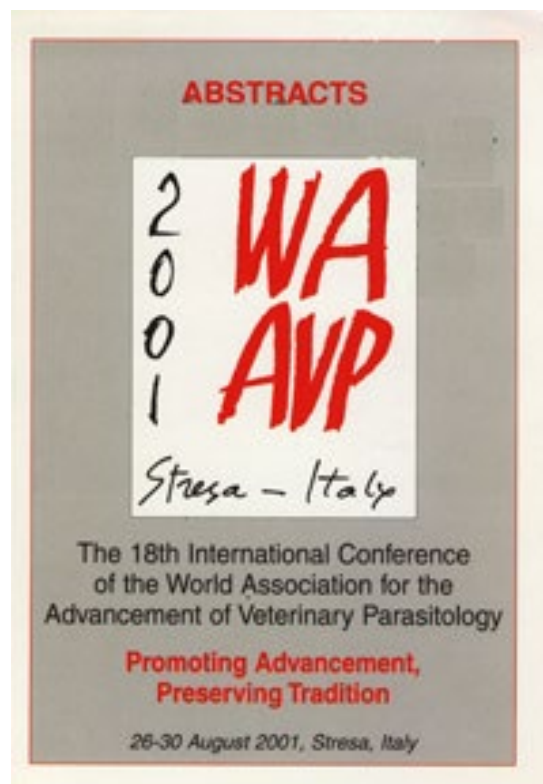


The 18th International Conference of the
**WORLD ASSOCIATION FOR THE
ADVANCEMENT OF VETERINARY
PARASITOLOGY**

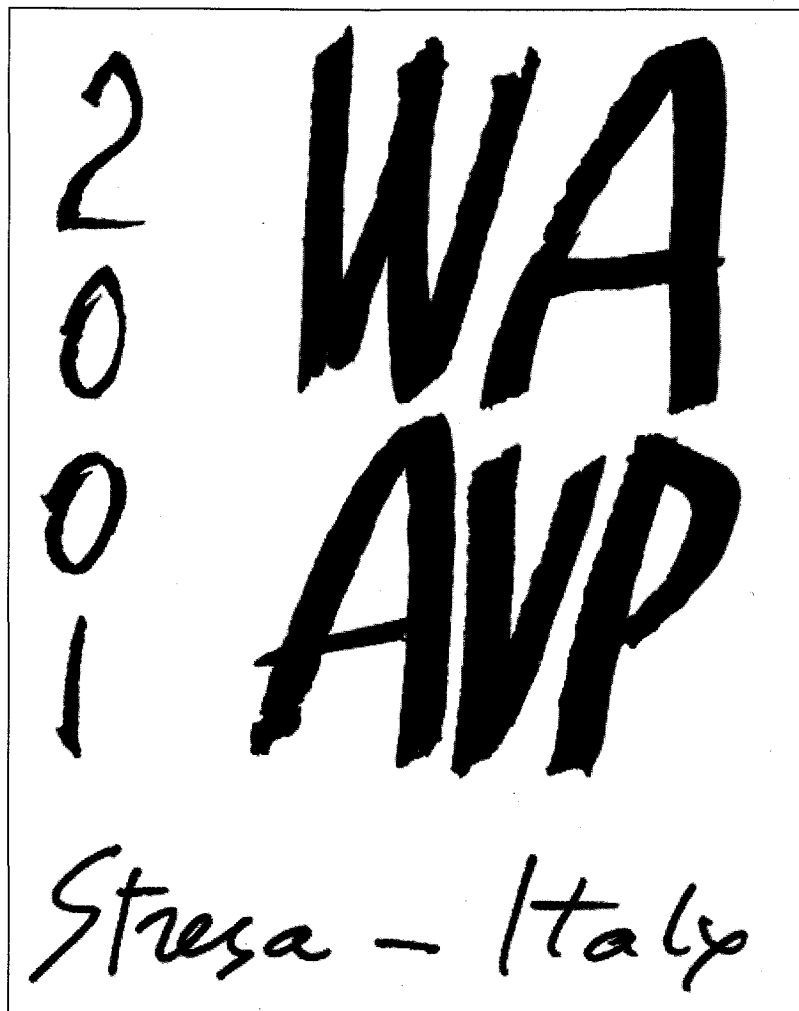
“Promoting Advancement Preserving Tradition”

August 26-30, 2001

STRESA, ITALY



ABSTRACTS



The 18th International Conference
of the World Association for the
Advancement of Veterinary Parasitology

**Promoting Advancement,
Preserving Tradition**

26-30 August 2001, Stresa, Italy

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Preface

Dear colleagues and friends,

This volume contains the nearly 600 scientific abstracts that will be presented here in Stresa at the 18th International Conference of the World Association for the Advancement of Veterinary Parasitology.

I would firstly like to thank all of our global colleagues for contributing to the Conference. The WAAVP has always strived to bring together the most recent and advanced results in research in the field of veterinary parasitology and I feel that this Conference continues this tradition. This volume contains abstracts of the oral and poster presentations from the various scientific sessions, workshops and corporate symposia, making it a complete collection of the scientific content of our meeting.

Many people have supported us in our efforts and I would like to thank the WAAVP 2001 Scientific Committee, Local Organising Committee, the WAAVP Executive Committee and NewTeam, for their help, together with our corporate sponsors who have made this Conference a reality.

