useful to test the hypothesis of a clonal structure of the parasite population which has been previously observed in other parasites.
Acknowledgements: Supported by a WHO grant (GL-Res-CTD-030-R8-98).

e.5.03 KARYOTYPIC DIFFERENTIATION IN GENUS PHYLLODISTOMUM (TREMATODA)

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Background: Genus Phyllodistomum Braun, 1899 is very large, cosmopolitan in distribution. Species of the genus are common parasites of Lithuanian fish. There is considerable taxonomic confusion within the genus. These uncertainties may be attributed to inadequate specific diagnosis, close similarity between species and the variability of many diagnostic characters some of which may change with development. Karyological date can define new characteristics which we intend to utilize in a taxonomy and comparative study of trematodes.
Method: The investigations were carried out on parthenites obtained from naturally infected molluscs collected in the Lithuania water bodies. Molluscs Dreissena polymorpha was infected with parthenites of Phyllodistomum folium, molluscs Sphaerium corneum, Pisidium amnicum - Ph. elongatum and P. amnicum -Phyllodistomum sp. Chromosomes of somatic cells of the parthenites were examined after air-drying of the cells and staining with 4% Giemsa.
Results: A diploid sets of all investigated species consists of 9 chromosome pairs gradually decreasing in length. The karyotype of Ph. elongatum is characterized by prevalence of uniarmed elements (8 pairs). Biarmed elements are prevailing in diploid sets of Phyllodistomum sp. (6 pairs) and Ph. folium (7 pairs).
Conclusion: Karyotypic differences between Phyllodistomum spp. indicates that chromosomal characters could improve the taxonomy of genus. The highest degree of karyotype symmetrization of Ph. folium indicates that as phyllogenetically more ancient, but this fact is in contradiction with fact that Dreissenidae become the intermediate hosts of cercariae of the gorgoderid kind later than other molluscs.

e.5.04 MOLECULAR CHARACTERISATION OF MONOSPECIFIC CYATHOSTOME INFECTIONS IN HORSES

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Adult small strongyles were isolated from the large intestine of a horse that belonged to an anthelmintic naive herd. Female worms were differentiated and the uteri of 87 females of *Cylicocyclus insignis* and 240 females of *C. nassatus* were dissected in phosphate buffer saline (PBS) and the eggs stripped out of the uteri. The uteri were suspended together with the eggs in PBS and cultivated for 12 days in horse faeces free of parasite stages. Approximately 300 larvae were isolated by Baermannization from each culture and given to one parasite naive foal, each. For the foal infested with larvae derived from the *C. insignis* culture egg counts were positive at 49 days post infection (d.p.i.). These eggs developed to *cyathostome* larvae with 8 midgut cells. The foal infested with larvae from the *C. nassatus* culture showed first positive egg counts at 63 d.p.i.. DNA was isolated from the *cyathostome* larvae which were cultured from both foals. ITS2-PCR using primers derived from the conserved regions of the 5.8 and 28 s rDNA gene of *Caenorhabditis elegans* resulted in a fragment of approximately 440 bp harbouring approx. 320 bp of ITS2 sequence. Sequence analysis revealed highest degree of identity with already published ITS2 sequence from other small strongyles.

The monospecific *cyathostome* infections described here for the first time are the basis for further studies on defined parasite material.

e.5.01-08 Use of PCR in molecular parasitology

e.5.05 A GALECTIN FAMILY FROM *HAEMONCHUS CONTORTUS*

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Background: Galectins are soluble β-galactoside-binding lectins that are broadly conserved amongst many organisms. They are divided into proto-, chimera- or tandem repeat-type, depending on the molecular architecture of their carbohydrate recognition domains. Tandem-repeat galectins have been described in nematodes including *Caenorhabditis elegans*, *Onchocerca volvulus* and *Teladorsagia circumcincta*. The aim of this work was to clone and characterise a family of these galectins from *H. contortus*.

Methods: A tandem repeat galectin cDNA from *H. contortus* was initially cloned by hybridisation with a cDNA clone from *Teladorsagia circumcinta*. The family of galectins was then isolated by PCR using redundant primers and subsequent library screening with PCR products.

Results: Analysis of PCR products revealed the existence of 3 families of galectins in *H. contortus*. Sequencing of cDNA clones showed that the different families were approximately 70% identical at the amino acid level, while clones within families were highly conserved. Developmental regulation was examined by Northern and Western blotting. Expression in adult parasites was localised to the gut by *in situ* hybridisation.

Conclusions: These results suggest that the 3 families of galectins may have different functions. Within families there is a high selection pressure for conservation of protein sequence, implying a crucial function.

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e.5.06 SURFACE ANTIGEN AND CARBOHYDRATE PATTERNS DURING DEVELOPMENT OF *OESOPHAGOSTOMUM DENTATUM*

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Background: It has been demonstrated earlier that *O. dentatum* undergoes changes in somatic and excretory/secretory protein patterns during its development from the free-living to the parasitic stages. It was hypothesised that developmental changes also occur on the surface of this nematode.

Method: Living and fixed worm specimen were labelled *in situ* with serum antibodies or a panel of biotin-labelled lectins.

Results: Antibody binding was observed in all parasitic stages -freshly exsheathed third-stage larvae (L3), third- and fourth-stage (L4) larvae cultured *in vitro* and L3 and L4 and adults isolated from pig intestines, but not in sheathed L3. Larvae cultured *in vitro* exposed serum-derived proteins on their surface which could be labelled with secondary antibody directed against the respective serum donor species. While freshly exsheathed larvae were recognised by *O. dentatum*-positive serum only, older larvae and adults cross-reacted with serum from pigs infected with *O. quadrispinulatum*, a closely related species. Lectin binding varied considerably between stages. While binding was not observed in preparasitic stages, ConA, SBA, WGA, RCA_{120} and PNA bound to developing larvae in varying degrees. DBA only bound to advanced (luminal) larval stages, while adults generally displayed only weak or partial lectin binding (except with ConA and WGA). UEA I only labelled larvae derived from cultures containing serum as described above. Cleavage of the carbohydrates by sodium periodate treatment resulted in reduction of antibody binding to cultured larvae, but not to freshly exsheathed L3. ConA, SBA, and PNA binding was also reduced by periodate treatment, while binding of the other lectins was unaffected.

Conclusion: The different lectin labelling patterns are related to the different stages of the nematode -infective, invasive, histotropic, and luminal- and may serve as a mode of adaption for the parasite to the host's immune attack by surface protein variation, together with antigen shedding (as demonstrated by labelling of motile larvae) and a possible acquisition of host molecules at the parasite's surface. Furthermore, this developmental variation in surface carbohydrates may play a role in parasite-parasite interactions.

e.5.07 ASSESSMENT OF SURFACE CARBOHYDRATES OF *NEOSPORA CANINUM* TACHYZOITES

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Neospora caninum is an apicomplexan parasite which is an important cause of bovine abortion and neuromuscular disease in dogs worldwide. The parasite is closely related to *Toxoplasma gondii*. However, they seem to exhibit marked antigenic differences. Receptor-ligand systems employing mainly glycoproteins, glycolipids and lectin binding sites have been shown to be responsible for parasite adhesion and invasion in *Trypanosoma*, *Plasmodium* and *Toxoplasma*. Adhesion to, and invasion of, host cells are crucial processes in the life cycle of *N. caninum* and they are influenced by specific surface molecules which could mediate the interaction with one or several host cell surface receptors. In our laboratory we have identified two surface antigens of 43 and 36 kDa molecular weight (Nc-p43 and Nc-p36, respectively) which could be involved in host cell interaction. While it has been suggested that Nc-p36 is a glycosylated protein, no further data exists on the presence and composition of other glycoproteins or glycoconjugates on the surface of *N. caninum* tachyzoites.

This study was performed in order to acquire some initial information on glycoconjugates on the surface of these parasites. Using the non-ionic detergent Triton-X-114, subcellular fractionation of purified tachyzoites was carried out, and these extracts were separated by SDS-PAGE. After transfer to nitrocellulose, specific bands containing carbohydrate residues were detected in the fraction containing predominantly membrane-associated proteins. A series of biotinylated lectins with specificities for the most common types of carbohydrates (mannosyl-, glucosyl-, fucosyl- and galactosylresidues, N-acetylglucosamine, N-acetylgalactosamine and sialic acid residues) were used to label these nitrocellulose-bound extracts and to gain some insight into the type of carbohydrates present. Fluorescence surface staining of tachyzoites employing the same biotinylated lectins followed by incubation with FITC-conjugated streptavidin was also carried out. These studies were extended by electronmicroscopical investigations, and were comparatively assessed with respect to *T. gondii* tachyzoites.

e.5.08 MOLECULAR EPIDEMIOLOGY OF *CRYPTOSPORIDIUM* AND *GIARDIA* INFECTIONS

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Background: Sensitive methods for detecting and characterising *Cryptosporidium* and *Giardia* in clinical and environmental samples are urgently required for the predictive epidemiology of infections with both these organisms. In particular, for determining sources of in infection in outbreak situations and the zoonotic potential of different genotypes. Available diagnostics are of limited value due to insensitivity, poor reproducibility, problems with interpretation and cost.

Method: We have developed a range of PCR-based DNA techniques and have applied these directly to isolates of *Cryptosporidium* and *Giardia* from humans, pets, livestock, native animals and birds.

Results: Several genotypes, possibly new species, of both parasites have been identified and appear to be maintained in different transmission cycles. Identification of these genotypes is possible in clinical or environmental samples and is indicative of the source of infection or contamination, particularly in the case of livestock and pets.

Conclusions: The availability of such molecular epidemiological tools will be of value in cryptosporidial and giardial outbreaks where determining the source of infection will limit transmission, particularly to those most at risk.

f.1.01-08 Epidemiology and control af ruminant helminths II

f.1.01 THE LANDSCAPE EPIDEMIOLOGY OF FASCIOLOSIS IN BUFFALOES IN BANGLADESH

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Background: Among the domestic animals, buffaloes are thought to be more vulnerable to fasciolosis in many Asian countries of the world. This may be due to traditional nomadic type of grazing of buffaloes than other animal species. Fasciolosis is also very common in buffaloes in Bangladesh. The present report is an appraisal on the landscape epidemiology of fasciolosis in this country.
Method: To investigate fasciolosis in buffaloes, faeces from live and liver from slaughtered buffaloes from both flood plain and highland areas of the country were examined. This was then correlated with the abundance of snail intermediate hosts of *Fasciola* and their habitats; presence of *Fasciola* cercariae in the snails, and metacercariae onto the vegetation.
Results: Both faeces and liver examinations revealed a high incidence of *Fasciola gigantica* in buffaloes of the flood plain areas than in the highland areas of the country. Two lymnaeid intermediate host snails (*Lymnaea auricularia* var. *rufescens* and *L. luteola*) of *Fasciola gigantica* were found to occur in rivers, irrigation canals, rice fields and ponds, with a high abundance in the rice fields. Both the snail species collected from different habitats contained *Fasciola* cercariae, except from the rivers. These snails were much more abundant in clay soil with clear water and vegetation. The pH of soil and water of these snail habitats ranged from 6.45 to 7.84 and 6.35 to 7.78, respectively. Among the vegetation, rice plant (*Oryza sativa*) was the most suitable medium for encystment of cercariae.
Conclusion: The present finding suggests that extensive grazing of buffaloes in the low lying areas, specially in the rice fields soon after harvesting of paddy make buffaloes more prone to *Fasciola* infection.

f.1.02 GASTROINTESTINAL HELMINTH INFECTION IN MIGRATORY SHEEP AND GOATS OF NEPAL

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In the high altitude villages of Nepal, mixed flocks of Baruwal sheep and Sinhal goats reared under migratory management migrate to the high altitude alpine pastures of Himalayas during the wet summer months and to the villages at the lower altitudes during the dry winter months.

Gastrointestinal nematode infection is regarded as one of the important health problem in these animals.Epidemiological and production effects studies conducted for a period of one year, showed that the main infection period was the wet summer months (June-September) while the flocks were in the upward migration and at the alpine pastures. Grazing areas around the flocking places and the alpine pastures constitute the main foci of infection.*Ostertagia nianquingtanggulaensis* (recorded for the first time outside of Tibet) followed by *Trichostrongylus* spp. were the predominant nematodes with *Haemonchus contortus* infection confined to early summer months at the lower altitudes. Pasture infection of different nematode species varied according to the seasons and altitude range. While mixed infection of *H. contortus, Ostertagia* spp. and *Trichostrongylus* spp. was recorded below 2300 meters above sea level (masl), only *Ostertagia* spp. were recorded above 3500m altitude.The faecal egg counts in the ewes were consistently higher than in nannies but the seasonal trend was similar.

The effects on productivity, studied in pair matched groups of lambs and goat kids, showed 110 and 87 percent higher body weight gain in lambs and kids maintained worm free by regular anthelmintic treatments than the untreated animals. Hence,the control strategy suggested is the strategic use of anthelminthics during the wet summer months to protect the most susceptible animals.

f.1.03 GASTRO_INTESTINAL NEMATODES OF CATTLE IN BURKINA FASO

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A survey was conducted to determine the worm species in abomasa, small, and large intestines of cattle in Burkina Faso.Digestive contents were collected and appropriately sieved from 94 cattle conveyed to the slaughterhouse of Ouagadougou from the central, eastern,and northern part of the country. Nine diferent worm species were collected and identified: Cooperia punctata, C. pectinata, Haemonchus contortus, Trichostrongylus colobriformis, Bunostomum phlebotomum, Moniezia expansa, Avitellina sp., Oesophagostomum radiatum, and Trichuris sp. Two species, C. punctata and C. pectinata, usually present in the small intestines, were also collected from the abomasa of the cattle in study area. By far, Cooperia sp. was the most prevalent (89.4%), followed by L4 (80.8%), H. contortus (66%), and O. radiatum (42.6%). The other worm species were much less prevalent. While only one cattle was free of parasites, it was noticed that polyparasitism was very common. This study confirmed that the rainy season constitutes a period of worm explosion. During the hot and dry season, H. contortus seemed to have a triple way of overcoming the very difficult season. Like the other worm species it was able to remain in the cattle at the adult stage; secondly, Haemonchus was able to undergo arrested development in the L4 stage; thirdly, it increased its prevalence in animal. Statistical analyses of levels of infestation did not show any significant diference according to age, sex, and weight of cattle.

f.1.04 BOVINE PARASITE EGGOUTPUT DURING A YEAR UNDER EXTENSIVE CONDITIONS

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Purpose: to determine during a whole year the normal parasite egg and larval output in the feaces of bovines maintained under primitive pasturing conditions without any antihelminthic treatment.
Methods: 17 cows with 17 calves (born in early spring) and 17 heifers were maintained together on a single pasture surface of 52 ha. from september 1996 to september 1997 in a farm of southern Chile. Once a month individual faecal samples for coproscopic techniques, grass samples and individual live weight of the animals were obtained. Daily macroclimatic data were registrated.
Results: No animal presented clinical parasitosis during the whole period. The highest trichostrongylid eggoutput (mean 245 epg), with 61% *Cooperia*, 36% *Ostertagia* and 3% *Nematodirus*, was produced by the calves during the summer and early autumn months, then declined to 50 epg in winter months. The heifers showed the same tendencies and proportions of larvae, with a maximal mean of 145 epg, and in cows 33 epg in absence of *Nematodirus*. *Eimeria* oocysts showed a similar tendency of trichostrongylids in the three groups with exception of two calves who presented over 2000 opg in winter. *Dictyocaulus* larvae were found first in 18% of the heifers and then in 14% of the calves during spring; then 17% of the calves were positive in early autumn months. Low counts (max. 45 epg) of *Moniezia* eggs were found in the heifers and the cows, during the spring months. Larvae population on the grass increased from 120 larvae/kg dry grass (L/kg dg) in the summer to 490 L/kg dg in the autumn, coinciding with the most rainy month.
Conclusion: The observed tendencies in the parasite eggoutput were normal in subclinic bovine parasitosis. Under extensive management it is not necessary to treat with antihelminthics.

f.1.01-08 Epidemiology and control of ruminant helminths II

f.1.05 EPIDEMIOLOGY OF GASTRO-INTESTINAL NEMATODES OF CATTLE IN CENTRAL IVORY COAST.

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The objective of the present study was to obtain epidemiological information on helminths of cattle in the central region of Ivory Coast. This region has a tropical hot humid climate with temperature 19-34°C and annual rainfall 1100-1600 *mm* with two peaks in May-June and September-October. Over a one year period each month 6 N'Dama cattle obtained from extensive system were necropsied for parasitological examination. All animals were infected; 95% were infected with *Haemonchus contortus*, 50% with *Cooperia punctata*, 35.5% with *Oesophagostomum radiatum*, 30% with *Trichostrongylus axei*, 8.1% with *Cooperia pectinata* and 1.6% with *Trichuris* spp. Total number of worms/animal varied from 25 to 5203. The intensity of infection was below 1000 in 63%, 1001-2000 in 23%, 2001-3000 in 5%, 3000-5000 in 7% and over 5000 in 2% of animals. Worm burden of all species fluctuated during the course of the study with 1 to 3 peaks. Overall burden showed 2 peaks, in July and October. Faecal egg counts also showed two peaks (p=0.002). Animals below 3 years of age had more worms than those over 3 years (1642 vs 791, p=0.028). Hypobiosis was not detected in any of the nematode species which may be due to favourable conditions for development and infection throughout the year. These results show that the level of infection is low to moderate, the animals below 3 years have higher infection and therefore it is recommended that only these animals should be treated twice a year during two rainy seasons.

f.1.06 EPIDEMIOLOGY OF PARASITIC GASTROINTESTINAL NEMATODE INFECTIONS OF RUMINANTS ON SMALLHOLDER FARMS IN CENTRAL KENYA.

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ABSTRACT

To establish the infection pattern with gastrointestinal nematodes in ruminants in the Central Kenya highlands, a study was carried out in 58 smallholder farms. The study involved monthly faecal examinations from sheep, goats and cattle and pasture sampling from 8 communal grazing areas. Each month 6 Dorper worm-free tracer lambs were introduced and 4 local cross-bred sheep were purchased for parasite recovery. The mean faecal egg counts (FEC) for cattle were low throughout the study period while those for sheep and goats showed a seasonal pattern, high levels of infection occurring during the two main rainy seasons, especially in March, April and October. There were significant differences in egg counts over time and among farms. *Haemonchus contortus* was the most prevalent nematode in the tracer lambs while the previously exposed local sheep had significantly lower numbers of *H. contortus* but significantly higher numbers of *Trichostrongylus* spp. The highest levels of infection in the tracers occurred in November 1995 and January, May and June 1996. Other months were characterised by lower infections. Based on this study, two strategic treatments for sheep and goats, one in January and a second in October were recommended for the study area and for other regions with similar climatic conditions.

f.1.07 A COMPARISON BETWEEN GOATS AND SHEEP AS TRACER ANIMALS

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Background: Tracer animals are a key component of epidemiological studies examining extensive production systems. Given the limited availability of worm-free animals in some countries researchers may find it easier to use different small ruminant species as tracers. Although long term comparative studies have shown that goats tend to have higher burdens there have been no studies examining the short term acquisition of infection in the two species under tropical conditions.
Method: 12 parasite free Small East African goats (SEAG) and Dorper sheep (DS) aged 4-6 months were split into 3 groups and every month 4 animals of each species were introduced into a known *Haemonchus contortus* infected paddock to graze for a period of one month when they were withdrawn, kept in worm free conditions for 3 weeks and then sacrificed to provide worm burden data.
Results: There were no significant differences between the mature and immature populations carried by sheep and goats or in the worm load between the three months of the study. *H. contortus* infections in both species were not statistically different. The respective mean total *H. contortus* worm counts (TWC) for sheep and goats were 454.6 (SE57.8) and 562.8 (SE1.39). Very low numbers of *Trichostrongylus* spp were recorded in 4 animals.
Conclusion: The results suggested that it may be possible to use either species roadside and/or communal area tracers since the short term acquisition of infection appeared to be similar in both species. However sheep may be preferred simply because of ease of handling.

f.1.08 THE IMPACT OF GASTROINTESTINAL HELMINTHS ON PRODUCTION IN GOATS

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Background: The impact of gastrointestinal helminths on health and production of goats may be substantial. The present study estimated this impact by comparing suppressively treated and untreated goats.
Method: During the rainy season, 44 Small East African goats aged 4 -5 months were allocated to 4 groups grazing separate paddocks of similar contamination in tropical lowlands. Groups A and C were treated with albendazole po every 2 weeks while groups B and D only received individual salvage treatments. They were sampled every 2 weeks and worm counts were performed after 6 months.
Results: The treated goats gained an average of 2.3 $\pm$ 0.6 kg while controls gained 1.6 $\pm$ 0.1kg. 2 goats from the control groups died and 2 others were given salvage treatments. The maximum average egg counts for the controls was 922 epg in month of October while the treated goats had positive faecal egg counts with a maximum average of 209 epg. The average worm count for the controls was 1141 $\pm$ 968 while the treated an average of 124 $\pm$ 45. There was a significant difference in the worm counts of the controls and the treated goats. *Haemonchus contortus* was the main nematode species.
Conclusion: Gastrointestinal helminths particularly *H.contortus* cause clinical disease and mortalities in weaner goats. Anthelmintic resistant worms may have been selected during the trial.

f.2.01-08 Molecular and biochemical parasitology: systematics

f.2.01 VALID AND INVALID TAXA OF TISSUE CYST-FORMING AND RELATED COCCIDIA

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Background: Historically, all coccidia with disporous, tetrazoic oocysts were classified into the genus *Isospora*. In the 1970's, the family Sarcocystidae was defined to comprise former members of this genus that are heteroxenous and form tissue cysts in an intermediate host. Based on phenotypic characters, up to 10 genera have been placed into this family, and the family sometimes is divided into two or three subfamilies. However, these taxa have not been generally accepted and the taxonomy of tissue cyst-forming coccidia has become one of the more controversial areas of protozoan classification.
Phylogenetic analysis: Recently, phylogenetic analyses based on small and large subunit ribosomal RNA gene sequences have provided new insights into the phylogeny of coccidia, and a more robust classification is emerging from these data. The major conclusions from these analyses are: (A) The coccidia with disporous, tetrazoic oocysts are a monophyletic group, to the exclusion of coccidia with different oocyst morphologies. (B) At least two lineages exist within this group: (1) One lineage encompasses homoxenous and heteroxenous *Isospora* species and the subfamily Toxoplasmatinae. This finding refutes a classification of heteroxenous *Isospora* species into a genus *Cystoisospora* and also refutes a classification of the genus *Isospora* into the family Eimeriidae. (2) A second lineage encompasses the subfamily Sarcocystinae. Further lineages appear to exist within the Sarcocystinae, however, a division of this subfamily into the genera *Sarcocystis* and *Frenkelia* is invalid.
Conclusion: I suggest that all coccidia with disporous, tetrazoic oocysts be classified into the family Isosporidae Minchin, 1903, with a division of the family based on phenotypic as well as molecular data.

f.2.02 CHARACTERISATION OF THE PLASTID DNA OF *NEOSPORA CANINUM*

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Background: *Neospora caninum* is an obligately intracellular Apicomplexan parasite, which causes abortion and hind-limb paralysis in cattle and dogs. In beef and dairy cattle, *Neospora* is emerging as the most significant cause of abortion, with combined annual losses estimated to exceed $100 million in Australia alone. Other members of the Apicomplexa, including *Plasmodium* and *Toxoplasma*, have been found to possess a relic organelle containing a circular genome of about 35 kb. Sequence from this genome has shown that the circle shares many features with plant plastids, and it appears that the plastid carries out essential functions for the parasite.
Method: To investigate whether the NC- Liverpool strain of *N. caninum* possesses a similar genome, PCR was used to amplify known plastid genes. To elucidate the physical characteristics of the *N. caninum* genome, two types of Pulsed Field Gel Electrophoresis, CHEF and FIGE, as well as electron microscopy of the isolated circular DNA, have been employed.
Results: Our studies on the physical characterisation of the DNA of *N. caninum* confirm that the organism does possess a plastid, with physical characteristics similar to those of the plastids of *Toxoplasma* and *Eimeria*. Like that in *Toxoplasma*, the plastid in *N. caninum* appears to be circular, as shown by twisted structures seen in electron microscopy, and by its tendency to migrate anomalously during PFGE. When linearised, the *N. caninum* plastid migrates at a rate corresponding to about 35 kb in size.
Conclusion: The knowledge on the plastid of *N. caninum* generated here is likely to lead to new insights into the evolution of plastids throughout the apicomplexa, and may reveal new targets for chemotherapy against plastid genes and gene products, distinct from the host equivalents.

f.2.03 MOLECULAR CHARACTERISATION OF ASCARIDOID NEMATODES AND DETERMINATION OF THEIR GENETIC RELATIONSHIPS

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Background: There are obstacles in the morphological identification of some ascaridoid taxa to species, which has led to some taxonomic problems and consequently controversies over the classification systems for the Ascaridoidea. The aim of this study was to use a DNA approach to characterise 15 ascaridoid taxa and to determine their genetic relationships.
Methods: The rDNA region spanning the first (ITS-1) and second (ITS-2) internal transcribed spacer (ITS), and the intervening 5.8S gene was amplified and sequenced. Pairwise comparisons were made of the level of sequence differences for each spacer, the 5.8S gene, as well as for combined sequence data sets. The unweighted pair group method using arithmetic averages (UPMGA) was used to determine genetic relationships among all taxa.
Results: The lengths of the ITS-1 and ITS-2 sequences of the taxa examined were 392-500 bp and 240-386 bp, respectively, whereas the 5.8S sequence was 157 bp. For all taxa, the G+C contents for the three rDNA regions ranged from 30-53.5%. While no nucleotide variation was detected in the 5.8S gene for any taxon where multiple samples were examined, variation of 0-2.9% was detected in the ITS-1 and/or ITS-2 sequences within some taxa. The inter-taxon differences in the 5.8S gene were significantly lower than those for the ITS-1 and ITS-2. UPGMA analyses of sequence data revealed in most cases concordance with previous groupings based on morphology.
Conclusions: This study demonstrated that the ITS-1 and ITS-2 rDNA sequences provide useful genetic markers for the identification of ascaridoids. These sequences as well as the 5.8S gene could be used effectively for establishing the genetic relationships among ascaridoid taxa, which should have important implications for investigating the systematics of the Ascaridoidea. Moreover, genetic markers in the ITS-1 and the ITS-2 will also be useful for developing PCR-based tools for the diagnosis of human and animal ascaridoid infections.

f.2.04 A SINGLE, MULTIPLEX PCR FOR THE DIFFERENTIATION OF 7 GENOTYPES OF *TRICHINELLA*

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Background: To date, the genus *Trichinella* has been partitioned into 5 species and at least 2 additional unclassified genotypes, T5 and T6. The characterization of T8 as a third unknown species remains an enigma. Other than for *T. pseudospiralis*, the absence of distinguishing morphological characters within the genus has necessitated biochemical differentiation of the genotypes by isoenzymes, DNA probes, or genotype specific or random (RAPD) PCR primers. However, the need has arisen to develop a simple test that can unambiguously and reproducibly differentiate all 7 well-defined *Trichinella* genotypes currently known.
Method: DNA sequence data was generated from the ITS1, ITS2 and expansion segment V (ESV) regions of the rDNA repeat from each *Trichinella* genotype.. Five different PCR primer sets were identified which, when used simultaneously, produce a unique agarose gel banding pattern for each *Trichinella* genotype.
Results: Primer sets chosen provide unequivocal differentiation of the 7 *Trichinella* genotypes. Banding patterns for each parasite group contain no more than 2 DNA fragments where the ESV-derived primer set functions also as an internal control for PCR integrity. The diagnostic character of each DNA banding pattern was verified with multiple isolates of each genotype and the method was successfully adapted to diagnosing individual larvae using a nested multiplex PCR.
Conclusions: We have developed and verified a simple technique for unequivocal differentiation of all 7 genotypes of *Trichinella* as well as 3 distinct geographical isolates of *T. pseudospiralis* and propose this method be tested and adopted as an International standard for the diagnosis of *Trichinella* isolates.

f.2.01-08 Molecular and biochemical parasitology: systematics

f.2.05 ITS 2 SEQUENCES OF *DICTYOCAULUS* SPECIES OF RUMINANTS IN SWEDEN

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Background: The lungworm *Dictyocaulus viviparus* has been reported over the last 20-30 years from a range of ruminants. The objective of this study was to study the genetical relationship between *Dictyoculus* species from cattle, moose and roe deer in Sweden.

Method: The ITS2 region was amplified with PCR and DNA sequences were determined from nine individual worms that all came from different hosts. The sequence data obtained were aligned and compared with similar data derived from German lungworm isolates from cattle and fallow deer.

Results: Specimens of *D. viviparus*, were almost identical irrespective of their geographical origin. When the ITS2 sequence of *D. viviparus* was compared with that of lungworms from moose and roe deer, major differences were noticed. Although lungworms from these cervids had identical ITS2 sequences, they proved to be genetically different from *D. eckerti* of German fallow deer, displaying a 66.5% similarity.

Conclusion: *Alces alces* and *Capreolus capreolus* in Sweden are parasitised with a *Dictyocaulus* species that is different from *D. viviparus* and *D. eckerti*, indicating that we are dealing with a new species in moose and roe deer.

Acknowledgement: This study was supported by the Swedish Council for Forestry and Agricultural Research.

f.2.06 ISOLATION OF PARTIAL cDNAs SEX-SPECIFICALLY EXPRESSED IN *OESOPHAGOSTOMUM DENTATUM*

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Background: Sexual differentiation and maturation of dioecious parasitic helminths, the pairing of male and female worms and the subsequent production of eggs represent important processes which occur in the host. In spite of their importance, there is almost no information on the molecular aspects of these processes for parasitic nematodes of animals. In this study, we isolated and characterised sex-specifically expressed gene transcripts using the technique of RNA arbitrarily primed-polymerase chain reaction (RAP-PCR).

Method: RAP-PCR was used to compare the differences in gene expression between adult male and female worms of the porcine nodular worm, *O. dentatum*. Sequence analysis was carried out on the cloned RAP-PCR products and database searches were conducted. Northern blot analysis was carried out on selected clones to confirm sex-specific expression.

Results: At the time of preparation of this abstract, 74 different cDNA products had been identified. Of these clones, 10 where found to be sex specifically expressed (9 male, 1 female). Sequence analysis showed that 5 clones had homology to hypothetical *Caenorhabditis elegans* proteins, while 5 had no significant homology to any other sequences contained in the databases.

Conclusion: The further characterisation of these genes should have important implications for understanding sexual differentiation and development in parasitic nematodes at the molecular level, and may lead to novel approaches for parasite control.

Acknowledgment: Project funding provided by Novartis Animal Health.

f.2.07 GENETIC VARIABILITY IN *ECHINOCOCCUS MULTILOCULARIS* ASSESSED BY PCR-SSCP

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The extent and pattern of genetic variability within the genus *Echinococcus* is the topic of controversial discussion. To date, there is only limited knowledge about potential genetic polymorphism in *E. multilocularis*. However, all of our working hypotheses are principally based upon a low intraspecific genetic variation of *E. multilocularis*, in contrast to *E. granulosus*, where more variability can been found. In order to tackle the genetic variability and sequence polymorphism in *E. multilocularis*, we analyzed samples of 33 *E. multilocularis* isolates from Europe, North America and Asia by PCR-SSCP and subsequent nucleotide sequencing. In parallel, sheep, cattle and pig strains of *E. granulosus* were characterized for comparison. As target sequences, coding regions (nuclear antigen B and mitochondrial NADH dehydrogenase genes) and non-coding regions (introns of actin and homeobox containing genes) of the parasite genome were chosen. We could show that the nucleotide diversity among genotypes of *E. multilocularis* were, in average, ten times lower than among the recognized different strains of *E. granulosus*. Consequently, we suggest that the conventional classification of *E. multilocularis* in two separated strains (European and North American) should be reviewed. Taken together, the low intraspecific genetic variation in this parasite species justifies the extrapolation of our research findings with regard to host immunity obtained with geographically defined isolates to other endemic areas.

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f.2.08 EST SEQUENCING OF TWO CAT FLEA (*CTENOCEPHALIDES FELIS*) cDNA LIBRARIES

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Background: In an effort to identify novel vaccine target antigens from the cat flea, *Ctenocephalides felis*, four tissue-specific cDNA libraries were created for the purpose of expressed sequence tag (EST) sequencing.

Method: Both conventional cDNA libraries and cDNA libraries enhanced for differentially expressed tissue-specific message by suppressive subtractive hybridization PCR were generated. The libraries were constructed using mRNA from 26,000 dissected hindguts and Malpighian tubules from unfed and blood-fed adult male and female fleas, and from 4,000 dissected heads and nerve cords from unfed and blood-fed adult male and female fleas.

Results: Over 4,000 clones from the libraries generated for each tissue type were sequenced and analyzed for homology to published sequences in GenBank by BLAST. Sequences encoding proteins involved in osmoregulation and excretion, such as V-ATPase complex subunits, inorganic ion transporting proteins, and allantoinase, were identified in the hindgut/Malpighian tubule libraries. Sequences encoding proteins relating to sensory reception and neuronal functions, such as pheromone and odorant binding proteins and sequences having homology to mammalian and *Drosophila* neuronal and developmental genes, were identified in the head/nerve cord libraries. Approximately 8% of the sequences containing open reading frames of at least 50 amino acids did not have significant homology scores by BLAST.

Conclusion: Several of the clones identified by EST sequencing have been used as probes to isolate full-length coding regions from the conventional cDNA libraries. These clones are being used in Northern and Southern blots to study flea gene structure and regulation, and as templates to express recombinant protein.

f.3.01-08 Genetic resistance to parasitic infections

f.3.01 GENETIC RESISTANCE TO GASTROINTESTINAL NEMATODES OF CATTLE

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Background: Gastrointestinal nematodes remain a serious constraint on the efficient raising of cattle throughout the world. Although there are several safe and efficacious anthelmintics in widespread use, changing environmental and consumer concerns coupled with the potential for anthelmintic resistance requires that adjuncts to current control procedures be developed. One means to supplement current control programs is to use host genetics to reduce parasite transmission, thus requiring less intensive anthelmintic use.

Methods: Cattle identical across their major histocompatibility complex were secondarily bred for enhanced or diminished resistance to GI nematodes. Offspring were challenged by natural exposure over a 4 month period to pastures containing Ostertagia ostertagi, Cooperia oncophora, and Nematodirus helvetianus. Calves were extensively monitored both immunologically and parasitologically throughout the test period, and selected calves were killed to accurately assess parasite burdens.

Results: Immunity to the parasites can be manifested either as reduced numbers of worms or as reduced fecundity of the females (heritibility - 0.3). Bulls with increased risk of producing susceptible (non-responder) calves can be identified. Fecal EPG are a poor indicator of Ostertagia numbers, and in calves serum pepsinogen levels are more accurate. Genetic resistance in cattle functions differently than that reported in sheep and likely involves different immune effector mechanisms, due to the dominance of Ostertagia in this parasite-host system.

Conclusion: Host genetics can be used to substantially reduce transmission of GI nematodes in cattle.

f.3.02 GENETICS OF NEMATODE FAECAL EGG COUNT IN WEST AFRICAN N'DAMA CATTLE

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Heritability of faecal egg counts (FEC) of traditionally raised West African N'Dama cattle was estimated by offspring-dam regression. The study was conducted in the Central River Division in The Gambia with a savannah woodland type vegetation. In 1992, five monthly faecal samples were taken between June and October, and in1993, four monthly samples from July to October, ranging from 275 to 365 dams and their calves per month. After regression on age, month and year, normal scores of residual average FEC of calves were regressed against normal scores of residual average dam FEC, including herd as a random effect using restricted maximum likelihood estimation. A heritability of 0.28± 0.11 was estimated from 300 offspring-dam couples. We suggest that the heritability of faecal egg counts is rather related to the burden of hypobiotic larvae than to field infections, which confirms earlier suggestions that genetic control of egg output is most effective during periods of low parasite transmission. National breeding systems are a precondition for exploiting heritability traits. In view of the difficulties in establishing such systems in West Africa, integrated control using improved management and strategic chemotherapy remain the methods of choice to control gastrointestinal nematodes of cattle in the short term future.

f.3.03 DIRECT AND MATERNAL GENETIC EFFECTS FOR RESISTANCE TO STRONGYLES IN CREOLE GOATS

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Background: Breeding for resistance is one reliable way to control infections with gastro-intestinal strongyles (GIS) in grazing small ruminants. Economical interest of breeding depends on selection efficiency. In order to introduce or not resistance into breeding schemes of Creole goat, genetical parameters of this trait were estimated.

Methods: Genetic variability was assessed through the sire/offspring relationships in the Creole experimental flock of INRA-Gardel. Animals were naturally infected on irrigated pastures. Records of 979 kids sired from 54 bucks were analysed by a REML method, using an animal model.

Results: Estimates of direct heritabilities (standard errors) were 0.18 (0.05), 0.23 (0.05), 0.17 (0.04) and 0.33 (0.06) for fourth root transformed faecal egg counts, measured at 4, 6, 8 and 10 months of age respectively. The genetic correlations reached 0.88 (0.09) between 4 and 6 months, and 0.96 (0.05) between 8 and 10 months. Part of genetic variability was due to maternal effects. Maternal heritabilities were 0.07, 0.15, 0.03 and 0.10 respectively. A strong antagonism between direct and maternal effects was found: direct-maternal correlations varied from -0.39 to -0.53 except at 8 months of age where it was null.

Conclusion: Breeding for resistance to GIS in Creole goats is possible but maternal components of variability should be taken into account to achieve optimum genetic progress.

f.3.04 SELECTION OF TRICHOSTRONGYLID RESISTANT SHEEP IN ARGENTINE FARMS.

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Background: Increased sheep Trichostrongilids drug resistance and difficulties in field technology resources practise, encourage us to develop a selection plan based on naturally resitant animals to integrate it in a sanintary management program in Argentine north east farms. *Haemonchus contortus* and *Trichostrongylus colubriformis* are prevalent species

Method: The program was applied on a Polwarth breed population in a commercial farm with 3500 sheep, of Corrientes province, and including each year since 1995: 70-100 males and 250-400 females in a a 2-6 natural challenge and FEC evaluation for selection. Complementary faecal egg count (FEC) control in different flock and rational use of Closantel and broad spectrum drugs after resistance diagnostic, complete the management. Each year the high level of resistance group (HR) was the cuartil with better behaviour in all challenges, the worse cuartil was selected as low level of resistance (LR). Fec control was carried out with HR and LR sheep in 1st., 2nd and 3rd pregnancy and after parturition.

Results: The HR cuartil Show in challenges after weaning, median FEC between 22% and 51% of general population (GP) level. LR cuartil between 179 and 500%. When adult groups were evaluated after parturition the HR present 56% (1st parturition) and 42% (2nd and 3rd) of LR FEC level.

Conclusion: Selection of resistance is applicable, and economically possible in integrated management programs, Females must be included and professional intervention is nessesary.

Acknowledgement: Biogenesis S.A. and La Plata Univ. by economical support.

f.3.01-08 Genetic resistance to parasitic infections

f.3.05 THE SELECTION OF A RESPONSIVE LINE OF SCOTTISH CASHMERE GOATS.

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Background: The selection of multiple anthelmintic resistance in a herd of Scottish Cashmere (SC) goats led to the initiation of a research programme in 1992 examining the potential for caprine genetic selection and aspects of acquired resistance against gastrointestinal (GI) nematodes.

Method: The first two kid crops in the selection study came from 95 does mated with 3 'responder'bucks, selected from a pool of 100 male goats drawn from 12 cashmere herds. Subsequently breeding males were identified from within the selected line (SL). Yearlings from each generation of SL and unselected line (USL) kids were exposed to both natural and artificial challenge with GI nematodes to determine responsiveness. Anthelmintic disrupted challenge models using identified responder and non-responder animals have also been used to investigate the effects of sex and nutritional supplementation in pen studies on the expression of immunity.

Results: The most recently examined (F4) SL male and female yearling goats had average faecal egg counts that were 29.1 and 34.4% lower than USL yearlings. The initial estimate of heritability of responsiveness was 0.37 ± 0.18. Intact male goats were less well able to regulate GI nematodes than female goats and the expression of immunity was unaffected by supplementation with non-rumen degradable protein.

Conclusions: Genetic selection appears to offer a means of enhancing responsiveness against GI nematodes in SC goats and hence reducing reliance upon intensive chemoprophylaxis.

f.3.06 SELECTION OF SHEEP UPON THEIR RESISTANCE AVOID NEMATODE OUTBREAKS

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Background: It is possible to select sheep upon their resistance to gastro-intestinal nematode parasites by using faecal egg counts (FEC) measured after natural or experimental infection. The benefits in terms of regulation of nematode populations is poorly illustrated; a three-year survey including a very favorable year for the nematodes (1998) was conducted.

Methods: Two INRA 401 flocks of 30 sheep were selected from 200 male lambs after 2 experimental mixed infections with *Teladorsagia circumcincta* (Tcir) and *Trichostrongylus colubriformis* (Tcol). The Resistant flock R (with low FECs) and the Susceptible flock S (with high FECs) grazed separated pastures. The two species populations were assessed on pasture by the use of groups of tracer lambs grazing 3 weeks and in the rams R and S. Rams necropsied in July and November were replaced each new year after selection from a flock naturally infected, predominantly with Tcirc.

Results: Small differences were observed between Tcirc populations on R and S pastures as in R and S rams. Very few Tcol remained the 3rd year in R pasture and rams. In 1998, very high levels of the two nematode species were observed. They ranged on the S pasture from 4000 to 150 000 worms in rams and provoked the death of all tracer lambs in October. They harbored between 20000 and 99000 worms. On R pasture, populations were divided by 5 and 40 for Tcir and Tcol respectively in rams, by 1.5 and 3000 in tracer lambs.

Conclusion: Breeding sheep selected with low Tcir and Tcol FEC permitted a huge regulation of Tcol population, a lower limitation of Tcir one, but prevent outbreaks the favorable year for the nematode species.

f.3.07 COMPARATIVE STUDIES ON *OESTRUS OVIS* INFECTION IN GASTRO-INTESTINAL STRONGYLES (GIS) RESISTANT AND GIS NON RESISTANT SHEEP.

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Novel options for nematode control are compulsory in sheep production because anthelmintic resistances increase all around the world. One of these options is to exploit a natural resistance to nematodes through a selective breeding program nevertheless, the consequences of this selection on the resistance to other pathogens are widely unknown. Thus, we compared in this study the *Oestrus ovis* (sheep nasal bot fly) infection between a GIS resistant flock and a GIS non resistant flock. 35 GIS resistant sheep and 33 GIS non resistant sheep were selected, reared in two contiguous fields and exposed to *Oestrus ovis*, *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* natural infections. 25% of them were necropsied every six months at the beginning and at the end of the fly season. Whatever the slaughter period, percentages of infected animals with nematodes were similar but parasitic burdens were of course higher in GIS non resistant sheep. In contrast, *Oestrus ovis* was more prevalent ($P = 0,03$) in GIS resistant sheep (71% harboured *O. ovis* larvae) than in GIS non resistant sheep (45% only). *Oestrus ovis* larvae were slightly more numerous ($P = 0,08$) in GIS resistant sheep ($11,5 \pm 12$) than in GIS non resistant sheep ($7 \pm 6,7$). Moreover, GIS resistant sheep had i) more mucous mast cells and eosinophils in the mucosae of the upper respiratory tract and the digestive tract and ii) higher *Oestrus ovis* specific antibodies titers than GIS non resistant sheep. These first results claimed for further investigations, especially experimental infections.

f.3.08 INTEGRATED TRYPANOSOMOSIS MANAGEMENT AND DISEASE RESISTANCE TRAITS

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Decreasing efficacy of the trypanocidal drugs and difficulties of sustaining tsetse fly control, strengthen the importance of trypanotolerant livestock and the enhancement of disease resistance as option for livestock production. Continued increase in urban demand for livestock products in west Africa and recent expansion of markets are influencing breed choices towards increased size and milk production. In order to assess the influence of these conflicting trends on farmers decisions, studies of changes in breed compositions and farmers priority traits for breeding decisions were undertaken in mid-west Africa.