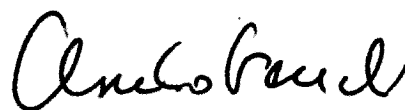
Lastly, I would like to express my gratitude to the following organizations for their patronage:

Società Italiana di Parassitologia (SOIPA)

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Claudio Genchi

Milan, August 2001

• MERIAL		
Innovations in the diagnosis and treatment of parasitic diseases	page	204
• NOVARTIS ANIMAL HEALTH		
Parasite vaccines	»	205
• PFIZER ANIMAL HEALTH		
Latest developments on Selamectin use and mode of action	»	206
Author Index	»	207

Scientific abstracts are assigned according to session letter and number.

Posters are followed by a small "p".

Table of contents

Scientific Session	page	1
A. Parasitic zoonoses	»	3
B. Neospora and other coccidial infection	»	13
C. Immunology and parasite pathology	»	27
D. Parasitism and aquaculture	»	43
E. Epidemiology of parasitic diseases	»	49
F. Genetics and parasitism	»	71
G. Ticks and tick-borne diseases	»	77
H. Parasite biology and systematics	»	89
I. Molecular and biochemical parasitology	»	103
L. Parasite control: surveillance and prevention	»	115
M. Diagnosis of parasitic diseases	»	137
N. Chemotherapy and pharmacokinetics	»	145
O. Helminth infection in horses	»	163
P. Echinococcosis and hydatidosis	»	173
Workshop	»	179
• Relationship between pharmacokinetics and anthelmintic efficacy of endectocides	»	181
• Harmonization of diagnostic methods in veterinary parasitology in the EU	»	183
• <i>Dirofilaria</i> infection in humans and animals	»	184
• Cryptosporidiosis	»	186
• Third WAAVP Workshop on the Systematics of the Cyathostominae of horses	»	187
• Nutrition and parasites: feasibility of dietary manipulation for parasite control	»	188
• Myiasis	»	190
• Psoroptic mange	»	192
• Pets, parasites and changing rules for travel	»	194
• Progress and problems of teaching of Veterinary Parasitology	»	195
• Wildlife and parasitism	»	196
Corporate Symposia	»	197
• BAYER ANIMAL HEALTH Current topics in veterinary parasitology	»	199
• FORT DODGE ANIMAL HEALTH Moxidectin: ten years of experience in the world	»	200
• INTERVET Prevention of Leishmaniosis in dog and man	»	203

A

Parasitic Zoonoses

A1

DETECTION AND SPECIATION OF CRYPTOSPORIDIA AND MICROSPORIDIA FROM SURFACE WATERS IN EUROPE

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Background: Little is known about the environmental occurrence of cryptosporidia and microsporidia, which are mainly enteric pathogens of a wide variety of mammals and which contain several species and genotypes with zoonotic potential.

Methods: Parasite stages were concentrated from various types of surface waters (rivers, creeks, lakes, sewage plant in- and outlets, swimming pools) by continuous-flow-centrifugation/immunomagnetic separation (cryptosporidia only). A novel nested PCR spanning a variable region of the 18S rRNA of Cryptosporidia and PCR specific for the microsporidian genus *Enterocytozoon* allowed to identify parasite species/genotypes after direct sequencing of amplicons.

Results: Thirty-three of the 83 samples investigated tested positive for *Cryptosporidium*. Nine isolates represented genotypes pathogenic to man, namely *C. parvum* "bovine" and "human" genotype. Furthermore, we identified *C. muris* (17 isolates), *C. baileyi* (2), *C. parvum* "pig genotype" (1) and 3 novel genotypes. *Enterocytozoon bienersi* (genotype described from cat) was detected in 2 water samples.

Conclusion: Our results confirm the ubiquitous presence of primarily *Cryptosporidium* in surface waters and demonstrate the importance of molecular characterization before drawing conclusions of epidemiological significance.

A2

TRANSPLENTAL TRANSMISSION OF MURINE CRYPTOSPORIDIOSIS AND AIDS IN HUMANS.

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Tuskegee University AL. U.S.A.

Background: Cryptosporidiosis is a zoonosis, its importance emerged with immunodeficiency syndrome (AIDS) in humans. The protozoan cause is an opportunist and affects neonates of many vertebrates. A possible transuterine transmission was hypothesized and murine models used to investigate this. **Method:** Pregnant and non-pregnant C57BL/6J mice with and without dexamethasone were used. Orally, 2m *C. parvum* oocysts/mouse and dexamethasone (125/ug/mouse/day) were given on day 13 of pregnancy. Oocysts were monitored alternately from 3DPI. Antigenemia was determined by antigen-ELISA, rat anti-cryptosporidium polyvalent sera being the primary antibody. Intestinal infections were evaluated by histology and electron microscopy.

RESULTS: Immunosuppressed mice shed more oocysts than the controls (P<0.01). Antigenemia developed faster in immunosuppressed mice but once infection was fully established, no difference was noted. Peak antigenemia followed after oocyst shedding started to decline. Foetal infection with *Cryptosporidium* was observed among mice with immunosuppression at day 18 of pregnancy. Characteristically, developmental stages appeared at the extracytoplasmic portion of the intestinal epithelium. They had prominent basophilic nucleus and eosinophilic cytoplasm. Further, electron microscopy revealed oocysts other stages within the foetal intestinal epithelial cells.

Conclusion: Transplacental infection by *Cryptosporidium* in mice was demonstrated, for the first time in any host. This may have serious transmission implications in relation to pregnant human AIDS patients among other vertebrates hosts. This calls for a thorough re-examination of the epidemiology of *Cryptosporidium parvum* in all hosts.

A3

SOIL CONTAMINATION WITH EGGS FROM NEMATODES OF ZOO NOTIC POTENTIAL

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Background: Prevalence of antibodies to *Toxocara spp* - nematodes of major zoonotic importance - has been found to be higher in rural than in urban populations. To estimate the risk of zoonotic helminth infections for a population conducting mixed farming in an area of central Italy, the peridomestic soil contamination with helminth eggs was investigated.

Method: Faecal (6) and soil (2) samples have been collected on each of 60 farms, randomly chosen from a total of 810, located in a sanitary district of the Marche Region (central Italy). The presence of helminth eggs in faecal and soil samples (2-5g and 250g, respectively), was revealed by a floatation technique based on saturated sugar-NaNO₃ solution (specific gravity: 1.3).

Results: Soil samples from 57% (CI₉₅ 44-70%) of the farms contained eggs from nematodes with zoonotic potential (NZP: *Toxocara spp*, *Ancylostoma spp*, *Strongyloides spp*). In particular, *Toxocara spp* eggs were demonstrated in 52% (CI₉₅ 39-65%) of the sites. Faecal samples were positive for NZP in 35 farms (58%, CI₉₅ 46-71%), showing a mean egg abundance of 68 e.p.g. (CI₉₅ 39-117). *Toxocara spp* eggs were recorded in faeces from 27% (CI₉₅ 15-38%) of the sites, at a mean density of 43 e.p.g. (CI₉₅ 19-97).

Conclusion: The considerable level of soil contamination with eggs from NZP in the peridomestic area may constitute a zoonotic risk. Awareness should be raised among the inhabitants on the possible infection routes: for children and adults egg intake can be the consequence of soil related activities, or of contaminated vegetable consumption.

Acknowledgement: This study was conducted in collaboration with the veterinary service (ASL 10) and supported by Ministero dell'Università e della Ricerca Scientifica e Tecnologica (cofinanziamento 1998 & 1999).

A4

DOMESTIC AND SYLVATIC TRICHINELLA SPP. IN EXPERIMENTALLY INFECTED FOXES.

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Background: Carnivores are believed to be universal hosts for most *Trichinella*, but although extensively studied in pigs and mice, no studies have compared the infectivity, persistence, antibody response, and freeze tolerance of the multiple species and geographical isolates recognized presently.

Methods: A 108 foxes (*Vulpes vulpes*, 10 wks old) were inoculated orally with 9 genotypes of *Trichinella* (10,000 larvae per fox). Blood samples were collected at inoculation and at slaughters. The foxes were slaughtered 10, 20, and 40 wks p.i. Muscle tissue from 10 different muscles were digested by HCl-pepsin digestion. Infected fox muscle tissue was stored at +5, -5, and -18 and after 1 and 4 weeks the viability of recovered larvae was determined in mice.

Results: All the genotypes of *Trichinella* had a high infectivity and good persistence in the foxes, and for all, strong antibody responses were detected. The tongue and the lower extremities were generally found as predilection sites. Moderate freeze tolerance was found for *T. britovi* and *T. murrelli* and expressed freeze tolerance for *T. nativa* and *Trichinella* T6. The remaining genotypes were sensitive to freezing.

Conclusion: It was evident that both encapsulation and non-encapsulating species of *Trichinella* has a high and uniform infectivity to and good persistence in carnivores rendering these host the most important sylvatic reservoirs. This is in contrast to findings in pigs and rats where only the domestic species *T. spiralis* is highly infective and persistent. Carnivores are thus an obvious choice for studies on the characteristics of the members of the *Trichinella* genus.

Acknowledgement: The Danish Research Councils and the Danish National Research Foundation is acknowledged for financial support

A5

AN OUTBREAK OF TRICHINELLOSIS IN PEOPLE DUE TO THE CONSUMPTION OF BEAR MEAT.

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Background: *Trichinella* species infect warm-blooded vertebrates and are world-wide in distribution. In recent years there have been increasing reports of trichinellosis in people. In the outbreak described here, forty-three people from two adjacent communities in northern Saskatchewan, Canada became ill and several individuals were hospitalized for tests and treatments.

Method: A coordinated multi-disciplinary investigation including epidemiology, clinical, serology, and parasitology, was conducted to determine the cause and source of the outbreak and to limit the spread of the disease.

Results: It was determined that meat from a wild black bear was consumed by 70 people including all 43 sick individuals, 14 children and 1 pregnant woman. Recovery and digestion testing of parts of the bear revealed the presence of *Trichinella* larvae which were identified by PCR as *Trichinella nativa*. The density of larvae in the musculature of the bear varied with location and ranged from 0 to 256 larvae per gram of tissue. The serology and clinical signs supported the diagnosis of trichinellosis and management of the outbreak.