Ten traditionally managed cattle herds were monitored in northern Cote d'Ivoire. Breed of calves at birth, tsetse challenge and trypanocidal drugs requirements, were recorded over a ten year period with changing disease risk influenced by various interventions. There was a slow trend towards more crossbreeding between tolerant and susceptible cattle in the first years. The trend was substantially accelerated following a tsetse control operation organised by the Government.

These results combined with trypanocidal drug usage, evolution of tsetse challenge and farmers's ranking of priority traits provide a good insight on farmers strategies. They indicate the importance of considering breed types in impact assessment of trypanosomosis control.

f.4.01-08 Nutrition - parasite interactions

f.4.01 DIETARY PROTEIN AFFECTS THE PERIPARTURIENT RELAXATION OF IMMUNITY IN EWES

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Background: The periparturient relaxation (PPR) of acquired immunity may be due to an increasing amount of metabolizable protein (MP) being directed to reproductive efforts rather than to immune functions. Therefore, we studied the effects of an increased MP-intake on faecal egg counts (FEC), mucosal mast cells (MMC), and globule leucocytes (GL) in the parasitized periparturient ewe.

Method: Twin-bearing ewes, trickle infected with 6,000 L_3 *Ostertagia circumcincta*, were fed at 85% (LP; n=30) or 130% (HP; n=30) of their MP requirements (MP-req) for late gestation. Six of each group were slaughtered at lambing (d_0); the rest were fed at 85% (LL; n=12) or 130% (HL; n=12) of their MP-req for lactation, and slaughtered at either d_{21} or d_{42} (n=6). FEC were done weekly. Abomasal folds were sampled at slaughter for MMC and GL counts.

Results: The LP-ewes had higher FEC in the last three weeks of gestation ($P<0.05$) and less GL at d_0 ($P<0.10$) than did the HP-ewes. FEC increased during lactation for each diet. However, this increase was smaller for the HL ewes than for the LL ewes, especially during the second three weeks in lactation ($P<0.05$). GL were lower in lactation than at d_0. However, the HL-ewes had more GL than the LL-ewes at d_{42} but not at d_{21} ($P<0.05$). The diet did not affect MMC counts.

Conclusion: We conclude that MP-intake above MP-requirement for late gestation diminished PPR at lambing (decreased FEC and increased GL). However, the PPR did occur during lactation, though less pronounced due to MP-intake above MP-requirement for lactation in the second three weeks.

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f.4.02 EFFECTS OF INITIAL LEVEL OF MILK PRODUCTION AND DIETARY PROTEIN INTAKE ON THE COURSE OF NATURAL NEMATODE INFECTION IN DAIRY GOATS

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Background : The level of dietary protein is known to alter the establishment and the pathophysiology of digestive nematodes in sheep.On the other hand, high-producing dairy goats are less resistant and/or resilient than low-producing ones to experimental nematode infection. During a 2-year study, we have investigated the course of a natural *T. colubriformis (T.c.)* infection in a high-producing dairy goat flock.

Methods : In year 1, 50 grazing goats divided in High (HP) or Low Producer (LP) were compared from April to October for parasitological and milk parameters. In year 2, the 25 HP goats were only considered and were allocated to 2 levels of dietary protein, high level (PR+) with 372 PDI or low level (PR-) with 337 PDI. They were monitered as above.

Results : In year 1, HP goats showed a greater nematode egg output (1856 vs 1000 epg) associated with higher values in *T.c.* IgG in autumn than LP ones whereas decrease in serum phosphate concentration was similar in both groups. In year 2, PR+ goats exhibited lower but non significant egg ouput in autumn (2219 vs 2817) vs PR- ones. Moreover, milk yield and fat content were significantly higher in PR+ vs PR- in the 2nd part of the study.

Conclusion : HP goats are less resistant to nematode infection in natural conditions. Resistance and resilience of HP goats may partially be improved by a protein supplementation in the diet.

Acknowledgements : This work was funded by FAIR 3CT96-1485 project.

f.4.03 SUPPLEMENTARY FEEDING AND RESILIENCE OF BROWSING GOATS TO GI NEMATODES

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Background: Pen studies have demonstrated that host nutrition can influence host-parasite relationships but few data are available on the effect of supplementation on grazing ruminants. Two trials (rainy and dry season) were performed to study this interaction in a sub-humid tropical environment.

Method: In each trial 4 groups of 8 parasite free kids were included in a factorial design. Two groups were drenched with moxidectin every 28 days (T-S and T-NS); two were supplemented (100g/day; 74% sorghum meal:26% soyabean meal)(T-S and NT-S); one remained as a control (NT-NS). Every second week body-weight, faecal egg counts (FEC) and selected blood parameters were measured.

Results: In both trials cumulative live-weight gain was improved by supplementation ($P<0.0001$) but FEC was not affected. PCV, Hb and albumin concentration showed that the pathogenicity of GIN was less severe in the NT-S group compared to NT-NS, especially in the rainy season, when the NT-S group had fewer diarrhoea-days than NT-NS. Supplementation was not sufficient to control the negative effects of GIN at the end of the wet season. Peripheral eosinophil counts (PEC) were higher in supplemented groups.

Conclusion: Supplementary feeding increased resilience of Criollo kids against GIN infections in both seasons. This trend was more evident in the rainy season, when the kids were exposed to higher challenge. Elevated PEC suggests that supplementation also increased resistance, although this was not reflected in FEC.

f.4.04 EFFECTS OF ANTHELMINTIC TREATMENT AND FEED SUPPLEMENTATION ON GRAZING TULI WEANER- STEERS NATURALLY INFECTED WITH GASTROINTESTINAL NEMATODES

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A study was carried out to determine the epidemiology of gastrointestinal nematodes in indigenous Tuli cattle and the effect of dietary protein supplementation and anthelmintic treatment on productivity in young growing cattle. Forty steers with an average age of 18 months were divided into four groups; 1) fenbendazole (slow release bolus) and cottonseed meal (FCSM group), 2) fenbendazole (FBZ group), 3) cottonseed meal (CSM group) and 4) control (no cottonseed meal and no fenbendazole) (CON group). Performance parameters measured included worm eggs per gram of faeces (EPG), packed cell volume (PCV), albumin and live-weight gain. Results showed that faecal worm egg counts were lower and PCV was higher in the FCSM and FBZ groups than in the CSM and CON groups ($p<0.01$). Weight gains were higher in the CSM and FCSM groups than in the FBZ and CON groups ($p<0.05$). The cost benefits of anthelmintic treatment and dietary supplementation were apparent in this study. The improved growth performance of FCSM, FBZ and CSM groups reflected financial gain over the controls on termination of the study. The dominant genera of gastrointestinal nematodes on faecal culture, pasture larval counts and necropsy were *Cooperia sp* and *Haemonchus sp*. The incidences of *Trichostrongylus sp* *Oesophagostomum sp* and *Bunostomum sp* were low.

f.4.01-08 Nutrition - parasite interactions

f.4.05 INTERACTIONS OF NUTRITIONAL PROTEIN AND ANTHELMINTICS ON THE CONTROL OF NEMATODES IN SHEEP

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Background: Gastrointestinal parasitism in sheep is one of the most pervasive challenges to their health, welfare and productivity. The use of anthelmintics for the control of nematodes offers only short-term economic benefits and has resulted to drug resistance in many parasites. The aim of this study was to use animal nutrition (protein) as a tool for the control of nematodes in sheep.

Method: Sixty female lambs, naturally infected with a mixture of local nematodes, were divided into 3 similar groups A, B & C (n=20). All lambs were grazing infected pasture and additionally lambs of group B were receiving anthelmintics and those of group C nutritional protein. Faecal epg, pasture larvae and adult worm counts were carried out.

Results: Lambs of group A, B & C had 632, 334.8 & 426.1 mean epg counts, respectively and 80.8, 25.9 & 25.1 mean adult worm counts, respectively.

Conclusion: Lambs receiving nutritional protein had significantly lower faecal epg counts and worm burdens than controls and similar counts to the animals receiving anthelmintic treatments.

Acknowledgement: The work was supported by EU (DGVI) Project No PL96 1485 as part of a collaborative program between UK, France, Greece and Spain.

f.4.06 THE EFFECT OF DIETARY COMPOSITION ON THE ESTABLISHMENT OF *OESOPHAGOSTOMUM DENTATUM* IN THE GASTROINTESTINAL TRACT OF PIGS

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Background: Earlier studies have shown that diets with high levels of lignin and insoluble non-starch polysaccharides (NSP) had a profound influence on the establishment of *Oesophagostomum dentatum* in the large intestine of pigs.

Objectives: The aims of the present studies was to investigate: (1) the influence of two diets with contrasting digestibilities on *O. dentatum* establishment and (2) the effect of these two diets on already established infections.

Results: The diet with high levels of lignin and NSP offered favourable conditions for establishment of *O. dentatum* in the large intestine, whereas the diet where the carbohydrate fraction was highly degradable reduced development of *O. dentatum*.

Conclusion: The investigations suggest that there seems to be an interaction between the content of dietary carbohydrates and the establishment and the reproductive capacity of *O. dentatum* in the pig.

f.4.07 EFFECT OF *OSTERTAGIA CIRCUMCINCTA* INFECTION ON ABOMASAL MICROBES

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Background: Decreased productivity in sheep with abomasal parasites has been linked to reduced feed intake and increased survival of microbes in the less acidic abomasal contents. Loss of appetite has been attributed to the concurrent hypergastrinaemia and the greater abomasal microbial populations to raised pH. In this study, abomasal aerobic and anaerobic microbe numbers during a moderate *O. circumcincta* infection have been related to concurrent changes in pH of abomasal fluid, serum gastrin levels and to feed intake.

Methods: Abomasal pH and serum gastrin were estimated twice daily from parasite-naive sheep infected via abomasal cannulae with 150,000 exsheathed L3 and 11 days later by 100,000 sheathed L3 given intraruminally. Abomasal contents were collected anaerobically for microbial culture at 2-3 day intervals.

Results: Aerobic counts remained between 10^4-10^5 cells ml^{-1}, while anaerobic counts increased from 10^5-10^7 cells.ml^{-1} before infection to 10^{10}-10^{11} cells.ml^{-1} in parasitised sheep. Anaerobic microbes increased with pH from pH 2.5-3.5 and were approaching rumen fluid levels above pH 3.5. Food intake decreased for a variable period and was poorly correlated with serum gastrin levels (R^2 between 0.12 and 0.48, mean 0.29, $P<0.01$ or <0.001).

Conclusions: Loss of appetite was not attributable to hypergastrinaemia. Failure of rumen microbes to be lysed even at relatively low abomasal pH (3 to 4) may lead to a significant reduction in the availability of digestible protein.

Acknowledgments: We wish to thank the C. Alma Baker Trust and E.&C.Thoms Bequest for financial support.

f.4.08 COPPER OXIDE WIRE PARTICLES FOR *HAEMONCHUS* CONTROL IN GRAZING MERINO LAMBS.

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Background: The emergence of the "super resistant strain" of *Haemonchus* in Northern NSW has meant that there is an urgent need to investigate new methods of control. Recent studies in New Zealand have established that use of copper oxide wire particles (COWPs) can reduce *Haemonchus* and *Ostertagia* numbers in infected sheep. This experiment was designed to test the efficacy of COWPs under Australian grazing conditions.

Method: 40 worm-free Merino lambs were divided into 4 equal groups and allocated to separate 1 ha pasture plots. 2 groups then received 2.5g COWP (Coopers Permatrace Copper, Schering-Plough Animal Health) while the other 2 groups were untreated. From one week after COWP treatment all lambs received weekly challenge with 2000 *Haemonchus* larvae. Liveweights, PCV and faecal egg counts were recorded and 2 lambs from each group were slaughtered at weeks 4 and 6 for postmortem worm burden estimation.

Results: Faecal egg counts and worm numbers were lower in the COWP lambs and after 6 weeks of infection all untreated control lambs had to be withdrawn from experimentation due to clinical haemonchosis. Faecal egg counts in the majority of COWP lambs remained low and clinical disease did not develop prior to the termination of the experiment at 10 weeks.

Conclusion: Use of COWPs did not eliminate all worms from these lambs but lowered numbers to levels that did not produce clinical disease. Through lowering faecal egg output COWPs should reduce larval availability on pasture and hence overall worm populations.

Acknowledgment: The support of Schering-Plough Animal Health is thankfully acknowledged.

f.5.01-08 Non-conventional treatment of helminths, and product development

f.5.01 APE SELF-MEDICATION: NEW SOURCES FOR THE TREATMENT OF PARASITOSIS

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Background: Parasite resistence to modern anthelmintics is an increasingly serious problem. The study of host-parasite relationships in nature provides alternatives and important insights into dealing with this problem.

Methods: Multi-disciplinary investigation of chimpanzee behavioral strategies in the wild for the control of parasite infection was conducted.

Results: Ingestion of the medicinal plant *Vernonia amygdalina* and the non-nutritional use of bristly leaves from other species by chimpanzees was shown to reduce burdens of *Oesophagostomum* and *Bertiella* worm loads, providing temporary relief from gastrointestinal upset. In vitro pharmacological assays demonstrated antischistosomal and antimalarial activities in ingested species.

Conclusion: Use of in vivo animal models for the R&D of promising anthelmintic plants identified from these studies is the next step. Continued study of self-medication in wild primates is a promising means of providing drugs and new strategies for the treatment of parasitoses in domestic animals.

f.5.02 ANTHELMINTIC ACTIVITY OF INDIAN MULBERRY FRUIT AGAINST *HAEMONCHUS CONTORTUS* IN SHEEP

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Background: Control of gastrointestinal nematodosis using anthelmintic in small holder sheep farming in Indonesia is seldom practiced due to high price of modern synthetic anthelmintics. The present study was designed to investigate anthelmintic effect of Indian mulberry fruit (*Morinda citrifolia*), a herbal medicine, against *Haemonchus contortus* in experimentally infected sheep.

Methods: Twenty-five sheep experimentally infected with 7500 L_3 *H. contortus* were allocated into five groups of each 4 animals. On week 3 after the infection four groups were given dried Indian mulberry fruit powder per-os at single doses of 0.4, 0.6, 0.8 and 1 g^{-1} kg BW, whereas another group served as non-treated controls. Fecal samples were taken at one-week intervals during the experiment. All animal were slaughtered on week 5 after infection for post-mortem worm counting.

Results : Following the treatment faecal egg counts in the treated groups reduced by 72.5 – 82.2%. Result of the post-mortem worm counting revealed that administration dried Indian mulberry fruit powder at single doses of 0.4, 0.6, 0.8 and 1 g^{-1}reduced *H. contortus* burden in the experimentally infected animals by 73.6, 19.0, 85.9 and 88.8% respectively.

Conclusion: Indian mulberry showed a potential anthelmintic effect against *H. contortus*.

f.5.03 EVALUATION OF LOCAL MEDICINAL PLANTS FOR THEIR ANTHELMINTIC ACTIVITY

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The ethnopharmacological data from the cross-sectional study on anthelmintic use in goats, revealed that over 52% of the farmers in the traditional systems (tethering and free range) are involved in the usage of herbal plants as remedies against helminthiasis.

Three of the most commonly used herbs were selected for validation studies. The powdered components of the plant in question were subjected to ethanolic extraction in a soxhlet apparatus and another part boiled with distilled water to produce aqueous/boiled extract.

In vivo trials with the extracts revealed significant activities (up to 60% efficacy) against gastro intestinal nematode for both or one type of extract depending on the plant in question.

f.5.04 EVIDENCE FOR DIRECT ANTHELMINTIC EFFECT OF CONDENSED TANNINS

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Background: It has been suggested that condensed tannins (CT), have an effect on the parasitic burden of infected lambs. One hypothesis is that condensed tannins have a direct anthelmintic effect against gastrointestinal parasites of ruminants. We investigated the anthelmintic effect on a intestinal parasite of sheep.

Method: Twelve, castrated, naive lambs, were infected with a single dose of 20,000 L_3 of *T. colubriformis* at the start of the experiment. Four weeks later, half of the lambs were drenched with an 8% (of their food intake) CT solution (*Quebracho* tannins solution) daily for a week. The other half were the parasitised controls. Faecal and blood samples were taken regularly to estimate the establishment of parasitism. At the end of the experiment (week 5) all lambs were killed, their small intestines were removed and the worm burdens were estimated.

Results: The faecal egg counts of the lambs that were drenched with the 8% solution were half as much as the non treated lambs, from about three days after the first CT dose. A similar pattern was also seen in the worm counts. The sex ratio between male and female in both groups was similar.

Conclusion: The effects of CT's may, at least in part, reside in their 'anthelmintic' properties

f.5.01-08 Non-conventional treatment of helminths, and product development

f.5.05 COMMERCIAL DEVELOPMENT OF *DUDDINGTONIA FLAGRANS* AS A BIOLOGICAL NEMATICIDE.

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The nematode trapping fungus, *D. flagrans*, has shown great potential as a supplement to the present methods of nematode control in livestock, in that it has been repeatedly shown that daily administration of chlamydospores can prevent clinical disease and increase daily weight gain.

To turn this exciting concept into a product, however, requires a great deal of further study and expenditure. The first step is to be able to produce, formulate and stabilise spores to allow "the product" to compete with the existing, efficacious and low cost anthelminthics. Leaving aside yeast, there are very few fungi produced commercially and therefore there is no readily acessible technology for large scale production of hyphomycete fungi. With *D. flagrans* it is essential to produce chlamydospores and these are difficult, or impossible, to produce economically in liquid fermentation, the method of choice.Secondly, the product must be shown to be safe for target animals, humans and for the environment and the data submitted to regulatory authorities for approval in all countries where it is wished to sell the product. Thirdly, the fungus must be tested in various animal species, production systems and climatic conditions and recommendations developed for the products use. It is essential to find ways of administering the product on a daily basis; the ideal solution would be to develop a slow release device. In addition to these technical issues, consideration must be given to commercial matters such as patents and sales and marketing. Only if sucess is achieved with **all** these tasks, can *D. flagrans* become a commercial reality.

f.5.06 MEDICATION OF CATTLE ON RANGE: MEASUREMENT OF INDIVIDUAL ANIMAL INTAKE OF DRUG, MINERAL, SALT AND PROTEIN

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Background: Chemotherapeutic agents have been provided to range cattle in mineral/salt mix or protein supplements to control parasitic infection in grazing cattle. Product consumption is based on the intake by the herd but the individual animal consumption may be highly variable.

Methods: Cows and calves were identified by a passive radio-frequency device located in the ear. A computerized radio-frequency mineral feeder system was used to measure attendance and mineral, salt, protein and drug (fenbendazole, lasalocid) intake of individual cows and calves. Studies were conducted throughout the year in free ranging cattle or animals restricted to a limited area.

Results: Individual intake were dependent on the product, salt concentration, location of feeder, herd size, time of year and age of animal. The individual animal intake of supplements containing fenbendazole and lasalocid did not differ from animals offered non-medicated products. Uniform delivery of supplement and drugs and parasite control was achieved in some feeding programs.

Conclusion: The muliplex radio-frequency mineral feeding system can assist in designing free choice medication programs which optimizes individual animal intake. The system can be also be used to address government regulatory issues with respect to toxicity, resistance development and efficacy.

Acknowledgement: Alberta Agriculture, Food and Rural Development.

f.5.07 LARVICIDAL EFFICACY OF DIFFERENT FENBENDAZOLE FORMULATIONS IN CATTLE

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A controlled bridging study was conducted in experimentally parasitized cattle in order to demonstrate bioequivalency of paste and premix formulations of fenbendazole with the reference formulation, the 10% suspension, thus harmonizing label claims for the three formulations. Thirty-two (32) Holstein or Holstein cross steer calves with experimentally induced infections with gastro-intestinal nematodes were randomly assigned to four treatment groups, each comprising eight animals. On Day 6, after infection when the parasites would be at the 4th larval stage of development, cattle in three of the groups were treated with different formulations of fenbendazole.

All formulations were administered at a dose rate of 5.0 mg fenbendazole / kg bodyweight. Cattle in the remaining Group 4 were water treated controls. Starting 21 days after treatment, 2-3 calves from each group were euthanized daily over a three day period and their parasite burdens determined at necropsy.

All three formulations of fenbendazole significantly reduced 4th stage parasite burdens by 90% or more. Analysis of variance of the parasite burdens of the treated calves indicated that there were no significant differences between groups thus demonstrating that the paste and pellet formulations were bioequivalent with the reference suspension formulation.

f.5.08 VICH HARMONIZATION OF VETERINARY ANTHELMINTIC GUIDELINES.

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In 1996 a new Intemational body was created: the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal products (VICH). The VICH will focus on harmonizing certain registration requirements for veterinary medicinal products in the EU, US and Japan (ad hoc: Australia and New Zealand). The international harmonization of registration requirements for veterinary medical products has political and economical consequences. The reduction, or the elimination of the requirements to provide different sets of data for the marketing approvals could markedly reduce R&D and regulatory review costs and have a positive impact on the product approval time. Animal welfare will also benefit by eliminating unnecessary duplication of studies. One working group focussing on efficacy requirements for anthelmintics started in January 1996. Until now major progress has been made on the General guideline, and the Bovine, Ovine and Caprine guidelines; guidelines for equine, swine, canine, feline and poultry are in preparation. Two major sections have been identified in the General Guideline: general elements, and specific evaluation studies. The General Elements section includes: good clinical practice, evaluation of effectiveness data, types of infection and parasite strains, product equivalence, recommendations for the calculation of effectiveness, standards of effectiveness and the definition of helminth claims. The Specific Evaluation Studies section describes: dose determination, dose confirmation, field efficacy and persistent efficacy studies. An important definition in the General Guideline is the Standards of effectiveness for an anthelmintic: A compound should be declared effective in principle when effectiveness against each parasite declared on the labelling stands at 90% or above, based on calculation of geometric mean worm counts using pooled data, and there is statistically significant difference in parasite burden between control and treated animals.

Bayer Workshop: Diseases Related to Protozoa and Possibilities for Treatment

EQUINE PROTOZOAL MYELOENCEPHALITIS (EPM): MYSTERY WRAPPED IN ENIGMA.

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EPM is a multifocal progressive neurologic disease of horses caused by *Sarcocystis neurona*. Although the disease was first recognized in 1964, prevalence and/or awareness of EPM has greatly increased since the late 1980s. Serologic evidence suggests that approximately half the horses in the US are exposed to *S. neurona*; however, only a small proportion develop EPM. Because cysts apparently do not develop in horses, they are regarded as aberrant, dead-end hosts. Congruence of 18S rRNA gene sequence from wild opossum-derived sporocysts and cultured *S. neurona* merozoites have implicated the opossum *(Didelphis virginiana)* as a definitive host. Both stages of the parasite can induce encephalitis in athymic ("nude") mice or interferon γ-knock-out mice, but not in congenic immunocompetent mice. Sporocyst challenge induces anti-*S. neurona* IgG in blood and cerebrospinal fluid of previously naïve horses, followed by neurologic signs and inflammatory changes in the CNS; however, protozoa have not been found in the CNS of experimentally infected horses.

The natural intermediate host(s) for *S. neurona* are not known. Multiple lines of evidence have shown that *S. falcatula*, a well-described parasite of opossums and birds, is different than *S. neurona*.

Significant further progress in understanding this disease will involve modeling *S. neurona* infection in horses and determining risk factors which allow progression of subclinical infection to EPM.

ROLE OF ACQUIRED IMMUNITY AND NATURAL RESISTANCE ON COURSE OF *ISOSPORA SUIS* COCCIDIOSIS IN PIGLETS

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Background: Coccidiosis in nursing piglets is a clinical disease syndrome caused by the coccidium *Isospora suis* which has been found in all types of farrowing facilities and under all types of management systems.
Method: Thirty-two piglets from three litters were experimentally inoculated with 200,000 sporulated oocysts of *Isospora suis* at 3 days of age and/or rechallenged at 19 days of age or primary inoculated at 19 days of age, to compare the role of acquired immunity and natural age resistance on the course of coccidiosis. Twelve piglets were not inoculated and served as a control.
Results: Following challenge, the signs of coccidiosis characterised by clinical symptoms, oocysts shedding and weekly weights were similar to those occurred in piglets primary inoculated at 19 days of age.
Conclusion: This comparison suggests that the maturation of non-specific components of the immune system plays a more important role in resistance of neonatal piglets to *I. suis* infections than specific immune mechanisms. Prospective vaccination against *I. suis* have to override obstacles of an immature immune system. The key to sufficient means of controlling of *I. suis* coccidiosis in nursing piglets is still improved sanitation and chemotherapy with anticoccidial compounds.

EPIDEMIOLOGICAL, CLINICAL AND CONTROL INVESTIGATIONS ON FIELD PORCINE COCCIDIOSIS

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Isospora suis is a major problem in confined intensive swine farrowing units over the world. However, knowledge on field situation is scarce.
Objectives are 1-Evaluation of the effect of clinical natural coccidiosis on pre- and post-weaning zootechnical performances; 2-To elucidate kinetics of oocyst excretion in relation to clinical signs and 3-Evaluation of different dosages of toltrazuril for the field control of pre-weaning porcine coccidiosis.
Methods. Objective 1: 77 litters have been used; 56 were treated with toltrazuril (20 mg/kg piglet), and 21 are control. Litters were weighed at day 2, 7, 14 and 21 (weaning) and at day 63. Litter ADG was calculated for each interval. Objective 2: rectal samples (OPG, consistency) were collected daily from 100 piglets between days 7 and 14. Objective 3: 87 litters were used (24 litters as control). Three levels of toltrazuril (10, 20 and 30 mg/kg piglet once at 3 days of age). OPG were performed with the formaldehyde-ether centrifugation technique (Martineau GP et al., Proc. Jour. Rech. Porcine en Prance. 1994; 26: 21-26)
Results and conclusions. Coccidiosis has a marked impact on pre- and post-weaning ADG. Correct diagnosis might be improved with collection of pasty faeces between day 10 and day 12. Although larger oocyst excretion was found in early fluid diarrhoea, piglets with normal faeces had larger excretion at 14 days of age. Collection of pooled faeces samples from several piglets belonging to different litters seems to be mandatory, since great variation in oocyst excretion between littermates is seen. A single oral treatment with toltrazuril at 10, 20 or 30 mg/kg piglet at 3 days of age is sufficient to reduce significantly oocyst excretion of *I. suis* and to control coccidiosis diarrhoea.

EXPERIENCES WITH TREATMENT OF I. SUIS IN DANISH SWINE HERDS

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For many years diarrhoea in piglets has caused problems with decreased weaning weight in herds with a high level of hygiene and a good management. These cases of diarrhoea typically appear in the 2nd week of life, and experience from several herds in east Jutland shows, that in spite of several measures such as antibiotic treatment and hygiene programmes, the results of the efforts have disappointed. Faeces samples from these herds have shown a very high frequency of occurrence of *Isospora suis*.
The measures in these herds have comprised: Inserting sows in a cleaned farrowing pen - *mechanical cleaning (broom, shovel, soaking) *high pressure cleaning of pens. Steam cleaning (water > 80° C). Singeing. Liming (stamped lime). Disinfection (Stalosan, Virkon-S).
Treatments: Sulfa inj. of piglets on day 3-5-7. Sulfa/DHS/Vismuth mixture orally at diarrhoea. Sulfa inj. at diarrhoea. Amprosol for piglets + sows. Baycox 5% suspension (toltrazuril) orally on day 4.
The different traditional measures to reduce the diarrhoea in piglets in their 2nd week of life show that the effect on diarrhoea has been limited. The farmer often experienced re-occurrence of diarrhoea after a while in spite of a large effort for a long period. The disappointing effect often caused frustration among farmers and consultants. In my practice, however, we have now used Baycox 5% for 3 years in 60 - 70 herds, where the described problem has been dominant in the farrowing section. In all herds we applied for a special license to use Baycox 5%, as it has not yet been registered. Treatment with toltrazuril has been extremely efficient in these herds, which often have a very high level of hygiene and optimum management. In the period when we have used toltrazuril, we have not seen signs of resistance or decreased efficacy. These diarrhoeas are simply not a problem in these herds. After a while a few of the herds have stopped the treatment, but after a problem period they have started with toltrazuril again.

TOLTRAZURIL TREATMENT OF CYSTOISOSPOROSIS IN DOGS UNDER EXPERIMENTAL AND FIELD CONDITIONS

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Background: Coccidia of the genus *Cystoisospora* cause mild to severe diarrhoea in dogs. The effects of toltrazuril treatment on cystoisosporosis were studied under experimental and field conditions.

Methods: 24 puppies were experimentally infected each with $4x10^4$ oocysts of the *C.-ohioensis*-group. 3 groups of 6 puppies were treated 3 dpi with 10, 20 or 30 mg/kg body weight of toltrazuril suspension (5%), the remaining 6 puppies served as non-treated controls. Toltrazuril suspension or microgranulate were given once in a dose of 10 or 20 mg/kg body weight, respectively to naturally infected puppies in conventional dog breeding facilities depending on the coproscopical evidence of infection. Oocyst excretion and clinical data were recorded.

Results: Under experimental conditions the non-treated puppies excreted oocysts beginning with 6 dpi and suffered from catarrhalic to hemorrhagic diarrhoea. Until 12 dpi 4 of 6 non-treated puppies had died. Irrespective of the dose toltrazuril treatment totally suppressed oocyst excretion and no diarrhoea or other signs of disease were observed in the treated groups. Natural *Cystoisospora* infections were regularly found during the third or fourth week of age in dog breedings although not always associated with diarrhoea. A single oral application of toltrazuril abrogated oocyst shedding and the treated puppies remained generally coproscopically negative during the following 2 to 4 weeks.

Conclusions: *Cystoisospora* is pathogenic for puppies and can induce severe disease. Natural infections are common in conventional dog breeding facilities. Toltrazuril treatment is suited to control cystoisosporosis under experimental and field conditions. A single oral treatment of puppies in the third or fourth week of age is recommended.

HUMORAL IMMUNE RESPONSES AND SAFETY OF EXPERIMENTAL FORMULATIONS OF KILLED *NEOSPORA* VACCINES

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Neosporosis is the leading diagnosed cause of bovine abortion among dairy cattle. Abortion and stillbirth due to *Neospora* has been reported worldwide and causes significant economic loss to the dairy industry. Because of the lack of pharmaceutical and biological control measures for neosporosis, this study was conducted to determine the humoral immune responses and safety of experimental formulations of killed *Neospora* vaccines. Cattle procured for this study had *Neospora* titers from 40 to 160 as determined by indirect fluorescence antibody (IFA) test. All eighteen heifers were randomly assigned to three experimental groups with five animals each and one control group with three heifers. Cattle of the experimental groups were injected twice four weeks apart, with killed *Neospora* vaccine formulated with the same quantities of *Neospora* antigen but different adjuvants. One control group was not vaccinated and served as a contact control. Sera samples collected bi-weekly from these heifers showed in the IFA test that all vaccinated animals generated a detectable level of specific antibody for *Neospora* antigens, especially after the second dose of vaccine. Antibody titers to *Neospora* antigens ranged from 80 to 5120 two weeks after first dose of vaccination, and 320 to 40960 two weeks after second (booster) vaccination. The injection site reactions were recorded bi-weekly during this study and the results were satisfactory for the purpose of demonstrating safety in vaccinated cattle.

IMMUNITY TO COCCIDIOSIS IN CHICKENS AFTER TREATMENT WITH TOLTRAZURIL.

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Early experiments with toltrazuril in the control of poultry coccidiosis indicated, that therapeutic medication with this drug does not interfere with the development of immunity. Therefore medication with toltrazuril should be particularly useful also for treatment after vaccination with live vaccines where problems due to stress factors or immunovariant coccidia strains may cause a drop in bird performance. In the present floor pen studies SDS-PAGE was combined with Western blotting, IFAT and ELISA to investigate the humoral immune response in chickens after vaccination with the attenuated live vaccine Livacox® and treatment with toltrazuril, 12.5 and 25 ppm toltrazuril were administered with the drinking water and given on different days after vaccination with the live vaccine. Specific humoral immune responses were detected in serum samples collected 7 days after challenge with a serum antibody concentration > 1:250. These antibodies recognized the entire spectrum of parasite proteins on the immunoblot after SDS-PAGE. High antibody titres in the vaccinated and toltrazuril treated groups correlated with a significant increase of IgG in the ELISA assay. In addition, birds that had been immunised with a live vaccine and were shuttle-medicated with toltrazuril, were significantly protected against challenge by heterologous *Eimeria* strains and had significantly higher weight gains and lower intestinal lesion scores than immunised non treated controls. Intracellular stages of *Eimeria* which have been damaged by toltrazuril probably stimulate the immunosystem of the host for a prolonged time. Thus, the combination of vaccination and toltrazuril treatment might be an alternative approach to an improved coccidiosis control.

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g.6.01-08 Epidemiology and control of swine parasites

g.6.01 SURVEY ON ENDO- AND ECTOPARASITE INFECTIONS IN SOW FARMS IN SOUTHERN HESSEN, GERMANY

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The aim of the study was to collect representative data on the prevalence of endo- and ectoparasite infections in breeding sow farms. A sample size of 111 farms, randomly selected by a stratified modus according to the herd size, was calculated to estimate the prevalence of intestinal parasites with a precision of 5% at the 95% confidence level assuming a true prevalence of 70%. Between January and August 1997, sows (n= 2750) were examined by coproscopic methods (McMaster, SAF), visual inspection (lice) and ear scrapings (mites). Serum samples (n=2050) were analysed by ELISA techniques for antibodies to *Toxoplasma gondii* and *Sarcocystis miescheriana*.

Infections with *Oesophagostomum*, *Ascaris*, *Trichuris*, *Eimeria spp.*, *Balantidium*, *Entamoeba*, *Jodamoeba*, *Chilomastix* and *Giardia* were detected in 88%, 43%, 36%, 45%, 100%, 95%, 92%, 54% and 7% of the sow herds, respectively, and in 50%, 8%, 4%, 17%, 92%, 55%, 32%, 6% and 0.4% of the sows, respectively. Sows of only one farm harboured *Hyostrongylus*. *Haematopinus* and *Sarcoptes* infections were found in 11% and 45% of the farms. Antibodies to *T. gondii* and *S. miescheriana* were present in 2% and 29% of the sows (15% and 72% of the herds), respectively. An explorative statistical analysis of data shows only few significant correlations between parasite infections and management factors.

g.6.02 ENDOPARASITES OF SWINE IN DIFFERENT MANAGEMENT SYSTEMS

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Background: Three independent investigations were undertaken on the occurence of endoparasites in swine in Northern Germany.
Methods: In trial A, fecal samples from sows were examined for endoparasites, especially the excretion of coccidia. In trial B, fecal samples of litters from piglet production farms were investigated during the suckling period for the occurrence of *Isospora suis*. In trial C, the endoparasitic infection rates on pig fattening farms with different management systems (all-in-all-out management system or continuous fattening) were investigated over three fattening periods.
Results: A: On most farms endoparasites were found. Animals from farms with a history of *Eimeria* infections were examined during their reproductive cycle. *E. polita*, *E. debliecki*, *E. suis*, and *E. scabra* were the most common *Eimeria* spp. B: On all farms *I. suis* was found to be a common endoparasite with infection rates being highest in litters of 3-4 weeks of age. By the end of the third sampling the cumulative infection rate was 53.8 % of the litters. During the suckling period the infection rate increased from 18.6 to 37.7 %. *Isospora suis* infections were often associated with diarrhea. Above-average hygiene measures and mainly perforated pen floors seemed to lower the risk of isosporosis. C: At the beginning of the fattening period 1/3 of the samples contained stages of endoparasites, the most commonly being eggs of gastro-intestinal strongyles (*Oesophagostomum* spp., mostly *O. dentatum*). In 10.5 % of the fecal samples eggs of *Ascaris suum* were present, while *Trichuris suis* and coccidia were only rarely found. The number of farms the piglets originated from positively correlated with the helminth infection rate. Immediately after deworming all samples were free of helminth eggs. During fattening the infection rates increased again to 40.8 %, with *A. suum* being the most abundant (33.0 %). The infection rate with *A. suum* was significantly higher in groups that had been negative before treatment than in those positive for *A. suum*. The infection rate with *A. suum* at the end of fattening was significantly higher in cases of groupwise exchange systems and in older pig housings.
Conclusion: Endoparasites are still common in pigs even under intensive rearing conditions. Their occurrence varies with age and management of the animals.

g.6.03 NOSE-RINGS AND TRANSMISSION OF HELMINTH PARASITES IN OUTDOOR PIGS

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Background: The environment of outdoor reared pigs generally provides favourable conditions for parasite transmission. As excessive medical treatment is not desirable, more knowledge about epidemiology and possible alternatives for control of helminths is needed, e.g. the effect of nose-rings, which change pig behaviour from rooting to grazing.
Method: In June 1996, pigs infected with low doses of *Ascaris suum*, *Trichuris suis* and *Oesohagostomum dentatum* were turned out together with helminth-naïve pigs on three pastures for 16 weeks. The following year, helminth-naïve pigs were turned out on the contaminated pastures in May and August for 12 weeks. Both years the pigs on one of the pastures were nose-ringed. All pigs and pastures were followed parasitologically and the grass cover monitored.
Results: Pasture infectivity and transmission levels of all three parasites were low both years. The nose-rings reduced rooting considerably. However, there were no overall significant differences in the parasitological data obtained from the ringed and non-ringed pigs.
Conclusion: In the present study nose-rings did not significantly influence the uptake of infective helminth parasite stages by pigs on pasture. This may partly be explained by the way pigs utilize the pastures and by the complex host-parasite relationships.
Acknowledgement: The study was funded by The Danish National Research Foundation and The Danish Environmental Research programme.

g.6.04 AGE RELATED OCCURENCE OF *Balantidium coli* ON A DANISH PIG FARM

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Background: In faecal samples for investigation of eggs from experimental *Schistosoma japonicum* infections in pigs, cysts of the intestinal ciliate *Balantidium coli* were found. It was decided to study the prevalence and intensity of this infection in the pigs.
Method: Faeces was suspended in saline, sieved and the content washed and centrifuged, resuspended and a subsample was counted in a Sedgewick-Rafter chamber and the number expressed as cysts per gram faeces (CPG).
Results: Cysts were nearly circular. Measurements showed the range of cyst size being 47-101 µm in length and 44-91 µm in width The prevalence of *B. coli* increased from 57% in suckling pigs to 100% in pigs ≥ 4 weeks old. The mean CPG of pigs aged 12 weeks and younger were ≤ 200, whereas pigs aged 28 weeks and > 52 weeks had significantly higher counts of CPG the mean± S.D. being 2102± 913 and 865± 701 respectively.
Conclusion: *B. coli* cysts is identifiable on their great size; the diameter being 3 times or more the size of other protozoan cyst found in pig faeces. The infection was found in all age groups of pigs but at a lower prevalence rate and much lower intensity (CPG) in suckling pigs.
Acknowledgement: This work was done in corporation with J. Andreassen, L. Willingham, M. Bendixen, M.A. Nielsen and N.O. Nielsen.

g.6.01-08 Epidemiology and control of swine parasites

g.6.05 PREVALENCES OF INTESTINAL HELMINTHS IN SWINE IN RELATION TO MANAGEMENT

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A total of 826 weaners, which were given for fattening test to 4 Pig Testing Stations in Poland, were examined during 1994-1995 on the occurrence of helminths by means of faecal sampling. A questionnaire about herd variables was carried out in 82 breeding herds of swine origin in order to obtain information concerning those aspects of management practices which are of importance in relation to parasite infections.

Only 23,2% of the breeders owned herds with 25 or more sows, whereas 76,8% possessed smaller ones (5 to 24 sows per herd). All farms had solid floor with straw bedding, however only 37,8% had pens cleaned daily. Most of the herds (81,1%) were regularly treated with anthelmintics and mainly (59,7%) ivermectin was used; 18,9% of the respondents applied drugs randomly. Weaning age of 5-6 weeks was practiced in 29,1% of the herds, whilst the age of 7-8 weeks in the remaining 70,9%. Disinfection at least once a year was realized in 97,7% of the observed herds.

The occurrence of four helminths genera was recorded: *Ascaris suum*, *Oesophagostomum* spp., *Trichocephalus suis*, and *Strongyloides ransomi*, with the prevalences of 13,4%, 10,2%, 1,6% and 1,5%, respectively. In general, the occurrence of helminths was 22,5% and the mean intensity equalled to 1230,4 EPG (SD=3294,7). The greatest difference in helminth prevalences related to the herd size, with the higher incidence of helminths in small herds (26,5% vs 16,4%), and to the anthelmintic regimen (21,0% in herds with the regular use of anthelmintic vs 34,9% in those with random or no treatment). In both cases the differences referred mainly to *A. suum* infection.

g.6.06 TRANSMISSION RATES OF *ASCARIS SUUM* IN PIGS ON CONTAMINATED PASTURES

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Background: Infection of pigs with *Ascaris suum* results in small overdispersed adult worm burdens that are only partly dependent on transmission rates/inoculation doses. Little information exists about the range of natural transmission or whether experimental inoculation doses reflect reality.

Method: In 1995, pigs contaminated a pasture with *A.suum* eggs. In May 1996, the pasture was divided into 'house' (without floor), 'feeding area', 'pasture close to the house' and 'pasture distant to the house'. Groups of 10 helminth-free pigs were grazing each area for 7 days and necropsied 10 days later. This protocol provides an intestinal recovery of ~50%. The study was repeated in May 1997.

Results: In 1996, the mean worm burdens were 1406, 59, 7 and <1 larvae for the 4 areas, respectively, while the comparable 1997 figures were 451, 1343, 3985, and 3362 larvae. Thus, while especially the house was strongly infective in 1996, the pastures were most infective in 1997. These differences were highly significant, but unexplainable. A mean worm burden of approx. 3500 larvae for the two pasture areas in 1997 corresponds to a mean establishment of 500 larvae per day, i.e. a daily uptake of ~1000 infective eggs (the highest individual transmission rate was 3200 eggs daily).

Conclusion: Transmission rates of *A.suum* on pastures may be very high, and the infective eggs are unevenly distributed.

Acknowledgement: The study was supported by the Danish Environmental Research Programme.

g.6.07 THE INFLUENCE OF STOCKING RATE ON HELMINTH TRANSMISSION IN OUTDOOR PIGS

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Background: Higher demands for outdoor pig products necessitate investigation into helminth epidemiology and factors influencing their transmission, e.g. stocking rate (SR).

Method: Pigs infected with low doses of *Oesophagostomum dentatum*, *Ascaris suum* and *Trichuris suis* were turned out in June 1996 together with helminth-naïve pigs on each of 4 pastures with 3 different SR. In October, pasture infectivity was measured using helminth-naïve tracer pigs. In 1997, new groups of pigs were turned out in May and August, respectively. Both years pigs and pastures were followed parasitologically and the grass cover was recorded.

Results: In 1996, *O. dentatum* infection levels were significantly higher on the high SR pasture compared to the other pastures. The grass cover was reduced proportional to SR, however, except for the highest SR, pigs did not utilize the entire area. Transmission of *A. suum* and *T. suis* was not significantly influenced by SR. Examination of grass and soil samples did not reveal differences in pasture infectivity.

Conclusion: The results indicate that outdoor transmission of pig helminths may be less influenced by SR than observed in many ruminant trichostrongyle infection studies.

Acknowledgement: The study was funded by The Danish National Research Foundation and The Danish Environmental Research Programme.

g.6.08 THE PREVALENCE OF SWINE PARASITES AND THE EFFICACY OF SOME DRUGS IN KOREA

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Background: The swine industry is very important and some parasites are important for that in Korea. Coccidiosis, *Ascaris suum*, *Trichuris suis*, *Balantidium coli* and ectoparasites are some popular in the swine farms, so that we investigate the prevalence of swine parasites.

Methods: We investigated the swine parasites by flotation method of feces and scraping method of ectoparasites. And then we investigated the drug efficacy for them.

Results: In the survey of swine parasites in Korea, total parasites infection rate was 15.8% of 8,429 pigs. The infection rates of coccidia, *A suum*, *T suis* and *B coli* were 7.2, 6.5, 1.2 and 1.1%, respectively. Those of sow, growing pigs and piglets were 28.5, 16.2 and 15.0%, respectively at the 1st year (1996) of experimental period. Total parasites infection rate was 8.7% of 4,716 pigs. The infection rates of *A suum*, coccidia, *B coli* and ectoparasites were 4.8, 1.4, 1.4 and 1.0%, respectively. Those of sow, growing pigs and piglets were 20.1, 7.4 and 9.0%, respectively at the 2nd year(1997) of experimental period. Total parasites infection rate was 9.7% of 1,838 pigs. The infection rates of *A suum*, coccidia, *B coli* and ectoparasites were 3.8, 2.7, 1.6 and 1.6%, respectively. Those of sow, growing pigs and piglets were 11.6, 9.4 and 7.7%, respectively at the 3rd year(1998) of experimental period.

In the experiment of drug efficacy, almost of all the drugs had a good effect on *A suum* and *T suis*. The drug I and D had very good effective to *A suum*, *T suis* and ectoparasites. In lactating piglets, *Isospora* coccidiosis is some problem. Drug B was very effective to swine *Isospora* coccidiosis. The body weight gain and diarrhea were improved in the lactating piglets treated with Drug B.

Conclusion: The prevalences of swine parasites in this studies were lower than those of previous studies, because the feed additive anti- parasitic drugs were used in the growing pigs. If the effective drugs were frequently used in sows and piglets, the prevalence of swine parasites would be reduced quickly.

g.6.09-22 Epidemiology and control of ruminant helminths in temperate climates

g.6.09 NEMATODE PARASITES OF ADULT DAIRY CATTLE IN BELGIUM.

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Over a period of one year, from November 1997 to October 1998, the abomasa, blood and faecal samples of 121 dairy cows in Belgium were collected and examined for nematode infections. Coprocultures of 73 animals were positive and the genera recovered were *Ostertagia* (100%), *Trichostrongylus* (42%), *Oesophagostomum* (32%), *Haemonchus* (29%) and *Cooperia* (16%). Nematodes were present in the abomasa of 110 animals. *Ostertagia* was found in all 110, *Trichostrongylus* was seen in 65 abomasa and *Haemonchus* in 14. Overall, ninety-one percent of all trichostrongyles recovered were *Ostertagia.* The geometric mean total number of *Ostertagia* was 2750, with an average of 45% inhibited larvae (EL4). Between November and Februari >85% of the *Ostertagia* worm burden were EL4 stages. Ninety-two cows (64%) had a low to moderate *Ostertagia* burden (100-10,000) and 18 cows (16%) a high worm burden (> 10,000). The highest number of worms was 58,700 with 98% EL4 stages. A seasonal pattern was evident for serum *Ostertagia* –specific antibodies and for serum pepsinogen concentration, with the highest levels during the grazing season, and low values during the housing period. *Dictyocaulus viviparus* specific antibodies were detected in the serum of 8 (7%) animals.

g.6.10 CONTROL OF NEMATODES IN FIRST SEASON GRAZING CALVES WITH EPRINOMECTIN

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A study was carried out in Belgium to evaluate the effects of strategic early-season treatments with eprinomectin on first-season grazing calves exposed to nematode infections on a naturally contaminated pasture. Two groups of first grazing season calves were turned out in mid May on 2 plots that were similar with respect to size and herbage infectivity. They grazed separately until housing at the end of October. One of these groups was given eprinomectin pour-on at turnout and 8 weeks later, while the other group served as untreated controls. The epidemiological pattern in this study was that of a low to moderate gastrointestinal strongyle infection. The results showed that the treatments reduced gastrointestinal strongyle infections throughout the season, as evidenced by lower faecal egg counts and serum pepsinogen levels compared with the controls. Furthermore, the results of herbage larval counts and post-mortem worm counts in tracer animals demonstrated that the treatment had successfully suppressed herbage infectivity on the 'treated' plot. Lungworm infection was detected in some animals of both groups. The chemoprophylactic treated calves had a better weight gain of 20 kg during the study than the untreated controls.

g.6.11 MONITORING GASTROINTESTINAL NEMATODE INFECTIONS IN CATTLE

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Background: A wide variety of parameters can be applied to monitor gastrointestinal nematode infections in cattle. The aim of this paper is to discuss the relevance of these parameters.

Discussion: To evaluate the relevance of parameters it is necessary to define what is meant with 'infections'. When this only implies worm numbers which are present at a certain time the best, though impracticle, parameter is obviously worm counts from a sufficient number of animals. As an alternative faecal egg counts correlate fairly well with *Cooperia* spp. numbers and serum pepsinogen values with *Ostertagia* spp. numbers in young cattle. However, for herd health monitoring measuring the level of immunity is much more important than actual worm numbers. Unfortunately, measuring immunity against nematode infections is not yet possible but immunity levels will somehow relate with exposure to these infections. Cumulative pasture larval counts and sequential tracer worm counts, preferably combined with sentinel worm counts, would be the most direct, but impracticle, parameters to measure exposure. A correlation between exposure and faecal egg counts is rapidly lost during the first grazing season. As a consequence molecular techniques which have been developed to differentiate eggs to species level in cattle faeces will not be a suitable method to monitor nematode infections. The best parameters at the moment are serum pepsinogen values and an ELISA using a specific recombinant *Cooperia oncophora* protein. However, additional data on the results of these tests and the level of resilience of cattle against the pathogenic effect of the nematodes are needed before these parameters can be implemented for herd health monitoring.

g.6.12 PREVALENCE OF INFECTION WITH *TOXOCARA CANIS* IN DOGS IN THE WARSZAWA AREA

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Toxocara canis is recorded as common dogs' parasite in the world. The humans, especially children are paratenic hosts with visceral larva migrans (VLM) or ocular larva migrans (OLM) syndrome present.

The evaluation of *Toxocara canis* infection in stray dogs from two shelters and and house dogs kept as pets was the aim of this study. In 1998 samples from 266 dogs in two shelters and from 234 dogs in private houses were examined. The number of *Toxocara canis* eggs per gram of faeces was estimated using flotation method in sodium chloride. The homeless dogs were found more infected than those kept as pets. *T. canis* was recorded in 3.4% and 8.8% stray dogs from the shelters and in 0.4% animals from flats.

The higher prevalence of infection in homeless dogs was due to high density of dogs' population, worse environmental condition and irregular anthelmintic treatment in the shelters.

g.6.09-22 Epidemiology and control of ruminant helminths in temperate climates

g.6.13 INTESTINAL STRONGYLE INFECTIONS IN SHEEP IN THE HANKAVAN MOLYBDENUM BIOGEOCHEMICAL PROVINCE (ARMENIA)

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One of the problems in the geochemical ecology having a practical significance is the study of animal diseases under conditions of the deficiency or excess of microelements in the geochemical environment. The experiments showed that trace elements influence the host-parasite relationship, decrease the parasitic burden and are effective in the therapy of helminthosis (Davtian Edw., Biological Journal of Armenia, 1977, #4, p.3-12). The objective of our study was to investigate the naturally infected with intestinal strongyles in sheep in the Hankavan molybdenum biogeochemical province (Kotayk District). Here the composition of molybdenum in the soil, water and herbage is exceeding the norm at 10-100 times. The control was the sheep from Norashen farm, situated as well as Hankavan in the mountain-forest landscape zone in the neighboring Tavush District. In July-August the sheep were necropsied and adult strongyles were collected from the intestines. The results of investigation show that in the control sheep the parasite burden is more than in the sheep from Hankavan (see Table). These investigations will be continued, that's why the resistance of parasitic helminths to anthelmintics is an increasing problem (Boray J. C., Rolfe P. F., IX International Congress of parasitology, 1994, Abs. vol.1, p.27) and trace elements may be used as an alternative to the anthelmintics in the therapy of *helminthosis*.

TABLE

The site of investigation	Chabertia ovina			Bunostomum trigonocephalum			Oesophagostomum (H.) venulosum		
	Prevalence %	Range, specimens	Mean intensity, specim.	Prevalence %	Range, specimens	Mean intensity, specim.	Prevalence %	Range, specimens	Mean intensity, specim.
Hankavan	54.5	1-8	3	36.3	1-2	1.5	-	-	-
Norashen	22.2	1-59	30	44.4	3-577	209	22.2	1.45	23

g.6.14 SHEEP GASTROINTESTINAL STRONGYLATOSES IN ESTONIA

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Background: Gastrointestinal strongylatoses are the most spread parasitic sheep diseases in Estonia. Every year during 1984—1998 about 200—1200 sheep have been coprologically investigated. The purpose of this work was to estimate the viability of strongylate larvae on pastures in the climatic conditions of Estonia.
Method: Nematode-free lambs born from ewes keeping indoor conditions during the winter period, allowed under separate grazing during the pasture period. In 3 trials the lambs were grazed on the pastures contaminated with parasites of the previous year. The lambs of the 4th trial were grazed on pastures not contaminated with strongylate larvae. All lambs examined coprologically once a week.
Results: According to our data, the lambs of trials 1—3 began to pass strongylate eggs at 1—2 months after the beginning of grazing period. The mean extent of invasion was below 10%. The lambs of the 4th trial did not pass strongylate eggs during the whole time of observations (5 months).
Conclusion: The results of our trials and observations show that in spite of relatively low degree of pasture contamination, the pastures contaminated with strongylate larvae of the previous year represent natural invasion sources next spring. According to our hypotheses, the strongylate larvae can be transferred to deep soil layers by earthworms and there can be remained viable during whole winter period.

g.6.15 THE PATHOGENESIS AND CONTROL OF DIARRHOEA AND BREECH SOILING ADULT MERINO SHEEP

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Background: Diarrhoea and soiling of the breech with faeces ('winter scours') is a serious problem of adult Merino sheep grazing improved pastures in south-eastern Australia. This occurs even on farms where gastro-intestinal nematodes are effectively controlled.
Method: Observational and pathological studies were conducted on 3 farms over 2 years. Comparisons were made between the following groups of 2-years-old ewes: 1) those with and without treatment with controlled-release anthelmintic capsules, and 2) untreated ewes with and without severe breech soiling.
Results: It was shown that winter scours was associated with the ingestion of trichostrongylid larvae, and host factors were important in determining susceptibility to this syndrome. Affected sheep had a hypersensitive inflammatory reaction in the pylorus and upper jejunum, characterised by the infiltration of significantly more eosinophils and changed lymphocyte populations. The latter included reduced numbers of $CD8^+$ cells, an increased $CD4^+$:$CD8^+$ T-cell ratio, and significantly reduced numbers of cells reacting to interferon-gamma (IFN-γ). High doses of infective larvae (20,000/week of *O. circumcincta* and *T. vitrinus*) did not induce diarrhoea in sheep not susceptible to winter scours. In contrast, only low doses (2000/week) initiated scouring in sheep selected as being susceptible.
Conclusion: Considerably improved worm control programs, including the selection of sheep with increased resistance to gut nematodes, will not prevent breech soiling. Rather, removing the sheep susceptible to the inflammatory response, by culling and genetic selection, is the best long-term control strategy.

g.6.16 *NEMATODIRUS BATTUS* - A POTENTIAL PROBLEM TO ORGANIC SHEEP FARMING IN SWEDEN

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Background and method: In a coprological survey in 1998 of parasites of sheep from organic farms throughout Sweden, eggs of the intestinal nematode parasite *Nematodirus battus* were demonstrated for the fist time in this country. The survey comprised 100 flocks and included approx. 3000 faecal samples. At a follow-up post mortem examination of lambs adult worms of *N. battus* were identified.
Results: *N. battus* was found in 3 sheep flocks located in the south-western part of Sweden more than 200 km apart. No primary contact between the flocks could be established. One of the farms also raised cattle of which the calves were found to be infected with *N. battus*. On this farm annual rotation of sheep and cattle on the pastures had been applied for a few years.
Conclusion: Before rotation of pastures between sheep and cattle is recommended as a strategy to control parasites on organic farms, freedom of *N. battus* has to be demonstrated in both flocks although sheep is the most susceptible host.
Acknowledgment: The study was financially supported by the Swedish Board of Agriculture.

g.6.09-22 Epidemiology and control of ruminant helminths in temperate climates

g.6.17 ROLE OF DOMESTIC ANIMALS IN DISSEMINATION OF COXIELLOSIS IN CENTRAL EUROPE

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Manifestation of coxiellosis (causative agent Coxiella burnetii, C.b.) in humans is always associated with infected domestic animals, their products and discharges. The infection is spread predominantly by aerogene route. Parturient and aborting animals eliminating C.b. in high numbers into environment represent the main source of infection. Coxiellosis is ascertained by detection of the agent in spleen, placenta and milk and antibody response in incriminated animals.

Cattle play the main role in dissemination of coxiellosis in Central Europe. However, this way of C. b. spreading may result in attenuation of its virulence which leads to silent infection demonstrated by antibodies only. This way confirmed C. b. isolates from cow milk and antibody presence in cattle, namely in Czech and Slovak Republics. At present, other domestic animals seem to be outsiders in C. b. ecology in Central Europe, though their import, when animals infected, may lead to the introduction of highly virulent C. b. strains from abroad as found in 1993 in Slovakia in epidemic flared via infected goats from Bulgaria.

The most probable way of C. b. dissemination inside of Central European countries is the veterinary uncontrolled movement and transport of cattle from coxiellosis contaminated to coxiellosis free areas as demonstrated namely in Slovakia. For this reason the veterinary control (screening of antibodies, detection of C. b. in milk) of movement and transport of domestic animals into and within these countries is recommended.

g.6.18 STUDIES ON THE SEASONAL DYNAMICS OF *FASCIOLA HEPATICA* IN SNAILS USING GENE PROBES

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A 114 bp pepetitive DNA fragment was used as *Fasciola hepatica*-specific gene probe to examine the occurence of liver fluke developmental stages in the intermediate host snail *Lymnaea truncatula*. Specificity of the gene probe was examined using different trematodes, snails and specimens of the genus *Fasciola* from different origins (Africa, Australia, Cuba, China, USA, Europe). Four cattle or sheep farms in Northern Germany with a proven history of *Fasciola hepatica* were examined between May and November. Snails was collected on each site and examined for developmental stages of *F. hepatica* using the gene probe. A mean percentage of 9.7 % of the examined snails was positive for *F. hepatica*. Highest infection rates were found in May (20.2 %) and August (15.6 %). No correlation between size of the snails and infection rate could be found.

g.6.19 EPIDEMIOLOGY OF GASTROINTESTINAL PARASITISM OF SHEEP AND GOATS.

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Background: Greece has the highest number of sheep and goats (animals which are known to be highly infected with gi-nematodes) per head of population in Europe. The objective of this survey, which lasted a year and took place in two selected areas of Central and Northern Greece, was the collection of information regarding the extent of gi parasitism, the parasitic species involved and the seasonal effects on parasitic intra-populations dynamics. **Materials and Methods:** A total of 4 flocks (2 of sheep and 2 of goats), were used in both areas. The study was consisted of faecal and post-morten examinations. Faecal samples were collected monthly from the rectum of 30 animals of each flock and examined using the mod. McMaster technique. Coprocultures were also performed. Additionally, a total of 96 animals were serially euthanised (2 animals/month/flock) and their gi-tracts were examined for the presence of nematodes. For this, standard necropsy procedures were followed. **Results:** The parasitic genera found were *Teladorsagia, Haemonchus, Trichostrongylus, Cooperia, Nematodirus, Bunostomum, Oesophagostomum, Trichuris* and *Chabertia.* The most commonly found genus was *Teladorsagia* and the rarest were *Oesophagostomum* and *Chabertia.* Both animal species were infected thoughout the year with parasites mainly *Teladorsagia, Haemonchus, Trichostrongylus* and *Chabertia.* Regarding the faecal egg output pattern thoughout the year it seems to be more or less similar to both species, although sheep expelled much more eggs. On the contrary, goats had higher burdens of adult worms than sheep, i.e. the numbers of *Teladorsagia* and Chabertia in goats were found to be 4 and 5 times more than in sheep respectively. **Acknowledgement:** This work was supported by EU (DG VI) project no PL 96-1485 as part of a collaborative program between the U.K., France, Greece and Spain.

g.6.20 LARVAL PENTASTOMIASIS IN SMALL RUMINANTS

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Background: Larval pentastomiasis is caused by the larval stages of *Linguatula serrata.* The adult, is a parasite that occurs in the nasal and respiratory passages of the dog and other canids. The eggs of *L. serrata* are ingested by small ruminants (intermediate hosts), hatch in the alimentary canal, and the larvae migrate to the mesenteric lymph nodes, in which they develop to the infective nymphal stage. This parasite has been known to exist in sheep and goats in Greece, causing quite important economics losses to animal production but without ever knowing the real extent of it. It is worth mentioned that, in living animals it cannot be diagnosed and it is not treatable. This survey was carried out in order to determine the prevalence of this disease in a sample of sheep and goats. **Methods:** 291 animals (144 sheep and 147 goats) slaughtered at 6 different abattoirs in N. Greece were examined. The intestinal lymph nodes and liver of each animal was obtained and examined. The parasites, where found, were collected and counted. From the areas found infected, faecal samples were taken from a number of dogs and examined with a sedimentation technique. **Results:** The prevalence of infection based on necropsy findings was 2.1% for sheep and 43.5% for goats. The parasitic burdens found were ranged from 10 to 30 nymphs/animal for sheep and from 10 to 460 nymphs/animal for goats. A number of small round lesions was a common finding on the liver surface of the infected animals. The faecal examinations failed to reveal any *L. serrata* eggs. **Conclusions:** From the samples examined it is shown that the infection was almost restricted to goats. The infected sheep were limited having also very low numbers of parasites per infected animal. Could it be that goats are more sensitive to this parasitism?

g.6.09-22 Epidemiology and control of ruminant helminths in temperate climates
g.6.23-33 Epidemiology and control of ruminant helminths in the subtropics and tropics

g.6.21 SEASONAL VARIATION OF GASTROINTESTINAL NEMATODES OF SHEEP IN THE REGION OF JOANNINA, GREECE

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Background: Epidemiological studies of gastrointestinal parasites in naturally infected sheep in Greece have not been carried out previously. The aim of the present study was to investigate the seasonal variation of gastrointestinal parasites in naturally infected sheep, and the effectiveness of anthelmintic treatment as applied by farmers under local management practices in the region of Joannina, which is an important livestock production area in NW Greece.

Method: Parasitological and growth studies on two groups of naturally infected sheep, with or without anthelmintic treatment, from the age of 3 months to 2 years were carried out. A split-plot design was used so that each group, consisting of seven pure-bred Butsiko (mountain type) and seven cross-bred Butsiko with Karamaniko (low plain type) (F_1) lambs, grazed separate parasitologically equivalent pasture plots. Faecal egg counts, pasture larval counts, plasma pepsinogen levels and liveweight were recorded monthly.

Results: Infective larvae on each pasture plot increased during autumn and winter. Mean faecal egg counts for strongyle-type eggs were higher in the non-treated than the treated group and in the cross than the pure-bred sheep. Mean plasma pepsinogen levels were higher during autumn of the second year.

Conclusion: The factors affecting the epidemiology of gastrointestinal nematodes of naturally infected grazing sheep in the region of Joannina include anthelmintic treatment, host genotype and season, while the effectiveness of anthelmintic treatment, as applied in the area, is questionable.

g.6.22 HELMINTHOFAUNA IN RUMINANTS IN UPLAND AREAS OF BOSNIA AND HERZEGOVINA

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The ruminants (sheep and cattle), due to the intensive keeping throughout the grazing season are daily exposed to invasions by various species of endo-parasites. Each region has different climate conditions, keeping and feeding of animals, so that each region has a specific environment requiring other than uniform measures for the control and prevention of economically important parasitoses in ruminants.

As a matter of fact, no comprehensive investigations, especially of endohelminths were carried out up to date. The objective of the investigation was: On the basis of the results of parasitologic sections and laboratory analysis of feces to make an inventory of helminthofauna in sheep and cattle in the investigated upland areas in Bosnia-Herzegovina; to establish the extensiveness and intensity, with respect to the age and breed of the ruminants.

Methods: During the 4 years investigations performed were complete parasitologic sections on 5.805 ruminants (1.908 sheep and 3.897 cattle) from 29 localities in this country. With the aim of getting better insight into helminthofauna in ruminants in Bosnia and Herzegovina collected were 19.291 samples of feces from sheep and cattle of 28 localities. The animals belonged to different age groups. For endoparasites examination standard parasitologic methods were used: the method after Whitlock, flotation after Filleborn and sedimentation.

Results: A total number of detected species of endoparasites is 46. The most frequently encountered were the species: of Trematoda class: Dicrocoelium dendriticum 28,0-76,25%, Fasciola hepatica 37,1-67,7% and Paramphistomum spp.24,5-64,1%, of Eucestoda class larval forms: Cysticercus tenuicolis 17,0% and Echinococcus polymorphus 23,3-27,7%, and of Nematoda class stomach-intestinal trihostrongylata with total extensiveness of ruminants is 74,0-94,2% for cattle. Of this class the most common were the species from the genera of: Trichostrongylus, Ostertagia, Cooperia, Nematodirus and Haemonchus. Coprologic examination of 19.291 samples from sheep and cattle feces showed that 71,0-88,4% of them were positive. The most common were species of Nematoda class for both kinds of animals.

Conclusion: Of the total number of examined ruminants, invaded by endoparasites were 5.084 or 87,6% animals. The number of positive sheep was 1.412 or 74,0%, and that of cattle was 3.672 or 94,2% animals. A total of 46 species of endoparasites were identified, belonging to 22 genera, 15 families and 3 classes.

g.6.23 SEASONAL VARIATION OF *Boophilus decoloratus* TICKS FROM FOUR COMMUNAL GRAZING AREAS IN SOUTH AFRICA

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Background: *Boophilus decoloratus* and *Boophilus microplus* are the two most important "blue ticks" found on cattle in South Africa. The distribution of these ticks is limited by rainfall and the degree of tick control. In drought conditions, and with intensive dipping, the *Boophilus* populations are severely restricted, but with good rains and poor dipping management, the populations expand.

Method: Four communal grazing areas (CGA) in South Africa, three in North West Province (Rietgat, Madinyane and Bethany), and one in Mpumalanga Province (Geluk), were visited during a two-year project (1991 - 1993). The cattle were cast with ropes and 13 different anatomical sites sampled. The goats were restrained on the kraal floor and similar sites sampled. Free-living ticks were also collected with a flannelette apparatus.

Results: A total of 16 128 ticks were collected off cattle during the project, and 974 (6%) were *B. decoloratus* and 16 (0,09%) were *B. microplus*. A peak abundance of *B. decoloratus* was recorded in the summer of 1991 and 1993. In 1992, very low numbers of blue ticks were collected at Rietgat and Madinyane, due to a severe drought. Very few *B decoloratus* ticks were collected off the goats (total n = 17), or off larval drags (average one larval tick per drag). In the CGA where dipping had been reduced (Geluk), *B. decoloratus* populations increased sharply in 1993.

Conclusion: The very low and erratic populations of *B. decoloratus* found on cattle in these CGA should have resulted in a serologically unstable situation, especially after the drought of 1992. This was not the case, and in most of the CGA, large changes in the serological status of the cattle were recorded without clinical diseases. Antibodies to *B. bovis* were also recorded in all four CGA, despite the very low numbers of B. microplus..

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g.6.24 THE DISTRIBUTION OF NEMATODE EGG COUNTS IN GRAZING GOATS AND ITS IMPLICATIONS FOR HELMINTHS CONTROL

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The frequency distribution of nematode egg counts in 246 naturally infected Small East African (SEA) goats was studied in Morogoro. Animals were grazed on dominantly *Haemonchus contortus* contaminated pastures at Sokoine University of Agriculture. Faecal samples from all goats were taken at different months from 1996 to 1998 basing on the epidemiology of gastrointestinal nematodes in the study area. Faecal egg counts from each goat was determined by the modified McMaster method. Results showed that the majority of goats had low faecal egg counts (FEC) at all sampling times. The frequency distribution of FEC followed the negative binomial pattern with the variance to the mean ratio greater than unity ($S^2/\bar{x} >> 1$) and the negative binomial parameter below unity ($k << 1$). The results suggested highly over dispersed worm burdens in goats and that a relatively small part of the herd was responsible for contamination of pasture with worm eggs. It is concluded that effective and economical control of parasites can be achieved by eliminating animals with high FEC ('wormy' animals) in the herd either through selective breeding or selective anthelmintic treatment as opposed to mass treatment.

g.6.23-33 Epidemiology and control of ruminant helminths in the subtropics and tropics

g.6.25 EPIDEMIOLOGY OF GASTRO-INTESTINAL NEMATODES OF SHEEP IN CENTRAL COTE D'IVOIRE

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From August 1994 to July 1996, a total of 142 randomly selected sheep from the central area of Côte d'Ivoire, were examined at post mortem, to study the epidemiology of gastro-intestinal parasites. Each month six animals, three of Djallonke and three of Sahelian breed were necropsied, parasites collected and examined. All the animals were infected. The spectrum of parasites consisted of 15 helminth species, 11 nematodes, 3 cestodes (*Moniezia expansa, M. benedeni, Cysticercus tenuicollis*) and 1 trematode (*Paramphistomum* spp). The prevalence of nematodes was: *Haemonchus contortus* (77.4%) *Trichostrongylus colubriformis* (76.7%), *Strongyloides papillosus* (61.2%), *Oesophagostomum columbianum* and *Gaigeria pachyscelis* (24.6%),*Cooperia curticei* (23.9 %) and *Gongylonema pulchrum* (0.7 %). *G.pulchrum* is found for the first time in West Africa. There was no significant difference between the worm burden of two breeds. Season, sex and age had no significant effect on the infection. Hypobiosis was not detected. It is concluded that the infection level is low to moderate and persists throughout the year in sheep on extensive system in Central Côte d'Ivoire.

g.6.26 EPIDEMIOLOGICAL STUDY OF NEMATODE PARASITES IN DAIRY CATTLE IN SOUTHEAST BRAZIL.

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Tracer calves were used to assess the seasonality of infections of gastrointestinal parasites in dairy cattle raised at a farm in the State of Minas Gerais- Brazil. Tracer calves acquired infections during all months of the year, however, highest worm burdens were observed at beginning and at of the end the rainy season. The following nematode species were recovered: *Cooperia punctata, C. spatulata, C. pectinata, Haemonchus similis, H. placei, Oesophagostomum radiatum, Trichostrongylus colubriformis, T. axei, Bunostomum phlebotomum, Dictyocaulus viviparus and Trichuris discolor. Cooperia* was most prevalent, followed by *Haemonchus Oesophagostomum, Dictyocaulus, Trichostrongylus, Trichuris and Bunostomum.* The recovery of *Trichuris* was greater in the dry season. 40 Zebu x Holstein calves calves were used to evaluate the dynamics of the infections from first ,up to the 26th month of age. *Strongyloides* was the first infection observed in the calves. The peak egg count was observed in the 3rd month; however, all *Strongyloides* egg counts became negative in the 10th month. Strongylate nematode egg counts were observed in calves from the 2nd month of age onward, and increased gradually until calves were 6th month. These counts remained high until the 18th month. A gradual reduction then occurred until the end of the trial. From the 2nd month onward *Cooperia* and *Haemonchus* infective larvae(L_3) were most prevalent in fecal cultures. Fecal cultures after the 3rd month also resulted in recoveries of *Oesophagostomum, Trichostrongylus. Bunostomum* L_3 were recovered only after 10th month, in low percentages.

g.6.27 BOVINE FASCIOLOSIS SURVEY IN UGANDA

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ABSTRACT
Faecal samples from 650 cattle kept under traditional management were obtained from Bugiri, Pallisa, Masindi and Ntungamo districts. In addition, 380 livers of slaughtered cattle were obtained over a period of 17 months from Mbale, Tororo and Busia districts in Uganda. Of the 650 cattle examined, 174 (26.8%) were infected with *Fasciola gigantica.* The prevalence of fasciolosis increased with age as follows: 12.5% in calves below 6 months, 21.6% in calves of 6-12 months, 22.6% in cattle of 13-14 months and 30.3% in cattle older than 24 months. The prevalence of fasciolosis was highest in cattle in Masindi district (45%), followed by Bugiri district (29.3%), Pallisa district (19.2%) and Ntungamo district (14.6%). Of the 380 livers, 113 (29.7%) were infected with *F. gigantica* in all cases. The high prevalence of fasciolosis in traditionally managed cattle in Uganda calls for urgent attention in terms of an established control programme, given that farmers keeping cattle under traditional management do not practise routine control of fasciolosis.

g.6.28 EPIDEMIOLOGY OF GATRO-INTESTINAL NEMATODOSES IN SHEEP OF RAJASTHAN, INDIA.

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Epidemiological studies on gastrointestinal (GI) nematode infections of sheep kept by nomads of Thar desert of Rajasthan were conducted by monthly examination of faeces for a period of one year from July 1997 to June 98. Out of 1200 faecal samples examined, 936 (78%) were found positive for GI parasitic infections. The incidence of strongyles was the highest (72.06%) followed by Strongyloides (9.50), Trichuris (5.25) and Moniezia (2.35%). The maximum (92%) incidence was reported in post-rainy season with minimum incidence of 65.75% in summer.
Peak faecal egg counts (1776.75 ± 167.74) were recorded in post rainy season (October and November) followed by rainy season (July, August and September ; 928.50 ± 77.01) and lowest (460.50 ± 50.60) in winter (December, January and February). On coproculture exmanination, the infective larvae of Haemonchus spp., Trichostrongylus spp., Oesophagostomum spp., Bunostomum spp., Cooperia spp., Strongyloides spp. and Trichuris spp. wre recorded.
The respective per cent compostion of the larvae was 67.41% (Haemonchus spp), 14.12% (Trichostrongylus spp.), 7.97% (Oesophagostomum spp.), 4.46% (Bunostomum spp.), 1.75% (Cooperia spp.), 3.43% (Strongyloides spp.) and 0.80% (Trichuris spp). The Present study indicated that the season and age of the animal had significant influence on the prevalence and intensity of GI parasitoses in sheep.

g.6.23-33 Epidemiology and control of ruminant helminths in the subtropics and tropics

g.6.29 MODELS OF CHEMOTHERAPEUTIC CONTROL AGAINST *Fasciola hepatica* IN CATTLE

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Background: It is very important to develop control models for fasciolosis in Mexico. The aim of the study was to evaluate three models of treatment against *F. hepatica* using triclabendazole (tbz) at 12 mg/kg/body weight.
Method: three groups each of 23 cattle, were used. Treatments were given as follows: Group 1 was treated in january, Group 2 in january and june and 3 in january, june and october. Coprological examinations were carried out during one year at 45 day intervals. **Results:** At the beginning the percentage of positive samples were 100% for all groups. Group 1, the average of eggs per gram (epg) at the beginning was of 13.2 $\pm$ 3.14 was reduced in 5 samplings from 0.56 $\pm$ 0.26 to 12.1 $\pm$ 2.02 while in 4 samplings it was increased from 14.6 $\pm$ 3.87 to 21.1 $\pm$ 3.06 ($p \leq 0.01$) between the first sampling and the others. In Group 2, the average epg recorded at the beginning of the study was of 15.04 $\pm$ 3.62 being reduced in the following 8 samplings from 0.17 $\pm$ 0.13 to 14.10 $\pm$ 2.13, except in sampling 5 showing statistical differences between samplings ($p \leq 0.01$). In Group 3, the average of epg at the beginning of the study was 13.13 $\pm$ 2.24 decreasing in the following samplings from 0.27 + 0.13 to 11.55 $\pm$ 4.56, being different the first sampling to all others ($p \leq 0.01$).
Conclusion: The percentage of improvement in egg reduction for group 1 was of 0%, for group 2 of 8.19% and for group 3 of 59.15% suggesting that group 3 was the best.
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g.6.30 SUPPRESSIVE TREATMENT EFFECTS ON NEMATODE INFECTION IN FATTENING CATTLE

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Background: The effect of previous suppressive anthelmintic treatments after weaning on parasitological parameters and weight gain of cattle was studied in the Pampeana region of Argentina. The study was carried out in two grazing fattening periods, GFP: GFP1: 1995/96 and GFP2 1997/98
Method: During both GFP, 60 weaned calves that grazed contaminated pastures, were divided into 3 groups during the first part of the GFP: GY1 group was treated every 2 weeks with avermectins while GY2 and GY3 groups remained untreated. During the second part of the GFP, GY1 and GY2 remained untreated and GY3 were treated every 2 weeks. Groups of yearlings grazed together with a new group (GNC) of 20 weaning naive untreated calves. Egg counts epg, coprocultures, herbage larvae L_3, serum pepsinogen Pep, eosinophils Eo and live weight gain LWG were recorded monthly.
Results: *Ostertagia, Trichostrongylus, Haemonchus* and *Cooperia* were the predominant species. In GFP1, in spite of the low levels of previous infection during the first part of GFP, slight differences of epg and Eo ($P<0.05$) between GY1 and GY2 were detected in the second part of the GFP. In GFP2 moderate infection levels during the first part of GFP was observed. During the second part of GFP2, GY1 and GNC showed higher ($P<0.01$) epg than GY2 and only GY3 and GNC had ($P<0.05$) lower Pep and Eo levels. Also during the second part of 2FGP, LWG responses of GY3 were greater ($P<0.01$) than those of GY1 and GY2 groups and LWG advantage of GY2 over GY1 was 19 kg.
Conclusion: Higher epg and lower LWG of GY1 suggest that suppressive treatments affect the level of the immune response of yearlings, but these effects were influenced by previous levels of nematode infection.

g.6.31 CAPRINE GASTROINTESTINAL PARASITISM OF SMALLHOLDER FARMS IN ZIMBABWE

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Background: Gastrointestinal parasitism (GIP) in goats is considered to be a problem of considerable magnitude to smallholder farmers. A survey in order to study the prevalence and geographical pattern of the occurrence of GIP in goats was carried out in three Zimbabwean provinces.
Method: Faecal samples from three age groups of goats from 237 flocks were collected during May and June and examined for nematode, cestode and trematode eggs and coccidial oocysts. Strongyle larvae were identified following the culture of pooled faeces.
Results: The flock infection rates determined from presence of eggs/oocysts in faeces were as follows: nematodes (85%), paramphistomes (34%), *Fasciola* (9%), *Schistosoma* (1%), cestodes (18%) and *Eimeria* (28%). The mean prevalence of trichostrongylid infection was 68%. From 100 to 400 eggs per gram were found in 20% of the positive samples, the rest being less than 100. *Haemonchus* and *Oesophagostomum* prevailed in kids, whereas *Trichostrongylus* predominated in older groups. The faecal oocyst counts were significantly higher in young animals.
Conclusion: Gastrointestinal parasitism is a real threat to the health of the young goats in the wet regions and needs systematic prophylaxis. The low rate of GIP in old animals is not a major constraint to goat production and a special prophylactic programme is unnecessary.

g.6.32 WIREWORM IN GOATS ON A SMALL-SCALE FARM: INCIDENCE AND CLINICAL EVALUATION

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Background: The FAMACHA© method of clinical evaluation of wireworm (*Haemonchus* spp.) infection in sheep was developed in South Africa; the colour of the mucous membranes of the eyes is classified into 5 categories: 1 (healthy) to 5 (severely anaemic).
Purpose: To determine the predominant worm species and investigate the importance of wireworm as a cause of anaemia and poor body condition in goats on a resource-limited farm in Gauteng Province, South Africa.
Method: During summer, the period of heaviest wireworm infection, the colour of the conjunctivae was compared with haematocrit determination, faecal nematode egg count (FEC) and body condition scoring as means of appraising the degree and effect of wireworm infection. Only those animals that were considered to be in FAMACHA© categories 4 and 5 were treated with anthelmintics.
Preliminary results: Differential larval counts indicated that wireworm predominated on the farm in question. FECs rose during spring and early summer, the period covered by these initial observations. Mean haematocrit remained relatively constant. The body condition scores steadily increased into the summer.
Conclusion: The increase in body condition scores may be related to an increased availability of forage following summer rain. Correlations between FAMACHA© score and haematocrit and the effect of selective treatment in maintaining haematocrit require further investigation.

g.6.23-33 Epidemiology and control of ruminant helminths in the subtropics and tropics

g.6.34-41 Epidemiology and control of companion animal parasites

g.6.33 THE EPIDEMIOLOGY OF GASTROINTESTINAL NEMATODES OF DAIRY CATTLE IN KENYA

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The epidemiology of gastrointestinal nematode infections in dairy cattle was studied in two farms in the central Kenyan highlands during a 13 month period from April 1993 to April 1994. In each farm, 32 weaned calves were given a single dose of albendazole and then placed on contaminated experimental pastures. Two parasit-free tracer calves were also grazed alongside the resident weaner calves each month throughout the study period. Data from faecal nematode egg counts, pasture larval recoveries, and worm counts from the resident and tracer calves were used to investigate the seasonal infectivity pattern. Four nematode species of economic importance: *Haemonchus placei*, *Oesophagostomum radiatum*, *Trichostrongylus axei and Cooperia* spp, were recorded with *H. placei* being the predominant nematode present in young cattle of both farms. Faecal egg counts from resident cattle and necropsy worm counts revealed that pasture larval levels were directly related to the level of rainfall. Total worm burdens present in the animals were highest during the rainy seasons (March/June and October/December) and lowest during the dry seasons (July/September and January/February). These findings suggest that strategic anthelmintic treatments are required 3-4 weeks into the long and short rains, respectively.

g.6.34 SURVEY ON PREVALENCE OF *DIROFILARIA IMMITIS* IN DOGS ON REUNION ISLAND

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Background: *Dirofilaria immitis* has a worldwide distribution but its importance greatly varies. The aim of this study is to evaluate the ditribution, characteristics and prevalence of infestation in dogs in the island La Reunion.
Methods : Three groups of dogs were evaluated : 1) Stray dogs were examined just after euthanasia. and heart and pulmonary arteries carefully dissected. and blood samples were drawn 2) A retrospective study was performed using the records from dogs seen in the veterinary clinics 3) A prospective study was organized on dogs in 5 veterinary clinics. Their characteristics were recorded and blood samples collected. On blood samples the presence of microfilariae was determined by direct examination or methods of concentration (Knott's method or DIFILTEST) and research for antigens using the VETRED Test.
Results : in 124 dogs stray dogs from 4 localities the overall frequency of infestation was 19,4% (7,4 to 25%). Parasitism burden varied from 1 to 17 filariae. Direct examination of blood and concentration methods revealed respectiveley 37,5% and 79,2% of infested dogs. Serological test detected all the dogs harbouring 3 adults *Dirofilaria* or more.The retrospective examination of records from 317 dogs revealed a percentage of infestation of 22,4% when DIFILTEST was used and 4,6% with routine direct examination. Infection rates on 100 group 3 dogs from 5 veterinary clinics varied from 0 to 45%.
Conclusions : The results from 541 dogs show that overall prevalence of *Dirofilaria immitis* is probably 20% (5 to 45% according the areas). When comparison was possible the results were similar in stray or companion animals. Necropsy revealed also a high prevalence of *Spirocerca lupi* (63.7%).

g.6.35 *OTODECTES CYNOTIS*: EPIDEMIOLOGICAL CLINICAL AND PARASITOLOGICAL, RESULTS FROM A STUDY ON 180 CASES IN THE DOG

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Background : Ectoparasitoses other than flea infestations are rarely related in the literature. The aim of this study is to precise the characteristics of *Otodectes cynotis*.infestation in dogs.
Methods : A retrospective study was conducted on records from dogs presented to the parasitology (subsequently dermatology) clinics in the E.N.V. Nantes during the period 1983 - 1993. Informations on the animals, clinical signs, results of laboratory procedures were computerized. Diagnosis of Otodectic infestation was based on the isolation of parasites or evidence of infestation (association of epidemiological and clinical observations, presence,"audito-pedal" reflex, response to topical antiparasitic therapy). Pruritus was scored on a 0 to 4 scale
Results : Of 5595 dogs, ectoparasitoses (not flea related)were diagnosed in 5,7 % and *Otodectes cynotis* the most frequently diagnosed (180 = 3,2%) (56,7% of these ectoparasitoses). More infestations were observed in april than in any other month. Average age was 2,3 years (1 month to 13 years). Infestations occurred significantly more frequently in dogs with non-erect ("floppy") ears. The mites were found in 95 % of the cases. Erythema was present and cerumen brown to black in respectively 81.4% and 93,2% of the cases. In 12 % abundant *Malassezia pachydermatis* were observed. In 21% of cases the pruritus seemed absent or very mild. In other cases the infestation was highly pruritic but never at the highest level (4) and not correlated to the presence of yeasts when *Otodectes* are seen. The characteristics of the dogs with otodectic mange were compared to the population of dogs seen in the clinics.

g.6.36 THE PREVALENCE OF CANINE DIROFILARIA IN TAIPEI

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Background: Heartwarm disease, one of the most important epidemic diseases in canine population, has an increasing prevalence over the last 15 years in Taipei. The purpose of this study was to investigate the prevalence of canine dirofilariasis in Taipei.
Methods: 1099 dogs which were admitted for routine physical examinations, or various illness during January to December 1996, were enrolled for this study using Canine Heartworm Antigen Test Kit (Snap®, Idexx Laboratories Inc, U.S.A.). Among these 1099 dogs, 590 were dogs, 509 were bitches; the mean age of the group was 4.9 ± 3.8 years. Apart from mongrels (386/1099), 49 breeds were recorded.
Results: The prevalence of 1099 dogs was 23.57% (259/1099). Rottweilers (19/25; 76%), German Shepherd Dogs (14/42; 33.33%), and Shiba Inus (9/27; 33.33%) were most vulnerable. No Shih-Tzu (0/30) was found to be affected by dirofilariasis. Miniature Poodles (3/35; 8.57%) and Yorkshire Terriers (4/39; 10.26) showed lowest prevalence among these 49 breeds.
Conclusion: The prevalence of canine dirofilariasis was raised in 1996, compared to that of 1995. High prevalence of canine dirofilariasis in strays was believed to contribute to the result.

g.6.34-41 Epidemiology and control of companion animal parasites

g.6.37 ENDOPARASITES IN DOGS FROM BOSNIA AND HERZEGOVINA

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In consideratation of epidemiological and epizootiologial significante of endoparasites in dogs, we would like to present results examinations of dogs endoparasites in Bosnia and Herzegovina.

The big number of the registered and stray dogs from various localities in Bosnia and Herzegovina were examinited by coprologic feces examination as well as by parasitologic section, over the period 1952-1998.

In parasitological examinations in dogs were detected 24 species of endoparasites belonging to the classes of: Sporozoa, Trematoda, Eucestoda, Nematoda and Pentastomidea.

The following species have been determinated: Isospora canis, I.ohioensis, I. wallacei, Babesia canis, Leishmania donovani, Sarcocystis spp., Echinochasmus perfoliatus, Opistorchis felineus, Metagonimus yokogawai, Dipylidium caninum, Mesocestoides lineatus, Taenia hydatigena,T. pisiformis, T. multiceps, Echinococcus granulosus, Toxocara canis, Toxascaris leonina, Ancylostoma caninum, Uncinaria stenocephala, Crenosoma vulpis, Trichuris vulpis, Capillaria aerophila, Trichinella spiralis and Linquatula serrata.

The most significant detection is the presence of Echinococcus granulosus and the species causing the complex of "larva migrans".