Conclusion: Although serology and clinical signs may indicate the presence of trichinellosis, an early diagnosis can be facilitated by the recovery of *Trichinella* larvae in the muscles of animals consumed. However careful interpretation should be placed on the amount of muscle tested and the location on the host from which the sample was collected.

A6

TRICHINELLA LARVAE IN WALRUS MEAT, A SIGNIFICANT FOOD SAFETY HAZARD

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Background: Outbreaks of human trichinellosis in the arctic are often associated with the consumption of walrus meat, either undercooked, or in traditional preparations such as igunaq. Igunaq means "aged and fermented meat" and walrus igunaq is prepared by placing walrus meat inside a walrus skin and blubber bag that is laced shut and stored at ambient temperatures in a sheltered stone cache outdoors. Igunaq may be consumed as early as 3 - 8 weeks after preparation, but is usually stored for longer periods of time.

Method: *Trichinella* larvae were recovered from traditionally prepared walrus igunaq and from fresh frozen walrus muscle tissue by pepsin/HCl digestion. Coiled larvae were orally inoculated into mice and/or guinea pigs to determine infectivity. Doses ranged from 6 to 1000 larvae.

Results: The larvae recovered from 4, 10 and 20 month old igunaq preparations were not infective for mice or guinea pigs. Larvae recovered from meat stored frozen for 1 or 20 months at -20 C were infective for mice and guinea pigs. Larvae from fresh frozen walrus meat were infective for guinea pigs after a single 20 month passage in mice.

Conclusions: Fresh frozen walrus meat stored at -20 C remains a food safety hazard for a least 20 months (the duration of this study). Fresh and under aged igunaq were not tested, but human outbreaks of trichinellosis indicate that some preparations retain infectious larvae. In this study, 4 month old igunaq did not contain viable larvae indicating that killing does occur under certain conditions. However, the variable methods of preparation and storage of igunaq could extend the duration of infectivity and only meat that is digestion test negative for trichinellosis should be used to prepare igunaq.

A7

TRICHINELLOSIS IN FINNISH DOGS

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Background: In Finland, trichinellosis is endemic and highly prevalent in wild carnivores. High prevalence is reflected in annual meat inspection condemnations of farmed wild boar and in-door raised pigs. The five million Finns own approximately 0.5 million dogs. Finnish lifestyle allows close contact to wilderness for pet animals, and also wild raccoon dogs and foxes are often seen in urban areas. Experimental trichinellosis causes clinically only mild disease in the related species, raccoon dog - even with heavy exposure.

Methods: Surplus serum samples gathered during normal routine work to the veterinary hospital of University of Helsinki were randomly tested for trichinella antibodies. Excretory-secretory antigen ELISA was used for 262 serum specimens. Known positive and negative raccoon dog serums were used as controls.

Results: In serum dilution 1:200 absorbances higher than six-fold the average absorbance of negative raccoon dogs were seen in specimens of 38 dogs (15%). These positive dogs represented different breeds and ages.

Conclusions: The endemic infection of wildlife affects also pet animals. The veterinary significance is open, for *Trichinella* parasite dogs are often dead-end-hosts of the infection cycle. Four different *Trichinella* species have been detected in Finnish fauna and all of them could be involved also in infections of dogs.

A8

TAENIA SOLIUM CYSTICERCOSIS IN NORTHERN VIETNAM

Dorny P.^{1,2}, Holland W.^{2,3}, My L.N.³, Erhart A.⁴, Nguyen Van De⁴, Le Dinh Cong⁴, Geerts S.¹, Brandt J.¹, Vercruysse J.²¹Institute of Tropical Medicine, Antwerp, Belgium²Department of Veterinary Parasitology, Ghent University, Belgium³National Institute for Veterinary Research, Hanoi, Vietnam⁴National Institute of Malariology, Parasitology and Entomology (NIMPE), Hanoi, Vietnam

Background: Neurocysticercosis is regularly diagnosed at the hospital of NIMPE in man living in rural areas around Hanoi, Northern Vietnam. However, little is known about the epidemiology of *Taenia solium* in these regions. Therefore a survey was undertaken to study the prevalence and importance of cysticercosis in man and pigs in rural communities.

Method: Surveys were conducted in humans and pigs in villages in Bac Ninh province, north-east of Hanoi. Infection was measured by an ELISA that detects circulating antigen, CT scan and biopsy of subcutaneous nodules in humans, and by lingual examination and autopsy in pigs.

Results: Twelve out of 209 people (5.7%) showed a positive ELISA result, including five persons with epilepsy. Their age ranged between 13 and 76 year. Diagnosis of cysticercosis was confirmed in 9 of these 12 persons by biopsy, CT scan or both. In pigs 5 (1.8%) out of 272 were positive by lingual examination. In 4 of 28 pigs selected from an endemic area cysticerci were found on autopsy.

Conclusions: *T. solium* cysticercosis is a common infection in humans and pigs in rural areas around Hanoi. Local pig husbandry conditions, lack of meat inspection and feeding habits in man favour transmission.

A9

CYSTICERCOSIS IN ZAMBIA

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- ³ Faculty of Veterinary Medicine, RUG, Merelbeke, Belgium.
- ⁴ Danish Centre for Experimental Parasitology, Frederiksberg, Denmark.