The high percentage infected dogs with dangerous antropozoonoses agents presents danger for infestation of the population and live stock on the territory of Bosnia and Herzegovina.

g.6.38 FIRST REPORT OF INDIGENOUS CANINE BABESIOSIS IN BELGIUM

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Background : Canine babesiosis caused by *Babesia canis* and transmitted by the ticks *Dermacentor reticulatus* and *Rhipicephalus sanguineus* is a fairly common and severe hemoprotozoan infection in some European countries, especially around Mediterranean sea, but it has never been reported in Belgium. However, since 1990, it seems that some practitionners encountered clinical canine babesiosis acquired in Belgium (Mons area-province of Hainaut). It was therefore attempted to confirm this hypothesis by scrutinizing suspected cases referred to our Laboratory.
Method : Anamnestic, clinical, parasitological, haematological and biochemical data were collected and analysed from three dogs with suspected canine babesiosis between October 1997 and February 1998.
Results : Microscopic examination of stained blood smears revealed the presence of *Babesia canis* in all three cases. The dogs were severely affected and one died. Main haematological and biochemical findings consisted of anaemia, decreased PCV's, leukopenia and hyperuremia. A *Dermacentor reticulatus* tick was identified on one dog. The animals had never been abroad.
Conclusion : The occurrence of three indigenous cases of canine babesiosis in Belgium is reported for the first time.
Acknowledgment : We thank Drs F. Avez, F. Malaise and J. J. Mollet for providing the documented clinical cases.

g.6.39 A LONGITUDINAL STUDY OF HELMINTH PARASITES IN DOGS FROM BOKSBURG, SOUTH AFRICA

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Purpose: The incidence of helminths in dogs in highly populated informal settlement areas was studied.
Method: Samples were collected from a total of 208 dogs; these included 72 postmortem intestine samples, 164 faecal samples and 148 adhesive tape swabs. The owners were also interviewed to establish the level of parasite control.
Results: Postmortem analyses indicated that 93% of the dogs were infected with *Ancylostoma caninum*, 45% *Dipylidium caninum*, 40% *Toxocara canis*, 24% *A. braziliense*, 15% *Spirocerca lupi*, 10% *Joyeuxiella pascualei*, 8% *Toxascaris leonina*, 8% *Taenia* spp. and 6% *Trichuris vulpis*. Eggs of *Ancylostoma* spp. were confirmed in 66% of the faecal samples, 20% contained *T. canis* eggs, 4% *D. caninum* and 2% *Taenia* spp. eggs. Of the 85 dog owners interviewed, only 13% were aware of the possibility that internal helminth parasites could affect the health of their dogs and treated their dogs with anthelmintic remedies. Only 3% adhesive tape swabs tested positive for eggs of *Taenia* spp., 2% for *Dipylidium caninum*, and 1% for *Toxocara canis*.
Conclusion: Levels of helminth parasite infections in dogs in the Boksburg area are high and since most of these helminths are potentially zoonotic, the impact on public health in the area should be considered.

g.6.40 TOXOCAROSIS AMONG PETS AND *TOXOCARA* EGGS IN SOIL IN POZNAN AREA, POLAND

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Background: *Toxocara canis* and *T. cati* are zoonotic nematoda causing toxocarosis among people. The role of each species in human infections is unknown. The prevalence of toxocarosis among dogs and cats and soil contamination with *Toxocara* spp. eggs on the same areas could show the epidemiological situation in the region and suggest which pets are more important in soil contamination.
Methods: In 1997/1998, 445 of dogs faeces , 105 of cats faeces and 365 of soil samples were examined by the flotation technics (faeces by Fülleborn's method and soil samples by Dada's method). Different public places were sampled (parks, backyards, streets and playgrounds). Age and sex of the pets were considered.
Results: Both in dogs and cats toxocarosis was the most common parasitosis. *Toxocara* spp. eggs were detected in faecal examination of 140 dogs (31,5%) and 41 cats (39,1%). The highest prevalence of *Toxocara* spp. was found in dogs up to the third month of age (51,1%) and in cats between 3-6 month of age (64,3%). Among adult pets, cats were more infected (29,7%) than dogs (2,5%). The range of soil contamination with *Toxocara* spp. eggs was 10 % the samples examined. The most heavily contaminated areas were urban backyards (21% positive samples with mean egg density 1,23 eggs/100g of soil).
Conclusion: In Poznan region the exposure of human population to *Toxocara* spp. infection may be higher in the city area than in the province. Cats may play a more important role than dogs in the contamination with *Toxocara* spp. eggs.

g.6.34-41 Epidemiology and control of companion animal parasites
g.6.42-49 Chemotherapy of companion animal parasites

g.6.41 CANINE LEISHMANIOSIS: AN AUTOCHTHONOUS CASE IN GERMANY

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Background: Canine leishmaniosis (Cal) caused by L. infantum is a protozoan disease which is usually transmitted by sandflies of the genera Phlebotomus and Lutzomyia. Here, we give a report on a case of non-vector transmitted leishmaniosis in a dog and the outcome of the treatment.

Case history: A 21-month old native-born German Stafford terrier was referred to the Veterinary Hospital of the Justus-Liebig University in Germany with Cal (AB-positiv, PCR-positive). The dog had never been in endemic areas but the mother had also suffered from Cal. Clinically the dog showed lameness, alopecia, ulcerative dermatitis, lymhadenopathy, anemia and hyperglobulinemia.

Material: Therapy consisted of the iv-administration of meglumine antimonate for 10 days (Days 1-2: 50 mg/kg bw; Days 3-10: 100 mg/kg bw) followed by prolonged daily administration of allopurinol (2x10 mg/kg bw). The success of therapy was assessed clinically and using PCR of the blood and bone marrow.

Results: 13 months after starting the therapy, the dog has so far been clinically healthy and likewise PCR-negative in both blood and bone marrow.

Conclusion: Although mechanical transmission from the bitch to the puppy cannot be ruled out completely, the most likely mode of transmission in this case appears to be intrauterine during pregnancy.

g.6.42 EFFICACY OF UK-124,114 AGAINST NEMATODES IN CATS

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Objectives: To determine the efficacy of a novel avermectin (UK-124,114) against natural infections of nematodes in cats presented as veterinary patients in France, Germany and Italy.

Methods: On the basis of pre-treatment faecal egg counts, 119 cats with ascarid infections were enrolled. Of these, 8 cats had concurrent infections of hookworms. Cats were allocated randomly in a 2:1 ratio to treatment with either UK-124,114 at a minimum dose of 6 mg/kg, or a positive control of pyrantel pamoate in France and Germany, and flubendazole in Italy. UK-124,114 was administered topically on day 0 (defined individually for each cat), and on day 30. Reference product was administered orally, in accordance with the manufacturers' label recommendations, starting on day 0. The investigating veterinarians did not know treatment allocation throughout the study. Efficacy was assessed by faecal egg count reductions on days 30 and 60, compared with samples taken pre-treatment.

Results: There were significant reductions (P=0.0001) in faecal ascarid egg counts for both UK-124,114 and reference product on days 30 and 60 compared with day 0. Cats treated with UK-124,114 showed reductions in geometric mean egg counts of at least 99.9% on days 30 and 60, compared with day 0, whilst cats treated with reference product showed reductions of >99.4% and >96.5%, respectively. There was no significant difference (P>0.05) between treatments in France, but in Germany and Italy, UK-124,114-treated cats showed significantly lower day 60 egg counts than cats treated with reference product (P=0.0013). Only 8 of the 119 cats had patent hookworm infections pre-treatment; none had patent infections after treatment with UK-124,114 (5 cats) or reference product (3 cats). Other studies in veterinary patients conducted to similar protocols have shown that UK-124,114 is highly effective (98-100%) against hookworms in the cat (P=0.0001).

Conclusion: UK-124,114 administered topically at monthly intervals was safe and highly effective against nematodes in cats presented as veterinary patients.

g.6.43 *NOTOEDRES CATI* INFESTATION IN A NEW NON FELID HOST *PAGUMA LARVATE TAIVANA*.. AND TREATMENT WITH MILBEMYCIN-OXIME.

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Background: Amongst about twenty species of *Notoedres*, *N. cati cati* is a Parasite of Cat and also reported in other felids like Ocelot, Florida panther, Siberian tiger and Bobcat. We report here an infestation in a pet Formosan Masked Civet.

Case summary: A five-month-old male Formosan Masked Civet (*Paguma larvate taivana*) had a 3 weeks' history skin disease. The lesion was a 5x10cm size alopecia with crusting and lichenification and with very mild pruritus. It was localized on the dorsal aspect of cervical to scapula area and slowly extensive. Skin scrapings revealed eggs and adults forms of mites. These mites were identified as a *Notodres* species. Microscopic slides of theses parasites were compared to *Notoedres cati cati* reference slides and found morphologically identical. The civet was successfully treated with milbemycin oxime (INTERCEPTOR ®) 1 mg/kg orally weekly for 3 weeks. The owner had only 2 civets as pet but no cat . The other household female civet received the same treatment although clinically healthy and with negative skin scrapings.

Conclusions: Formosan Masked Civet is a Viverridae endemic species in Taiwan. Now it turns to be a popular exotic pet. This was the first known case of *Notoedres cati* in this species .This conduces to the question to a Viverridae subspecific adaptation (like *N.c. cuniculi* in the rabbit) or the sensitivity to the subspecies in Felidae. There is also few reports of the use, tolerance and efficacy of milbemycin-oxime in species other than dogs.

g.6.44 MICROMORPHOLOGICAL CHANGES OF *Toxocara canis* UNDER THE ACTION OF PYRANTEL PAMOATE

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The micromorphological changes in the structures of *Toxocara canis* under the action of 14.4 mg/kg of pyrantel pamoate *in vivo* have been studied on the 8th, 12th, 24th and 36th hours post treatment. Eight naturaly infected pupies have been treated with pyrantel pamoate and eliminated parasitic nematode have been collected for histological studies.

The results show that the anthelmintics applied cause serious alterations in the structures of intestinal cells, hypodermis and muscle cells of *T. canis*. The changes may be described as destructive, degenerative and necrotic processes. The intestine are predominantly affected by the drug. The micromorphological changes in intestinal cells have been found on the 8th hour of the action of pyrantel pamoate, and on the 36th hour the intestinal cells have been completely destroyed. The muscle cells schowed vacuolization in the cytoplasmic portion on the 12th hour under the action of pyrantel pamoate, whereas the fibrilian region of the muscle cells remaind unchanged. The cuticle and the endothelial cells of the sexual tubes of both sexes indicated only a weak vacuolisation and maintened their structure during all experimental time. The fine structure of the nerve cords and the sexual products themselves showed no noticeable changes at all.

It's concluded that *T. canis* worms had taken up pyrantel pamoate via an oral route, leading to initial intestinal changes.

g.6.42-49 Chemotherapy of companion animal parasites

g.6.45 ANTIFEEDING ACTIVITY OF A DELTAMETHRIN SHAMPOO AGAINST FLEAS ON DOGS.

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Purpose : Assess the antifeeding effect of an application of a 0.07% deltamethrin shampoo against fleas in dogs.

Method : Twenty beagles, housed in separate cages, were randomly allocated into two groups of 10. Dogs were treated with 25 ml of a shampoo containing 0.07% deltamethrin (group 1). Dogs in group 2 were controls. Three hours later, dogs were infested with 50 *Ctenocephalides felis* each, and one hour later dogs were careffully combed using a fine-toothed comb (13 teeth/cm) : collected fleas were swatted to detect blood in their abdomen. Dogs were reinfested with fleas on days 3, 7, 10 and 14. Parasites were counted one hour after each reinfestation.

Results : This study showed that the application of 25 ml of shampoo containing 0.07% deltamethrin to beagle dogs weighing between 10.2 and 12 kg was very well tolerated. Among the 2500 fleas deposited on control animals, 1419 have been recovered on dogs one hour after infestation and 99.9% were engorged. The mean number of alive engorged fleas collected from treated dogs was respectively 0.4, 0.9, 2.8, 15.2 and 21.1 one hour after infestations realised 3 hours, 3, 7, 10 and 14 days after shampoo application. 0.07% deltamethrin shampoo prevents fleas' feeding during the hour following infestation with an efficacy >98% between day 0 and day 3, >90% until day 7. Then, results are lower but interesting : 46.9% on day 10 and 30.1% on day 14.

g.6.46 ADULTICIDAL ACTIVITY OF A DELTAMETHRIN SHAMPOO AGAINST FLEAS AND TICKS ON DOGS.

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Purpose : Assess the adulticidal efficacy of an application of a 0.07% deltamethrin shampoo against fleas and ticks in dogs.

Method : Twenty beagles, housed in separate cages, were randomly allocated into two groups of ten. The dogs were infested with 50 Ctenocephalides felis and 50 Rhipicephalus sanguineus each, and parasites were counted 24 hours (fleas) and 72 hours (ticks) later (day 0). Dogs, when state, were then treated with 25 ml of a shampoo containing 0.07% deltamethrin (group 1). Dogs in group 2 were controls. Fleas and ticks were counted 24 hours (d 1) and 48 hours (d 2) after the shampoo was applied, and parasites were then removed. All dogs were reinfested with fleas and ticks on days 2, 7, 9, 14, 16 and 20. Parasites were counted 24 and 48 hours after each reinfestation. Effectiveness against fleas was calculated 24 hours after infestation, and against ticks 48 hours after infestation.

Results : The first study showed that the application of 25 ml of shampoo containing 0.07% deltamethrin to beagle dogs weighing between 10.2 and 12 kg was very well tolerated. It controlled the parasites present on the animals at the time of application : with an efficacy of 100% against fleas 24 hours after treatment and an efficacy of 95% against ticks 48 hours after treatment. The treatment protected against flea reinfestations with an efficacy of 100% during the first week, > 98% in the second week and > 95% in the third week. It also gave ≥99% protection against tick reinfestations in the first week and >96% in the second week.

g.6.47 EFFICACY OF SELAMECTIN, FIPRONIL, AND LUFENURON AGAINST *CTENOCEPHALIDES FELIS* IN CATS

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Objective: to evaluate the efficacy of monthly administration of selamectin, fipronil, and lufenuron against *Ctenocephalides felis* in cats housed for 3 months in a flea-infested environment, and subsequently against weekly challenges of cats with *C. felis* for 2 months.

Design: laboratory study using a mixed-model, repeated-measures ANOVA for treatment comparisons.

Animals: 26 healthy male and 26 healthy female cats.

Procedure: Cats free of fleas were experimentally infested with 100 adult unfed fleas on day -28 (28 days before first treatment) and on day -21. On days 0, 30, 60, 90, and 120, cats were treated with selamectin (6 mg/kg of body weight, topically), fipronil (7.5 mg/kg, topically), or lufenuron (10 mg/kg, PO). On day -6 and every 2 weeks after the first treatment, comb counts were performed to detect viable adult fleas. Fleas that were removed (up to 50%) were then replaced onto the cat from which they came. On day 89, fleas were not replaced. On day 91 and every 7 days until the end of the study, cats were challenged with 20 adult fleas. Flea counts were compared among treatment groups.

Results: Fourteen days after treatment, geometric means of flea counts rose 518% (over day -6 values) in the lufenuron-treated group, but were reduced 71% in the fipronil-treated group and 35% in the selamectin-treated group. Flea counts remained elevated in the lufenuron-treated group until day 59, at which point they were reduced by 64%, and then reduced from 71 to 84% from days 89 to 150. Selamectin and fipronil treatments resulted in a 97% reduction in mean flea counts on day 29 and a 99 to 100% reduction through the end of the study.

Clinical implications: Selamectin is as effective as fipronil, and more effective than lufenuron, in ameliorating *C. felis* infestation in cats.

g.6.48 A CLINICAL FIELD STUDY WITH LUFENURON INJECTABLE SUSPENSION (PROGRAM® 100 IJS) FOR CATS

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A clinical field trial in cats according to GCP was conducted in the USA to demonstrate the efficacy and safety of PROGRAM 40/80 (Injectable Suspension for Cats) under practical field conditions.

Two injections were given six months apart of PROGRAM 40 or 80 at the intended dose level of ≥ 10 mg/kg or of a placebo formulation. A total of 466 cats from 242 households were enrolled into the study. PROGRAM 40/80 was effective in preventing the build-up of flea infestations over a period of 6 month following initial dosing. PROGRAM 40/80 was also effective in controlling pre-existing flea infestations in households over a period of 6 months following initial dosing. Based on adult flea counts on the animals, between 75% and 100% control was attained in 57 out of 87 households, 100% control was obtained in 22 households.

The product was well tolerated by the cats. 60 incidents of transient reactions to the injection (mostly due to the needle sting) were recorded amongst a total of 845 doses administered. Following injection a few cats developed a slight subcutaneous swelling which resolved after a short time. No test article related adverse events were observed in any animals that received concomitant treatment with standard vaccination.

It is concluded that PROGRAM 40/80 administered at the recommended minimum dose rate of 10 mg lufenuron per kg bodyweigh effectively prevents and controls flea infestations under practical use conditions for a period of 6 months, is well tolerated by cats and is compatible with concurrent medications.

g.6.42-49 Chemotherapy of companion animal parasites
g.6.50-83 Chemotherapy against protozoan and ectoparasitic infections

g.6.49 EFFICACY OF THE COMBINATION OF FEBANTEL + PYRANTEL EMBONATE AGAINST NEMATEDS: A MULTICENTER FIELD STUDY ON PUPPIES AND YOUNG DOGS

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A multicenter field study was performed at 17 different locations in Germany to test a combination of febantel (15 mg/ml) and pyrantel embonate (14.4 mg/ml) for efficacy and tolerability against *Toxocara canis, Ancylostoma canium, Uncinaria stephanocephala* and/or *Trichurs vulpis*. The results of the treatment were compared with those of the pyrantel embonate on its own.420 puppies and young dogs of various breeds ageing up to one year entered in the study following positive coprological testing for at least one type of nematode. 212/208 dogs were randomly allocated to treatment group I (febantel + pyrantel embonate) or group II (pyrantel embonate) respectively. Both products were provided in the form of suspensions and were administered orally at a dosage of 1 ml/kg b.w. at day 0.

Nematode eggs were counted before treatment (day –7 to 0) and once after treatment(day 5 -12) using a modified McMaster-method. Cases where *T.vulpis* was detected before treatment underwent a second follow-up examination on day 14, 15 or 16.

Infection with T.canis was diagnosed in 88.1 %, infection with hookworms in 35 % and infection with T.vulpis in 3.3. % of dogs. 74.7 % of the animals were infected with only one species of nematodes, 24.1 % with two species and 1.2 % with all 3 species.

Following one single treatment with the combination of febantel + pyrantel embonate or with pyrantel embonte only caused following reduction in egg shedding occured: T. canis – 97.2 % and 83.7 %, hookworms – 96.8 % and 65.2 %, T. vulpis – 85.4 % and 13.9 %. The reduction in egg counts of T.canis, hookworm and T.vulpis was significant ($p<0.01$) higher following treatment of the dogs with combination of febantel + pyrantel embonate than after treatment with pyrantel embonate alone. Both products were extremly well tolerated by the puppies and young dogs.

g.6.50 *COCHLIOMYIA HOMINIVORAX*: PERSISTENT ACTIVITY OF DORAMECTIN AND IVERMECTIN

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A study was conducted to evaluate the duration of single administrations of doramectin or ivermectin against induced infestations of *Cochliomyia hominivorax*. Twenty four Holstein bull calves were allocated to 4 groups of 6 animals each and treated with saline , doramectin 1 % or 1 of 2 formulations of ivermectin 1% . On day 12 after treatment each calf was anesthetized and 2 full thickness skin wounds were created on the left side shoulder and hindquarters. Two hours later, each wound was implanted with 100 *C. hominivorax* newly hatched larvae. On day 15 after treatment, the procedure was repeated on the right side and each new wound infested according to the process previously described. Wounds were examined daily for 8 days and evidence of live larvae was note. For each challenge day, the number of doramectin-treated calves with active myiasis was significantly ($P < 0.0001$) lower than that for calves treated with saline. Doramectin efficacy was 90.9 % and 83.3 % when larvae implants were performed 12 and 15 days after treatment, respectively. Under these experimental conditions, there were no significant differences in the number of calves with myiasis between the ivermectin and control groups.

g.6.51 PILOT INVESTIGATIONS USING IVERMECTIN TO SELECT MITES PATHOGENIC TO SHEEP (*PSOROPTES OVIS*) FROM POPULATIONS OF THE RABBIT EAR MITE (*P CUNICULI*).

Bates, P.G.

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Background: Early work in the development of ivermectin demonstrated complete eradication of *P cuniculi* but incomplete control of *P ovis* in rabbits. Evidence suggests that there are variations in virulence between isolates of the scab mite (*P ovis*) infesting sheep. Single injections of ivermectin can eradicate isolates producing chronic infestations but two injections are required to eradicate isolates producing acute disease. Could this information be helpful in the speciation of *Psoroptes* mites?

Method: 4 isolates of *P cuniculi* (2 "typical ear canker" and 2 "extra auricular") were cultured in rabbits. Mites were harvested from the ears and used to challenge sheep (2 sheep per isolate). Rabbits were treated with ivermectin and residual mites allowed to resurge. Resurged (ivermectin exposed) mites were harvested and used to challenge sheep as before.

Results: Mites from the original harvest (pre ivermectin exposure) did not produce clinical sheep scab. Ivermectin was 100% effective in eradicating the "typical ear canker" isolates but mites resurged in the rabbits infested with the "extra-auricular " isolates and these produced clinical sheep scab on challenging sheep.

Conclusions: Some "extra auricular" populations of *Psoroptes* infesting rabbits may therefore contain sub-populations of *P cuniculi* (non infestive to sheep) and *P ovis* (infestive to sheep) and these populations can be selected for by ivermectin.

g.6.52 VARIATIONS IN VIRULENCE AND ACARICIDE SUSCEPTABILITY BETWEEN ISOLATES OF THE SHEEP SCAB MITE (*PSOROPTES OVIS*) IN GREAT BRITAIN.

Bates, P.G.

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Background: Sheep scab is a severe welfare condition characterised by irritation and wool loss caused by the mite, *Psoroptes ovis*. Work carried out in the USA in the 1970s demonstrated variations in virulence and susceptability between isolates of *P ovis*. The studies documented here investigated similar variations in isolates of *P ovis* in Great Britain.

Method: Between 1987 and 1997 groups of between 5 and 10 full fleeced, close wool breed, yearling sheep were challenged with 25 adult female *P ovis* originating from one of 16 geographical isolates. The relative virulence (rate of lesion and mite population growth with time) and susceptability to organophosphate (OP) and synthetic pyrethroid (SP) plunge dips and s/c injections of ivermectin were compared to similar sheep challenged with the CVL reference isolate.

Results: There was considerable variation in the relative virulence of the isolates. Some low virulence (chronic) isolates taking 8 to 10 weeks, or even longer, to cover sheep. Other high virulence (acute) isolates taking only 4 to 5 weeks to cover the sheep. Low virulence isolates could be eradicated with a single injection of ivermectin, but highly virulent isolates required two injections to achieve eradication. 4 isolates resistant to SP dips and 1 isolate resistant to an OP dip were identified.

Conclusions: There is considerable variation in virulence and susceptability to acaricides between populations of *P ovis* and this may be an important epidemiological factor in the control of sheep scab in Great Britain.

g.6.50-83 Chemotherapy against protozoan and ectoparasitic infections

g.6.53 PROPHYLACTIC EFFICACY OF AN IVERMECTIN LAI FORMULATION AGAINST *P. OVIS*

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A study was carried out in cattle to compare the prophylactic control of *Psoroptes ovis* infestations provided by an ivermectin long-acting injectable (IVM-LAI) formulation with a doramectin (DOR) injection and untreated controls (UC). Thirty Holstein steers were used. Animals were allocated by restricted randomization based on Day 0 body weight forming six replicates of five animals. On Day 0, within each replicate one animal was randomly allocated to one of the following treatments: UC; IVM-LAI at 630 mcg/kg on Day 0, Day 14 or Day 21; DOR at 200 mcg/kg on Day 21. Starting on Day 49, animals were housed in individual pens. On Day 56, steers were infested with *P. ovis* in the area between the shoulders. Live mites were counted from scrapings of two sites on each animal on Days 70, 77 and 84. Live *P. ovis* mites were found in 33%, 67% and 83% of the UC cattle on each evaluation day, respectively. Live *P. ovis* mites were found in 33%, 67% and 67% of DOR treated steers on each respective evaluation day. No *P. ovis* mites were found in any IVM-LAI treated steers. These results indicate that ivermectin LAI can prevent infestation of *P. ovis* for at least 56 days after treatment and that doramectin injection did not prevent infestation 35 days after treatment.

g.6.54 EFFICACY OF AN IVERMECTIN LAI FORMULATION AGAINST *B. MICROPLUS*

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A study was conducted to compare the therapeutic and prophylactic efficacy of an ivermectin long-acting injectable (IVM-LAI) formulation with a generic ivermectin long-acting (g-IVM-LA) injection or doramectin (DOR) injection on the cattle tick *Boophilus microplus*. Twenty penned Holstein steers were used. All steers were artificially challenged with 2500 unfed tick larvae three times a week from Days -44 up to -1. On treatment day (Day 0), steers were allocated by restricted randomization based on tick counts from Days -3 to -1 to the following treatments: untreated controls (UC); IVM-LAI at 630 mcg/kg, *sc*; g-IVM-LA at 200 mcg/kg, *sc*; or DOR at 200 mcg/kg, *sc*. After treatment, cattle were challenged with 5000 unfed tick larvae at approximately 2-week intervals from Day 14 up to Day 126. IVM-LAI treated steers had significantly ($p<0.01$) lower tick counts and index of reproduction than the controls for all intervals evaluated from Day 5 to Day 151. Reductions for IVM-LAI were >90% for total tick count up to Day 116 and for index of reproduction up to Day 129. Compared to g-IVM-LA treated steers, IVM-LAI treated animals had significantly ($p<0.05$) lower tick counts and index of reproduction for all intervals from Day 34 to Day 151. In addition, cattle treated with IVM-LAI had significantly ($p<0.05$) lower total tick counts (intervals from Day 48 to Day 143) and lower index of reproduction (intervals from Day 62 to Day 151) than animals treated with DOR. These results indicate that ivermectin LAI effectively reduces tick burden, prevents reinfestation for a longer period of time and can have a stronger impact on reducing pasture contamination than generic ivermectin LA or doramectin injections.

g.6.55 *IN VITRO* RESPONSE OF *PSOROPTES OVIS* TO PYRETHROID INSECTICIDES.

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Background: Detection of pyrethroid resistance in *P.ovis* currently requires infestation and dipping of sheep or measurement of insecticide concentrations on wool. A simple contact test for susceptibility was investigated to make testing for resistance quicker and easier.

Methods: Mites of a susceptible isolate of *P.ovis* from Ireland were collected from sheep and enclosed in 'tea bags' made from heat sealable paper. Bags were immersed in insecticides for known periods of time.

Results: All mites failed to die twenty four hours after a one minute dip in working concentrations of pyrethroid sheep dips or ten times concentration of flumethrin. Inclusion of paper impregnated with olive oil, or lanolin, or inclusion of wool or pieces of sheep skin into the 'tea bags' failed to improve the efficacy of flumethrin.

Discussion: The low activity of pyrethroids against *P.ovis* for one minute or prolonged contact on papers or skin suggests that mites do not absorb pyrethroids through their surface, but rather ingest them. This is in marked contrast to many insects, *e.g.* fleas. Preliminary data suggests that activity may be increased by prolonged immersion of mites or keeping them off sheep for one day before testing.

Supported by Bayer plc.

g.6.56 SHEEP LICE: LEARNING FROM INSECTICIDE RESISTANCE IN *PEDICULUS CAPITIS.*

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Background: Insecticide resistance in *P.capitis* suggests what could happen with sheep and cattle lice in the UK if insecticides are used incorrectly.

Methods: Response of lice was determined by contact tests of lice on impregnated paper (*in vitro*) or treatment of patients (*in vivo*). The domain II region of the para-type sodium channel gene was PCR-amplified to investigate the molecular basis of pyrethroid resistance

Results: Lice from Bristol school children were resistant to permethrin (pyrethroid) and malathion (organophosphate) both *in vitro* and *in vivo*, but not carbaryl (*in vitro* only). The sodium channel gene in this head lice sample encodes a single amino acid mutation that has been implicated in conferring resistance to pyrethroids in other insects. Lice were fully susceptible to imidacloprid, but a few survived treatment with fipronil (*in vitro* tests).

Discussion: Resistance in sheep and cattle lice is poorly documented in the UK. Data from head lice suggests that resistance in the order Anoplura can develop easily to both pyrethroids and organophosphates and is probably a major cause for recent increases in the incidence of head lice. Resistance to pyrethroids can result from point mutations in the sodium channel target, whereby the addition of synergists to extend activity is not beneficial. Both fipronil and imidacloprid could be useful for lice control unless there is evidence for prior resistance to lindane, when resistance to fipronil may develop rapidly.

g.6.50-83 Chemotherapy against protozoan and ectoparasitic infections

g.6.57 EFFICACY OF MOXIDECTIN AGAINST *GASTEROPHILUS INTESTINALIS* AND *GASTEROPHILUS NASALIS:* A GLOBAL SUMMARY

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Background: Moxidectin is a macrocyclic lactone in the milbemycin chemical family. It has endectocidal activity against a broad spectrum of endo- and ecto-parasites of livestock and is highly effective against equine nematodes when administered as an oral gel formulation. The dose-limiting species in equines are *Gasterophilus* spp.
Method: A total of 14 studies have been conducted globally evaluating the efficacy of 0.4 mg moxidectin/kg b.w. (n=113) versus untreated control horses or ponies (n=113) naturally infected with *Gasterophilus intestinalis* and/or *Gasterophilus nasalis*. Presence of *Gasterophilus* spp. was determined at necropsy 14 days posttreatment with moxidectin. Treatments occurred at various times throughout the year, with a majority of studies treating horses in late winter.
Results: Six or more control horses were infected with either 2nd or 3rd instars of *G. intestinalis* in 13 studies. Efficacy (based on arithmetic means) of 0.4 mg moxidectin/kg b.w. (commercial dose) averaged 90.9% against this species, with a range of 56.1 to 100%. Moxidectin was equally effective against both instars of *G. intestinalis*. A total of 42 control horses were infected with either 2nd or 3rd instars of *G. nasalis* in 10 studies. Efficacy (based on arithmetic means) of 0.4 mg moxidectin/kg b.w. was 100% against this species in all studies. No 1st instars of either species were found in any animal in this series of 14 studies.
Conclusion: Moxidectin, administered as an oral gel at 0.4 mg/kg b.w. to horses is effective against 2nd and 3rd instars of *G. intestinalis* and *G. nasalis*.

g.6.58 EFFICACY OF FLUAZURON *pour on* AGAINST TICKS IN ARGENTINA.

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Background: Fluazuron, is a tick growth inhibitor that is administered *pour on* to cattle. *Boophilus microplus* in Argentina, is currently controlled mainly using synthetic pyrethoids and cyclic amidines. However, failures of efficacy due to resistance and management problems, show that new compounds applied as *pour on* formulations could be the best choice for anti-tick treatments.
Methods: Sixteen cattle Hereford breed, raised in a free tick area of Argentina, were divided into four groups of 4 animals each, and held in confinement during a 50 days period. On Day 0, animals from groups 1, 2 and 3, were treated with Fluazuron 2.5% at 3, 2, and 1 mg/kgbw, using a *pour on* formulation. Group 4, was considered untreated control. From Day 5 through Day 21, each animal was experimentally infested with 3500 infective larvae of *B. microplus* along the body, three times a week. From Day 25 through Day 41 all engorged tick females dropped from the cattle, were collected, identified, counted and weighed. Of the tick dropped each day, a sample was incubated to determine levels of oviposition and fertility.
Results: Based on the number of ticks collected, and the reduction of the oviposition and fertility, the overall efficacy of Fluazuron 2.5% vary from 99-100% in Group 1, 82-98% in Group 2 and 54-95% in Group 3.
Conclusions: While there was a lower ($P<0.05$) number of ticks in in all the groups receiving Fluazuron 2.5% in comparison with the control group, Group 1 treated with Fluazuron at 3 mg/kgbw had a higher efficacy ($P<0.05$) in relation to Group 2 (2 mg/kgbw) and Group 3 (1mg/kgbw). No significant differences ($P>0.05$) in the percentage of efficacy was observed between Group 2 and 3. No adverse effect due to Fluazuron, were observed after treatment.

g.6.59 PERSISTENT EFFICACY OF DORAMECTIN AGAINST *P. ovis* INFESTATIONS IN SHEEP

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Background: Psoroptic mange in sheep is a widespread ectoparasitic disease in Argentina. The present study, was designed to evaluate the persistent efficacy of doramectin against experimentally induced infestations of *Psoroptes ovis*.
Methods: A total of 36 sheep, free of mange lesions and with no parasitic treatments for the last 2 months prior to the study, were selected on the basis of uniformity and common origin. Fifteen days before infestation (day -15), all sheep were uniquevely identified, weighed and randomly assigned to 6 groups of 6 animal each. On days -14, -9, -7, -5 and -3 before *P. ovis* infestation, sheep of groups 6, 5, 4, 3 and 2 respectively, were treated with a 1% solution of doramectin administered intramuscularly at a dose rate of 200 mcg/kg bodyweight. On day -3, sheep of group 1 received an injection of saline solution. On day -1, two aluminium cells were implanted on each side of each animal. On day 0, each cell was infested with *P. ovis* obtained from donor animals harboring active mange lesions. On day 14 after infestation, all cells were opened and inspected for the presence of mange.
Results: No mites were found in any cell of the doramectin-treated animals (groups 2 to 6). In contrast, live mites and active mange lesions were detected in all 24 cells of the 6 control animals (group 1), confirming the infectivity of the challenge. There was a significant difference between the number of mange infested cells in the treated groups, compared to that for the non-treated group ($P<0.05$). No adverse effects due to the treatment were observed.
Conclusions: The persistent efficacy of a single injection of doramectin prevented induced *Psoroptes ovis* infestations for a period of 14 days.

g.6.60 DRUG TARGETS OF PROTOZOA: COCCIDIA -STATE OF PRESENT KNOWLEDGE AND PERSPECTIVES.

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Drug activities against Coccidia of the genus *Eimeria* and *Toxoplasma* can be subdivided into different groups according to the biochemical target: 1. The anticoccidial quinolones decoquinate and methylbenzoquate, clopidol and the guanidine robenidin inhibit the oxidative phosphorylation in mitochondria. 2. Sulfonamides, ethopabate and pyrimethamine block the folate metabolism. 3. Nicarbazin, the thiamine analog amprolium and the purine derivative arprinocide interfere with nucleic acid synthesis. 4. Polyether antibiotics like monensin, salinomycin, lasalocid, narasin, maduramicin and semduramicin destroy ion crossmembrane gradients. 5. Halofuginone inhibits the mammalian collagen synthesis. 6. The symmetrical triazinone derivative toltrazuril inhibits not only enzymes of the mitochoindrial respiration but also enzymes of the pyrimidine metabolism. Paramomycin, an aminoglycoside, is currently the most effective drug for the treatment of cryptosporidiosis, halofiginone is effective against cryptosporidiosis in calves. No specific and effective treatment is available for infections in the intermediate host caused by *Sarcocystis spec.* and *Neospora caninum*. Resistance problems, especially in poultry coccidiosis, play a very important role and constantly require new anticoccidials but the discovery of new active lead structures for the indication is rare. In the future advances in vaccination may lead to combined prophylaxis programs.

Acknowledgement : the poster supplements the BAYER workshop „Diseases Related to Protozoa and Possibilities for Treatment".

g.6.50-83 Chemotherapy against protozoan and ectoparasitic infections

g.6.61 DRUG TARGETS OF PROTOZOA: KINETOPLASTIDA - STATE OF PRESENT KNOWLEDGE AND PERSPECTIVES.

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Drugs used against *Trypanosoma brucei* are : 1. suramin, which is suggested to interfere with carbohydrate metabilism by inhibiting different glycolytic enzymes and glycosomal protein import thus slowing down energy metabolism; 2. melarsoprol which is suggested to inhibit trypanothione reductase, but also different kinases involved in glycolysis ; 3. diamidines (diminazene aceturate, quinapyrine, pentamidine) which interfere with kinetoplast minicircle DNA and possibly trypanothione metabolism and 4. eflornithine, which is suggested to interfere with trypanothione metabolism by inhibiting ornithine decarboxylase.

Drugs used against *Leishmania spp.* are 1. the antimonials glucantime, sodium stibogluconate and stibophen are suggested to inhibit enzymes with essential SH-groups, among these presumably trypanothione reductase; 2. diamidines (pentamidine, stilbamidine) which interfere with the trypanothione metabolism; 3. allopurinol inhibiting the purine salvage resulting in impaired RNA/DNA synthesis; 4. amphotericin B which disturbs the integrity of membranes.

There are only two drugs used against *Trypanosoma cruzi* : nifurtimox and benznidazole are believed to act as a so-called subversive inhibitor of trypanothione reductase and as generators of reactive oxygen radicals.

Although there exist a diversity of specific drugs for each protozoal group within the kinetoplastida trypanothione metabolism and trypanothione reductase seem to be a common target of the majority of the drugs.

Acknowledgement : the poster supplements the BAYER workshop „Diseases Related to Protozoa and Possibilities for Treatment

g.6.62 DRUG TARGETS OF PROTOZOA: HAEMOSPORIDIA – STATE OF PRESENT KNOWLEDGE AND PERSPECTIVES.

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In the group of Haemosporidia too, drug targets of the folate metabolism and the DNA-synthesis are effective: 1. Sulfonamides, pyrimethamine and cycloguanil interfere with enzyme activity of the enzyme dihydropteroate-synthetase and/or the dihydrofolate reductase. 2. The artemisinine derivatives artesunat, arteether and artemether inhibit the cytochrome-oxidase and presumably by alkylation of different malaria proteins. 3. Besides these two groups there are substances which act mainly on ***Plasmodia*** species due to the invasion of bloodcells: chloroquine, mefloquine and quinine accumulate in the food vacuoles of growing parasites and inhibit the process of digestion. Iron ferriprotoporphyrin IX, which is released upon digestion of haemoglobin, is supposed to be the receptor. 4. Clindamycin, spiramycin and erythromycin act specifically on ribosomes and inhibit proteinsynthesis. 5. The hydroxynaphtoquinone atovaquone and the 8-aminoquinolines pamaquine and primaquine damage Haemosporidia through generation of free radicals and oxidative stress.

For prophylaxis chloroquine and mefloquine are the drugs of choice at present. Because of worldwide increasing resistance problems prophylaxis must be performed in a country specific way. There is an urgend need for new drugs. Atovaquone and artemisinin derivatives are therefore of great interest, because they are active against multi drug resistant strains of ***Plasmodium falciparum***.

Acknowledgement : The poster supplements the BAYER workshop „Diseases Related to Protozoa and Possibilities for Treatment".

g.6.63 DRUG TARGETS OF PROTOZOA: -GIARDIA; TRICHOMONAS AND AMOEBA -STATE OF PRESENT KNOWLEDGE AND PERSPECTIVES.

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Drugs active against the anaerobic protozoa ***Giardia***, ***Trichomonas*** and ***Entamoeba*** can be subdivided into several groups : 1. carbasone (***Trichomonas***, ***Entamoeba***), glycobiarsole (**all three protozoa**) and iodoquinol (***Entamoeba***) interfere with carbohydrate and/or energy metabolism; 2. metronidazole and other 5-nitro-imidazoles (**all three protozoa**), furazolidone (***Giardia***), nifuratel (***Trichomonas***) and presumably etofamide (***Entamoeba***) are activated by pyruvate-ferredoxin-oxidoreductase generating free nitro anion radicals; 3. diloxanide (***Entamoeba***), quinacrine (***Giardia***) and chloroquine (***Entamoeba***) presumably affect DNA-synthesis; 4. emetine (***Entamoeba***) is an inhibitor of protein synthesis; 5. benzimidazoles such as mebendazole, albendazole or fenbendazole (***Giardia***) are known to interfere with the polymerization of microtubuli; 6. the antibiotics erythromycin, paromomycin, tetracyclin and azithromycin (***Giardia***) presumably act indirectly by killing intestinal bacteria serving as food supply.

Human ***Giardia-***, ***Trichomonas-*** and ***Entamoeba*** infections can be easliy controlled by metronidazole and other 5-nitro-imidazoles. After a correct and quick diagnosis parasites can be eliminated to a high degree. For the control of ***Acanthamoeba*** and ***Naegleria***, however, there is no drug of choice available. Here treatment is performed only symptomatically.

Acknowledgement : the poster supplements the BAYER workshop „Diseases Related to Protozoa and Possibilities for Treatment".

g.6.64 LONG TERM EFFECTIVENESS OF TWO INJECTABLE TREATMENTS AGAINST PIG MANGE

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Background: Treatments that provide long term control of *Sarcoptes scabiei* infection will offer economic advantages to the pig farmers.

Method: A controlled randomised field trial was conducted in a fattening building in South Eastern Poland with mange history. Groups of 12 pigs each were used: 2 groups were medicated once by IM injection in the neck on D0 with Long Acting (L.A.) Ivermectin* (200 µg/kg) or Doramectin** (200 µg/kg) and one group was left untreated. Pigs were included in the trial based on two *S. scabiei* positive counts on D-7 and D0. They were allocated to groups according to D-7 mite counts. Earwax and skin scrapings counts (adults live/dead+larvae+eggs), total clinical score (pruritus+lesions score) were performed individually every fortnight until D56.

Results: The two treated groups'geometric means were significantly different from untreated group for the mite counts, clinical score and daily weight gain until D56 included ($p<0.0001$). An earlier decrease of the clinical score was observed in the LA.Ivermectin group. The proportion of pigs with nil mite counts was significantly different in this group compared to untreated group on D14 ($p=0.018$), D28 and D56 ($p<0.0001$), and difference could be shown between Doramectin and untreated group on D28 and D56 ($p<0.0001$). 100 % animals in untreated group remained positive until D56 (mean=51.7).

Conclusion: A single treatment yielded average daily weight gain increased by 200g compared to untreated animals over the two months trial period. The better growth is due to the decrease in parasite numbers by at least 93% at D14 and 99% at D28/D56 in Doramectin group or 95% at D14 and 100% at D28/D56 in LA.Ivermectin group. Ivermectin in this L.A. formulation was safe and efficacious when injected intramuscularly.

*VIRBAMEC® L.A. **DECTOMAX®

g.6.50-83 Chemotherapy against protozoan and ectoparasitic infections

g.6.65 TOLTRAZURIL CONTROL OF ISOSPORA SUIS COCCIDIOSIS IN PIGLETS IN THE CZECH REPUBLIC

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Neonatal porcine coccidiosis caused by *Isospora suis* is recognized as a disease responsible for major economic losses. Coccidiosis is characterized by pasty diarrhea in 7-day old piglets. The principal source of infection is the enviroment with highly resistent oocysts. In 1995-98 trials in Moravia (Czech Republic), the *Isospora suis* prevalence was found in the range from 3.9 to 20.0%.

The present trial was carried out in autumn and winter 1998 in sow herds where coccidiosis had been confirmed by clinical symptoms, necropsies and parasitologic examination before the trials. Faecal samples were collected from Day 7 to Day 14 and on Day 21. The presence of *Isopora suis* oocysts were ascertained by a modified McMaster technique (OPG). Faeces of each litter were taken from the farrowing house floor for a direct determination of *Isospora suis* oocysts by using a coprological test with Sheather solution. The diarrhea score was defined (normal to liquid), a number of animals affected, mortality and weaning weight. A single peroral application of toltrazuril (Baycox 5%) at 20 µg/piglet to 3 or 4 day-old piglets seems quite sufficient to significantly reduce oocyst shedding of *Isospora suis* and to control coccidiosis diarrhoea. These results indicate that the intensity of disease depends on good animal management and health on the farm. During the trial two different disinfectants were tested (Interkokask, Oo – Cide) and their effectiveness in controlling isosporosis infections was confirmed.

g.6.66 TESTING THE EFFICACY OF POTENTIAL DISINFECTANTS AGAINST COCCIDIAL OOCYSTS

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Background: Coccidia are ubiquitous parasites found in all animals and are of particular concern in intensive farming systems such as the poultry industry. Very few disinfectants that are currently on the market are effective against coccidial oocysts and there is no "standard test" available for assessing new products. The effectiveness of a disinfectant is dependent on several criteria; the level of parasite contamination, environmental conditions, and stability of the compound. Safety considerations for application and disposal of the product are also important. The disinfectant needs to be highly effective against both the unsporulated and sporulated oocysts.

Method: Unsporulated and sporulated oocysts are exposed to the disinfectant under conditions that mimic the intended use. Viability of the treated oocysts is then determined either *in vitro* or *in vivo*.

Conclusion: Determination of the efficacy of a disinfectant must be tailored to its intended use.

g.6.67 TICKICIDAL ACTIVITY (BOOPHILUS MICROPLUS) OF FOUR ENDECTOCIDES

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Background: Chemical treatments such as Pyrethroids and Amidines are major means of tick control but are exposed to resistance threat. The endectocide family is a highly efficacious alternative and provides long term effectiveness.

Method: A field trial was conducted in Brazil, from January to March. Hereford cattle (Rio Grande do Sul) were purchased and kept in paddocks of native pastures under high natural Boophilus challenge during the overall study. 50 heifers aged between 15 and 18 months were included, with counts of 60 ticks as geometric means (GM) on D0. Both engorged (>8mm, full of blood) and semi-engorged female ticks (4.5 to 8mm, half full of blood) were counted. 10 animals were randomly allocated to a Doramectin *, an Ivermectin **, a long acting (L.A.) Ivermectin ***, a long acting (L.A.) Abamectin **** and a control group, according to D0 tick counts. All treatments were administered S.C at 0.2 mg/kg.