Background: Infections with *Taenia solium* cysticercosis in pigs and *T. saginata* cysticercosis in cattle are widely prevalent in Eastern and Southern Africa. However, nothing is known on the prevalence of these infections in Zambia. The objective of this study was to determine the prevalence and importance of porcine and bovine cysticercosis in Zambia.

Methods: In pigs, samples were collected at a slaughter slab in Lusaka (n=1316) and in villages in Southern (n=98) and Eastern (n=151) Provinces. Cattle were sampled in abattoirs in Central and Western Provinces (n=228) and on traditionally reared farms (n=227), feedlots (n=207) and dairy cattle farms (n=200). Parameters used were lingual examination (pigs), meat inspection (abattoir), ELISA for measuring circulating antigen, and a questionnaire survey.

Results: In pigs, lingual examination revealed the presence of cysticerci in more than 10%; on meat inspection 20.6% of the carcasses were positive; and ELISA showed circulating antigen in 20.6–54.8% of the samples. The prevalence was low in cattle slaughtered near Lusaka and on dairy farms; in feedlots and traditionally reared cattle the prevalence was near 10%.

Conclusions: *T. solium* cysticercosis is very common in free ranging pigs. The absence of meat inspection makes it a very serious public health problem. The prevalence of bovine cysticercosis is affected by husbandry conditions.

Acknowledgements: VLIR and ENRECA projects for financial support.

A10

PREVALENCE AND FACTORS ENHANCING TRANSMISSION OF PORCINE CYSTICERCOSIS IN NORTHERN TANZANIA

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Background: The objective of this study was to determine prevalence and factors enhancing transmission of porcine cysticercosis in northern Tanzania. **Method:** Between November 1997 and November 1999, 770 live pigs between two months and five years old were examined for presence of *Taenia solium* larvae by lingual palpation in 21 villages in Mbulu district. 198 (45%) of the selected pig owners were interviewed to gather information on the presence of important factors enhancing transmission of *T. solium* infections in the area. Hospital records of human epilepsy and taeniosis cases were examined for the years 1992–1997 in Mbulu district hospital. Data were analysed by Bayesian logistic regression model in WinBugs statistical package. **Results:** Overall 388 female and 382 male pigs were examined in dry (482) and wet (288) seasons. 16.4% of the pigs were below six months while 83.6% were above six months old. 134 (17.4%) of the pigs were infected with porcine cysticercosis, ranging from 3.2–46.7% in the different villages. There was significantly lower prevalence of the disease in pigs raised in households using latrines than in pigs raised in households not using latrines (POR = 0.37, 95% CI = 0.20–0.67). There was significantly higher prevalence of porcine cysticercosis in the dry than in the wet season in pigs less than six months old suggesting higher incidence rate in the dry than in the wet season (POR = 4.85, 95% CI = 1.23–19.21). 94.4% of the farmers allowed their pigs to free range and 40.7% did not know how a pig could acquire cysticercosis. Hospital records indicated prevalence of taeniosis and increase in the proportion of epilepsy from 19.8–36.8% among neurological conditions diagnosed in Mbulu district hospital. **Conclusion:** The observed prevalence was relatively high and public health education was suggested to be a priority in order to reduce the risk of this important zoonosis. Future plan is directed in designing and testing effectiveness of an educational program in reducing the incidence rate of porcine cysticercosis in Mbulu.

A11

PREVALENCE AND RISK FACTORS FOR TAENIA SOLIUM TAENIOSIS AND CYSTICERCOSIS IN PIGS IN CHUNYA, TANZANIA.

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Background: This study aimed to determine the prevalence and risk factors for *Cysticercus cellulosae* in pigs in Chunya district of Tanzania.

Method: Prevalence studies were done by ante mortem lingual examination of pigs while risk factors were evaluated by the use of questionnaire survey.

Results: Visual inspection of tongues of 692 pigs revealed cysticerci in 35 (5.05%) pigs. Interviews of farmers in 231 households keeping pigs revealed that majority (94.9%) of the respondents were ignorant of the mode of transmission of *Taenia solium* taeniosis/cysticercosis. Respondents who allowed pigs to roam were 45.2% while those who did home slaughtering of pigs and pork not officially inspected were 36.4%. Those who preferred their pork fried or barbecued were 43.1 and 25.9 respectively. Households lacking latrines were 3.5% only. Households regarding epilepsy in humans as main health problem in the community were 82.1% while those who ranked *C. cellulosae* in pigs as problem number one hindering production were 40.7%.

Conclusion: We concluded that there is *C. cellulosae* in Chunya and the population is very much ignorant of the mode of transmission of the parasite. Thus, effective and long lasting control of the transmission of *T. solium* from humans to pigs in Chunya must include community education on mode of transmission of the parasite, total confinement of pigs, radical improvement on pork inspection and enforcement of existing laws.

A12

COMPARISON OF LINGUAL PALPATION, NECROPSY AND SEROLOGICAL ASSAYS FOR THE DIAGNOSIS OF PORCINE CYSTICERCOSIS

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Background: This study aimed to compare lingual examination, necropsy and serological assays (ELISA 16 and ELISA 17) for the diagnosis of porcine cysticercosis in Northern Tanzania.

Method: A total of 374 pigs from Mbulu District were examined by lingual palpation method, and 10 pigs out of them were further examined during routine postmortem meat inspection. Fifty positive and 50 negative pigs confirmed by lingual palpation method were compared for correlation of the tests.

Results: Results from immunoblot, ELISA 16 and ELISA 17 were highly correlated ($r = 0.8$) and moderately agreed with those of lingual palpation method. All serological methods gave more positive responses than lingual palpation method; however, the difference in the proportions of positive responses were only significant with ELISA 16 ($P = 0.0053$). When all 374 samples were analysed, the prevalence by lingual palpation, ELISA 16 and ELISA 17 was 23.3%, 53.5% and 38.2% respectively. ELISA 16 was 86% sensitive and 56% specific while the sensitivity of ELISA 17 was 82% with 66% specificity. Three pigs confirmed negative at postmortem meat inspection gave strong positive responses in both ELISA 16 and ELISA 17. Presence of hydatid cyst or *Ascaris suum* infection seemed to have no cross reactivity in ELISA.

A13

STUDY OF HUMAN CUTANEOUS DIROFILARIOSIS IN NORTH TERRITORIES OF IRAN IN 2000

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Background: *Dirofilaria repens* is a filarial worms of canine, but also can be an incidental parasite of human being.

Method: In order to study the human cutaneous dirofilariosis, 750 cases of subcutaneous nodules dissected in general hospitals of northern cities of Iran were chosen to be studied by histopathological and parasitological methods.

Results: 7 cases out of 750 ones were diagnosed as *Dirofilaria repens* (0.93%).

All of nodules had a abscess structure with outer layer made of fibrotic tissue. Inside the nodule was filled by sections of the immature worms and a purulent exudate. The exudate was made of dead, degenerated and live neutrophils, lymphocytes and macrophages. In the sections of the worms, large uteri observed empty of microfilaria.

Conclusion: According to this fact that transmission of *Dirofilaria repens* can be occurred in north of Iran, local parasitologists and pathologists should pay more attention to subcutaneous and lung nodules diagnosed in native patients.

A2 p

CURRENT SEROPREVALENCE OF TOXOPLASMA GONDII INFECTIONS IN SOWS IN TWO REGIONS OF GERMANY

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Background: Two cross-sectional studies were carried out to determine the current seroprevalence of *Toxoplasma gondii* infections in sows from two different regions of Germany.

Methods: Serum samples of 2,044 breeding sows from 95 herds in Hesse and of 1,500 sows from 75 herds in Northrhine-Westphalia were collected in 1997 and 1999, respectively. All the samples were examined for antibodies to *T. gondii* using an ELISA technique and, in Hesse, also using IFAT. Data on farm management and individual sows were collected by a questionnaire in the Hessian survey.

Results: *T. gondii* antibodies were found in 18% and 9% of the sows ($p < 0.001$) and in 69% and 51% of the herds ($p < 0.05$) from Hesse and Northrhine-Westphalia, respectively. The explorative analysis of data from Hesse showed that the seroprevalence was positively correlated with the age of sows and negatively with the herd size ($p < 0.05$). Both the age of sows and herd size-associated management practices were the only risk factors for *T. gondii* infections found. Significantly ($p < 0.05$) higher within-herd prevalences of *Toxoplasma* antibodies were found on farms with reproductive problems than on those without problems.

Conclusion: *T. gondii* infections are present in sows in Germany to a remarkable degree. Their role in fertility problems needs to be further studied.

A1 p

ACQUIRED TOXOPLASMOSIS: PATHOLOGY OF DEEP CERVICAL AND INTRAPAROTID LYMPH NODES

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Clinic of Parasitic and Tropical Diseases¹, Clinic of Otolaryngology², Karol Marcinkowski University of Medical Sciences and Department of Pathology³, Centre of Oncology of Wielkopolska³, Poznań, Poland. The study aimed at detection of deep cervical and intraparotid lymph nodes at various stages of toxoplasmosis and at evaluating activity of the pathological process.

Methods. The studies were performed on 72 patients, including 37 adults, aging 18 to 58 years, and 35 children, 5 to 17 years old. The diagnosis of toxoplasmosis in early period, during regression, and chronic transformation was confirmed by positive ELISA tests (Vidas, BioMerieux). Ultrasonography of lymph nodes was performed using ALOCA SSD-500 apparatus, equipped with the head of 7.7 frequency.

Results and Conclusions. Involvement of deep periparotid and intraparotid lymph nodes of various sonographic density (low, heterogeneous, high density, or with presence of fascicular densities) was demonstrated in 55 (76.4%) patients. Among the 35 patients with enlarged periparotid lymph nodes, in 12 (34.2%) patients, enlarged intraparotid lymph nodes were also detected. High IgG class antibody levels were noted in 97.2% of examined patients and high IgM class antibody titres were detected in a total of 82.0% patients. The high antibody titres were detected not only in the early stage and during regression of the disease (in 90.0% and 88.2% cases, respectively) but also in its late period (more than 5 months to over a year after beginning of the symptoms, in 60.0% cases). This indicated a protracted activity of the pathological process, including intraparotid lymph nodes.