Results: The Control increased and remained at very high levels of infestation until the end of the trial period (GM= 402.2 on D35), indicating constantly high parasite pressure. The Control had 82.5 ticks and was statistically different from all treated groups on D4 (Kruskal-Wallis). The L.A. Ivermectin (GM=11.8) was statistically lower than the Ivermectin group (GM=21) on D4, with 85.8% and 74.6 % reduction respectively. All treated groups had more than 90% reduction from D8 until D28 included (all >99.7% on D21). The effectiveness reduced on D35 from 63.4% (Ivermectin group) to 83.6 % (L.A. Ivermectin), all treated groups still being statistically significant from Control.

Conclusion: The Controls suffered from high ticks' burden within the entire study duration (heavy rainfalls and high temperature registered). For all products tested, a satisfying activity (>90%) was observed from D8 until 4 weeks post treatment. The knockdown effect (assessed on D4) varied according to groups.
*DECTOMAX®, **IVOMEC®, ***VIRBAMEC® L.A., ****VIRBAMAX® L.A.

g.6.68 PERSISTENT EFFICACY OF FOUR ENDECTOCIDES AGAINST NATURAL DERMATOBIA INFECTION

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Background: The tropical warble fly is a widespread and critical problem for animals continuously exposed during the grazing season.

Method: Among 1200 naturally infected cattle, 125 were selected for this field trial on the basis of minimum number of 5 nodules. The trial was held in Brazil (SP) from February to May. They were allocated to 5 groups of 25 animals each from D-1 individual nodules counts (both sides): a Control, a long acting Ivermectin*, a long acting Abamectin**, a Doramectin*** and a classical Ivermectin**** group. All treatments were injected S.C. on D0 (0.2 mg/kg). All live nodules of Dermatobia hominis were counted on D7, D14, D21, D28, D35, D42, D49, D56 and D70.

Results: At D0 the mean number of live nodules was similar in each group (ranging from 19 to 22.16), with no significant difference ($p>0.05$). The Control group remained at average levels of infection until the end of the trial period (AM= 8.24 on D70). All treated groups had mean counts significantly lower than Control until D35 included (>96% efficacy). From D42 included, the classical Ivermectin dropped its efficacy to levels of 67.1% to 30 % at D70, whereas other groups remained statistically different from Control and classical Ivermectin. On D70, L.A. Abamectin was still at 91.6 % efficacy, compared to 83.6 % in the two others. On D70, percentage of parasite-free animals was 28% in Control, 36% in classical Ivermectin, 60% in Doramectin, 68% in L.A. Ivermectin and 84 % in L.A. Abamectin.

Conclusion: The value of long acting formulation is demonstrated in the treatment of Dermatobia hominis when long acting and classical Ivermectin groups performances are compared.
*VIRBAMEC® L.A.,**VIRBAMAX® LA., *** DECTOMAX®, ****IVOMEC®

g.6.50-83 Chemotherapy against protozoan and ectoparasitic infections

g.6.69 SODIUM HYPOCHLORIDE AS NEW REMEDY IN TREATMENT OF RABBITS PSOROPTOS.

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Method: First laboratory experiments in vitro were carried on t icks Alveonasus lahorensis, ants Formica rufibarbis and terrestrial snails. Different concentations (< 15 %) of the preparate were shown to produce acaricide and insecticide effects. 60 animals were in treatment group and 10 ones served as control. Sodium hypochloride appeared o be highly efficacious in this experment. Next experiments were carried out on rabbits. Total 40 psoroptos deseased rabbits were treated with single dose of 20% sodium hypochloride solution. Each aural cochlea was supplied with 1,5-2 ml solution. The improvement was observed in 6 - 8 days pt. Moreover 34 rabbits were found to be tick-free and 6 animals had single specimen of live ticks, the affections of the aural cochlea being significantly reduced. The experiments were caried out to spray rabbits with cages and forage. The drug given was unlikely to produce unfavourable effect on animals.
Results: Sodium hypochloride does not effect negativly on physiogical condition of rabbits and may be used without limitations.
Conclusion: Sodium hypochloride is preferable owing to its untoxicity and ecological purity as NaCl, H_2O and O_2 being formed in splitting. Hence it is not a source of environment contamitation and is much cheaper than other.

g.6.70 EFFICACY OF AN IVERMECTIN BOLUS AGAINST SHEEP SCAB

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To determine the therapeutic efficacy of an ivermectin intraruminal controlled-release (CR) bolus, releasing a *minimum* dose of 20 µg ivermectin kg/day for 100 days, 14 sheep of mixed breeds and weighing between 37 and 57kg were infested with *Psoroptes ovis*. Subsequently they were divided into 2 groups of 7. In one of these groups each sheep received one ivermectin CR bolus appropriate to its weight, the other group remained as an untreated control. All mites were eliminated from the group receiving the bolus, as determined by visual examination and counting mites at predeliction sites on the animals.The control group remained infested and all but one sheep required treatment for psoroptic mange before the end of the experiment. A second lot of 14 sheep of similar mixed breeds, weighing between 26 and 47kg but free from *P. ovis*, were divided equally into 2 groups to determine the prophylactic efficacy of the ivermectin CR bolus. In one group, each sheep was given an ivermectin CR bolus according to body weight and the sheep in the other group received no medication and served as untreated controls. 21 days later 2 sheep infested with psoroptic mange were introduced into each of the groups. These donor sheep were removed 10 days later. The group treated with the ivermectin CR bolus remained mange-free and did not harbour any mites. All of the sheep in the control group developed psoroptic mange and required treatment to control the infestation at the end of the experimental period. Sheep that received the ivermectin CR bolus had greater weight gains than the control groups in these experiments. The prolonged activity of the ivermectin CR bolus provided 100% efficacy against existing *P. ovis* infestations and 100% prophylactic activity against challenge, indicating its potential as a valuable tool in the treatment and eradication of scab.

g.6.71 THE PERSISTENT PROPHYLACTIC EFFICACY OF MOXIDECTIN AGAINST *SARCOPTES SCABIEI* IN SHEEP

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Background: Sarcoptic mange is a contagious parasitic disease of sheep caused by *Sarcoptes scabiei*. Affected animals suffer and their productivity is significantly reduced. Its effective control is paramount for the welfare and the optimal production of sheep. The objective of this study was to evaluate the possible persistent efficacy of moxidectin against *S. scabiei* in ewes.
Method: One-hundred ewes were divided into 2 equal groups (n=50): those in group A were injected once with moxidectin (0.2 mg/kg sc) and those in B remained untreated and were controls. Ten ewes naturally infested with *S.scabiei* formed group C and were used as challenge animals divided into subgroups CA and CB (n=5). Two weeks post treatment, 10 ewes from group A and 10 from group B were housed separately for 5 days with the 5 ewes of subgroup CA and CB, respectively, i.e. until the 18th day post treatment. This procedure was repeated with other ewes from group A or B, 21, 28, 35 and 42 days post treatment. Clinical examination and skin scrapings for recovering the mites were carried out throughout the experimental period.
Results: No skin lesions were observed nor were mites recovered in ewes challenged 14 to 18 or 21 to 25 days post treatment with moxidectin, for 84 days after challenge. Skin lesions were observed in and mites were recovered from untreated ewes and the remaining treated ones.
Conclusion: The persistent prophylactic efficacy of moxidectin against *S. scabiei* in ewes was found to be at least 25 days, but less than 32 days.

g.6.72 EFFICACY OF MOXIDECTIN AGAINST HEAVY SARCOPTIC MANGE INFESTATION IN GOATS: CRITICAL FIELD TRIAL

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Background: Sarcoptic mange of goats in not very common in Italy and usually occurs with mild symptoms and is disregarded by breeders. Moxidectin has never been used in goats but showed to be effective against sarcoptic and psoroptic mange in many others animal species.
Method: In a flock of 650 goats, Ionian breed, of a traditional breeding farm in Basilicata (South-Italy), showing heavy lesions of sarcoptic mange, 30 goats, 4-5 years old, were selected and randomly allocated to 3 groups of 10. On day 0 and day 14 group 1 received a subcutaneous injection of moxidectin at 0.2 mg/Kg b.w., group 2 received a subcutaneous injection of ivermectin at 0.2 mg/Kg b.w., and group 3 were the untreated controls. Efficacy was assessed by taking skin scrapings from each animal, counting viable mites and evaluating skin lesions on days -2, 0, +19, +26, +35, +42, +56, +81.
Results: Group 1, treated with moxidectin showed clinical improvement begining from day +26 and no viable mites were found in skin scrapings from day +35 to the end of the trial. The same results were obtained in animals of group 2 but later (days +35 and +56 respectively). Animals of group 3, the untreated controls, showed active skin lesions and remained positive at microscopic examination for mites until the end of the trial.
Conclusion: On the basis of these results it may be concluded that moxidectin 1 per cent, at the dose of 0.2 mg/Kg b. w. injected subcutaneously and repeated after 14 days, is efficacious for the control of sarcoptic mange in goats.

g.6.50-83 Chemotherapy against protozoan and ectoparasitic infections

g.6.73 EFFICACY OF MOXIDECTIN AGAINST *Sarcoptes scabiei* var. *ovis* MANGE IN NATURALLY INFESTED SHEEP

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Background: Sarcoptic mange of sheep is still present in breeding farms in Italy; clinical symptoms usually limited and the long course of the disease increases the difficulties of cleaning up. The need for effective broad spectrum drug is strongly felt.

Method: Field efficacy was evaluated in naturally infested sheep. In a flock of 450 heads of Gentile di Puglia breed, of a breding farm in Basilicata (South-Italy) 36 sheep 4-5 years old were selected; all showed active lesions and were positive for living *Sarcoptes scabiei* specimens under microscopic examination. Two groups of 27 and 9 sheep were randomly formed and kept separate until the end of the trial. Group 1 received a subcutaneous injection of moxidectin at 0.2 mg/Kg b.w. on day 0 and day 14. Group 2 were the untreated control. Efficacy was assessed by taking skin scrapings from each animal on days –2, 0, +12, +21, +28, +42, +67 post 1st treatment, counting viable mites and on the bases of clinical findings.

Results: In the treated group the signs of itching and clinical lesions began to disappear on day +21, and starting from day +42 skin scrapings were negative for mites. All untreated animals were observed to be suffering, showed active skin lesions and remained positive for viable mites until the end of the trial. No adverse reactions, local or general, were observed.

Conclusion: On the basis of these results it may be concluded that moxidectin 1 per cent, at the dose of 0.2 mg/Kg body weight injected subcutaneously repeated after 14 days, is efficacious for the control of sarcoptic mange in sheep.

g.6.74 CONTROL OF *HAEMATOBIA IRRITANS* (L.) BY CHLORPYRIPHOS 10% w/v POUR-ON.

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Background: *Haematobia irritans* (Linnaeus) was introduced to Argentina from Brazil at the end of 1991 and rapidly spread within Argentina from Northern regions to Chubut river valley in Patagonia. To control this fly, synthetic pyrethroids as pour-on treatments were used but last year a wide distribution of resistance was detected.

Method : To evaluate the efficacy of organo phosphate compounds to control *H. irritans* resistent populations to synthetic pyrethroids a Chlorpyriphos 10% pour-on was tested from February to April 1998 in a farm near Bolivar (Province of Buenos Aires) using 50 steers, 24 months old, 300/330 kg. weighing and the fly population on cattle counted before pour-on treatment were exceeding 235 *Haematobia irritans* /animal. The steers, identified by numbered ear tags, were randomly allocated in an untreated group (25 steers) and in a treated group (25 steers), which were pour-on treated by Chlorpyriphos 10% w/v at the dosage rate of 10 ml /100 kg. b.w.

Cattle were placed in two paddocks wich were separated by a 2 km.stock free area, and *Haematobia irritans* counts were conducted before pour-on treatment on day -1 and 0 and after the treatment on day 1,4,7 and then at weekly intervals to day 41.

Results : *Table N°1.* Reduction of *Haematobia irritans.*

Group	Day -1	Day 0	Day +1	Day + 4	Day + 7	Day +14	Day +21	Day +28	Day +35	Day +41
Control	236	238	241	253	274	283	279	273	241	177
Treated	249	247	0	0	7	21	37	53	65	59

Table N°2. Average effect on removing *Haematobia irritans.*

Group	Day 0	Day +1	Day +4	Day +7	Day +14	Day +21	Day +28	Day +35	Day +41
Treated	——	100%	100%	97%	92.5%	86.7%	80.5%	73%	66.6%

Conclusions: According to the results Chlorpyriphos 10% w/v pour-on, at the dosage rate of 10 ml/100 kg b.w., provided a very satisfactory control of *Haematobia irritans.*

g.6.75 CONTROL OF BITING AND SUCKING LICE ON CATTLE BY CHLORPYRIPHOS 10% W/V.

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Background : Three species of bloodsucking lice as *Haematopinus eurysternus, Linognathus vituli, Solenoptes capillatus* and one species of biting lice as *Bovicola bovis* are commonly infesting cattle in Argentina during winter and spring months. Pour-on treatments, using synthetic pyrethroids, were used to control them but last years resistance to these compounds was detected in some cattle producing areas of Buenos Aires province and heavy lice infestation may cause losses in meat and milk production.

Methods : To evaluate efficacy of an O.P. compound as Chlorpyriphos 10% pour-on to control resistent lice, a study was carried out from October to November 1998 in a farm near Balcarce (Buenos Aires province) using 50 Aberdeen Angus heifers, 30 months old, 430-450 kg weighing which were identified by numbered ear tags and randomly allocated in an untreated group of 10 heifers and in a group of 40 heifers, which were pour-on treated by Chlorpyriphos 10% w/v at the dosage rate of 10 ml/100 kg b.w.

Cattle were placed in two non adjiacent paddocks and lice were counted before and after treatment at 10 cm lenghtwise surface situated on head, neck, back, hip and tail; these counts were carried out on days 0,3,7,15,21,28,35, and during these inspections also some specimens were collected in order to be classified.

Results: Table N° 1 - Biting and sucking lice reduction

Groups	Day 0	Day +3	Day +7	Day +15	Day +21	Day +28	Day +35
Treated	21	0	0	0	0	0	0
Control	20	18	18	24	23	25	26

Table N° 2 - Average efficacy on removing biting and sucking lice.

Group	Day 0	Day +3	Day +7	Day +15	Day +21	Day +28	Day +35
Treated	——	100%	100%	100%	100%	100%	100%

Conclusions: According to this study Chlorpyriphos 10% w/v pour-on, applied at dosage rate of 10 ml/100 kg. b.w., eradicated on treated heifers for 35 days biting and sucking lice population.

g.6.76 PREVENTION OF BLOWFLY STRIKE ON COARSE-WOOL SHEEP WITH DICYCLANIL

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Background: Dicyclanil, a new pyrimidine derivative with IGR activity, showed promising *in-vitro* and *in-vivo* efficacy against *L. sericata* and *L. cuprina* in the past. In the current studies the preventative effect against blowfly strike was investigated in field trials with coarse-wool sheep.

Method: In a series of multi-centered field trials animals treated with dicyclanil were compared for up to 22 weeks with untreated controls to establish the length of protection of a 5% ready-to-use pour-on formulation. 9'674 lambs were involved in three consecutive trials during the 95, 96 and 97 blowfly seasons in the United Kingdom. 3'743 mix-aged sheep were involved in one trial in New Zealand during the 95-96 blowfly season.

Results: Consolidated results for all the trials showed >88% reduction in the incidence of blowfly strike in dicyclanil treated sheep for the whole duration of the UK trials, and >80% reduction for the whole duration of the New Zealand trial. In the United Kingdom trials the consolidated cumulative incidence of blowfly strike reached >2% by weeks 19 to 20. In the New Zealand trials the 2% limit by weeks 13 to 14.

Conclusion: Under United Kingdom field conditions a 5% pour-on formulation of dicyclanil protects lambs from blowfly strike for 16 weeks and beyond. Under New Zealand conditions the same formulation protects mix-aged sheep for 12 weeks and beyond.

g.6.50-83 Chemotherapy against protozoan and ectoparasitic infections

g.6.77 LONG-TERM PREVENTION OF BLOWFLY STRIKE IN MERINO SHEEP WITH DICYCLANIL

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Background: Under Australian conditions protection against blowfly strike offered by most currently available products does not last beyond 14 weeks, significantly less than the length of the blowfly season. Dicyclanil a new IGR with promising *in-vitro* efficacy against *L. cuprina* was tested in environmental house and field trials.

Method: The lengths of protection of two formulations of dicyclanil (a 50% jetting and of a 5% ready-to-use spray-on) against artificial infestations of *L. cuprina* were studied at different dose rates in environmental house trials involving a total of 479 merino ewes. Based on the results obtained the 5% ready-to-use dicyclanil spray-on formulation applied on long wool and off-shears was tested in multi-centered field trials by comparing it with a cyromazine ready-to-use spray-on formulation. During two consecutive years 42 mobs totaling 34'635 merino sheep were involved in these trials.

Results: Under environmental house conditions the spray-in formulation applied at a dose of 1.5 g A.I. / sheep (50 kg) completely prevented body and breech strikes for 30 and 21 weeks respectively. In field trials the limit of protection was considered to be reached when >1% of the sheep were struck. This limit was reached by the cyromazine treated sheep between weeks 12 and 13. Dicyclanil treated sheep reached this >1% limit between weeks 21 and 22.

Conclusion: Under Australian conditions a 5% dicyclanil spray-on formulation can protect merino sheep from blowfly strike for 20 weeks and beyond.

g.6.78 STRATEGIC CONTROL *OF AMBLYOMMA CAYENNENSE* POPULATIONS WITH FLUAZURON

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Background: Fluazuron is a new IGR with high efficacy against ticks. In the last years it has been introduced in Australia, South Africa and Latin America for the strategic control of single-host ticks (*Boophilus sp.*) on fattening and breeding cattle. Its suitability for strategic population control of the multi-host tick *Amblyomma cayennense* was investigated under field conditions in Mexico.

Method: The first year (1994) a group of 10 cross-bred (Cebu - Brown Swiss) cattle was treated on May 14 with a 5% ready-to-use fluazuron formulation at a dose volume of 5 ml/ 50 kg bodyweight (corresponding to 2.5 mg A.I./kg bodyweight). A second treatment was applied twelve weeks later (July 24). This group was compared with a group of 10 cattle left untreated. Each group was kept in separate paddocks. The second year, another group of 10 animals received three fluazuron treatments (February 15, May 11, and August 3) and was compared with a group of untreated cattle. Both groups remained in the same paddocks as the year before. Nymphs and adult ticks were counted fortnightly on one side of the animals.

Results: During the first year the infestation with *Amblyomma cayennense* adult ticks was reduced for an average of 70% during a period of 24 weeks. During the second year, reduction of the infestation reached an average of 91% during the first 24 weeks, and dropped to 51% for the last 12 weeks.

Conclusion: Fluazuron applied strategically to cattle can substantially reduce *Amblyomma cayennense* field populations under Mexican conditions.

g.6.79 PATHOLOGY AND THERAPY IN NATURALLY *EIMERIA STIEDAI* INFECTED RABBITS

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Background: An outbreak of hepatic coccidiosis was reported in experimental NewZealand white rabbits (n=24) of either sex (aged 1-2 months) maintained in the Department of Veterinary Parasitology, Punjab Agricultural University, Ludhiana, India.

Methods: The clinical signs of the infected rabbits included anorexia, depression, diarrhoea, emaciation, rough hair coat, reluctance to move and death in 3-4 days. At necropsy enlarged liver with irregular whitish nodules (0.2-0.5 cm) scattered on its surface and deeper parenchyma were seen. The bile ducts were dilated, with extensive proliferation of the biliary epithelium. The granulomatous tissue encircled the bile ducts with infiltration of inflammatory cells. Schizonts and oocysts of *E. stiedai* were found in the epithelial cells and bile ducts. Oocysts were also isolated from the faeces with oocysts per gram of faeces varying between 800 and 15,200. The infected rabbits were divided into two groups. In group I (n=18) rabbits were treated with 2.5% toltrazuril (Baycox) @ 25 ppm in water for 2 days. The rabbits of group II (n=6) were kept as infected and untreated control.

Results: The oocysts disappeared in the faeces on day 6 after treatment and not recorded thereafter. No mortality was recorded in treated rabbits while all the infected untreated rabbits died within 7 days of observation.

Conclusion: *E. stiedai* is highly pathogenic coccidia in rabbits. Toltrazuril @ 25 ppm is highly effective for its treatment.

g.6.80 EXPERIMENTAL MODELS FOR EVALUATING ENDECTOCIDE PERSISTENT EFFICACY AGAINST *LICE* INFESTATIONS OF CATTLE

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Background: Experimental designs for assessing therapeutic efficacy of endectocides against lice infestations of cattle and interpretation of these data are well recognized. Guidelines for evaluating persistent efficacy of these compounds against ectoparasites have not been proposed.

Method: Methods for evaluating persistent efficacy include experimental infestation and repeated exposure (natural infestation) models. The experimental unit is either the animal or pen. An adequate number of experimental units for statistical analyses is essential to test the null hypothesis. Defined predilection sites are examined at predetermined intervals (weekly or biweekly) for estimating the density of the louse population. For a valid assessment of the duration of persistent efficacy, a definition of infestation, such as the presence of lice or a minimum number of lice in a body region(s) for consecutive observation days, should be predetermined.

Results: Lice count data may be presented as arithmetic or geometric means with percentage reduction in counts compared to untreated controls at each time point evaluated. The presentation of the data from the consecutive lice counts definition may be reported as a cumulative percentage of louse infestation (when infestation occurs) or survival analysis (how long is the interval of no louse infestation).

Conclusion: The interpretation of lice count data to determine the protective efficacy of the endectocides against reinfestation depends upon the criteria used to define infestation and the experimental model used.

g.6.50-83 Chemotherapy against protozoan and ectoparasitic infections
g.6.84-88 Non-conventional treatment of helminths, and product development

g.6.81 USE OF *Boophilus microplus* ACARICIDE-SUSCEPTIBLE STRAINS AS REFERENCE FOR LARVAL RESISTANCE TESTS.

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Background: Acaricide resistance is a major concern for the cattle raising industry, then there is a need to develop reliable diagnostic techniques, for early detection of resistant strains. Larval tests are widely used and recommended by FAO. Determination of the LD_{50} and LD_{99} for two acaricide-susceptible strains of *B. microplus*, for use as reference values in the calculation of a Resistance Factor (RF), was performed.

Method: Two pyrethroid-susceptible strains (Mozo, from Uruguay, and Salta, from Argentina) were used, together with one field strain from Brazil. Batches of 108,9 ± 29 larvae aged 15days were placed in impregnated papers supplied by FAO. Papers were sealed and incubated for 24h, then mortality rates were assessed. After probit analysis, the LD_{50} was calculated and the RF was determined.

Results: For both susceptible strains, LD_{50} and LD_{99} could be determined for all compounds but Flumethrin. The field strain values for LD_{50} and LD_{99} were significantly higher than reference values of sensitive strains for all compounds tested.

Conclusion: Both susceptible strains confirm their susceptibility. Mortality rates reached 100% at much lower doses than those used under field conditions. They can therefore, be used as reference strains, however, results confirm that both strains had a previous contact with organophosphates. The adequacy of the concentrations available in the FAO standard kits for the determination of reference values is questionable, since for 2 acaricides (Flumethrin and Deltamethrin) the lowest concentration available killed 50% or more larvae. The field strain was resistant to all compounds tested. The analysis of the 95% confidence limits proved to be a valuable tool in resistance diagnosis management, with wider intervals indicating a higher probability of rapid resistance establishment.

g.6.82 IVERMECTIN 1% L.A.: EFFICACY OFFICIAL TESTS AS CATTLE TICKICIDE.

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Background : Cattle production is one of the most important productive activities in Argentina and among ectoparasites *Boophilus microplus (Can.)* is the most important in the sub-tropical regions where about 12.5 millions of cattle are grazing.

To prevent spread to southern regions from 1938 a compulsory eradication program was established by Animal Health National Service and up to day *B. microplus* was eradicated over 290.000 Km^2

All tickicides must be approved by an official control test; the efficacy for endectocide must be > 95% (IEC).

Methods : Animal Health National Service evaluated by severe tests a commercial long-acting injectable ivermectin 1%.

For these tests a group of cattle, artificially tick infested, were used and tickicide action on treated cattle was compared to tick infestation on untreated group of cattle during four months.

Results : After the treatment ivemectin 1% L.A. eradicated on treated cattle a 99,7% of *B. microplus* infestation and for 12 days protected the animals from tick reinfestation.

Conclusions : According to the results obtained in these tests the long-acting inyectable ivermectin 1% was authorized to be used for the compulsory tick eradication program.

g.6.83 ANTICOCCIDIAL EFFECTS OF SEMDURAMYCIN FOR BROILER CHICKENS

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Background: Avian coccidiosis is a very important disease in the poultry industry in Korea. Recently, Pfizer Ltd. Co. developed a new anticoccidial drug, Semduramycin (Aviax), and we evaluated its efficacy before it was supplied to the broiler farms in Korea.

Methods: To evaluate the anticoccidial efficacy of Semduramycin (S: 25 ppm) and to compare it with that of Clinacox (C: 1 ppm), Monensin (Mo: 100 ppm) and Maduramicin (Ma: 5 ppm) for broiler chickens, we investigated the survival rate (SR), average body weight (BW) in the final period, degree of diarrhoea, lesion score (LS) of dead chickens, the number of excreted oocysts in faeces, and feed conversion rate (FC).

Results: In the 1st, 2nd, 3rd and 4th trials, SR was 96.0%, 89.4% in S, 91.1% in C; 95.9% in S, 96.4% in Mo; 92.6% in S, 93.7% in Ma, in the final period. There was no diarrhoea related to avian coccidiosis. 133;68,000 OPG of oocysts were detected at 3, 4, 5 weeks' age; 13,000 and 17,000 OPG at 4 and 5 weeks' age in S; 400, 48,800 and 5,200 OPG in C at 3, 4, 5 weeks' age; 77,000, 150,000 and 124,000 OPG in S, and 58,600, 136,600 and 141,200 OPG in Mo at 3, 4, 5 weeks' age; 3,400 OPG at 4 weeks' age in S; 200, 46,600 and 800 OPG in Ma at 3, 4, 5 weeks' age. LS of all birds that had died of coccidiosis was not detected every weekend. BW in the last period was 1,430.2 g, 1,640.0 g in S, 1,597.7 g in C; 1,580.0 g in S, 1,600.0 g in Mo; 1.935.2 g in S, 1.916.9 g in Ma. FC in the last period of the experiment was 1.88, 1.82 in S, 1.83 in C; 1.728 in S, 1.73 in Mo; 1.93 in S, 1.95 in Ma.

Conclusion: These results revealed that the anticoccidial efficacy of Semduramycin was better than that of Clinacox, Monensin and Maduramicin for broiler chickens in the 1st, 2nd, 3rd and 4th trials.

g.6.84 EFFECTS OF CONTINUOUS INTAKE OF CONDENSED TANNINS ON PARASITISED LAMBS

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Background: It has been shown that condensed tannins, have an effect on the development of parasitism in lambs. One hypothesis is that they may help the development of the immune response to gastointestinal nematodes, through their ability to increase dietary protein availability in the host. The effect of condensed tannins contained in *'Quebracho Extract' (QT),* on the aquisition and the expression of immunity in lambs infected with *Trichostrongylus colubriformis (T.c.),* was investigated.

Method: Forty-two, castrated, naive lambs were allocated to seven groups. They were dosed five days a week with 3,000 L_3 of *T.c.*. Six groups were fed diets containing either 3 or 6% w/w *QT* from either week 0-5, or from week 6-10 or throughout the whole period (week 0-10). Faecal samples were taken once a week to estimate the progression of the parasitism. At week 10 all lambs were killed, and worm burdens were estimated.

Results: Lambs fed 6% *QT* diet had lower faecal egg counts(FEC) and worm burdens compared to those fed the 3% *QT* diet and to those fed the tannin-free diet. There were no significant differences on the development of the immune response when tannins were provided different periods during the study.

Conclusion: At concentrations above 3%, *QT*'s clearly influence the size of the established burden and the fecundity. However the underlying mechanism(s) associated with these effects, remain to be elucidated.

g.6.84-88 Non-conventional treatment of helminths, and product development

g.6.85 COPPER OXIDE WIRE PARTICLES : AN ALTERNATIVE TO THE USE OF ANTHELMINTICS IN DAIRY GOATS ?

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Background : The spread of benzimidazole resistant nematodes in dairy goat farms is of a great concern as probably more than 70 % of the flocks are involved. The other anthelmintic options being very few during the lactating period, we have evaluated the efficacy of copper oxide wire particles -COWP- (Copinox®, Bayer, Ireland) in experimental and natural infections.

Methods : The curative effect of COWP (4 g) on existing worm burdens was assessed on goats experimentally infected with *Teladorsagia circumcincta*, *Haemonchus contortus* and *Trichostrongylus colubriformis* compared to controls. The preventive effect of COWP on worm establishment was monitored for 3 to 6 months in two farms exhibiting either predominant *T. colubriformis* or *T. circumcincta*/*Oesophagostomum venulosum* infections.

Results : The efficacy of COWP was nil against *Teladorsagia* and *Trichostrongylus* both in experimental and natural infections and against *Oesophagostomum* in natural infections. In contrast, the efficacy of COWP against *Haemonchus* was clearly established in eliminating worm burden (65.1 %) as well as in preventing the establishment of new infections during several weeks.

Conclusion : COWP may represent an alternative to conventional anthelmintics in the control of *Haemonchus* infection in dairy goat farms. This option has to be evaluated in a larger scale field study.

Acknowledgements : This work was partially funded by EU project FAIR 3CT96-1485.

g.6.86 ANTHELMINTIC EFFECTS OF PROANTHOCYANIDINS AND RELATED POLYPHENOLICS

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Background: Feeding of plants containing condensed tannins may influence nematode infections in sheep. The present study investigated the relative content of condensed tannins in different legumes and evaluated their anthelmintic activity in vitro.

Method: Larval development assays were set up according to Hubert and Kerbouf (1992) to determine the direct effects of condensed tannins on development of larvae *in vitro*. Trichostrongylid eggs were exposed to tannin extracts from *Hedysarum coronarium*(Sulla), *Onybrychus viciifolia* (Sainfoin), *Trifolium repens* (White clover) and *Phleum pratense*(Timothy). A two fold dilution from 1mg/ml to 0,0009mg/ml was used. The eggs were incubated at 27°C for 7 days then larval counts were done to determine LD50.

Results: At the same concentrations, soluble condensed tannins (CT) and related polyphenolics from plants containing high concentrations (Sulla and Sainfoin) were less effective (LD50=99mg and 71mg respectively) than plants containing low concentrations (Timothy grass and clover, LD50= 54 mg and 50mg respectively) in reducing the *in vitro* development of larvae..

Conclusions: These results confirmed the potential use of plants containing CT in reducing parasitic burdens in animals.

g.6.87 SARCOPTIC MANGE IN DROMEDARY CAMEL AND ITS CONTROL WITH HERBAL TREATMENT

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Sarcoptic mange casused by Sarcoptes scabiei var. cameli is a wide spread highly contagious and debilitating skin disease; ranking among the most serious and economically important disease of camel. Mange in camel usually seen during winter season, though sporadic cases occur throughout the year. One thousand five hundred, one humped dromedaries (Camelus dromedarius) kept by nomads living in Trypanosoma evansi endemic areas of Thar Desert of Rajasthan, India were selected to study the prevalence of sarcoptic mange. Clinical observations and parasitological diagnosis were selected as field techniques to ascertain the prevalence. The study was carried out between September 1997 to August 1998. Efficacy of herbal preparation "Ectozee" was evaluated in the control of naturally occurring sarcoptic mange in camels.

Out of 1500 camels individually examined for mangy lesions 433 (28.86%) had mite infestation. The causal agent was found to be Sarcoptes scabiei var Cameli. The lesions were commonly observed on neck, brisket, inner surfacr of the thigh and perineum. The affacted areas were hairless, thickened, corrugated, dry and toughy on touch. Complete cure with elimination of mites was effected by 3 topical application os the drug. Itching, scratching, biting and rubbing of body ceases on 20th day of the treatment. The skin regained almost normal appearance 28 days after the first application of "Ectozee". Climate and season of the year had a great bearing on the occurrence and spread of mange, being more rapid in winter than in summer.

g.6.88 DEVELOPMENT OF A FLUORESCENT BASED *C. ELEGANS* ASSAY

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Background: Screening various substances for anthelmintic properties is commonly done with tests that require visual readings, and as such, these tests are labour intensive. We have developed a fluorescence-based assay for *C. elegans* development and have used this assay to test plant extracts for anthelmintic properties.

Method: This assay utilises the high fluorescent intensity (FI) of green fluorescent protein (GFP) that has been expressed in *E. coli*, which served as a food source for *C. elegans*. FI of duplicate samples was measured in 96well plates. The decline in FI corresponded well to the development of *C. elegans*. The fluorescence-based assay was validated by comparison with a visual screening procedure over a culture period of 5 days at 20 °C.

Results: 141 plant extracts have been tested for anthelmintic properties at a wide range of sample concentrations. Identical results between the fluorescence-based and visual screening assays have been observed.

Conclusion: The fluorescence-based assay has the potential of a high throughput-screening assay.

g.6.89-94 Nutrition - parasite interactions

g.6.89 EFFECTS OF UNDER-NUTRITION OF BLACK BELLY LAMBS INFECTED WITH *HAEMONCHUS CONTORTUS*

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Background: It is well known that energy and nitrogen nutrition can modify resistance and resilience of sheep to gastro-intestinal nematodes (GIN). However, under-nutrition of the host is poorly studied. Therefore, the objective of this study was to assess the effects of under-nutrition on Black Belly sheep, a resistant hair sheep breed, infected with its main tropical parasite i.e. *Haemonchus contortus*.

Methods: 42 6-months Black belly naive ram lambs were allocated in a 2 x 3 experimental design : (a) lambs fed for a growing rate of 110 g/d *vs.* lambs fed for the maintenance of body weight during 3 months ; (b) control uninfected lambs *vs.* infected lambs with 16000 L3 of *Haemonchus contortus* 2 times at 2 weeks interval *vs.* lambs infected with 1150 L3/d during 28 d. Lambs were fed with Pangola hay and alfalfa pellets for a ratio of 9.6 g protein digestible in the intestine/MJ of metabolizable energy.

Results: Geometric means (GM) of fecal egg count of underfed lambs were markedly higher than those of growing lambs ($P<0.01$). Packed Cell Volume of lambs was more affected by under-nutrition than by infection with *Haemonchus contortus*. Circulating eosinophils response to infection was totally inhibited by under-nutrition. Establishment of parasites remained low but it was higher in underfed lambs than in growing lambs (GM: 7.2 *vs.* 1.9 % ; $P<0.05$). Percentage of L4 in worm population of underfed lambs were lower than in growing lambs (46 *vs.* 78 % ; $P<0.05$). Prolificacy of female worms of underfed lambs was lower than that of the others (GM: 844 *vs.* 5095 eggs/female/d ; $P<0.05$).

Conclusion: Under-nutrition of resistant sheep affects both reactions of the host and *Haemonchus contortus* population and its biology.

g.6.90 CHARACTERISATION OF DIETS LEADING TO HIGH AND LOW ESTABLISHMENT OF *OESOPHAGOSTOMUM DENTATUM* IN THE LARGE INTESTINE OF PIGS

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Background: It is well known that diets varying in carbohydrate (CHO) composition may have a profound influence on the metabolism in the pig large intestine and consequently the physiology and health of the animal.

Objective: The aim of the investigations was to study the impact of type and levels of CHO fermented in the pig large intestine on the establishment and reproduction capacity of *Oesophagostomum dentatum* in pigs

Results: Diets were formulated to provide variable amounts and types of CHO potentially available for fermentation in the large intestine. Low levels of fermentable CHO or readily fermentable CHO provided conditions leading to low establishment, whereas diets with high levels of resistant CHO deriving from lignified cell walls provided more favourable conditions. Neither pH nor short-chain fatty acids seems to play a role.

Conclusion: The results suggested that establishment of *Oesophagostomum dentatum* in the large intestine is influenced by type and amount of CHO fermented in this gastrointestinal compartment.

g.6.91 PROTEIN AND LEUCINE METABOLISM ACROSS THE GI TRACT OF *T. COLUBRIFORMIS* INFECTED LAMBS

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Background: Subclinical *T. colubriformis* infection in sheep can markedly impair protein metabolism leading to reduced liveweight gain, wool growth and protein deposition in the carcass. It has been shown that rates of protein synthesis in the intestine of guinea pigs is increased by *T. colubriformis* infection but there are no comparable data available for parasitised ruminants.

Method: Worm-free lambs were prepared with indwelling catheters into the aorta and the mesenteric and portal drained viscera at 4-5 months of age. Lambs were then infected with 2500 *T. colubriformis* L3/day for the duration of the experiment. Uninfected control lambs were fed a range of intakes to span the intakes of infected animals. Mass isotope tracer kinetics ([1-^{13}C] and [5,5,5-$^{2}H_3$] leucine) were used to examine leucine metabolism across the GI tract of infected lambs at weeks 5-7 and 11-13 of dosing and in control lambs.

Results: Infection had no detectable effect on whole body leucine flux but it markedly increased total leucine sequestration into the GI tract tissue proteins (24%) and GI tract oxidation of leucine (21-39%). Net release of 'absorbed' leucine for use by peripheral tissues was reduced (33%).

Conclusions: Sheep infected with *T. colubriformis* seem to repartition their protein metabolism to provide more amino acid to service the requirements of the infected gut tissues (repair damage and develop the immune response). As a consequence the availability of amino acid for protein anabolism in other tissues is reduced.

g.6.92 DIETARY PROTEIN INTAKE AND RESILIENCE OF CALVES TO *Haemonchus placei* INFECTION

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Background: Gastrointestinal parasitism is predominantly a problem in grazing ruminants, and there is a paucity of information on the influence of nutrition on the ability of growing calves to accommodate parasitic infection. The experiment was conducted to investigate the interactions between dietary protein intake and trickle infection, on some parasitological and immunological parameters in calves. **Method:** Thirty, 2-3-month-old worm-free male Holstein calves, were assigned to three groups each containing ten animals. Each group was offered one of three diets: High (HP), Medium (MP) and Low (LP) protein groups, balanced for energy and minerals. After an initial period of 4 weeks on the diets, the calves from each group were subdivided into 2 sub groups of 4 and 6 calves. A trickle infection of 5,000 *Haemonchus placei* L3 was given twice a week for 9 weeks to the sub group of 6 calves (I). The remaining 4 calves from each dietary group were used as non-infected control (C). Four weeks after the last infection, all calves were slaughtered and their worm burdens determined. Blood, faecal samples and bodyweights were recorded once a week. **Results and Conclusions:** The MP group had significantly higher mean total worm counts (13437 ± 9194) when compared with HP (9046 ± 4938) and LP (8550 ± 8430). Despite lower worm burdens resilience was reduced in the LPI calves, the serum total protein, albumin, PCV and haemoglobin values being lower than in the HPI and MPI calves. There was a dietary effect as these parameters and body weight gain were also lower in the LPC calves. There were no significant differences in IgG levels, measured by ELISA, between the three infected groups throughout the experiment. **Acknowledgement:** To FAPESP – São Paulo, Brazil, for financial support (Process 95/3654-1).

g.6.89-94 Nutrition - parasite interactions
g.7.01-14 Pharmacokinetics of parasiticides

g.6.93 GENETIC AND NUTRITIONAL CONTROL OF NEMATODE PARASITISM IN PERIPARTURIENT EWES.

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Background: Protein supplementation has been shown to enhance the ability of housed periparturient ewes to resist infection with nematode parasites. This experiment was designed to test the effects of short-term protein supplementation on the ability of periparturient ewes to resist nematode infection while grazing at pasture.

Method: 120 single-bearing Merino ewes selected either for resistance to *Haemonchus contortus* (n = 60) or at random (n = 60) were divided into subgroups (4 replicates for each dietary treatment) and maintained in 0.8ha pasture plots. Ewes were fed either 250g/hd/d cottonseed meal pellets for 6 weeks prior to the start of lambing, 6 weeks after lambing or were not fed.

Results: Faecal egg counts (FEC) of resistant ewes were significantly lower than those for random bred ewes throughout the experiment. Supplementation prior to lambing lowered FEC in random bred ewes to a level equivalent to resistant ewes but supplementation after lambing had no effect. Supplementation had no effect on FEC in resistant ewes throughout the experiment. There were no differences in bodyweight between the selection lines but supplementation increased bodyweight during the period of availability.

Conclusion: Protein supplementation of random bred ewes prior to lambing may assist to reduce pasture contamination with nematode larvae. Supplementation may provide increased resistance during pregnancy and lambing until similar benefits of long-term selection can be achieved.

Acknowledgment: This experiment was partly funded by Australian Centre for International Agricultural Research Project 9422.

g.6.94 THE EFFECT OF DIETARY PROTEIN LEVELS BEFORE TURNOUT ON THE SUBSEQUENT FAECAL EGG OUTPUT OF STRONGYLE-TYPE NEMATODES OF GRAZING SHEEP IN THE REGION OF JOANNINA, GREECE

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Background: The effect of dietary protein level during the postweaning period on faecal egg output of gastrointestinal nematodes was investigated in grazing sheep, with or without anthelmintic treatment as applied by farmers under local management practices in the region of Joannina, in NW Greece.

Method: Lambs were given a low protein (LP) or a high protein (HP) diet for three months (December-March) until grazing started. Half of the animals in each diet group were given anthelmintic treatment (AT) in March and June. The four groups (LP-AT, LP-NT, HP-AT and HP-NT) each consisted of pure-bred Butsiko (mountain type) and cross-bred Butsiko with Karamaniko (low plain type) lambs and grazed separate plots that were initially parasitologically and nutritionally similar. Faecal egg counts were recorded monthly.

Results: Faecal counts for strongyle-type eggs were higher in the LP-NT group than all the other groups and in the LP than the HP groups. This effect was intensified in the absence of anthelmintic treatment. No genotypic differences were detected.

Conclusion: The study indicates that faecal egg counts during grazing are influenced by levels of dietary protein before turnout.

g.7.01 INVOLVEMENT OF Mdr P-GLYCOPROTEIN IN THE FATE OF ENDECTOCIDES IN MICE AND RATS

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Purpose: The disposition of endectocides in animals is a major problem related to drug efficacy and toxicity .The involvement of the Mdr P-glycoprotein in the tissue distribution of ivermectin and moxidectin was assessed by use of Mdr P-glycoprotein deficient mice.The effect of verapamil (Mdr reversing agent) on the absorption of a pour-on formulation of ivermectin was evaluated in rats.

Methods: The Mdr (-/-) and wild type mice received ivermectin or moxidectin at the same dose of 0.2 mg/kg by oral route.Mice were euthanized at 24 h post-dosis. A pour-on formulation of ivermectin was topically applied to rats at a dose of 0.5mgkg, with or without verapamil at a dose of 5mg/kg. Plasma and tissues were analysed for parental drug levels by using an HPLC method with fluorescence detection.

Results: Deficient Mdr (-/-) mice exhibited increased concentrations of ivermectin or moxidectin in all tissues, with special emphasis in the brain . In rats receiving ivermectin after topical administration, the absorption of the drug was enhancedof 40% by the presence of verapamil.

Conclusion: Our results describe that Mdr P-glycoprotein take charge of both ivermectin and moxidectin with equal affinity. However, the consequences in terms of distribution and toxicity are different in view of the different pharmaco kinetic profiles of both drugs.The enhanced absorption of ivermectin associated to verapamil suggest that the P-glycoprotein is involved in the mechanism of absorption and that verapamil act as a competitive inhibitor.This hypothesis is consistent with early observation describing verapamil as a blocking agent of the P-glycoprotein mediated efflux of ivermectin in resistant selected strains of *Haemonchus contortus*. (Molento .M.B, Prichard R.K, 1997) This first experiment highlights a new way of pharmacological approach of research in order to improve the efficiency of the formulations of endectocides.

g.7.02 PHARMACOKINETIC AND EFFICACY OF EPRINOMECTIN IN GOAT

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Background : Eprinomectin is a new member of the avermectin class of anthelmintics that is licensed for use in lactating cows as a pour-on formulation. There is some evidence that eprinomectin is being used « off-licence »in dairy goats in France. Our objective was to determine the plasma pharmacokinetic profile and the efficacy of this new endectocide in goat.

Methods : 11 non lactating not pregnant Saanen goats were experimentally infected with *T. circumcincta*, *H. contortus* (abomasum) and *T. colubriformis* (small intestine). On day 28 post infection, 6 animals were given eprinomectin (Eprinex®, Mérial, France) topically at a dose of 0.5 mg/kg. Blood samples were collected at predetermined times over a period of 30 days and plasmas analysed by using an HPLC method with fluorescence detection. 30 days post-treatment, treated and control goats were necropsied for worm recovery.

Results : The observed peak plasma concentration (Cmax) of 5.60 ng/ml appears 2.55 days post dosing. The area under the plasma concentration curve (AUC) which refers to the extent of absorption was 72.31 ± 11.5 ng.d.ml. The mean residence time (MRT) was 9.42 ± 0.43 days. Anthelmintic efficacy was 100% for *T. circumcincta* and *H. contortus* and 98.2% for *T. colubriformis*.

Conclusion : It is generally accepted that the effect of a drug is better represented by the systemic AUC than by the actual dosage administered .The lower AUC in goats compared to cows (72.31 vs 239.07) and the less than complete efficacy against *T. colubriformis* indicate that the dose rate of 0.5 mg/kg when given topically could be considered as a suboptimal dosage in goats.

	Hc	Oc	Tc
C	364	1212	1100
T	0	0	3

1 animal until 20 wks

g.7.01-14 Pharmacokinetics of parasiticides

g.7.03 PLASMA AND MILK PHARMACOKINETIC OF EPRINOMECTIN IN DAIRY COWS

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Purpose: Eprinomectin is a menber of the avermectin class of compound,this new endectocide is licensed for use in lactating cows as a pour-on formulation there is no information regarding it's pharmacokinetic parameters.The present study was designed to determine the plasma profile and the milk excretion.
Methods: Five prim-holstein cows(570-830 kg) were kept outdoor and milked twice a day, the daily milk production was 19.9 ± 8.1 kg. A commercial formulation of eprinomectin (EPRINEX, MERIAL) was applied topically at a dose of 0.5 mg/kg.Blood and milk samples were collected at predetermined times over a period of 40 days.Plasma and milk were analysed for parental drug by using an HPLC method with fluoresence detection.
Results: The both plasma and milk concentrations increased progressively to reach maximal peak concentration of 43.76±18.23 ng/ml and 5.14±2.53 ng/ml respectively.The milk / plasma ratio was 0.102±0.048 and the amount of the drug recovered in the milk during this period was 0.109 % of the total dose.
Conclusion: It is generally accepted that the effect of a drug is better represented by the systemic area under the plasma concentration curve than by the actual dosage administered.The AUC observed for eprinomectin 239.07 ng.d.ml was significantly greater than the AUC observed for ivermectin 115.50 ng.d.ml or doramectin 168.00 ng.d.ml in animals receiving the same dose of 0.5 mg/kg by topical administration.The most sriking result of the present experiment is the low level of milk /plasma partitioning(0.1) by comparison with values obtained for others compounds of the avermectin /milbemycin family(1.0), in fact minor changes to the structures of these complex molecules can change their solubilities menbrane interactions involved in both absorption and elimination process. This first experiment emphasize the characteristic and original pharmacokinetic profile of eprinomectin in comparison with those obtained with others compounds of the endectocide's family.

g.7.04 CHLORPYRIFOS PLASMA AND MILK CONCENTRATIONS AFTER ITS POUR-ON ADMINISTRATION TO HOLANDO ARGENTINO COWS.

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Background: The arrival of Hematobia irritans to the south cone created the necessity of efficient and safe products for its control. The residual and toxicologic profile of organo phosphate based pharmaceuticals is a key issue and must be carefully considered.
Method: Chlorpyrifos (CPF) in 10 % solution (Clorpirifós 10 ® Biogénesis S.A. Argentina) was administered pour-on to 8 Holando Argentino cows in production. Blood and milk samples were obtained at different post-administration times. CPF was assayed by HPLC. Plasma cholinesterase levels were determined photometrically.
Results: Plasma and milk concentration profiles were irregular, confirming that pour-on absorption is a not an easily predictable process. Plasma concentrations were lower than milk concentrations. There was a decrease in plasma cholinesterase, but not significant statistically.
Conclusions: Plasma concentrations lower than milk concentrations are considered normal because of the lipophilicity of CPF. There was a good correlation between plasma concentrations and cholinesterase inhibition. No toxic symptoms were seen.

g.7.05 PHARMACOKINETICS OF DORAMECTIN AND IVERMECTIN TOPICAL FORMULATIONS

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Background: The present study was designed to compare the plasma concentration profiles of pour-on ivermectin and new topical formulation of doramectin when applied to cattle as a pour-on preparation.
Method: According to a parallel design, 12 young male beef cattle were given a single 500 $\mu g \cdot kg^{-1}$ topical application of a 0.5 % doramectin solution (Dectomax®, Pfizer Inc., USA) while 12 cattle were given a topical application of the same dose of a 0.5 % ivermectin solution (Ivomec® Pour-on Bovin, Merck AgVet Division, USA). Blood samples were taken regularly until 50 days post-administration. Doramectin and ivermectin were analysed by HPLC.
Results: The Cmax and Tmax of doramectin and ivermectin were not significantly different. In contrast, the AUC of doramectin (168.0 ± 41.7 ng·day·mL^{-1}) was significantly greater than that of ivermectin (115.5 ± 43.0 ng·day·mL^{-1}). Furthermore, the range of AUC values calculated for ivermectin was wider than that obtained for doramectin, extending from 51.3 to 182.3 ng·day·mL^{-1} for ivermectin *vs* 104.3 to 228.7 ng·day·mL^{-1} for doramectin.
Conclusion: This experiment demonstrated that a 500 $\mu g \cdot kg^{-1}$ pour-on administration of doramectin led to a 45 % higher overall exposure over ivermectin, as reflected by the mean area under the curve and that the relative variability in bioavailability was lower for doramectin than for ivermectin.

g.7.06 PHARMACOKINETICS OF IVERMECTIN, DORAMECTIN AND MOXIDECTIN AFTER ORAL ADMINISTRATION IN HORSES

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The comparative plasma disposition of Ivermectin (IVM), doramectin (DRM) and moxidectin (MXD) were determined in horses following oral administration (Table 1). Twenty-four (24) horses (490-880 kg) were allocated into three groups of eight. Animals in each group received either IVM (Eqvalan paste, 1.87% w/v), MXD (Equine gel, 2% w/v) or DRM (Dectomax 1% w/v, injectable solution for cattle) orally as a single bolus dose at 200 µg/kg. Blood samples were collected up to 80 days post treatment and at 188 and 197 days for MXD. The plasma samples were extracted, derivatized and analyzed by high performance liquid chromatography. The parent compounds were detectable for 11 days for IVM, 39 days for DRM and 188 day for MXD (under the limit of quantification). Large interindividual variations were observed in each group. The longer retention time and higher concentrations found for MXD in comparison to IVM and DRM may be explained by its more lipophilic nature.
Table 1: Pharmacokinetic parameters (mean ± SEM) of IVM, DRM and MXD following oral administration in horses at 200 µg/kg.

Parameters	IVM	DRM	MXD
AUC_{last}	46.41±8.20	76.54±15.57	86.81±10.45
MRT_{last} (d)	2.40±0.15	4.00±1.05	16.31±2.45
C_{max} (ng/ml)	23.50±4.15	26.98±5.46	30.064.47
t_{max} (d)	0.40±0.09	0.44±0.12	0.31±0.04

g.7.01-14 Pharmacokinetics of parasiticides

g.7.07 PHARMACOKINETICS OF OXFENDAZOLE, FENBENDAZOLE AND OXIBENDAZOLE AFTER ORAL ADMINISTRATION IN HORSES

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The pharmacokinetics of oxfendazole (OFZ), fenbendazole (FBZ) and oxibendazole (OBZ) were determined in plasma following oral administration to horses. Twenty-four (24) horses (390-720 kg) were allocated into three groups of eight. Animals in each group received the commercially available equine formulation of OFZ, FBZ and OBZ at a dose of 10 mg/kg. Blood samples were collected up to 120 hours post-treatment and plasma analyzed by high performance liquid chromatography. The estimated concentrations of OBZ from all samples were below the limit of quantification (0.005 µg/ml) and significantly higher concentrations of sulphone metabolites than parent molecules of OFZ and FBZ were detected. Significantly higher C_{max} and larger AUC of parent molecules were obtained for OFZ (C_{max}: 0.35±0.07 µg/ml, AUC: 4.40±0.90 µg.h/ml) compared to FBZ (C_{max}: 0.04±0.01µg/ml, AUC: 0.61±0.01 µg.h/ml). Such differences could be a reflection of the lower solubility of FBZ.

Table 1: Pharmacokinetic parameters (mean ± SEM) of OFZ, FBZ and their metabolites following oral administration to horses at 10 mg/kg.

	OFZ			FBZ		
Parameters	OFZ	FBZ	$FBZSO_2$	FBZ	FBZSO	$FBZSO_2$
AUC_{inf} (µg.h/ml)	4.4±0.9	1.5±0.4	13.2±1.7	0.6±0.0	0.2±0.0	1.1±0.2
MRT_{inf} (h)	19.62±4.3	18.7±3.0	18.7±3.0	14.7±1.8	13.3±1.3	18.2±2.2
C_{max} (µg/ml)	0.4±0.1	0.1±0.0	0.8±0.1	0.04±0.0	0.01±0.00	0.06±0.01
t_{max} (h)	8.9±3.0	13.5±2	12.00±3.	6.8±2.3	9.5±3.5	10.5±3.2

g.7.08 MODIFIED IVERMECTIN 1% INJECTABLE: PHARMACOKINETICS AND FIELD EFFICACY

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In Argentina, Brasil and Uruguay, injectable SC ivermectin 1% formulations are widely used, characterized by their lyphophilic profile, a long half-life of elimination, high bioavailability and an extended persistency of effect. New formulations based on excipient changes, have been developed to improve these characteristics. In 1997, Biogénesis S.A. patented a long-acting formulation with a Modified Absorption slow release excipient (MABS[R]), the first in its field. The pharmacokinetic profile in cattle showed marked advantages: Cmax was 30-42 ng/ml and minimum concentrations reached 2.81 ng/ml at 30 days PT; the conventional formulations averaged 0.9 to 1 ng/ml. Half-life of elimination reached nearly 9 days, an improvement on the previous 5-6 days. In sheep the formulation showed a slow absorption phase, with a Cmax at 3 days with a high AUC; mean residence time was 6.36 days and half-life of elimination 5.27 days. These changes were reflected in critical field trials: in cattle, against *Psoroptes ovis* var. *bovis*, *Dermatobia hominis* and *Haematobia irritans* (adulticide, 28 days) and in Persistency of Activity against GI nematodes (*Cooperia* spp, 35 days) ; in sheep, against *Psoroptes ovis* and *Melophagus ovinus* (with a single 300 mcg/kg dose).

g.7.09 PLASMA AND TARGET TISSUES AVAILABILITIES OF IVERMECTIN ADMINISTERED AS AN OIL-BASED FORMULATION TO CATTLE

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Background: Slight differences in formulation account for changes to the plasma kinetics and ecto-endoparasiticide activity of endectocide compounds. This work reports on the plasma disposition kinetics and target tissues availability of ivermectin (IVM) after the subcutaneous (SC) and intramuscular (IM) administration of an oil-based long-acting formulation (IVM-LA) to cattle.
Method: Parasite-free Aberdeen Angus calves (n=44) (240 kg) were treated (200 µg/kg) with either IVM-LA (Bayer Argentina S.A.) given by SC (group 1) and IM (group 2) injections or the IVM-control formulation (Ivomec®, MSD AGVET) (group 3). Blood samples were taken over 35 days post-treatment. Two animals (groups 1 and 3) were sacrificed at either 20, 30, 35 or 40 days post-treatment, and abomasal/small intestine mucosas, lung and skin samples collected. Plasma/tissue samples were analyzed by HPLC with fluorescence detection.
Results: Prolonged IVM absorption half-life and delayed peak plasma concentration were obtained following the SC administration of the IVM-LA compared to the IVM-control formulation. No differences in total plasma availability were observed among treatments. However, IVM mean plasma residence time and elimination half-life were significantly longer after injection of the IVM-LA formulation. Plasma concentrations were above 0.5 ng/ml during 20 (control) and 27.5 days (IVM-LA), respectively ($P<0.05$). IVM concentrations in target tissues (days 20 to 40 post-treatment) were higher for the IVM-LA compared to the control treatment. Marked higher concentrations were obtained in abomasal mucosa (11-fold), intestinal mucosa, (4.8-fold), lungs (4.9-fold) and skin (1.75-fold) 35 days after the IVM-LA treatment.
Conclusion: The modified kinetic behaviour of IVM obtained after the administration of this oil-based formulation, may positively impact on its strategic use in cattle.

g.7.10 THE EFFECTS OF PF4 ON *ASCARIS* MUSCLE CELLS: AN ELECTROPHYSIOLOGICAL STUDY

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Background: PF4 is a neuropeptide which was originally isolated from *Panegrellus redivivus*, a free-living nematode. This study investigated the effects of PF4 on *A. suum*.
Method: The investigations were carried out on *A. suum* somatic muscle cells, using two microelectrode current- and voltage-clamp techniques. PF4 was compared with PF1 and GABA.
Results: PF4 caused an increase in chloride-ion conductance through the muscle cell membrane. Its speed of action was similar to that of GABA and faster than that of PF1.
Conclusion: The observations were consistent with the hypothesis that PF 4 directly gates chloride ion channels in *A. suum* somatic muscle cells.
Acknowledgement: Thanks are due to Pharmacia and UpJohn Inc., Kalamazoo and the Department of Preclinical Veterinary Sciences, Edinburgh for funding the work.

g.7.01-14 Pharmacokinetics of parasiticides

g.7.11 THE PATCH-CLAMP TECHNIQUE IN STUDYING ANTHELMITIC DRUG ACTION

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Background: Many anthelmintic drugs are known to act on membrane ion-channels. Levamisole and pyrantel act on muscle nicotinic acetylcholine receptors; the avermectins act on a glutamate-gated chloride channel in nematode pharynx; and piperazine acts on GABA-gated chloride channels.
Method: Enzymatic treatment of nematode muscle with collagenase causes the formation of vesicles from the muscle surface. These vesicles are composed of sarcolemma but also contain numerous membrane proteins including ion-channels. These membrane vesicles are suitable for use in the patch-clamp technique which permits the study of individual ion-channel molecules within a small area of membrane.
Results: Patch-clamp studies have described the properties of the sites of action of several anthelmintics. More recent studies have furthered our understanding of the mechanism of resistance to levamisole. Additionally the patch-clamp technique has described the properties of a novel chloride channel that is a potential target site for future anthelmintics.
Conclusion: The patch-clamp technique offers a method of investigating anthelmintic action and the mechanism of resistance to certain anthelmintics. This technique has identified ion-channels that are potential target sites for new therapeutic compounds.

g.7.12 EFFECTS OF IVERMECTIN ON GLUCL CHANNELS IN *ASCARIS* PARYNX

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Background: Avermectins are very potent anthelmintics. The mechanism of their actions is not fully elucidated. Recently, effects of ivermectin on glutamate-gated chloride (GluCl) channel currents were established. In the present study we are investigating these effects at the single-channel level.
Method: Single-channel currents were recorded from membrane vesicles derived from *Ascaris suum* pharyngeal muscle. The effects of glutamate and ivermectin on the glutamate-activated chloride channels were studied using isolated inside-out patches.
Results: Ivermectin, applied to the cytoplasmic surface of the membrane, was found to increase the probability of opening of channels activated by 1 mM glutamate, applied to the extracellular surface of the membrane via the pipette solution. Ivermectin by itself, also opened chloride channels. 1nM ivermectin was regularly found to produce effects. The glutamate- and ivermectin-gated channels showed a spread of conductances between 10 - 50 pS. No regional differences in the patch sensitivity to the agonists were observed, suggesting that the glutamate receptors are present throughout the pharyngeal muscle of *Ascaris suum*.
Conclusion: Ivermectin, applied to the inner membrane surface, was found to activate a heterogenous population of chloride channels. It also increased the probability of opening of channels activated by glutamate in the pipette.

g.7.13 PLASMA KINETICS AND TISSUE DISTRIBUTION OF RICOBENDAZOLE ENANTIOMERS IN CATTLE

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Background: Ricobendazole (RBZ), the sulphoxide derivative of albendazole, exhibits a pattern of efficacy similar to its parent compound. RBZ is a chiral molecule and its (+)/(-) enantiomeric forms have been identified in plasma. Interpretation of the enantioselectivity of a pair of enantiomers is relevant to understand drug action. The impact of the chiral behaviour of benzimidazole sulphoxides on their kinetics and efficacy needs to be evaluated. The plasma disposition and pattern of tissue distribution of both enantiomers were characterised following the intravenous (IV) injection of racemic RBZ to cattle.
Method: Eighteen (18) Holstein calves (140 kg) received RBZ (racemic solution 150 mg/ml, Bayverm PI, Bayer Argentina SA) by IV administration (7.5 mg/kg). Blood samples (n=8) were collected over 48 h. Two animals were sacrificed at either 4, 12, 20, 28 or 36 h post-treatment to obtain liver, bile, abomasal/upper small intestine mucosa and abomasal/intestinal fluids samples. Plasma and tissue samples were extracted and analysed by HPLC using reverse/chiral phases.
Results: The (+) and (-)RBZ enantiomers and the sulphone metabolite were detected in plasma and tissues following the IV administration of the racemic solution. The plasma disposition kinetics of both enantiomers was markedly different. (+)RBZ was the enantiomer recovered at the highest proportion in plasma and target tissues/fluids. The (-) enantiomer was depleted rapidly from the bloodstream ($T_{1/2}el$= 2.8 h) compared to the (+) molecule ($T_{1/2}el$= 5.3 h). The availabilities of (+)RBZ in bile (36%), abomasal mucosa (748%), intestinal mucosa (328%), abomasal (25%) and intestinal (67%) fluids were higher than those measured for the (-) enantiomer. Both enantiomers were recovered in equivalent proportion from the liver tissue. Interestingly, albendazole was recovered from the liver and gastrointestinal mucosa of RBZ-treated calves.
Conclusion: An enantioselective disposition and tissue distribution of RBZ accounts for the higher plasma/tissues availability of the (+) and the faster depletion of the (-)RBZ enantiomer.

g.7.14 EFFECT OF SURFACTANTS ON ALBENDAZOLE ABSORPTION IN CATTLE

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Background: Albendazole (ABZ) is a widely used broad-spectrum benzimidazole (BZD) anthelmintic. Low hidrosolubility and poor/erratic gastrointestinal (GI) absorption are detrimental for the systemic availability and resultant clinical efficacy of BZD compounds. Different strategies are currently investigated to improve their bioavailability and efficacy in different animal species and humans. Surfactant agents facilitate dissolution of lipophilic drugs and increase membrane permeability. The influence of amphiphilic surfactants on the pattern of absorption and systemic availability of ABZ and its metabolites in cattle was characterised.
Method: Twenty (20) parasite-free Holstein calves (100 kg) were randomly allocated into 4 groups and treated intraruminally (10 mg/kg) using one of the following ABZ suspensions (100 mg/ml): *control* without surfactant (75/25 dimethyl sulphoxide/saline solution) (group A), *5 mM sodium taurocholate* (STC) in saline solution (group B), *8.27 mM sodium lauryl sulphate* (SLS) in saline solution (group C) and a *commercial micronized formulation* (Valbazen ®, Pfizer Inc. SA) (group D). Blood samples were taken over 72 h post-treatment and plasma analysed by HPLC.
Results: Albendazole sulphoxide (ABZSO) and sulphone were the metabolites found in plasma. Increased ABZSO peak plasma concentrations (26-158%) were obtained when ABZ was co-administered with both surfactants. ABZSO plasma availability was significantly greater after the ABZ-STC (33%) and ABZ-SLS (164%) co-administrations compared to that obtained in the control group. A similar ABZSO plasma availability was obtained following the treatments with the ABZ-SLS and the commercially available formulation.
Conclusion: Surfactant-mediated enhanced dissolution and absorption of ABZ accounted for the observed increased systemic availability of the active ABZSO metabolite in cattle. These results should be considered among other strategies to improve the use of BZD anthelmintics.

g.7.15-27 Parasite biochemistry

g.7.15 MOLECULAR CHARACTERIZATION OF CA2+-BINDING PROTEINS IN TRITRICHOMONADS

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Protozoa of the species *Tritrichomonas foetus* are parasites important for veterinary medicine by causing problems of bovine infertility and abortion. They are highly motile through three anterior flagella and a posterior flagellum forming an undulating membrane, thus allowing the parasite to colonize the urogenital tract of male and female cattle. For the tissue destructive effect of *T. foetus*, attachment to the host mucosa and direct contact to the host cells mediated by parasite surface molecules are regarded to be necessary.

In order to identify proteins with membrane and thus potential surface localization crude cell fractionation using the detergent Triton X114 was performed. By immunoblot with a polyclonal antiserum against intact *T. foetus* parasites, immunoreactive bands in a fraction potentially enriched in membrane proteins were identified and used as substrate for the affinity purification of monospecific antibodies. With two of the affinity purified antibodies, screening of a parasite cDNA library was done resulting in the identification of three different groups of clones. One series of clones corresponding to a 66kDa protein of *T. foetus* displayed homology to the Calreticulin protein family of Ca2+-binding proteins. With antibodies corresponding to a 42kDa antigen, two groups of cDNA clones were isolated which turned out to be closely related but not identical. Both groups contained 6 socalled EF-hands, sequence motifs which are known to be able to bind Ca2+ with high affinity. All three *T. foetus* proteins were expressed in *Escherichia coli*. Binding assays with a radioactive ^{45}Ca isotope on native and recombinant proteins were performed. Monospecific antibodies were affinity purified on the recombinant antigens and used for immunofluorescence. These studies revealed that the 66kDa Ca2+-binding protein presumably is localized in the endoplasmic reticulum of the parasite. Consequently, it potentially may have the function of a molecular chaperone. In contrast, the 42kDa Ca2+-binding protein was localized on the flagella of *T. foetus*. The abundance of the protein, its presumable Ca2+-binding properties and flagellar localization suggest that it participates in molecular processes associated with the high motility of the parasite, like analogous Ca2+-binding proteins in the flagella of trypanosomes.

g.7.16 ANALYSIS OF A DIROFILARIA IMMITIS AROMATIC AMINO ACID DECARBOXYLASE

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Background: Aromatic amino acid decarboxylase (AADC) is the key enzyme for synthesis of monoamine neurotransmitters and trace amine neuromodulators in higher eukaryotes. In insects, AADC has been reported to have other roles, such as peritrophic membrane synthesis, exoskeletal cuticle formation and maturation, and female fertility. In *C. elegans*, an AADC-like protein can rescue the *let-56* mutation, a mutation resulting in larval death or slow development. AADC in the parasitic nematode *D. immitis* was investigated.
Method: Adult extracts and excretory/secretory products of third and fourth stage larvae were evaluated for the presence of AADC using 7-amino-4-methyl-coumarin (AMC)-conjugated amino acid substrates in a fluorimetric assay. The enzyme was purified using different chromatography methods followed by isoelectric focusing and SDS-PAGE. Enzyme activity was also assessed by dopamine formation and CO_2 formation assays. The effect of AADC inhibitors and protease inhibitors on purified *D. immitis* AADC activity was evaluated.
Results: The enzyme had a molecular mass of 48 kDa, a pI of 5.6 and had very specific activity towards phenylalanine, tyrosine and tryptophan substrates. The enzyme could catalyze formation of CO_2 from phenylalanine and dopamine from L-dopa. The enzyme was susceptible to inhibition by Pefabloc® and p-chloromercuribenzoic acid but not to diisopropyl flurophosphate. The K_m of the enzyme for H-Phe-AMC, H-Tyr-AMC and H-Trp-AMC were 32.1 µM, 35.1 µM and 29.1 µM, respectively.
Conclusion: Adults and larvae of *D. immitis* have an AADC, and the enzyme is secreted by larval stages. AMC-conjugated aromatic amino acids, usually used to assess protease activity, can be substrates for AADC. Protease activity must be distinguished from AADC activity when using these substrates.

g.7.17 PROTEOLYTIC ACTIVITIES OF LARVAL STAGE OF *ECHINOCOCCUS MULTILOCULARIS*

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Background: Alveolar hydatid (*Em*) develops and metastasizes within the tissue of the intermediate hosts as a malignant cancer. Metallomatrix proteinases (MMP) excreted from the tumors do act important role in their spreading within tissues. The MMP proteolytic activities of Em is firstly demonstrated.
Methods & Results: Gelatin substrate zymography to examine extracts derived from larval stage of *Echinococcus multilocularis* for proteolytic activity.
Em were isolated from natullarly infected *Clethrionomys rufocanus bedfordiae*, laboratory maintained *Meriones unguiulatus* and a *Sigmodon hispidus*. Extracts derived from these materials contained several proteins with high proteolytic activity, including a 20 kDa proteinase which was inhibitable by EDTA. Using anti- human mammary cancer MMP 9 monoclonal antibody , positive pattern was found on the space substance of brood capsule and surface of protoscolices of the tissue sections of Em from the *S. hispidus*.
Conclusion: We demonstrated proteolytic activities from extracts of Em and this enzyme seems to be a MMP. And also demonstrated its localization within the Em tissue and this proteinase possesses same epitope of MMP 9 from human mammary cancer.
Acknowledgments: This study was supported in part by the Cooperative Research fund (1993) and the Gakujyutsu Frontier Cooperative Research in Rakuno Gakuen University (1998).

g.7.18 TWO-DIMENSIONAL PROTEIN AND ANTIGEN PROFILES OF *TOXOPLASMA* AND *NEOSPORA*

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Background: *Toxoplasma gondii* and *Neospora caninum* are very closely related phylogenetically and share a broad range of phenotypic characters. Both parasites may infect a similar range of intermediate hosts in which they may be transmitted congenitally. However, while congenital toxoplasmosis is known to be of particular importance in humans, pigs and small ruminants, *N. caninum* is now recognised as a major cause of abortion in cattle. Thus far, little is known of the biological or immunological basis responsible for this difference.
Method: Here we used two-dimensional polyacrylamide gel electrophoresis to examine the protein and antigen profiles of *T. gondii* and *N. caninum* tachyzoites which were grown using the same line of host cells and culture conditions. Proteins were first resolved on immobilised pH gradient gels, and subsequently on SDS pore gradient gels under denaturing conditions. Gels were stained with silver and analysed by densitometry. Consensus protein profiles were constructed from the data derived from ten gels of each species obtained by the same electrophoresis conditions.
Results and conclusion: Significant differences were revealed between the protein profiles of *T. gondii* and *N. caninum* tachyzoites. While some protein spots were shared by the two parasites, the majority of the protein spots observed gave a specific pattern for either *T. gondii* or *N. caninum*. These latter spots will now be further evaluated by Western Blotting using polyclonal sera obtained from sheep and cattle experimentally infected with either *T. gondii* or *N. caninum*.

g.7.15-27 Parasite biochemistry

g.7.19 GUT EXPRESSED PROTEINS WHICH CONFER PROTECTION AGAINST HAEMONCHOSIS IN LAMBS

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Background: Lambs are significantly protected against haemonchosis following vaccination with two distinct peptide complexes which are expressed in the gut of the blood-feeding parasite stages and contain significant peptidase activity. Here, we describe progress on the molecular and functional definition of the peptides comprising these complexes.

Method: The protein complexes have been defined using SDS-PAGE, N-terminal sequencing, peptidase activity determinations and lectin binding properties. Antisera to the whole complexes and various subfractions have been used to isolate lambda ZAP clones expressing cDNAs encoding individual peptide components subsequent sequence analyses of which have enabled functional identification.

Results: H-gal-GP contains at least 3 distinct metallo endopeptidases, a pepsinogen, a galectin and 2 peptides which may act as protease inhibitors. TSBP are enriched for cysteine protease activity and this can be ascribed to 3 distinct cysteine protease gene products. The prominent peptide in TSBP is a glutamate dehydrogenase homologue which does not contribute to protection.

Conclusion: This work has provided an insight into potential function and possible means for further fractionation which are necessary to facilitate recombinant vaccine production.

g.7.20 *HAEMONCHUS CONTORTUS* CYTOCHROME P450; SUBSTRATE SPECIFICITY STUDIES

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Background: Cytochrome P450 monooxygenases catalyse the oxidation of endogenous and exogenous substrates, including steroids, fatty acids and drugs. They are found in bacteria, fungi, plants, insects and vertebrates. They have recently been detected in the nematode *H. contortus*, and the present paper reports on efforts to characterise their substrate specificities and their potential role in anthelmintic resistance.

Method: Microsomes prepared from adult and larval stages of *H. contortus* were examined for their ability to catalyse a number of oxidation reactions. Activities were measured in a macrocyclic lactone (ML) -resistant and a -susceptible isolate.

Results: Cytochrome P450 monooxygenase activities (epoxidation and O-deethylation) are readily detectable in microsomes prepared from adult and larval *H. contortus*. Peroxygenase activity supported by cumene hydroperoxide is also detectable, however, the significance of this pathway *in vivo* is unknown. Activities were equivalent in a ML-resistant and a -susceptible isolate.
No ecdysone 20-monooxygenase activity could be detected in microsomes prepared from various larval stages.

Conclusion: *H. contortus* possesses cytochrome P450 capable of a number of monooxygenase and peroxygenase activities *in vitro*. Enzyme levels appear to be equivalent in ML-resistant and -susceptible isolates. The low levels of activity detected in adult nematodes means that extensive substrate specificity studies will most likely need to await the generation of larger quantities of cytochrome P450 protein using molecular expression systems.

g.7.21 CUTICULAR HYDROCARBON DIFFERENCE BETWEEN *CULICOIDES ARAKAWAE* AND *C. SCHULTZEI*

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Background: The utility of cuticular hydrocarbons in chemosystematic studies has been demonstrated in mosquitoes. Our objective was to use cuticular hydrocarbons and lipids to investigate specific and sexual difference between *C.arakawae* and *C.schultzei*.

Method: *C.arakawae* and *C.schultzei* were collected from a poultry farm in Guangzhou, China. These midges were divided into four populations according to species and sex. Pooled samples of 60 midges from each population and three replicates per population, were immersed in 1 ml of hexane two consecutive times for 3 min each. The combined hexane extracts were then evaporated to dryness at 50 ℃ and redissolved in 10 ml of hexane, then two microliter of extracts were injected with a splitless injector into a gas chromagraphy-mass spectrometry apparatus for analysis.

Results: *C.arakawae* and *C.schultzei* had similar spectrum of cuticular hydrocarbon and lipid. Forty cuticular hydrocarbons and 11 cuticular lipids were identified. The cuticular hydrocarbons consisted of 36 alkanes, 3 alkenes and 1 cycloalkane. The alkanes ranged in length from 16 to 34 carbons. Of these n-pentacosane, n-heptacosane, n-octacosane, n-nonacosane and tricontane differed significantly($p<0.01$) between *C.arakawae* and *C.schultzei*. Among the five alkanes *C.arakawae* and *C.schultzei* could also be differentiated with principal components analysis. Of these 2,5-dimethyl-phenathene was characteristic alkane of *C. schultzei;* 1-octodecene and (E)-3-eicosene were characteristic alkanes of *C. arakawae*.

Conclusion: Cuticular hydrocarbons differ significantly between *C.arakawae* and *C. schultzei* and therefore may be used as biochemical markers to differentiate the two species.

g.7.22 Inhibition of trypanosome growth by classical inhibitors of protein farnesyltransferase.

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Protein prenylation or attachment of a prenyl group such as farnesyl to certain eukaryotic cell proteins by protein prenyltransferase is required for the functioning of a number of cellular processes including signal transduction. Protein prenylation is a recently discovered post-translational modification that occurs in many eukaryotic cells. The three structural classes of protein prenylation that have been identified are c-terminal farnesyltranslation (15-carbon isoprenoid) and c-terminal geranylgeranylation (20-carbon isoprenoid) and c-terminal digerenylgeranylation. We studied the effect of classical inhibitors of protein farnesyltransferase on trypanosome growth in culture and on the purified enzyme. Some of the inhibitors substantially retarded trypanosome cell growth and the farnesylation activity of the purified enzyme in vitro. Our studies suggest that protein farnesyltransferase is a potential target for the development of novel drugs against trypanosomiasis.

Acknowledgment: This investigation received financial support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR).

g.7.15-27 Parasite biochemistry

g.7.23 INTESTINAL GLYCOPROTEINS FROM *HAEMONCHUS LONGISTIPES* AND *H.PLACEI*

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Background: The intestinal microvillar membrane of the parasitic stages of *Haemonchus contortus* is a rich source of hidden antigens, such as the membrane aminopeptidase H11, useful in vaccination against this nematode.Different populations of *H. contortus* may have different genetic characteristics resulting in antigenic variation in these proteins. It is unlikely that the variation would be greater than that between species, so to assess the extent of possible variation we analysed cross-reactions with the closely related *H.placei* and the more distant *H.longistipes*.

Methods: Detergent extracts of washed endotube-brush border preparations from each species were i) fractionated by chromatography and SDS-PAGE and the binding of peptide and carbohydrate epitope-specific antibodies determined on blots, ii) assayed for aminopeptidase A and M activities and the effect of anti-H11 antisera determined.

Results: The detergent extracts of *H. longistipes* and *H. placei* each yielded SDS-PAGE patterns and contained membrane aminopeptidase activities comparable to those of *H. contortus*. Antibodies to gut surface proteins from *H. contortus* cross-reacted strongly with those from the other species and anti-H11 antibodies inhibited the aminopeptidase activity.

Conclusions: Any genetic (strain) variation within *H.contortus* is unlikely to be sufficient for evasion of vaccination-induced antibodies. The H11-based vaccine is potentially effective against all species of *Haemonchus*.

g.7.24 MONOCLONAL ANTIBODIES AGAINST *NEOSPORA CANINUM* TACHYZOITES: CHARACTERIZATION OF ANTIGEN FOR INVASION INTO HOST CELLS

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Background: *Neospora caninum* (*N. caninum*) is an apicomplexan parasite that was originally identified as an etiological agent of neurological diseases in dogs and has subsequently been found to associate with abortions in dairy cattle. The surface proteins of *N. caninum* are prime candidates for mediating adhesion and invasion into host cells by a receptor-ligand system which is most likely based on protein-protein interactions. The aim of this study is to produce monoclonal antibodies (MAbs) against *N. caninum* and to identify the antigen which might play a crucial role during invasion into host cells.

Method and Results: Hybridoma-producing MAb against *N. caninum* tachyzoites was produced from splenocytes of infected BALB/c mouse. Confocal laser microscopic study showed that antigens recognized by these MAbs were located on not only the surface, but also the inside or the apical end of the parasite. Some MAbs, which recognized 70, 42 and 36 kDa proteins of the parasite, significantly inhibited the invasion of the parasites *in vitro*. The MAbs, which recognized 42 and 36 kDa proteins of the parasite, reacted with Nc-p43 and Nc-p36 expressed by vaccinia virus and *E. coli*, respectively.

Conclusion: These results suggested that 70 kDa protein, Nc-p43 and Nc-p36 were involved in the entry of the parasite into host cells *in vitro*.

g.7.25 CYTOCHROME OXIDASE I GENE IN LARVAE OF *OESTRIDAE* FAMILY CAUSING MYIASIS: A PRELIMINARY APPROACH).

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Background: In the past few years mitochondrial DNA (mtDNA) has been commonly employed as a molecular marker for evaluating intergenomic heterogenicity and phylogenetic studies in insect populations. Cytochrome oxidase I gene (COI) of many insects (Order Ortoptera, Diptera, Hymenoptera, Lepidoptera) and some arachnida, were characterized by using preserved primers. The aim of this paper is to obtain more information concerning the most diffused larvae of the Oestridae family causing myiasis (*Gasterophilus* spp., *Oestrus ovis, Hypoderma bovis, H. lineatum, Przhevalskiana silenus*) to investigate the taxonomic relationship in this group.

Method: The DNA of third instar larvae of *Hypoderma bovis, H. lineatum*, (bovine hypodermosis), *Przhevalskiana silenus* (goat warble fly infestation), *Gasterophilus* spp. (equine gasterophilosis) and *Oestrus ovis* (oestrosis) was extracted. PCR was prepared by using 5 primers (UEA1, UEA3, UEA5, UEA8, UEA10) in different combinations.

Results After optimization of the PCR set-up the reaction performed with the above mentioned primer combination produced different molecular weight bands for each primer combination as described in the bibliography.

Conclusion The results obtained represent an advance in the detection of specific sequences of the larval genomes causing the most important myiasis; sequencing and comparing the amplification profiles with others of other families causing myiasis (Calliphoridae and Sarcophagidae) will allow us to identify the origin of the parasitic behaviour and the taxonomic relationships among the diptera causing myiasis.

g.7.26 GENETIC VARIABILITY IN SUSCEPTIBLE AND RESISTANT *OESOPHAGOSTOMUM* LINES

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Purpose: During the selection for resistance, quantitative changes in the population gene pool usually occur. To obtain more information about these processes, the population genetic structure of *Oesophagostomum dentatum* (Od) and *O. quadrispinulatum* (Oq) lines susceptible and resistant to anthelmintics (levamisole, pyrantel, flubendazole) were analysed using allozyme analysis.

Method: Isoelectrofocusing technique was used to investigate a gene composition of adult worms. LKB Ampholine of pH 4.0-6.5 was added to the polyacrylamide gel solution. The polymorphic *Mpi* locus which codes for mannose-phosphate isomerase enzyme (MPI, EC 5.3.1.8) was found to be suitable for scoring genotypes.

Results: Significant changes in gene frequencies have been observed in resistant and susceptible lines. In resistant isolates, allele 100 decreased frequency whereas allele 104 occurred more frequently when compared to susceptible populations. For the flubendazole-resistant line GIBZ, a unique allele 97 was recorded and thus can be associated with the resistance status. Heterozygous genotypes have been almost exclusively possessed by females (1.2% in males vs. 54.3% in females).

Conclusion: Based on the data obtained, the *Mpi* locus seems to be involved in the acquisition of resistance. Resulting genotypes suggest that a recessive form of resistance appears to predominate in the *Oesophagostomum* isolates examined.

g.7.15-27 Parasite biochemistry
g.7.28-33 Use of PCR in parasitology

g.7.27 GLUTATHIONE-S-TRANSFERASES FROM *TELADORSAGIA CIRCUMCINCTA*

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Background: Glutahione-S-Transferases (GSTs) are one of a number of enzymes that are believed to be involved in parasite defence against host immune attack. GSTs are thought to detoxify lipid peroxidation products arising from free radical attack in response by the host to the parasite. As a result they have been identified as potential targets for chemotherapy and immunotherapy.

Native GST subunits are normally found as dimers and vary in size from 23-26 kDa. In mammals GSTs exist as isoenzymes and are grouped into 4 families, alpha, mu, pi and theta, however, structural and biochemical analyses of parasite GSTs to date, indicate that these enzymes don't fit into any one of the mammalian classes.

Method: In this study native GSTs have been purified from whole worm extracts of adult *Teladorsagia circumcincta* by affinity chromatography using glutathione agarose. Purified GSTs have been analysed by SDS-PAGE, NEPHGE and immunoblotting.

Results: Specific activities of the GSTs purified from *T. circumcincta* have been determined and 2 isoenzymes have been identified with a molecular weight of 24 kDa which are recognised strongly by anti *Heligmosomoides polygyrus* GST antisera. The 2 isoenzymes of GST identified have very similar molecular weights and isoelectric points.

Acknowledgements: We wish to thank Dr D.P. Knox, Moredun Research Institute, Edinburgh for generous supply of parasite material.

g.7.28 DIFFERENCES IN THE ITS-2 AND GENUS-SPECIFIC PCR FOR THE DIFFERENTIATION OF EGGS OR LARVAE FROM GI NEMATODES OF RUMINANTS

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Genetic differences in the nucleotide sequence of the second internal transcribed spacers (ITS-2) among *Trichostrongylus axei, T. colubriformis, Ostertagia ostertagi, Cooperia oncophora, C. punctata, Nematodirus helvetianus, N. filicollis* and *Haemonchus contortus* are described. The ITS-2 sequences of the 8 species ranged between 230 and 241 bp in length. Sequence similarities between the different genera varied between 60% and 80%. Identities between the different species within a genus varied between 99% for *C. oncophora* and *C. punctata*, 95% for *T. axei* and *T.colubriformis*, and 89% for *N. helvetianus* and *N. filicollis*. The ITS-2 sequences proved to be useful for species differentiation. Except for the species of *Cooperia* (2.07% intraspecific variations for *C. oncophora* and 0.83% for *C. punctata*) the degree of intraspecific variations (*N. filicollis* 0.85%, *T. col.* 1.26%, *T. axei* 1.27%, *H. contortus* 2.60%, *O. ostertagi* and *N. helvetianus* no variation) was markedly lower than the interspecific variations allowing a reliable differentiation within the ITS-2 region between single species.

Using sequences of the ribosomal second internal transcribed spacer (ITS2) PCR primers were designed for the differentiation. Single eggs or larvae from faeces could be differentiated without previous DNA extraction. Quantification of the PCR result proved to be difficult as the DNA content of eggs varied considerably between eggs from fresh or 24 h old faeces.

g.7.29 EVALUATION OF METHODS FOR THE DIAGNOSIS OF TRITRICHOMONOSIS IN CATTLE

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Tritrichomonas foetus is the causative agent of tritrichomonosis, a sexually transmitted disease leading to infertility and abortion in cattle. Several methods for the detection of bovine *T. foetus* infection in routine diagnostics are available. (i) Parasitological methods are involving microscopial examination of samples directly or after in vitro cultivation e.g. in the commercialized InPouch™TF test (Biomed Diagnostics). (ii) Two different molecular diagnostic tests based upon the polymerase chain reaction (PCR) have been independently established which are targeting different parasite sequences. A PCR test recently developped in our laboratory (TFR-PCR) is using primers derived from the rRNA gene unit of *T. foetus*, another PCR test (TF-PCR) is directed to a *T. foetus* gene probe sequence. We determined the performance characteristics of these two tests using diagnostic and clinical samples, and compared them with in vitro cultivation.
Methodical sensitivity of the InPouch™TF test was very much dependent on the sample composition, i.e. presence of preputial washings or vaginal secretions, and on the *T. foetus* isolate inoculated. The same variability was observed with several *T. suis* strains studied. Furthermore, the in vitro cultivation was not selective for tritrichomonads, but supported the growth of other trichomonadid organisms. The in vitro cultivation technique was comparatively assessed with the two PCR assays with diagnostic substrates obtained by routinely applied sampling techniques. Methodical sensitivity of both PCR assays principally is comparably high. However, the TF-PCR was more frequently hampered by unspecific amplicons when using diagnostic and clinical samples as a substrate.
In conclusion, the InPouch™TF test is suitable for routine diagnostic in vitro cultivation allowing the direct etiological proof for the presence of the parasite. Its methodical sensitivity, however, seems to be limited and much dependent on the biological sample composition. Both PCR assays may be applied as confirmation test for culture sediments, and are also suitable as a screening test for *T. foetus*. However, the TF-PCR potentially may cause difficulties in daily diagnostic routine. In contrast, the TFR-PCR exhibited a good diagnostic performance, and we suggest to further evaluate it for routine veterinary diagnostic application.

g.7.30 SPECIES-SPECIFIC AMPLIFICATION BY PCR OF RIBOSOMAL DNA FROM SOME EQUINE STRONGYLES

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The first and second internal transcribed spacers (ITS-1 and ITS-2) sequences of 28 morphologically-defined species of horse strongyle were characterised, and specific oligonucleotide primers were designed based on the nucleotide differences for some species. Utilizing these primers, a PCR approach was developed for the specific amplification of ribosomal DNA of *S. vulgaris, Cy. catinatum, Cc. nassatus, Cs. longibursatus* and *Cs. goldi*. The method allowed the species-specific amplification of parasite DNA derived from faecal samples and/or copro-cultures, demonstrating the potential of the approach for the diagnosis of equine strongyloidosis. The establishment of this PCR assay also has implications for studying the biology and epidemiology of equine strongyles and anthelmintic resistance using faecal egg count reduction tests.