A3 p

PREVALENCE OF GIARDIA SPP. IN DOGS AND CATS IN GERMANY

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Flagellates of the genus *Giardia* are ubiquitous in their distribution and found in the intestinal tract of humans and animals of several species throughout the world. The prevalence data in dogs and cats quoted in the literature indicate that the incidence of *Giardia* spp. varies considerably and depends to a large extent on the methods used and the composition of the studied population. The objective of the study presented was to determine the prevalence of *Giardia* infections in dogs and cats in Germany.

Between January 1998 and March 2001 fecal samples of 6534 dogs and 2234 cats were examined for the presence of *Giardia*. All fecal samples were obtained from private-owned dogs or cats presented at local veterinary practitioner for either a medical problem, or for routine examination and vaccination. The specimen were examined for *Giardia*-specific antigen by means of the coproantigen test ProSpect[®] *Giardia* Micoplate Assay or for cysts of *Giardia* by means of MIFC (Merthiolate-Iodine-Formaldehyd-Concentration) technique. The evaluation of the coproscopical examination showed 14.0% *Giardia* positive dogs in the year 1998, 16.7% in 1999, 20.5% in 2000 and 23.7% in 2001. A total of 1150 of 6534 dogs (17.6%) were proved to be infected with *Giardia* spp. The evaluation of the cat data revealed 6.5% positive cats in the year 1998, 7.9% in 1999, 13.9% in 2000 and 14.7% in 2001. The average prevalence over the 4-year period was 10.2%.

A4 p

AVIAN GIARDIA ISOLATE INFECTS LAMBS AND KITTENS

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Background: *Giardia* species have been considered host specific for different vertebrate classes such that different species were thought to infect birds and mammals. *Giardia* isolated from a moribund sulfur-crested cockatoo (*Cacatua galerita*) successfully infected mice. The objective of the study was to determine if this isolate would also infect domestic lambs and kittens.

Method: SPF lambs (3, 14 days old) and kittens (5, 6-8 weeks old) were challenged by intraduodenal injection of *Giardia* trophozoites (BRIS/95/HEPU/2041) initially isolated from sulfur-crested cockatoo. Control lambs (n=2) and kittens (n=3) received saline. Clinical signs and cyst output were evaluated in the animals for 8 days post challenge.

Results: All *Giardia* challenged lambs and kittens shed viable cysts from day 4-5 until the end of the study (day 8). On day 8, up to 10⁷ trophozoites per cm of small intestinal segment were counted. Four of 5 infected kittens had abnormal stools four day post challenge until day 8. Control animals were not infected with *Giardia* trophozoites and never passed cysts in their feces.

Conclusion: This study confirms that birds may harbour *Giardia* which are infectious to a variety of mammalian species including companion animals and domestic ruminants. Possibly birds may infect humans. As waterborne transmission is common, these results raise important implications for water management.

A5 p

TRICHOMONADS OCCURRING IN ORAL CAVITY OF DOG, CAT AND HORSE

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Background: The data concerning trichomonad species of animals are imprecise and incomplete; three species were described: canine parasite - *Tetratrichomonas canistomae*, feline parasite - *Tetratrichomonas felistomae* and equine parasite - *Trichomonas equibuccalis*. Conventional methods have proved insufficient for the identification of trichomonad species; therefore PCR - RFLP technique has been established.

Method: A pair of primers was designed to amplify a variable ITS region. The PCR products were digested with enzymes Dde I and Msp I.

Results: We obtained PCR products of following length: 393bp, 394bp and 410bp. The 393bp product was characteristic of *T. tenax* - a parasite of human oral cavity. Two other products were unknown. The microscopic study of trichomonads of the 410bp isolate showed a posterior flagellum present only in *Tetratrichomonas* (no data available in GenBank). The 394bp product was sequenced and the comparison with *T. tenax* showed its nucleotide sequence to differ in about 5% though morphological study gave it as similar to *T. tenax*; these isolates were called *Trichomonas tenax cani*. The infection of animal oral cavity caused by trichomonads was: 3 dogs infected by *Trichomonas tenax*, 4 dogs - *Trichomonas tenax cani*, 1 dog - *Tetratrichomonas canistomae*; 2 cats infected by *T. tenax*; 1 horse infected by *T. tenax*.

Conclusions: *Trichomonas tenax*, observed as a human specific parasite, can also inhabit the oral cavity of dog, cat and horse. *Tetratrichomonas canistomae*, described as a parasite of dog, was detected only in 1 dog. *Trichomonas tenax cani* - a trichomonad morphologically identical to *Trichomonas tenax*, but genetically different, was observed in the oral cavity of dog.

A6 p

Trichinella spp. IN WOLVES IN FINLAND

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Background: *Trichinella* species are prevalent in wild carnivores e.g. raccoon dogs, lynxes and foxes in Finland. The population of wolves varies nowadays from 100 to 120 animals. They live mainly in the eastern and northern parts of the country. Hunted or illegally killed wolves were studied for *Trichinella* in 1998-2000.

Methods: Muscle samples (50- 100 g) from 31 wolves were analysed with a digestion method. The species detection by RAPD-PCR was performed on individual larval DNA or pooled samples of 2-6 pcs. The number of analysed larvae per host ranged from 4 to 11 larvae (median 7); altogether 78 larvae were analysed.