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g.7.28-33 Use of PCR in parasitology
g.7.34-41 Parasite systematics

g.7.32 DISCRIMINATION OF ANAPLASMA MARGINALE AND BABESIA DIVERGENS INFECTIONS BY PCR

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Background: Infections with the tick borne blood parasite *B. divergens* occur occasionally among cattle in certain parts of Lower Austria and Styria. These areas also have a history of infections with *A. marginale*, a tick borne rickettsial pathogen that infects erythrocytes. Diagnosis relies widely on examination of Giemsa stained blood smears, however, low levels of parasitaemia often remain undetected.
Method: Based on the published sequences of the *B. divergens* specific rhoptry associated protein-1 (rap-1) gene and the major surface protein 5 (MSP-5) gene which is present on all subspecies of *A. marginale* we developed a PCR assay for both pathogens. We intended to used the PCR assay to identify latently or chronically infected cattle. Measuring the product size and comparison with a known positive control tested specificity. Sensitivity of both PCR assays was determined by using ten-fold dilutions of infected erythrocytes in normal blood.
Results: For both pathogens sensitivity was as low as $1.2x10^3$ to $1.2x10^4$ infected erythrocytes/ml blood. So far one *B. divergens* infected cow has been identified. 65 healthy cows from an area with a history of anaplasmosis did not show a MSP-5 amplicon.
Conclusion: In vitro low levels of parasitaemia can be detected. Further testing of field samples is indicated for a final evaluation.
Acknowledgement: This work was kindly supported by a Bundesministerium fuer Gesundheit und Umweltschutz.

g.7.33 *Detection of viscral leishmaniosis in Reservoir by PCR in Iran*

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Visceral leishmaniasis (kala -azar) is one of the vector born disease in Iran. Canines are the reservoir of parasite. The disease is transmitted from the reservoir by sandflies bite to human and dogs and spread through the body via the lymphatic system. In the light of this situation recognition of reservoir of disease is fundomental to control of disease in any contral program. In this study we used a highly sensitive PCR assay for the detection of lishmania DNA in lymph node, bone marrow aspirates and blood samples from 80 dogs obtained from endemic areas of Iran. leishmania DNA was detected in the blood and lymph node of 20 out of 80 (25%) dogs. The PCR results were confirmed by conventional methods. It was found that under optimal condition the PCR assay is fast, reproducible, and more convenient and sensitive method for the detection of leishmane in the resorvoir animals.

g.7.34 THE SYSTEMATICS OF EQUINE STRONGYLES BASED ON RDNA SEQUENCE DATA

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Background: Different hypotheses have been proposed for the evolutionary relationships of strongylid nematodes of horses based on morphological characters. Therefore, sequences of the two rDNA internal transcribed spacers were determined for 30 species of equine strongyle in order to examine questions relating to their systematics (taxonomy & phylogenetic relationships).
Methods: Sequence data for the two spacers were analysed, either separately or combined, using four different tree-building methods. As the length of the ITS-2 varied among species, a secondary structure model was constructed to provide a more reliable alignment of the sequences.
Results: The 12 trees derived from the different analytical methods had a similar topology. Phylogenetic analyses of the molecular data provided support for the hypothesis that genera with large subglobular buccal capsules are ancestral to genera with small cylindrical buccal capsules. However, the sequence data did not provide support for the current division of the two subfamilies (based on morphological characters), as some species within the Strongylinae always clustered with species of the Cyathostominae. Moreover, the sequence data failed to provide support for the grouping of some cyathostomes into different genera.
Conclusions: The molecular data obtained in this study indicate that the systematics of this important group of parasitic nematodes, as it currently stands, may be inappropriate. The rDNA sequence data, in combination with morphological data, provides the basis on which to re-examine the taxonomic framework and the phylogenetic relationships of equine strongyles.

g.7.35 DEFINITION OF MICROSATELLITE VARIABILITY IN THE ITS-2 PRE-rRNA OF PORCINE LUNGWORMS

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The aim of this study was to analyse sequence variation in the second internal transcribed spacer (ITS-2) of ribosomal DNA within individuals representing three species of *Metastrongylus* and to relate this variation to the predicted secondary structure of the pre-rRNA for this spacer. The ITS-2 (470-550bp) was amplified by PCR from individuals of *M. elongata*, *M. pudendotectus* and *M. salmi* and subjected directly to single-strand conformation polymorphism (SSCP) analysis. For individual taxa, representative bands were excised, reamplified by PCR and subjected to direct sequencing. For all three taxa, intraindividual variability in the ITS-2 was significantly higher than for any bursate nematode thus far examined, and was related to the presence of six microsatellites. The following variable microsatellites were defined: $(TG)_8$ and $(TCG)_{4-5}$ in *M. elongata*; $(TG)_7$, $(TCG)_{4-27}$, $(TA)_{4-5}$ and $(TACA)_{2-4}$ in *M. pudendotectus*; and $(A)_{4-5}$, $(TG)_7$, $(TCG)_{7-16}$ and $(TATG)_{2-4}$ in *M. salmi*.. Excluding the variable regions, interspecific differences were estimated at 2-22%. The microsatellites related to specific stem or loop regions of the predicted ITS-2 pre-rRNA secondary structure. For example, the microsatellite $(TG)_n$ formed part of a stem and interspecific differences occurred with compensatory base changes to maintain the stem structure. The results suggest that slipped-strand mispairing contributes to ITS-2 variability under structural constraint as a consequence of microsatellite location in the pre-rRNA.

g.7.34-41 Parasite systematics

g.7.36 CHARACTERISATION OF A *GRA* 2 HOMOLOGUE IN *NEOSPORA CANINUM.*

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Background: The isolation of genes coding for antigens of *N. caninum* was attempted by immunoscreening a cDNA expression library constructed in *Escherichia coli.*

Method: cDNA was synthesised from mRNA of in-vitro cultured tachyzoites of *N. caninum* (NC-Liverpool) and cloned into the bacteriophage expression vector lambda ZAP; packaged into viable phage and plated for single plague forming units on *E.coli.* The cDNA library was screened with preabsorbed bovine anti-*Neospora* antisera previously characterised by IFAT and western blotting. Northern blotting, PCR and DNA sequencing were performed using standard procedures.

Results: One clone isolated shared protein homology (from data base searching) to Gra2 of *T. gondii,* while several other clones possessed sequence similarity to uncharacterised ESTs of *T. gondii.* Northern blotting showed the *GRA* 2 homologue was expressed in cultured *N. caninum* tachyzoites as a single transcript approx. 1300 bases in length. The gene was isolated from genomic DNA and sequenced. It contained 2 exons and a single intron and the sequence was identical in 2 strains of *N. caninum* (NC-Liverpool and NC-SweB1).

Conclusion: Clones coding for antigens of *N. caninum* have been isolated from a cDNA expression library and one clone (a *GRA* 2 homologue) characterised in detail. The structure and expression of this gene is similar to that described for *T. gondii,* providing further evidence which supports the hypothesis that these two taxa evolved from a common ancestor.

g.7.37 COMPARATIVE GENETIC ANALYSIS OF TRICHOMONADS OF VETERINARY IMPORTANCE

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Two species of the genus *Tritrichomonas* represent parasites of veterinary importance. *T. foetus* is the causative agent of bovine tritrichomonosis, a primarily sexually transmitted disease which may result in problems of infertility and abortion in cattle. *T. suis* can be found in the nasal cavity and digestive tract of pigs, however, it is regarded to exhibit only low pathogenicity for this host. Based upon cross infection experiments and biochemical characteristics it was hypothesized that *T. foetus* and *T. suis* represent the same species.

We tackled this open question by two different molecular biological approaches. (i) The 5.8S rRNA gene and the flanking internal transcribed spacer regions (ITS1 and ITS 2) of different isolates of the two species in question were amplified by PCR and subcloned. Subsequently, sequence analysis of the cloned fragments was used to compare the parasite isolates. All *T. foetus* and *T. suis* isolates had identical sequences thus exhibiting an extremly high degree of homogeneity. In contrast, different species of the genus *Trichomonas* which were used as an outgroup shared more diversity. (ii) Furthermore, same isolates were assessed by the RAPD (random amplified polymorphic DNA) technique. Using this method with 20 different primers, all *T. foetus* and *T. suis* isolates resulted in identical genomic fingerprints. In contrast, the species *T. mobilensis,* an intestinal parasite from the squirrel monkey,which was used for comparison is genetically distinct and can be clearly discriminated from the other tritrichomonads.

Consequently, the results obtained with both molecular methods argue against the separation of the tritrichomonads from cattle and swine on the species level, and support a future revision of the taxonomic classification in the genus *Tritrichomonas.*

g.7.38 *Cryptosporidium parvum* classification following heterologous host transmission

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Background: *Cryptosporidium parvum* is the species most frequently cited as the cause of clinical cryptosporidiosis in Man and domesticated mammals. It's now accepted that at least two different genotypes of *C.parvum* exist (type 1 from human, type 2 from animal and human origin) and suggestions are being made that they are two distinct species. The aim was to determine whether pathogenic strains of *C.parvum* isolated from human infections are the same as, and can be maintained in farm animals, and identify molecular markers to aid epidemiological studies.

Method:*C.parvum* isolates were genotyped using PCR of the COWP, DHFR and TRAP-C1 genes and a characteristic fingerprint of the isolate produced using a protozoan specific lower eukaryote typing system, before and after transmission. Different fingerprints have been examined, and a genetic database established to assess if any DNA fingerprint changes occur associated with passage in heterologous hosts, and used to identify stable molecular markers associated with species, genotype, host, geographical area etc. Human isolates including genotype 2, mixed genotype 1 and 2, and genotype 1 have been passaged in calves and lambs.

Results: Studies so far indicate that only isolates of genotype 2 or containing genotype 2 can establish an infection.

Conclusion: The genotype is a probable indicator of transmission potential. Molecular classification shows some major markers within the fingerprints are maintained following transmission and infection of heterologous hosts. Fingerprints of different isolates of *C.parvum* share markers not seen with other protozoan parasites. Genotype specific markers may distinguish between type1and 2.

g.7.39 HOST SPECIFICITY, PATHOGENESIS AND MOLECULAR CLASSIFICATION OF *GIARDIA* SPECIES.

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Background: Enteric flagellates of the genus *Giardia* are the most commonly reported protozoan parasites in man worldwide. The role that livestock and companion animals may play in the potential zoonotic spread of Giardiasis is subject to much controversy and widespread investigation.

Method: Characteristic DNA fingerprints for both human and animal isolates were produced using a protozoan-specific lower eukaryote typing system. The subsequent effects that passage through heterologous hosts had on specific molecular markers within these fingerprints was then examined.

Results: Although host-specific markers were evident in many of the isolates so far investigated, a high degree of heterogeneity was observed within many of these host groups. Additionally, many of the isolates demonstrated a highly specific host range.

Conclusions: These preliminary findings highlight the benefits that such a typing system may have on our understanding of prevalence, pathogenicity and host specificity of *Giardia* isolates.

g.7.34-41 Parasite systematics
g.7.42-48 Genetic resistance to parasitic infections

g.7.40 *TELADORSAGIA CIRCUMCINCTA* SPECIES COMPLEX IN GOATS

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Background: Using isozymes, we have previously studied the genetic structure of different populations of *T. circumcincta*, a frequent nematode parasite found in French goats and sheep. A very strong deficiency in heterozygote was observed at the Malate dehydrogenase locus (MDH-2). Morphological studies and experimental infection of goats and sheep have clearly shown the existence of two lines of *T. circumcincta*, one adapted to goat, and another one adapted to sheep and goats. Other deficiencies in heterozygotes in other isozymes did suggest that there was a complex of species or semi-species. In the present study, using molecular biology techniques, we evaluated the level of differentiation between these two lineages. We also investigated other putative genetic entities in this complex on a larger sampling area.

Methods: In parallel to isozyme characterization we have studied the polymorphism of three genes: the ND4 gene of the mtDNA, the β-tubulin gene (nuclear DNA) and the ITS-2 of the ribosomal DNA.

Results: Firstly, we characterised 907 males of *T. circumcincta* originating from 9 goats farms by allozyme studies at the MDH-2 locus which permitted to distinguish goat and sheep lines. Secondly, the polymorphism of the β-tubulin gene was studied on 190 of these parasites and the one of the ITS-2 on 32 worms. These three approachs clearly showed the existence of 2 distinct genetic entities (species) that corresponded to the two lineages observed by the morphology and infestation essays. Finally, a third molecular study on the variability of the ND4 gene (70 males) has shown the presence of one additional entity to the two other previously defined, which must be more characterized.

Conclusion: Undertaken works revealed the existence of several genetic distinct entities within *T. circumcincta*.

g.7.41 Effects of drug selection and inbreeding on the genetic diversity of the parasitic nematode *Haemonchus contortus*

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Changes in the genome of the sheep parasitic nematode *Haemonchus contortus* were studied during selection for drug resistance and during inbreeding. We used amplified fragment length polymorphism (AFLP) analysis to analyse the genetic variation during consecutive levels of benzimidazole resistance, levamisole resistance and inbreeding. The drug resistant and inbred populations were selected and bred in our own fascilities. It appeared that the *Haemonchus contortus* genome is extremely variable. Selection for resistance to both drugs had only little effect on the heterogeneity of the genome. Two inbred strains were bred to a homozygosity of about 90% and inbreeding clearly decreased the genetic diversity.

g.7.42 Title: VARIATION IN ACQUIRED IMMUNITY OF WEST AFRICAN DWARF (WAD) GOATS TO EXPERIMENTAL *HAEMONCHUS CONTORTUS* INFECTIONS

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Background: Immunogenetic aspects of gastrointestinal nematode infections of ruminants are important and growing areas of research into alternative strategies for the control of caprine and ovine parasitic gastro-enteritis, aimed at selective breeding of parasite resistant genotypes. This field of research has received little attention in many countries in the humid tropics, such as Nigeria, where Haemonchosis poses a serious threat to profitable production of small ruminants in intensive and semi-intensive systems of production.

Method: Variations in acquired immunity and host resilience to experimental H . contortus (Hc.) infections and the immunological correlates of infection were studied in Nigérian WAD goats in order to assess the possible existence of Hc - resistant genotypes in this trypanotolerant breed of goat.

Results: The results showed that most 7 - 8 month-old WAD kids are capable of developing good acquired resistance to primary infection with either 2000 or 5000 L3 of Hc. This level of primary exposure to infection elicited significant ($z = 2.952$, $P = 0.002$) resistance to challenge with 5000 L3 given on D35 after infection, but the immunised challenged goats were generally unable to reject a substantial proportion of their persisting adult worms from the immunising infection. The development of the latter worms were however retarded and their growth stunted, more so in female than in male worms. These responses were subject to considerable individual variation. Faecal egg counts (FEC), eosinohilia, PCV, body weight and parasite-specific serum IgG responses also showed marked within - and between - group variability, and there were no consistent associations between any of these nor with parasite burdens

Conclusion: Overall, our results especially worm burdens and FEC, suggest that resistant genotypes exist among the Nigerian WAD goat population.

g.7.43 NATURAL GASTROINTESTINAL PARASITE INFECTION IN CORRIEDALE AND CRIOULA SHEEP

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Background: The gastrointestinal parasites are a major cause of economic loss in sheep throughout the world. Among them, it must be emphasised *Haemonchus contortus* in tropical, subtropical and temperate regions, during the warm and wet summers. In Rio Grande do Sul State, Brazil, high-grade of worm resistance is present and alternative methods are required for nematode control, such as resistant breeds utilisation. The present research sought to compare two sheep breeds regarding resistance to endoparasites: Crioula Lanada (a local breed) and Corriedale. **Method:** During nine weeks, fifteen pos-weaning lambs that were maintained in a grazing area and monitored through Faecal Eggs Counts (FEC), Packed Cells Volume (PCV), total serum protein and albumin levels. After this period, eight animals from each breed were slaughtered for worm burden evaluated. **Results:** The faecal cultures showed the predominance of *H. contortus* through the trial. The regional breed, Crioula, presented significantly lower FEC and higher PCV and serum protein and albumin. The Corriedale group was hypoalbuminaemic and hypoproteinaemic at the end of the experiment. At necropsy, Crioula sheep presented lower adult worm burden 376 (70-1190) than Corriedale 2391 (370-6330) ($p<0.01$). **Conclusion:** The results suggest that the local breed, Crioula Lanada, can offer an effective alternative for sheep helminth control.

Acknowledgement: To CNPq and Embrapa for financial support.

g.7.42-48 Genetic resistance to parasitic infections

g.7.44 GENETIC RESISTANCE TO HELMINTHS IN TRADITIONAL HUSBANDRY SYSTEM IN SENEGAL

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Background : In tropical areas, severe damages in small ruminants are due to nematode parasites, so the use of resistant breeds or individuals could contribute to limit their effect. The genetic parameters have been estimated in experimental farms or large flocks, but never in traditional husbandry system with its difficulties. This is the aim of this study conducted on local breeds of sheep and goats in Senegal.
Methods : Faecal egg count (FEC) and packed cell volume (PCV) were measured in 1 to 5 ewes or goats and in their offspring from 140 flocks during the rainy season, three times for FEC and twice for PCV, this two successive years. The heritability and the repeatability were estimated using an animal model.
Results : Within-year estimates of repeatability were 0.19 for FEC and 0.27 for PCV. They were lower between years : 0.12 for FEC and 0.21 for PCV. The estimation of the heritability was difficult because the existence of a non genetic effect either between the dam and her offspring or between animals of the same flock due to the environment shared by animals when grazing. Estimates of the heritability were generally low for two traits.
Conclusion : The pedigree structure and the small size of the flocks were constraints to evaluate the part of variance of genetic origin in resistance mechanisms.

g.7.45 GENETIC RESILIENCE TO STRONGYLES IN CREOLE GOATS OF GUADELOUPE (F.W.I)

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Background: Gastro-intestinal strongyles (GIS) induce high mortality before weaning and depressed production during fattening in small ruminant flocks of the humid tropics. From an economic point of view, low production losses due to infection to GIS (resilience) is a more acute breeding objective than resistance to infection. The present study was designed to quantify the genetic relationships among resistance, resilience and production traits, to verify that breeding for resistance in Creole goats would not increase production losses.
Methods: Genetic variability was estimated in the Creole experimental flock of INRA-Gardel. Growth of kids was recorded between 3 (weaning) and 11 months of age. In order to prevent non infected kids from larvae aggression, they grazed on separated larvae-free paddocks and were drenched each 21d with moxidectin. Resilience was defined for each buck as the weight difference between infected and non infected offspring, at the end of fattening. Records of 553 kids sired from 18 bucks were analysed.
Results: Worm free kids did not excrete any GIS egg while the egg output of infected kids was 4400 epg at 10 months of age. The detrimental effect of infection was on average 16% of the live-weight at 338d of age. A seasonal variation was observed. The impact of GIS is the most important for kids born during the humid season (-5kg).
Conclusion: Experimental evaluation of resilience is possible on Creole goats. More sires are required to estimate genetic variability on this trait.

g.7.46 EVOLUTION OF 2 LINES OF *HAEMONCHUS CONTORTUS* BRED ON SUSCEPTIBLE OR RESISTANT LAMBS.

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Background: *Haemonchus contortus* is a very adaptative parasite and breeding for resistance in small ruminants could induce a new selective pressure for the parasite. In order to investigate the response of this parasite to the host immune status, we allowed 2 parasite populations to evolve in 2 host environments.
Methods: Black Belly female lambs of 6 months old were allocated into 2 groups: a) Susceptible (n=12) lambs immunosupressed with a corticoid 3 days before infection (I) ; b) Resistant (n=12): lambs preliminary infected (PI) 6 weeks before I. 13 500 L3 were given in 3 occasions for PI and 1 dose of 5 000 L3 was given once for I. This experimental design was repeated for 9 parasite generations. The parasite fitness was estimated for 4 out of the 9 generations
Results: The eosinophil response always showed that 2 host environments were really created. The development rate of L3 into adults, the fitness index (No of L3 produced per day /No L3 used to infect lambs), and the prolificity were estimated. The significant differences between the 2 groups were 28 %, 660 and 1 800 eggs/female/day, respectively. Significant differences in the duration of the prepatent period between the 2 groups were 3 days and 10 days at the generation 3 and 9 respectively. All the values of the traits seemed to diminish during acclimation in laboratory.
Conclusion: The two *H. contortus* populations had actually evolved in two different host environments and worm phenotypic differences were recorded.

g.7.47 *ASCARIDIA GALLI* INFECTIONS IN 4 DIFFERENT LAYER-LINES.

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Background: Studies have shown that infections with *Ascaridia galli* are common in laying hens kept in deep litter and free-range systems but not in cage systems. The possibility that there is a genetic component involved in the establishment of *A. galli* in different chicken lines was investigated since this could have a practical application in selecting a suitable line for free-range systems.
Method: Four different layer-lines, Isa Brown, New Hampshire, Skalborg (HH) and New Hampshire x Skalborg, were compared by inoculating 40 hens from each line with a single dose of 500 embryonated *A. galli* eggs. The individual faecal egg output was monitored weekly. The hens were slaughtered 3, 6 and 9 weeks post infection (p.i.) and their intestines examined for the presence of larvae and adult worms.
Results: The faecal output of *A. galli* eggs were found to be significantly higher in the HH line. Additionally, 9 weeks p.i. the female worms from the HH line were found to have significantly higher fecundity. The results also suggest that the development from larvae to adult was more rapid in the HH line.
Conclusion: The results indicate that a genetic component is involved in the establishment of *A. galli* and that it therefore will be possible to identify lines less susceptible to parasite infections and thus more suitable for free-range systems.
Acknowledgment: The Danish Ministry of Food, Agriculture and Fisheries and The Danish Council for Development Research are thanked for providing financial support.

g.7.42-48 Genetic resistance to parasite infections
g.7.49-55 Protozoan parasite zoonoses

g.7.48 BREED RESISTANCE TO TRICHOSTRONGYLID PARASITES OF GOATS IN THE U.S.

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Background: The meat goat industry in the U.S. has been expanding in recent years. Several breeds of U.S. goats with a variety of breed origins and selection histories are being examined for their value as meat goats. Potential variation in resistance to *Haemonchus contortus* is an important consideration, since infection is one of the principal health problems faced by U.S. producers.
Method: Fecal egg counts, packed cell volumes and total worm burdens were evaluated in naturally infected Nubian, Pygmy, Spanish, Myotonic and Virginia Brush kids. Fecal egg counts and packed cell volumes were also determined in adult goats. Goats used for the experiment were derived from several sources in the southeastern and south central U.S.
Results: The lowest worm burdens were found in Myotonic and Pygmy goats. Worm burdens in these 2 breeds were significantly lower ($p < 0.05$) than worm burdens of Nubian and Spanish goats. In adult goats, Spanish and Nubian breed goats had generally higher fecal egg counts and lower packed cell volumes.
Conclusion: Variations in breed resistance to gastrointestinal trichostrongylids appears to occur in breeds of goats in the U.S. These differences are consistent with different intensities of producer selection and appear to have arisen over relatively short periods.

g.7.49 TICK-BORNE PATHOGENS AND THE RISK OF INFECTION OF PETS AND LIVESTOCK

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Ixodes ticks are major pathogen reservoirs and vectors in the vicinity of St. Petersburg (Russia). By means of dark-field microscopy, IFA and PCR methods, spirochaetes, different *Borrelia* species, tick-borne encephalitis virus and *Ehrlichia* were determined in *Ixodes persulcatus* ticks collected in the recreational zones of St. Petersburg.
Brown tick, *Rhipicephalus sanguineus,* does not occur in the St. Petersburg region. Nevertheless, two cases of ehrlichiosis in pet dogs were found. The dogs had been infected through *Ixodes* tick bites in the park zone of the town. Also outbreaks of tick-borne encephalitis caused by infected cow's milk were found. Borreliosis due to infection (or contamination) of goat's milk with *Borrelia burgdorferi* s.l. was found in at least one member of a family.
The above-mentioned cases serve to emphasize the fact that the bites of *Ixodes* ticks constitute an infection risk for pets and livestock.

g.7.50 PREVALENCE OF *TOXOPLASMA GONDII* ANTIBODIES IN CHILDREN OF NORTHERN GREECE

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Background: *Toxoplasma gondii* – a coccidian protozoan parasite of cats - causes one of the most common parasitic diseases in man and animals. The infection is determined by serological analysis. The aim of this work was to investigate the occurrence of IgG and IgM antibodies against toxoplasmosis in serum of children with various diseases.
Method: 486 serum samples from children sent to the AHEPA Hospital of Thessaloniki with various chronic diseases, were used. The samples were divided into 3 groups according to the age of the patients. Group I consisted of 201 samples (age from 5 months to 5 years), Group II of 157 samples (age from 6 to 10 years), and Group III of 128 samples (age from 11 to 15 years). For *T. gondii* antibody identification a mouse-virulent toxoplasma strain (RH-strain) from RIVM (The Netherlands) was used and determined by the ELISA method.
Results: In Group I, 74/201 (36.8%) serum samples were positive (24 for IgG, 46 for IgM, and 4 for both IgG and IgM antibodies). In Group II, 65/157 (41.4%) were positive (9 for IgG, 52 for IgM, and 4 for both IgG and IgM). In Group III, 47/128 (36.7%) were positive (12 for IgG, 34 for IgM, and 1 for both IgG and IgM).
Conclusion: The incidence of *T. gondii* antibodies in sera from the examined children was high. A prevalence in male children was noticed. The seropositivity rate was highest in children below 5 years of age.

g.7.51 ISOLATION OF *Toxoplasma gondii* FROM TISSUES OF GOATS EXPERIMENTALY INFECTED

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Background: The goat is an important source of *Toxoplasma gondii* infection for the man. This agent, in goats, causes great economic impact with reproductive damages as abortion, weak products or stillborn and the symptoms are not specific, making difficult the clinical diagnosis. Among the laboratorial methods, the bioassay test in mice is the standard method. The objective of this study was to identify the best organ for the isolation of *T. gondii*, from goat tissues. **Method:** Nine goats with four to six months of age were inoculated "per os", with 10^5 oocysts of *T. gondii* (AS28). Those animals were slaughtered eight weeks pos-inoculation and fragments from 16 organs (spleen, brain, liver, adrenal, salivary glands, gut, cervical lymph nodes, mesenteric lymph nodes, spinal cord, heart muscle, diaphragma, skeletal muscle, pancreas, lungs, retina and kidneys) from each animal were sampled for the bioassay test in mice. The evaluation of the infection was made through the presence of tissue cysts, tachyzoites in peritoneal fluid or the presence of specific antibody in the experimental mice (IFAT). The goats and mice were maintained in cages disinfected daily with boiling water and receiving food and water " ad libitum ". **Results and Conclusion:** By the McNemar's statistical test the brain, spinal cord and skeletal muscle were the tissues of choice for *T. gondii* detection in goats.
Acknowledgement: We wish to thank the financial support by FAPESP-Brazil (Process 97/12609-5 and 97/01177-7).

g.7.49-55 Protozoan parasite zoonoses

g.7.52 TOXOPLASMOSIS IN HOUSE SPARROWS (*PASSER DOMESTICUS*)

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Background: The house sparrow may be the most abundant synantropic wild bird, and *Toxoplasma gondii* protozoan parasite that most frequently infects birds and mammals. Only a little is known about a prevalence of toxoplasmosis in sparrows and how *T. gondii* may affect their health.

Method: First, free-living sparrows were tested for antibodies to *T. gondii* by an indirect fluorescent antibody test (IFAT). Later, a total of 31 sparrows divided into 5 groups of 6 to 7 birds were infected per os with 1, 10, 10^2, 10^3 and 10^4 oocyst of *T. gondii* of the K1 strain, respectively. The general health, production of antibodies and isolation positivity were examined.

Results: Antibodies to *T. gondii* were found in 12.3 % of 227 sparrows. After experimental infection, no clinical signs of toxoplasmosis were observed. Seroprevalences ascertained in weeks 3, 7 and 12 post infection were 64 % (18 positive/28 tested), 95 % (21/22) and 70 % (7/10), respectively, when tested by IFAT. *T. gondii* was reisolated from 45 % of 29 infected sparrows.

Conclusion: House sparrows are resistant to toxoplasmosis and they do not seem to be a significant source of toxoplasmosis in environment.

Acknowledgement: This study was funded by Grant No. 524/98/0111 from the Grant Agency of the Czech republic.

g.7.53 *NEOSPORA CANINUM:* INFECTIONS IN SAND RATS AND COMMON GERBILS

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Background: *Neospora caninum* (*Nc*) is a protozoan parasite associated with neurologic and skin disease in dogs and abortions in ruminants. Immunocompetent rodent models exhibit clinical signs mainly after the administration of immunosuppressive treatment.

Method: Sand rats (*Psammomys obesus*) and common gerbils (*Meriones tristrami*) were inoculated intraperitoneally (ip) or subcutaneously (sc) with various doses of culture-derived *Nc* tachyzoites. Peritoneal exudate, blood films and histological preparations from organs were examined for parasites or lesions.

Results: Rats inoculated ip with 10 to 10^6 *Nc* or sc with 10 to 10^3 *Nc* succumbed to the infection, except for one rat that received 10 *Nc*. The surviving rat died upon reinfection with 10^5 *Nc*. All gerbils inoculated ip with 10^5 or more *Nc* succumbed. Survivors were observed among those inoculated ip with 10 to 10^4 or sc 10 to 10^2 *Nc* parasites. Neuromuscular signs (hind-leg paralysis, head tilt, circling movements) were observed among the gerbils. Multifocal pyogranulomatous encephalitis with intralesional *Nc* cysts were seen in gerbils that survived 4 weeks or more. Numerous *Nc* were observed in the peritoneal fluid of ip-inoculated animals but none were seen in blood films. Swiss mice inoculated with *Nc* from peritoneal exudate of rats or gerbils had no clinical signs, but were serologically positive to *Nc* antigen.

Conclusion: Sand rats and common gerbils are immunocompetent rodent models, highly susceptible to *Nc*.

g.7.54 CROSS-TRANSMISSION OF GIARDIA

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Dogs: We investigated the susceptibility of dogs with different immune status to cross-transmission of Giardia. One of the inoculated puppies in each group was immunosuppressed by dexamethasone. In first experiment, an isolate from lamb was used for the inoculations at a dose of 20,000 cysts pro toto, in second experiment an isolate from pig at a dose of 30,000 cysts pro toto. Feces were examined regularly for Giardia cysts, blood samples were analysed for specific antigiardia IgG and IgM antibodies by IFAT and parameters of non-specific immunity were determined. The attempt at cross-transmission failed even in markedly immunosuppressed puppies. Neither release of cysts nor seroconversion of circulating specific antibodies were detected.

Cats: We infected experimentally three cats using 66,000 cysts pro toto collected from feces of the girl and two cats using 20,000 cysts pro toto isolated from feces of the dog. In first case only one cat started to excrete cysts in 52 day p.i. intermittently for 12 days. In second case again one cat excreted cysts after 48 days only for two days.

Conclusion: Dogs and cats are specific hosts for giardia and these animals are not probably responsible for significant number of cases of human giardiosis.

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g.7.55 ISOLATION OF *Sarcocystis falcatula* FROM *Didelphis albiventris*

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Background: *Sarcocystis falcatula* uses avians as intermediate hosts and the North American opossum (*Didelphis virginiana*) as the definitive host. *S. falcatula* infection in South American opossum (*Didelphis albiventris*) was investigated.

Method: *Sarcocystis* sporocysts from the intestines of 2 opossums (*Didelphis albiventris*) from Argentina were given to three captivity-raised budgerigars (*Melopsittacus undulatus*). Tissue samples were examined after staining with hematoxylin and eosin and by immunohistochemical methods. Lungs of one budgerigar were inoculated in bovine monocyte cell cultures.

Results: Budgerigars died of acute sarcocystosis 8, 9, and 14 days after ingesting sporocysts. Schizonts and merozoites found in lungs and other organs of the budgerigars were identified as *S. falcatula* based on structure and immunoreactivity to anti - *S. falcatula* specific antibody. *Sarcocystis falcatula* was also isolated in bovine monocyte cell cultures

Conclusions: The results of this investigation indicate that *Didelphis albiventris* is another definitive host for *S. falcatula*. This is the first report of S. falcatula infection in South America.

Keywords: *Sarcocystis falcatula*; Budgerigars (*Melopsittacus undulatus*); *Didelphis albiventris*; Pneumonia; Schizonts; Merozoites; Argentina.

g.7.56-69 Helminth parasite zoonoses

g.7.56 HELMINTH ZOONOSIS IN IRAN

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Zoonoses or zoonotic diseases are the most important problems worldwide.Of parasitic zoonoses identified by WHO/FAO,about 37 helminths are reported in Iran.The most prevalent and important are classified as follow.

a) Echinococcosis/Hydatidosis:
Hydatidosis in man and animals,caused by cyst form of Echinococcus granulosus,has been reported from all parts of the country. The incidence of hydatid disease is estimated by hospital records,so it dose not give us the true incidence.According to hospital records (1946-1993),37 cases of multilocularis cysts are reported in Iran.

b) Taeniasis/Cysticercosis:
Taenia saginata is a common parasite in Iran,and high infections (35.6%)are reported from Mazandaran province.

c) Trematode zoonoses:
Sporadic human fasciolasis are reported from most parts of the country.Epidemic disease with 7000-10000 infections occured (1989)in Gilan province.Heterophydosis (0.8-1.9%)is reported from Khuzestan province.

d) Nematode zoonoses and larva migrans:
Infections with 6-7 species of Trycostrongylus genus and Toxocara larva are reported specially from the north area.

e) There are some other helminth zoonoses of different genera and species of those,human cases are reported rarely,or not reported yet.

g.7.57 THE DISTRIBUTION OF *Cysticercus cellulosae* IN CARCASSES OF NATURALLY INFECTED SLAUGHTER PIGS IN MBULU DISTRICT OF TANZANIA.

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The distribution of *Cysticercus cellulosae* among organs and muscles groups was determined by slicing the tongue, heart, internal and external masseters, triceps brachii and other muslces of the half carcass. Preliminary study (n=7 out of 20) has shown that total number of cysts per carcass ranged from 1257 to 35,854. In the muscle groups, normally considered predelection sites triceps brachii, masseters and psoas major harboured the highest proportion of cysts (mean 6.4%, 6.1% and 5.5% respectively of the total cysts in the carcass) while lower proportions were found in the heart, tongue, diaphragm, brain and lungs (2.2%, 2.8%, 1.6%, 0.3% and 0.2% respectively). The proportion of cysts expected to be found at the surfaces exposed by visual examination or incision at meat inspection was calculated using indirect method, which incorporated the area revealed by incision and visual inspection of organ and proportion of cysts located in particular organ. It was estimated that 7.3% of the cysts would be located at the inspected site if the regulations were followed carefully. Relative cyst density was calculated for different muscle groups by dividing the mean proportion of total weight of muscle groups into the mean proportion of cysts located in that site. The cysticerci in the examined tissues were found in the following order of relative density: heart (1.7), tongue (2.3), internal masseter (3.8), external masseter (3.5), triceps brachii (1.6), diaphragm (1.3), brain (0.6), head muscles (1.5), fore quarters (1.1), hind quarters (1.0), oesophagus (0.3), trunk muscles(0.6), abdominal muscles (0.4), lungs (0.1) and psoas major (3.4).

g.7.58 *TRICHINELLA PSEUDOSPIRALIS* TRICHINELLOSIS OF POULTRY: THE VETERINARY-SANITARY INSPECTION OF CARCASSES.

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The development of procedures for identification of *T.pseudospiralis* larvae in carcasses of poultry needs to be done with the aim to prevent the possible infection of humans and establishment of infection sources. 3 months cock-broilers aged were orally infected with 35-day larvae (10 larvae per 1 g of body weight). One examined separate muscles for determination of localization sites of *T.pseudospiralis* larvae on different post-infection periods. The investigation was carried out digestion muscle test. It has showed that on 71 day of infection one recovered the highest numbers of *T.pseudospiralis* larvae from *Musculus depressor mandibulae*, *M.masseter* and *M.transversus mandibularis* (553, 445 and 214 larvae/g of muscles respectively). Among muscles of neck *M.intertransversarius colli* took the first place on intensity of infection (683 larvae/g) as while as *M.biventer cervicis* - the second one (363 larvae/g). As a whole the intensity of infection of separate muscles of legs was low and the corresponding indices for *M.tensor fasciae latae; M.peronaeus, M.tibialis, M.gastrocnemius* and *M.sartorius* were 367, 226, 216, 182 and 105 larvae/g respectively. The recovery of larvae by digestion muscle test was increasing with their age up to 71 day of post-infection period and later those values gradually fell. In conclusion one should note that the preliminary data indicate that it is expedient to inspect muscles of head (especially *M.depressor mandibulae* and *M.masseter*) and neck (*M.intertransversarius colli*) on *T.pseudospiralis* trichinellosis.

g.7.59 PREVALENCE OF TOXOCARA CANIS ANTIBODIES IN CHILDREN OF NORTHERN GREECE

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Background: Toxocarosis is a zoonosis, which constitutes a serious public health problem in many developing countries. In Greece, there is a high incidence of the disease among dogs, which can infect humans and cause visceral larva migrans syndrome (VLM). The aim of this work was to investigate the occurrence of IgG and IgM antibodies against Toxocarosis in serum of children with various chronic diseases.

Method: 511 serum samples of children presented to AHEPA hospital of Thessaloniki with various chronic diseases, were used. According to the age of the patients, the samples were divided into 3 groups. Group I consisted of 192 samples (age from 5 months to 5 years), Group II of 186 samples (age from 6 to 10 years), and Group III of 133 samples (age from 11 to 15 years). For T. canis antibodies identification excretory-secretory (E/S) antigen was used, and determined by the ELISA method.

Results: In Group I, 24/192 (12.5%) serum samples were positive (6 for IgG, 16 for IgM and 2 for both IgG and IgM antibodies). In Group II, 24/186 (13%) were positive (5 for IgG, 16 for IgM and 3 for both IgG and IgM). In Group III, 16/133 (12%) were positive (5 for IgG, 11 for IgM).

Conclusion: Generally, in serum of children with various diseases, the incidence of T. canis IgG and/or IgM antibodies is high, with a prevalence in female. Seropositivity rate was highest in children below 10 years of age.

g.7.56-69 Helminth parasite zoonoses

g.7.60 WILD ANIMALS AS THE SOURCE OF INFECTION FOR HUMAN TRICHINELLOSIS IN ESTONIA

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Background: In Estonia almost every year the cases of human trichinellosis occur. There are about 600 bears, 10 300 wild boars, 300 wolves, 1200 lynxes, 11 000 raccoon dogs, 10 000 red foxes, 6000 pine martens and 3000 badgers in the Estonian forests.
Method: From 1992—1998 *Trichinella* infected muscle samples were collected from 64 of the 797 wild animals. The species of *Trichinella* were identified by RAPD analysis.
Results: The prevalence was very high in wolves (80.0%) and raccoon dogs (50.0%). 44.4% of lynxes, 42.1% of red foxes and 38.5% of bears were infected. Only 0.7% of wild boars were infected with *Trichinella spp.* The prevalence in brown rats was 11.1%. The number of muscle larvae per gram during the research period in red foxes was 0.1—213.0, in brown rats 50.0, in wolves 0.01—44.9 and in raccoon dogs 0.3—32.0. The intensity of *Trichinella* infection in wild boars and bears was low (0.5—12.0 and 0.05—0.2 respectively). *T. nativa* and *T. britovi* were identified in wild boars, wolves, raccoon dogs, red foxes and lynxes. Bears were infected with *T. nativa* and brown rats with *T. spiralis*.
Conclusion: The important reservoirs of *Trichinella* infection in nature were wolves, raccoon dogs and red foxes. The main source of direct infection for humans is bear meat, not inspected for *Trichinella*. The role of wild boars is relatively insignificant. Wild animals and rats may serve as carriers of sylvatic and synanthropic trichinellosis to domestic animals and man.

g.7.61 INFECTIVITY OF SYLVATIC AND DOMESTIC *TRICHINELLA* SPP. IN WILD BOARS AND PIGS

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Background: Trichinellosis is still a threat to human health in USA and EU. Infectivity and persistence of the domestic species, *Trichinella spiralis*, have been intensively studied in pigs, but comparable information do not exist for the numerous newly recognised sylvatic species of *Trichinella*.
Methods: Nine isolates of *Trichinella* were inoculated in pigs and wild boars. Blood samples were taken weekly. Pigs were slaughtered 5, 10, 20, and 40 weeks p.i. and wild boars at 5 and 10 weeks p.i.. In each animal, muscle larvae were released by digestion from 18 muscle groups. The morphological development of encysted larvae were studied by light microscopy. Muscle tissue with encysted larvae were exposed to freezing, and subsequently, the reproductive capacity of the larvae was measured in mice.
Results: *T. spiralis* established in high numbers, *T. britovi*, *T. nelsoni*, and *T. pseudospiralis* in moderate numbers, and *T. nativa* and the remaining sylvatic isolated only in low numbers. In the wild boars some sylvatic species established significantly better than in pigs. Only larvae of *T. spiralis*, *T. britovi*, and *T. nelsoni* persisted in the pigs after 40 weeks. For the remaining species, morphological examination revealed incomplete development of the muscle larvae. In contrary to larvae in mice tissue, larvae in pig tissue did not survive freezing.
Conclusion: It appears that muscle larvae of all *Trichinella* species can be found in the tissue of experimentally infected pigs but that only three species *Trichinella* are able persist in the muscles of pigs. Freezing of pork meat can be regarded as a safe method for inactivation of muscle larvae of both domestic and sylvatic species of *Trichinella*.

g.7.62 PORCINE CYSTICERCOSIS IN TANZANIA

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Background: Porcine cysticercosis, in Tanzania, was reported to be present in pigs reared under free range management system in 1980's. The regions reported to have porcine cysticercosis based on abattoir records are Arusha, Kilimanjaro, Iringa and Ruvuma.
Method: On farm studies were conducted in Mbulu district, Arusha region using questionnaire and visual tongue inspection to determine factors associated with *Taenia solium*/ cysticercosis.
Results: Out of 770 live pigs examined by visual tongue inspection 134 pigs were found to be infected with *Cysticercus cellulosae* (17.4 % prevalence). Out of 436 households visited during the study 107 (24.5%) households had at least one pig in the flock infected with *Cysticercus cellulosae*. Despite the presence of porcine cysticercosis in Mbulu district, records from Hydom Lutheran Hospital and Mbulu District Hospital show that taeniosis is constantly diagnosed annually and that cases of epilepsy are on the increase from 1993 to 1996. Furthermore, no attempt has been made at these hospitals to determine the extent of association between the cases of epilepsy and *Taenia solium* taeniosis / cysticercosis.
Conclusion: It is concluded that porcine cysticercosis is highly prevalent in Mbulu district and that poor knowledge on *Taenia solium* transmission, poor hygiene and poor pig management are responsible for the maintenance of the disease in the area.
Acknowledgement: The authors wish to thank DANIDA through the ENRECA funded Collaborative Livestock Helminth Research Project for financial support.

g.7.63 THE DISTRIBUTION OF ECHINOCOCCUS MULTILOCULARIS CARRIAGE IN THE RED FOX (VULPES VULPES) IN THE SOUTH OF BELGIUM

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Background : The presence of *E. multilocularis* was reported for the first time in Belgium in 1992 when 13 red foxes out of 85 (15.3 %) were found to be infested. All the animals originated from the Province of Luxembourg, a hilly area of the South of Belgium. This finding was confirmed in 1995 - 1996 but with a higher prevalence (51 %).
The present survey was conducted in order to define the distribution of *E. multilocularis* in other parts of the country.
Method : 48 and 73 red foxes were available in 1997 and 1998 respectively. They were collected from 6 provinces : Brabant (9), Hainaut (1), Oost Vlanderen (2), Namur (14), Liège (30) and Luxembourg (65). After freezing at - 80° C the gut content of each animal was examined for the presence of the cestode.
Results : 14.5 and 16.4 % of the animals were found to be infested in 1997 and 1998 respectively. With the exception of 2 positive animals from the East of the Province of Liège (a high plateau - mean altitude 500 - 600 m.) all positive animals had been killed in the Province of Luxembourg. The mean prevalence in this province for 1997 - 1998 was 26 %.
Conclusion : This study confirms the high prevalence of *E. multilocularis* in the Province of Luxembourg and reveals its presence in some other parts of the country where the climatic and geologic conditions are suitable for the maintenance of the life cycle.
Acknowledgement : Direction générale des Ressources naturelles et de l'Environnement (Ministère de la Région Wallonne).

g.7.56-69 Helminth parasite zoonoses

g.7.64 FURTHER STUDIES ON THE INCIDENCE OF *TRICHINELLA SP.* IN RED FOXES (*VULPES VULPES*) IN NORTH-EAST, CENTRAL AND SOUTH OF POLAND

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Malczewski et al. 1997 reported results of examination of 582 foxes for incidence of *Trichinella sp.* from 31 voivodeships in Poland. *Trichinella sp.* were detected by means of a compresor method using trichinelloscope projector in site as suggested by Kapel et al. (1994) in 32 (5.4%) of foxes in 12 voivodeships. In further studies carried by Cabaj et al. (1998) in 13 samples available for examination by RAPD method, 11 foxes from Northern, Central and Southern Poland harbored *T.britovi* and 2 from Central Poland were infected by *T. spiralis*.This was a second report on the occurence of *T.britovi* in Poland after the initial case described by Nowosad and Pozzio (1995).

In further studies conducted during 1997-1998 493 more foxes from 33 voivodeships were examined. *Trichinella sp.* were detected in 14 (2.8%) foxes from 13 voivodeships.

Until now, 1077 foxes were examined all together. *Trichinella sp.* was found in 46 (4.27%) from 13 voivodeships. Among foxes examined 11 were infected by *T.britovi* and 2 by *T. spiralis*.

The results of the present study confirm, that the more common infection sites are legs, than the diaphragm. The detailed study of diaphragmal muscles, however, are still in progress.

g.7.65 ULTRASOUND IN LIVESTOCK: A CONTRIBUTION TO EPIDEMIOLOGY OF HYDATIDOSIS

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Background: The prevalence report of hydatid cysts in Camel Sheep and goats in Kenya has been difficult to establish due to home Slaughter and poor reliability of serologic tests. In an attempt to Establish this prevalence, we present data based on ultrasound Examination of the three animal species.

Method: Hair was clipped from the thorax and abdomen and a coupling gel applied. Ultrasound examination of the liver and the lung was performed with a real time B-mode ultrasound scanner. The number, size and location of the cysts were noted.

Results: Hydatid cysts were visualized in 5.02% (96/1910) of the goats, 72.2% (13/18) of the Camels and 9.7% (29/296) of the Sheep.

Conclusion: Ultrasonography proved to be a quick and non-invasive Technique. Unlike slaughter surveys, this technique provided unbiased Results because whole flocks were examined.

Acknowledgements: We wish to thank AMREF - Kenya,DAAD of Germany, Pennsylvania State University for their assistance.

g.7.66 ESTABLISHMENT *ASCARIS SUUM* LARVAE IN PIGS FED A. SUUM INFECTED CHICKEN LIVERS AND LUNGS.

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Background: Lately, cases of visceral larvae migrans have been reported from Japan. Severe illness was described. Previously, a few cases of ascariosis in human due to *Ascaris suum* have been reported. Whereas *Toxocara canis* is well known to cause larva migrans, this phenomenon has not been clearly described for *A. suum*. In the latter case human patients were infected by eating raw chicken liver. In the present experiment we looked at the possibility of *A. suum* eggs to hatch and migrate in chickens. Subsequently pigs were fed infected chicken livers and lungs.

Method: Eighty 5-day old parasite naive chickens were orally infected with 2500 embryonated *A. suum* eggs each. Twenty four hours post infection 40 chickens were slaughtered and the liver and lungs were removed. In order to study the level of infection in the chickens, the liver and lungs of ten chickens were examined using the Baermann technique. All larvae were counted. The lever and lungs of the remaining 30 chickens were sliced finely and fed to 3 piglets. On day 7 post infection the same procedure was repeated for the last 40 chickens. The two groups of 3 piglets were slaughtered 7 days after feeding with the sliced lungs and livers. The liver, lungs and intestines of the pigs were examined for the presence of *A. suum* larvae.

Results: Numerous live *A. suum* larvae were recovered in the lungs and livers of the chickens. After feeding the piglets with infected chicken tissue, several larvae were recovered (on day 7) in the lungs.

Conclusion: It is documented that *A. suum* larvae can migrate in chickens in the form of visceral migrans. After feeding infected chicken liver and lungs to piglets it was furthermore, possible to recover these from their lungs. Based on these observations we suggest that the chicken could serve as a paratenic host for *A. suum* in pigs and for *A. lumbricoides* in humans if their livers are consumed raw.

Acknowledgement: The Danish National Research Foundation is acknowledged for the financial support of the study.

g.7.67 *ANGIOSTRONGYLUS CANTONENSIS* NOW ENDEMIC IN *RATTUS NORVEGICUS* IN THE USA

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Since its problematic arrival in the United States in the early 1980's and first reported from New Orleans in 1987 (Campbell and Little, Am J Trop Med Hyg 38:568-573, 1988) death of a non-human primate (Gardiner et al., Am J Trop Med Hyg 42:70-74, 1990) and a human infection (New et al., New Eng J Med 332:1105-1106, 1995) attributed to *A. cantonensis* have been reported from New Orleans. In 1996 numerous adult *A. cantonensis* were recovered from the spinal cord of a 5-month old minature horse with neurological symptoms that did not respond to treatment, euthanized at the LSU Veterinary Hospital in Baton Rouge. Infection of the animal probably occurred in Picayune, MS. In 1998 the deaths of a red lemur from New Iberia, LA and a wood rat (*Neotoma* sp.) from the outskirts of Baton Rouge were attributed to the presence of adult *A. cantonensis* in the spinal cord and brain. Of 113 rats (*R. norvegicus*, the definitive host) trapped over a 10-month period near large food markets in the Baton Rouge area, 22% were infected with *A. cantonensis*. None of the 60 young rats were infected, 48% of the 52 adult rats had 1 to 35 adult worms. First stage larvae from rat pellets developed to infective third stage in snails (*Physa* sp.) collected from drainage ditches in the trapping vicinity. One of 2 laboratory rats given 9 or 10 infective larvae by gavage began passing larvae within 10-14 days later and has continued to shed larvae in the feces for 34 weeks.

g.7.56-69 Helminth parasite zoonoses
g.7.70-77 General aspects of parasitic zoonoses

g.7.68 SLAUGHTERHOUSE SURVEY OF CYSTICERCOSIS AND TREMATODE INFECTIONS IN PIGS AND CATTLE, AND SNAIL COLLECTIONS RELATED TO TREMATODES IN HONDURAS

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Slaughterhouse survey: *Cysticercus cellulosae* was found in 2.68% of the investigated pigs (7912/294858) and *C. bovis* in 0.08% of cattle (297/381983) at the Tegucigalpa Slaughter House between 1990 and 1997. At the Catacamas Slaughter House, the figure was 1.45% (102/7054) for *C. cellulosae* between January 1997 and April 1998. No signs of infections with *Fasciola* or with Paramphistomatid trematodes were observed. **Snail collection**: Freshwater snails were collected to study their possible role as intermediate hosts for *Fasciola* or Paramphistomatid trematodes. Snails were collected at 36 locations, such as ponds, rivers, streams etc. A kitchen-net, 16 cm in diameter with 16 mm mesh was used for collection. In the laboratory, snails were picked out with the help of a magnifying glass. The following snails were found (between brackets: the number of positive locations and the percentage): *Lymnaea* (1; 2.8%), Planorbids (26; 72.2%), *Physa* (14; 38.9%), *Pila* (2; 5.6%), Viviparids (3; 8.3%), Pleurocerids (3; 8.3%), Thiarids (10; 27.8%) and Unionids (2; 5.6%). The lack of liver fluke is in line with the observation of very low numbers of *Lymnaea*. However, the lack of Paraphistomatid infections cannot be explained by the lack of planorbid snails, because they were frequently present.

g.7.69 VERTICAL TRANSMISSION OF TRICHINELLA SPIRALIS IN PIGS, GUINEA PIGS AND MICE.

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Background: Migratory *Trichinella spiralis* larvae are distributed via the lymph and blood circulatory systems to the whole body of the host. This study aimed to examine whether migratory larvae can cross the placenta and establish in the foetuses of experimentally infected animals.
Method: 12 pigs, 16 guinea pigs and 41 mice were inoculated orally with high or low doses of *T. spiralis* at different stages of their gestation period. Pre- and post-colostral blood samples were collected from the piglets and tested for presence of antibodies against *T. spiralis* using an ELISA. Muscle samples from all animals were digested using the HCl-pepsin method and the number of larvae per gram were counted.
Results: Vertical transmission was demonstrated in guinea pigs (50%) and in mice (23%) but not in pigs. Larvae were recovered from two aborted guinea pig foetuses. Pre-colostral sera from the piglets of two sows were positive for antibodies against *Trichinella*.
Conclusion: Transplacental transmission of the parasite was demonstrated in guinea pigs and vertical transmission was shown for guinea pigs and mice but not for pigs. The possibility of milk-borne transmission can not be excluded for mice. Antibodies against *Trichinella* in pre-colostral sera indicate some foetal contact with larvae or larval antigens.
Acknowledgement: The study was funded by The Danish National Research Foundation.

g.7.70 LEISHMANIA ANTIBODIES IN CHILDREN SERUM SAMPLES IN CORRELATION WITH THE DISEASE IN DOGS

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Background: Leishmaniosis is a zoonosis with worldwide distribution in the temperate, subtropical and tropical areas. In Greece there is a high incidence of the disease among dogs which may be the source of infection for humans. The aim of this work was to investigate the occurrence of IgG and IgM antibodies against Leishmaniosis in serum of children with various chronic diseases and in correlation with the incidence of the disease among the dogs.
Method: 489 serum samples from children presented to AHEPA hospital of Thessaloniki with various chronic diseases, were used. According to the age of the patients, the samples were divided into 3 groups. Group I consisted of 200 samples (age from 5 months to 5 years), Group II of 164 samples (age from 6 to 10 years), and Group III of 125 samples (age from 11 to 15 years). For L. infantum antibodies identification the strain MHOM/GR/78/LA4, MON1, isolated in Greece, was used and determined by the ELISA method.
Results: In Group I, 19/200 (9.5%) serum samples were positive (13 for IgG and 6 for IgM antibodies). In Group II, 12/164 (7.3%) were positive (6 for IgG, 5 for IgM and 1 for both IgG and IgM). In Group III, 11/125 (8.8%) were positive (4 for IgG, 6 for IgM and 1 for both IgG and IgM).
Conclusion: Generally, in children with various diseases the incidence of Leishmania IgG and/or IgM antibodies is high. Chronic Leishmaniosis (high titres of IgG) is more common in comparison with acute or subacute (high titres of IgM).

g.7.71 TICK-BORNE PATHOGENS IN IXODID TICKS FROM THE NORTH OF SPAIN

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Background: The aim of this study was to determine the prevalence of three tick-borne pathogens (*B. burgdorferi, Ehrlichia phagocytophila* group and flavivirus causing encephalitis in sheep) in the different species of Ixodidae present in the vegetation of the Basque Country, and to characterize the habitats where each agent and their potential vectors could be present.
Method: A collection of ticks composed by 7835 ticks of eight different species (4494 larvae, 2970 nymphs and 371 adults) captured from vegetation in 37 different geographic areas was analyzed by PCR.
Results and conclusions: The prevalence of *B. burgdorferi* in *Ixodes ricinus* was 1.5% in adults and between 0.05% and 0.4% in nymphs. Infection was not detected in larvae. The infection risk for *Borrelia burgdorferi* was associated with areas located in forests, in the atlantic and transitional side, with altitudes below 800 m., in recreational areas, mountain routes or forests, with livestock and preferably between March and July. *E. phagocytophila* group was also widespread in any time of the year, but preferably during the spring. It was detected in all the vegetation associations studied, all the altitudes, uses of the areas and livestock presence. This pathogen was present in five out of eight species of ticks examined. In *Ixodes ricinus*, the prevalence was of 8.2% in the adults and between 1.6% and 13.7% in the nymphs. Regarding Flavivirus, the infection was not detected in none of the species of ticks analyzed, although the estimation of the maximum percentage of infection would be below 0.7%.
Acknowledgements: Work supported by CICYT AGF94-0275.

g.7.70-77 General aspects of parasitic zoonoses

g.7.72 PREVALENCE OF ENVIRONMENTALLY IMPORTANT PARASITES AND BACTERIA IN ALBERTA HOGS

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Background: There is increasing concern about the generation of fecal waste by swine and the distribution of waste on fields. One of the major concerns is the transmission of certain parasitic (*Giardia lamblia, Cryptosporidium parvum, Ascaris suum, Isospora suis*) and bacterial (*E. coli* O157:H7 and *Salmonella spp.*) pathogens to humans and other animals. This research will provide information to assure the public that hog production is environmentally sustainable and does not place the public at risk.

Method: Alberta hog operations (n=50) were visited and samples were collected from all age groups of hogs. Fecal samples from individual pens and slurry samples from indoor fecal pits and outdoor lagoons were collected. Water provided to the hogs was sampled and soil from the site of most recent slurry spread was collected. Bacteria (*E. coli* O157:H7, *Salmonella spp.*) were isolated on selective media and serotyped. *A. suum* and *I. suis* were isolated using sodium nitrate flotation. *Giardia* and *C. parvum* were recovered by sucrose centrifugation and stained with monoclonal (FITC) antibodies.

Results: Preliminary results indicate that the parasites are age group dependant and that the bacterial isolates are not age group dependant. *Giardia* and *C. parvum* were both recovered from the slurry storage pits/lagoons however, no parasites were recovered from soil or water samples.

Conclusion: It appears that due to the overall low prevalence of the parasitic and bacterial pathogens, swine fecal waste and the distribution of it on fields may not pose a significant threat to humans and other animals.

Acknowlegement: Alberta Pork Producers Development Corporation.

g.7.73 ESTABLISHMENT OF A COLLABORATING CENTRE FOR PARASITIC ZOONOSES

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Parasitic zoonoses remain a global problem in spite of advancements in diagnostics, therapeutics, mathematical modelling and vaccine development. In many countries these diseases cause serious human suffering and considerable losses in agricultural productivity, thus posing a significant hindrance to development. In view of the situation the Danish Centre for Experimental Parasitology (DCEP) is establishing a collaborating centre for parasitic zoonoses which will utilize DCEP's close interaction with a worldwide network of parasitologists and the United Nations' Organizations for Food & Agriculture (FAO) and World Health (WHO). The activities of the collaborating centre will be directed at problems of national, regional and international importance with its major task to advise WHO, FAO and endemic countries on surveillance, epidemiology, prevention and control of major parasitic zoonoses. This will be achieved by: 1)participating in the elaboration of guidelines, manuals and strategies, 2)facilitating the planning, implementation and assessment of relevant research projects, 3)organizing scientific meetings and educational activities, and 4)providing information services, advice and support especially for developing countries. It is anticipated that the collaborating centre will encourage technical cooperation at both the country and inter-country levels and stimulate the formation of regional and sub-regional networks hopefully resulting in more effective use of resources. Activities of the collaborating centre will be supervised by an international advisory board of scientists.

g.7.74 STUDY OF *SARCOCYSTIS* INFECTION IN SLAUGHTER ANIMALS IN THE NORTH-WEST OF IRAN

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Sarcocystosis is a common disease among humans and animals, and its etiological agent is a protozoan called *Sarcocystis*.

Sarcocystosis is world-wide, and it is distributed in high levels in Iran. It causes complications such as lessened appetite, emaciation, anaemia, decreased milk production, abortion and death; and in humans it causes gastrointestinal disorders, diarrhoea, and convulsions.

The present research comprised 3,000 sheep (1,347 males and 1,653 females) which were studied according to race, age, involved organs, and season. The general infection rate in sheep was 27.5% (males 2.59%, females 47.79%).

The infection rate was higher in autumn than in the other seasons, and the age at which the sheep were infected, varied. Thus, most of the infected sheep were more than one year old, and the infection rate increased with the age.

In this study, the diaphragm was found to be the most frequently involved organ.

g.7.75 CONTAMINATION OF CATS WITH TOXOPLASMA GONDII IN NORTH WEST OF IRAN (1996-97)

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In this study 107 cats were selected radomly from North west of IRAN. Stool specimen s of these animals were tested by direct examination, using sucrose and the serologic dye test our main object was to detect the oocyst microscopically and by detecting antibodies against T. gondii, using sabin feldman dye test Our findings on the test cats were analysed according to the sex, age and the place from which they collected.

Oocysts were shown in 4.6% of the cats and 34.6%of them were positive after dye test(1/16) with regard of the age and the enviroment from which they collected (hospital and nonhospital collection)statistical meaningful differences were found ($p<0.05$) but significant differences were not encountered according to the sex and intensity of the - contamination.

g.7.70-77 General aspects of parasitic zoonoses
g.7.78 Teaching of veterinary parasitology

g.7.76 *ECHINOCOCCUS GRANULOSUS* IN STRAY DOGS OF TABRIZ, IRAN, 1995-1996

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Background: Between 1995 and 1996, 81 stray dogs from eight districts of Tabriz were selected and studied to estimate the rate of *E.g.* contamination.
Method: First, the stray dogs were poisoned with strychnine, and after autopsy all their intestines were sampled for examination. To prevent any possible contamination, the specimens were kept in 10% formalin for one month.
Results: Out of the total number of samples, 58 were contaminated with *Echinococcus granulosus*. The most contaminated area was the place which is used for disposal of rubbish (95%), and the least contaminated areas were the Hokmabad refinery and the old slaughterhouse of Tabriz (50%).
Conclusion: In this study no significant differences were found with regard to the sex of the dogs (P>5%). The rate of contamination approached 71.6% compared to that of 50% in a similar study in Tabriz in 1970, which indicates a statistically significant difference (P<5%).
A close relationship was noted between the hygienic level of the studied locations and animal husbandry, and the rate of contamination (P<5%).

g.7.77 PREDICTING SCHISTOSOMIASIS RISK IN CHINA USING REMOTE SENSING AND GIS

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Background: A predictive model based on Geographic Information Systems (GIS) and satellite surveillance was developed to 1) link control program databases to map data on climate and other environmental features that affect success of control programs for zoonotic *Schistosoma japonicum* and 2) to anticipate changes in disease pattern after the Three Gorges Dam is built.
Methods: A regional GIS was constructed using ArcView3 and ERDAS Imagine software. Data layers were referenced to Digital Chart of the World basemap and included: a May-October time series of data from the AVHRR satellite sensor on vegetation index and maximum surface temperature; elevation data; and risk index maps prepared from a climate-based parasite forecasting system using monthly water budget and growing degree day values.
Results: At 84 climate stations in southern China, a climate forecast risk index of >900 and a lowest monthly temperature of <4°C defined a zone that was consistent with the known distribution zone of *S. japonicum*. Analysis of values for 18 climate stations in Jiangsu province by logistic regression analysis revealed a significant ($p<0.05$) relationship between risk index and human infection prevalence, with an overall correct classification rate of 88.9% There was no relationship to vegetation index or maximum surface temperature.
Conclusions: Application of prediction models may provide a useful measure of anticipated resistance to control measures and possible changes in transmission patterns associated with the Three Gorges dam.

g.7.78 UNDERGRADUATE TEACHING OF VETERINARY PARASITOLOGY: AN AFRICAN PERSPECTIVE

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Background: The teaching of veterinary parasitology to undergraduates in an African perspective is reviewed. Aspects like the structure of the curriculum, year in which veterinary parasitology is taught and the different times allocated to different disciplines of veterinary parasitology (i.e. general parasitology, veterinary helminthology, veterinary protozoology and veterinary entomology) and the methods of teaching are discussed.
Method: Information on the undergraduate teaching of veterinary parasitology was gathered from several veterinary schools in Africa. To compare the different schools a standard format was designed on different aspects of the curriculum and the methods of teaching applied.
Results: The results reveal differences in structure of the curriculum of undergraduate veterinary parasitology in African Veterinary Schools and the teaching methods applied. Availability of teaching staff and the cost of running practical classes are the most limiting factors in the teaching of veterinary parasitology to undergraduates.
Conclusions: There is need to constantly review the curriculum of undergraduate veterinary parasitology and standardise the materials and methods of teaching in light of new knowledge.
Acknowledgements: I wish to thank all the colleagues from veterinary schools in Africa who provided me with course outlines of undergraduate veterinary parasitology in their respective schools.

g.7.79-81 Further examples of zoonoses

g.7.79 PATHOLOGY OF PULMONARY SCHISTOSOMIASIS AND ECHINOCOCCOSIS IN GOATS

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In the present study a total of 2217 goat lungs were examined and out of these, 571 specimens showing frank lesions were further processed for histopathology. In pulmonary schistosomiasis the eggs were seen in branches of pulmonary arterioles where they produced moderate to severe inflammatory reaction in the form of small granulomas. These granulomas were predominantly formed of mononuclears,polymorphonuclears with or without giant cells. In pulmonary echinococcosis the fluctuating cysts revealed from within outwards a germinal layer from which brood capsules arose containing scolices. Outside this a thick concentrically laminated elastic hyaline layer blending with surrounding connective tissue capsule infiltrated by mononuclears. The lung parenchyma in the vicinity of cysts showed atrophic changes.

g.7.80 FISH ANISAKIDAE IN KHUZESTAN PROVINCE OF SOUTH - WEST , IRAN

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Background:Fishes including: Barbus spp, Cyprinus carpio,Liza abu, Aspius vorax have very important role in the economic condition of rural area of khuzestan province.These fishes have been consumed as barbecued or roasted. These, inadequately cooked fishes, could be served as a source of infection in these communities.

Method :For this reasons, 1995 - 96,283 fishes were caught from 4 lagoons (Hooral azim, Shadegan, Al hai, Seid Naser) and transported aliveto the Health research center and their intestine, body cavity and muscle observed carfully.

Results: In 20.8% of fishes, infection with contracaecum sp (Anisakidae) and/or Rhabdocona sp (probably R. denudata) were obtained.

Conclusion: This results, which are new record for Iran, suggested that human anisakiosis couldbe Medical problem in this area.

g.7.81 STUDY ON THE PROTECTIVE IMMUNITY OF LASER-IRRADIATED TOXOPLASMA

Li Aifen, Lu Shaohong, Chen Caihua, Chen Rui

Background: Toxoplasmosis is a wide-spread veterinary parasitic desease. It has serious affection to the health of human and animal. So it is important to prevent and cure the desease. To solve the problem, we use laser to irradiate toxoplasma tachyzoites in order to study an attenuated live vaccination.

Method: 0~ 6.0walter(W) laser was applied to irradiate Toxoplasma tachyzoites, and then do the following experiment.① Observing the active ability and calculate number of active tachyzoites.② Using Flow Cytometry (FCM) to detect the alteration of DNA contents of irradiated and non-irradiated tachyzoites to testify whether laser weaken the activity and reproductive capacity.③ Laser-irradiated Toxoplasma tachyzoites were immuned to ICR mice. On 0, 15, 30, 45, 60, 90, 120 days, mice sera were collected to detect antibody, at the same time T lymphocyte CD_4+/CD_8+subgroup were detected to know whether laser-irradiated tachyzoites can induce humoral and cellular immune response.

Result: Laser made the active ability of tachyzoites abate. No. of active tachyzoites decreased from 98% to 20%. DNA contents of tachyzoites obviously decreased with the increasing of dose of laser. IgG antibody of immuned mice was positve and T lymphocyte subgroup CD_4+、 CD_4+/CD_8+ was higher than non-immune dmice. Except that, immuned mice challenged by live tachyzoites can prolong the survival time.

Conclusion: Laser can weaken the activity and reproductive capacity of Toxoplasma tachyzoites. Laser-irradiated tachyzoites can induce humoral and cellular immune of mice, and improve the ability of mice versus Toxoplasma infection.

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AUTHOR INDEX

This index contains names of all presenting authors and co-authors. Papers marked * indicate a presenting author. The numbers refer to the session time-slot and room number, followed by the order in which the paper is presented in each particular session.

Fossum, C	b.2.05
Franc, M	g.6.46*, g.6.45
Frank, GR	g.7.16*
French, DD	b.1.07
Friis, C	e.2.01
Frontera, EM	c.6.14*, e.1.03
Frydas, S	g.7.50*, g.7.59*, g.7.70*
Fthenakis, GC	e.3.07, b.5.03, g.6.71
Fuchs, N	e.5.07, b.2.03
Fujisaki, K	c.6.34, c.6.46, c.6.20, g.7.24
Fukumoto, S	g.7.17*
Fulton, LC	c.7.85
Fung, MC	d.2.02
Gaasenbeek, CPH	e.1.08
Gahlot, AK	c.6.10
Gaines, P	f.2.08
Galtier, P	g.7.01
Gamble, HR	e.4.08*, g.7.61
Gamme, L	e.4.07
Ganter, M	g.6.02
Garcia-Pérez, AL	g.7.71*
Gardner, IA	a.5.02*
Garossino, K	f.5.06
Garrick, DJ	a.1.08
Gasardgan, NP	g.6.69
Gasbarre, L	f.3.01*
Gasquet, M	f.5.01
Gasser, RB	g.7.30*, f.2.06, g.7.34, g.7.35, f.2.03
Gathuma, JM	g.6.33, g.7.65
Gatongi, PM	f.1.06
Gauchi, CG	d.2.04
Gawor, J	g.6.12*, c.7.66*
Gayrard, V	g.7.05*
Gbodjo Zakpa, L	f.3.08
Geary, TG	a.1.04, g.7.10
Geenen, PL	c.7.81*
Geerts, S	e.4.04, a.3.05
Geldhof, P	c.6.13
Genchi, C	c.7.10*, c.7.06*, e.2.08*, c.6.28
Gennari, SM	c.7.05*, g.6.92*, g.7.51*, c.6.44*, g.7.43*
Georgousi, K	c.7.63
Ghadersohi, A	g.7.33
Giangaspero, A	g.7.25
Gibson, DI	c.6.77
Gichigi, MN	c.7.01
Gil, H	g.7.71
Giles, M	g.7.38*
Gillespie, AT	f.5.05*, c.7.71, c.7.72, d.3.03
Gimenez, R	c.6.70
Gipson, T	g.7.48
Githigia, SM	f.1.08*
Githiori, JB	b.3.07
Giudici, CJ	c.7.96*
Gleeson, MT	f.2.02
Goddard, PT	g.7.39*
Gogolewski, RP	b.5.04, c.7.43
Gokbulut, C	g.7.06*, g.7.07*
Goldová, M	c.6.71*
Goldstein, M	g.7.74
Gomez, L	c.6.68, c.6.65
Gonçalves, J	c.7.71
Goossens, B	a.3.05
Gopal, RM	a.1.08*, c.7.31*
Górski, P	c.6.23
Gossellin, J	c.7.45, c.7.46
Gottschall, D	e.2.02
Gottstein, B	b.2.03*, f.2.07*, b.5.02, e.2.04, g.7.37, g.7.29, g.7.15
Goudeau, C	c.7.32
Gray, GD	g.6.93
Greco, JA	g.6.82
Grecu, I	c.6.83
Green, JA	g.7.38
Greenhalgh, C	e.5.05
Greif, G	g.6.60*, g.6.61, g.6.62, g.6.63
Greiner, M	g.6.41
Griffin, B	f.3.04
Griffiths, T	c.6.37, c.6.38
Grisi, L	c.7.16
Gross, SJ	b.5.04
Groves, B	d.2.01
Gruner, L	f.3.06*, g.7.44*, b.3.05, f.3.07, f.3.03, g.7.45
Grytner-Ziecina, B	c.7.82*
Grzych, JM	c.6.31
Grønvold, J	c.7.67*, d.3.04, c.7.65

Guglielmone, AA	c.7.03, g.6.50
Guidali, F	c.6.62
Guimaraes, MP	c.7.60*, g.6.26
Gunawan, A	c.7.78
Gundlach, JL	g.6.64
Gunnarsson, L	e.3.05
Guselle, NJ	g.7.72*, c.6.54
Gustafsson, MKS	c.7.90
Guthrie, AJ	a.3.04
Haag, K	f.2.07
Haberkorn, A	g.6.62, g.6.63, g.6.61, g.6.60
Hagman, B	e.3.05
Hakanson, R	a.2.02
Halvorsen, O	b.4.04
Hamet, N	c.7.15*
Hamid, A	e.4.05
Han, Q	c.7.47*
Hansen, JW	b.3.03
Hansen, NP	c.6.31, f.2.06, e.1.01
Hanson, T	a.5.02
Harder, A	g.6.61*, g.6.62*, g.6.63*, g.6.60, e.5.04
Harper, PAW	g.7.36
Hatzistilianou, M	g.7.50, g.7.59, g.7.70
Hay, FS	c.7.75
Heaney, K	e.3.08
Heath, DD	d.2.04
Heckeroth, AR	g.7.18*
Heine, J	b.1.05*
Heise, M	g.7.28
Heitman, L	e.4.07*
Hejmadi, MV	c.7.36*
Hellmann, K	e.3.06*
Helwigh, ABM	e.4.01*
Hemmingsen, W	d.4.04
Hempel, L	c.6.87
Hemphill, A	e.2.04*, e.5.07*, b.2.03, g.7.15
Henkle-Duhrsen, K	d.1.04
Hennessy, D	e.2.02*, b.5.06*, e.2.01
Henningsen, E	c.7.65, g.7.66
Henriksen, SA	c.7.30, d.3.04
Hereu, C	g.6.58
Hermosilla, CR	b.2.04*
Hertzberg, H	a.2.02*, c.7.58*
Hilali, M	c.7.04*
Hill, D	b.3.01*
Hill, RJ	c.7.23
Himonas, C	b.5.03, e.3.07, g.6.19, g.6.20, e.1.05, f.4.05, g.6.71
Hindsbo, O	g.6.04, c.6.48, g.6.07, e.1.04, g.6.03
Hirschlein, CA	b.1.04, a.1.05
Hirumi, H	e.5.01
Hirumi, K	e.5.01
Hoberg, EP	b.4.01*, b.4.02, c.6.60
Hoekstra, R	g.7.41
Höglund, J	f.2.05*, e.1.06, d.3.01, d.5.04, a.3.02
Holdsworth, P	f.5.08
Holm-Martin, M	e.3.08*
Holovský, R	g.6.65
Homer, DR	c.7.48
Hong, KO	g.6.83
Hong, YS	c.7.56, g.6.83, g.6.08
Hooshmand-Rad, P	b.2.05
Horohov, DW	a.2.05
Hoskin, BC	g.6.76
Hostache, G	c.7.76, c.7.77, g.7.46
Hoste, HF	c.7.32*, b.3.05*, g.6.85, f.4.02
Houdijk, JGM	f.4.01*
Houffschmitt, P	c.7.16*, g.6.64*, c.7.14, g.6.68, c.7.25, c.7.48, c.7.49
Hove, T	c.6.52*
Hovnanyan, G	g.6.13*
Hoza, SB	c.7.52
Huang, CC	c.7.08
Huang, HP	g.6.36*
Hubleur, M	d.1.02
Huffman, MA	f.5.01*
Humbert, JF	a.1.06*, g.7.40, c.7.34
Humbert-Droz, E	e.3.03
Hung, GC	g.7.30, g.7.34, g.7.35, f.2.03
Hung, P	e.5.05, c.6.38
Huntley, JF	f.4.01, f.3.05
Huong, LTT	a.4.01*
Husein, A	c.6.49, c.6.06
Hustead, D	g.7.74
Hutchinson, MJ	a.3.03
Huther, S	c.6.30
Ibarra, F	g.6.29

Kooyman, FNJ	a.2.07*, e.1.07, b.3.07
Kosuth, P	c.6.79*
Kotomski, G	e.2.07
Kotze, AC	g.7.20*
Koudela, B	c.6.26*
Koutsotolis, K	g.6.21, g.6.94
Koval, ZZ	c.7.64
Kowalik, S	c.6.27*
Krämer, F	g.6.18
Kramer, L	c.6.28*
Krecek, RC	c.7.97, g.6.32, g.6.39, a.3.04
Kristensen, T	f.5.05, g.7.77
Kristiansen, E	g.6.90, f.4.06
Kritas, S	e.3.07
Krupicer, I	a.2.04
Kublickiene, O	g.6.44
Kujanek, R	g.7.09
Kunz, M	d.1.02
Kurabayashi, N	c.6.09
Kusel, JR	g.7.17
Kutz, S	b.4.02, c.6.60, b.4.01
Kuverová, S	c.6.26
Kyriazakis, I	f.4.01, f.5.04, g.6.84
Kyvsgaard, NC	b.5.08, f.4.04, d.5.01
La Rosa, G	e.4.03
Laaneoja, L	g.6.14
Labruna, MB	c.7.05
Lachowicz, J	c.6.04*, c.6.59
Lainas, T	g.6.19
Lamberti, JC	c.7.03*, g.7.08*
Lamidey, C	c.7.15
Lanfredi, RM	c.7.80
Langholff, WK	c.7.43, g.6.54, c.7.42, c.7.39
Langworthy, N	d.1.04
Lanusse, C	d.1.03*, g.7.09*, g.7.13, g.7.14, d.1.01
LaRosh, M	g.7.74
Larsen, JWA	g.6.15*
Larsen, M	c.7.68*, d.3.02, d.3.04, c.7.65, c.7.66, c.7.67
Larsen, MN	c.7.59*
Larter, N	c.6.54
Laughton, DL	a.1.03
Le Stang, JP	c.7.53
Leak, SGA	f.3.08
Leathwick, DM	a.2.06, c.7.75
Leclipteux, T	a.3.07
Lee, CG	g.6.83*
Lee, JK	c.7.56, g.6.08
Lee, SH	c.7.08
Leemans, I	b.2.05*
Lefrileux, Y	c.7.32, b.3.05
Leighton, EA	f.3.01
Leignel, V	a.1.06, g.7.40*
Lenstra, JH	g.7.41
Leontides, L	e.1.05
Letková, V	c.6.71, c.6.79
Letonja, T	f.5.08
Levkut, M	c.6.71, a.2.04
Lewa, AK	c.7.17*
Lewis, BD	a.4.03
Li, G	g.7.21*
Li, L	c.6.36*
Lia, R	g.6.73, g.6.72
Liang, SL	g.6.36
Lichtenfels, JR	c.7.97
Lifschitz, AL	g.7.09, g.7.14, d.1.01
Lightowlers, MW	d.2.04
Lima, JD	c.7.89
Lima, WD	g.6.26*, c.7.60
Limet, A	b.1.05
Limouzin, C	f.3.06
Lin, H	g.7.21
Lind, P	g.6.01, e.4.01, c.7.02, g.7.69
Lindenstrøm, T	c.6.80*, c.6.81*
Lindquist, A	g.6.16
Lindström, E	a.2.02
Literák, I	g.7.52*, c.6.61*
Ljungström, BL	g.6.16*, a.3.02
Lombardero, O	c.6.68
Lonsdale, B	g.6.76
Loószová, A	c.6.71
López, C	c.6.45
Lorimer, S	g.6.88
Losson, B	g.6.38, a.3.07
Losson, BJ	g.7.63*, c.6.53*
Loui, C	b.2.01
Loukas, A	e.5.05
Loumos, V	c.7.99
Louvandini, H	g.6.92

Louw, JP	c.7.43
Louw, K	c.7.43
Lubega, GW	g.7.22*, f.5.03, b.2.06, e.2.05
Luckins, AG	c.6.57
Luempert, LG	g.6.48
Lukesová, D	c.7.18*, g.6.65*
Lukyanchenko, TA	c.7.69*, c.7.70*, c.7.64
Lundvig, RF	c.6.75
Luty, T	g.6.40
Lyon, S	c.7.13
Ma, X	d.2.02
Maass, D	g.6.88
MacDonald, V	g.6.89
Mach, J	c.7.18
Machuca, M	b.2.02
MacKenzie, K	d.4.04*
Mackeviciene, G	c.6.82
MacRae, JC	g.6.91
Madder, M	a.4.07*
Madeira de Carvalho, LM	a.3.01*, c.7.71*
Magaya, A	f.4.04*
Mage, C	g.7.03
Mägi, E	g.6.14
Magnino, S	c.7.06, c.7.10
Magona, JW	g.6.27*, c.7.33*
Magwisha, HB	d.5.01*
Mahieu, M	c.7.57
Mahoney, RH	g.6.77
Maingi, NE	c.7.01*, c.7.17, f.1.08
Maki, Y	c.6.20
Makundi, AE	c.6.05*
Malczewska, M	g.7.64
Malczewski, A	g.7.64*
Malecki, J	c.7.00, c.6.66
Maljutina, T	c.7.92, c.7.91
Mallereau, M-P	c.6.42
Mallon, TR	b.3.06
Malone, JB	g.7.77*
Mancassola, R	c.6.42
Mancebo, OA	c.7.03
Mandonnet, NMG	f.3.03*, g.7.45*, c.7.57
Manera, M	d.4.03
Manfredi, MT	c.6.62*
Manga-González, Y	g.6.29
Manteca, C	a.3.07
Marchand, A	g.6.34, g.6.35
Marecek, J	g.6.65
Margueritte, JA	g.7.08, c.7.03
Marshall, RN	g.6.66*
Martin, A	f.2.04
Martin, PJ	b.1.08*, c.7.48*
Martin, RJ	a.1.01*, g.7.11, g.7.12, g.7.10
Martin, S	g.6.78, a.3.08
Martinez, L	c.6.11
Martinez-Gonzales, B	c.7.99
Masake, R	c.6.43
Maselle, RM	d.5.01, c.6.05
Masuku, M	d.5.03
Mathis, A	a.5.04
Matsuyama, A	g.7.17
Matthee, S	a.3.04*
Mattos, C	g.6.82
Mattsson, J	a.5.05*, f.2.05
Maure, PF	c.6.01
Maxson, AD	g.7.65
Maynard, L	c.7.16
Mazeika, V	c.6.63*, c.6.82*
Mbati, PA	e.5.01*
Mbithi, PMF	g.7.65
McAllister, M	a.5.01
McAllister, TA	a.4.02, f.5.06
McBean, D	f.3.05
McCarvill, E	b.3.06
McClure, JJ	g.7.67
McDermott, J	c.6.43
McKellar, QA	g.7.06, g.7.07
McPherson, WB	b.5.04
Measures, L	c.6.54
Meckenstedt, U	d.1.04
Medley, G	c.7.79, a.4.05
Meeusen, E	c.6.38
Meitenawy, TM	a.3.06
Mejer, H	e.1.04*, g.6.03*, g.6.07
Mellor, DJ	f.1.06
Mellor, P	c.7.04
Méndez, M	c.7.11
Mendosa, PA	g.7.68
Mercier, P	c.7.25*, c.7.49*, g.6.67*, g.6.68*, c.7.41
Merino, G	g.7.14

Merlo, A	c.7.83
Mertens, C	b.3.04
Mestorino, N	g.7.04, e.2.03, g.7.08
Meteling, B	e.3.06
Meyer, C	g.6.02
Meyer, S	c.7.43
Mickeniene, L	c.6.82
Midtgaard, N	c.7.50*
Mieszczanek, J	d.2.03
Mignon, BRM	g.6.38*, g.7.63
Mijal, J	c.7.82
Mikami, T	c.6.34, c.6.46, c.6.20, g.7.24
Miller, I	g.7.60
Miller, JE	d.3.03
Milligan, D	f.5.06
Mimapan, S	c.6.57
Minjauw, B	b.5.01*
Minnaar, WN	g.6.39*
Mizgajska, H	g.6.40*
Mlengeya, MM	b.4.08
Mlengeya, TDK	b.4.08*
Mohammady, P	g.7.76*
Molento, MB	d.1.01*
Möller, L	e.3.05
Moltedo, H	g.6.59, g.6.50
Mondal, MMH	f.1.01*
Monicat, F	g.6.31
Monrad, J	b.2.08*, c.6.05
Monsen, K	b.4.01
Montenegro, M	c.6.68
Moore, RM	a.2.05
Mora, JA	c.6.14
Morck, DW	c.6.39
Moreau, E	c.6.12
Morgan, U	e.5.08, a.4.02
Moritz, A	g.6.41
Morriss, CJ	c.7.19
Morrondo, MP	c.6.15*, c.6.47*, c.6.45
Moskwa, BM	c.6.16*
Movsessian, S	g.6.69*, c.7.91*, c.7.92
Moya-Borja, GE	g.6.68
Moyses, EW	e.3.03
Msiza, G	g.6.32
Mukaratirwa, S	g.7.78*, g.6.86, f.4.04
Munn, E	g.7.23*, c.6.38
Munteanu, GM	c.6.83*
Munyua, WK	b.4.07, c.7.17, f.1.08, g.6.33
Murray, M	a.2.01
Murrell, KD	g.7.66, g.6.06
Musisi, G	c.7.33, g.6.27
Nagasawa, H	c.6.46, c.6.34, c.6.20, g.7.24
Nakao, M	e.4.05
Nakaya, K	e.4.05
Nansen, P	a.4.04, a.4.05, a.4.06, b.2.08, b.3.03, c.6.74, c.6.75, c.7.30, c.7.47, c.7.50, c.7.65, c.7.67, c.7.81, d.3.04, e.1.01, e.2.01, e.4.01, f.1.08, f.2.06, f.4.06, f.5.01, g.6.90, g.7.26, g.7.57, g.7.66, g.7.69, g.7.73
Napompeth, B	c.7.74
Näslund, K	a.5.05
Navarrete, I	c.6.14, e.1.03
Ndao, M	a.3.05
Ndlovu, LR	g.6.86
Newlands, GF	g.7.19
Newton, SE	c.6.37*, c.6.38*, e.5.05*, f.2.06, c.7.85
Nganga, JC	b.4.07
Ngatia, TA	c.7.17
Nginyi, JM	f.1.06*
Ngowi, HA	g.7.62
Nguyen, VK	f.3.07
Nielsen, CV	g.6.04*, c.6.84*, c.6.48*
Nielsen, ME	c.6.80
Nieminen, M	b.4.03
Niezen, JH	c.7.61*, c.7.75
Nikolaou, E	g.6.94, g.6.21
Nilsson, O	g.6.16, a.3.02
Nishi, J	b.4.02, c.6.44, c.6.60
Nishi, SM	c.6.44*, g.7.51
Nishikawa, Y	g.7.24*
Njenga, JG	c.7.01
Njie, M	f.3.02
Njoroge, EM	g.7.65*
Nnadi, PA	g.7.42
Nöckler, K	c.6.64*
Noh, JU	c.7.56, g.6.08
Nordenfors, HE	d.5.04*
Nosal, P	g.6.05*, c.7.84*
Nottingham, R	g.6.76
Nourian, AA	g.7.56
Novák, P	g.6.65

Wisnewski, N	f.2.08*
Wissocq, N	c.6.43
Wolstenholme, AJ	a.1.03*, c.7.36
Wolstrup, J	d.3.04, c.7.65, c.7.67, c.7.72
Woollard, DJ	d.2.04*
Wright, I	f.3.05
Wu, K	d.2.02
Xuan, X	c.6.34, c.6.46, c.6.20, g.7.24
Yagi, K	g.7.17
Yagi, Y	c.6.09
Yang, CH	c.7.08
Yang, HL	g.6.36
Yatsuda, AP	c.6.24*, c.6.22
Yelifari, L	e.1.01
Yokota, H	g.7.17
Yokoyama, M	c.6.20
Yoshihara, S	c.6.21
Youn, HJ	g.6.08*, c.7.56*, g.6.83*
Young, C	g.7.27*
Yu, F	g.6.91
Zaha, A	f.2.07
Zahner, H	c.6.30*, b.3.04, g.6.01, a.2.08, b.2.04, c.6.27
Zajac, A	g.7.48*
Zakrisson, G	e.3.05
Zalunardo, M	a.5.06*
Zariffard, MR	g.7.33*
Zarlenga, DS	f.2.04*
Zervas, G	b.1.02, g.6.94
Zeyle, EE	g.7.65
Zhang, Y	c.6.31
Zhou, X	g.7.77
Zhu, XO	f.2.03*, g.7.35
Ziela, M	d.5.03*
Zimmermann, GA	g.6.50
Zinsstag, J	f.3.02*, g.6.25, f.1.05
Zislin, A	g.7.74*
Zizlavský, M	g.6.65
Zugogiannis, D	f.4.05, g.6.19
Zuko, A	g.6.22*, g.6.37