Results: 14 animals of 31 were infected (45%) with intensity varying from 0.4 to 5 larvae per gram (LPG) of muscle tissue. The most common species was *T. nativa* (6/12). One mixed infection (*T. spiralis* / *T. nativa* or *T. britovi*) was found. The RAPD-PCR patterns of *T. nativa* and *T. britovi* looked so similar that in seven animals they could not be reliably distinguished from each other.

Conclusions: The high prevalence in wolves is in accordance with earlier reports of prevalence in lynx, raccoon dogs and other carnivores in Finland. Both sylvatic and domestic species of *Trichinella* have been detected in raccoon dogs, which may act as prey animals for wolves in Finnish ecosystem. Alternatively the wolf infected with *T. spiralis* could have become infected via other synanthropic animals

A7 p

CARNIVORA AS THE MAIN HOSTS FOR TRICHINELLA IN ESTONIA

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Background: Trichinellosis in man and domestic animals is partly determined by the size, density and level of infection of the game animal populations. The present study was conducted in order to define the possibilities of trichinellosis spread from sylvatic to domestic animals and man.

Methods: 136 muscle samples from 8 species of wild carnivorous hosts and 151 samples from 3 species of farm fur-animals were digested. The prevalence and the intensity of infection were determined. *Trichinella* species identification was carried out by RAPD analysis.

Results: 80,5% of wolves, 51,5% of lynxes, 45,4% of raccoon dogs and 42,9% of red foxes investigated were infected with *Trichinella*. The highest larvae burden was established in red foxes (up to 213 larvae/g) and raccoon dogs (up to 200 larvae/g). The number of raccoon dogs and red foxes among carnivorous hosts in Estonia is the biggest, likely about 10 000 animals. Most of game species investigated were infected with *T. nativa* and *T. britovi*. From farm fur-animals some blue foxes were infected with *T. nativa* and *T. spiralis*. The prevalence of *Trichinella* in bears about 29% and in wild boar only 1-3% has been registered.

Conclusion: The biomass of *Trichinella* in carnivorous hosts in Estonia seems to be big. The spread of sylvatic *Trichinella* species from game animals to domestic animals and man is realistic and occurs in Estonia. This must be taken into consideration in the organising of trichinellosis control.

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A8 p

TRICHINELLA SPIRALIS IN RED FOXES IN BELGIUM

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Background: The red fox population has increased dramatically in the last decade in Belgium. Because of the potential role of the fox as a reservoir for trichinellosis, a survey was carried out to assess the prevalence of *Trichinella* spp among the Belgian vulpine population.

Method: Between 1996 and 2000, 818 dead foxes (hunting or road casualties) from various areas in Flanders (n=179) and Wallonia (n=639) were collected for serological and parasitological analysis. Muscle samples were inspected by trichinostomy or artificial digestion. Where possible serum and/or muscle juice was collected for detection of antibodies by ELISA with excretory/secretory products of L1 of *T. spiralis* as antigen.

Results: Larvae of *Trichinella* spp. were demonstrated in none of the muscle samples. In contrast, specific antibodies were demonstrated in serum of 8 (4.5%) of 176 Flemish foxes and in 61 (46.9%) of 130 serum samples of foxes from Wallonia. ELISA on muscle juice showed antibodies in none of the 26 samples from Flanders and in 90 (18.8 %) of 478 samples from Wallonia.

Conclusions: Evidence of infection with *Trichinella* spp was shown by ELISA in a high proportion of Belgian foxes. Sero-prevalence was much higher in Wallonia where the fox population is denser than in Flanders. The absence of larvae in muscle samples may be due to the lower sensitivity of parasitological methods.

A9 p

ZOONOTIC PARASITES FROM DOGS : SURVEY ON VETERINARIANS OPINIONS IN FRANCE.

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Introduction: The objective of the study was to evaluate the relative importance of parasites in dogs and humans and their severity through opinions and experiences of a panel of veterinarians

Material and methods : A survey was distributed to 201 veterinary clinics (54.7% in urban areas) concerning 7 major potential parasites from dogs in the clinics' area. Evaluation on the frequency in dogs and in man was codified from 0 (non existant) to 3 (very frequent) and the severity from 1 (benign) to 3 (severe) and indexes of frequency (IF) and severity (IS) both in dogs and humans for each disease was calculated. Possible regional variations also were statistically evaluated.

Results : Information indicated IF/IC were respectively: *Toxocara* in dogs 2,6 / 1,6 and in humans 1/ 2,1 (significant increased prevalence in humans in the south of the country); sarcoptic acariasis in dogs 1,5/1,1 and in human 1,1/1,6; Leishmaniosis in dogs 1,4/2,9 and in humans 1,1/2,7 (significant prevalence for both dogs and humans in the south-east); Dirofilariosis was in dogs 0,3/2,6 in humans only frequency was evaluated as 0,03 for *Dirofilaria immitis* and 0.05 for *Dirofilaria repens*, *Ankylostoma/Uncinaria* in dogs 1,4/2 and 1,1/1,6 in humans. *Echinococcus* was possibly reported from 37 areas with a frequency of 1.7.

Conclusions : Veterinarians were highly concerned by the risks from *Toxocara* in 65,1% for their clients as opposed to only 21.9% for themselves. These findings underline the reality of concern of French veterinarians on the problem of parasitic zoonoses from dogs in France.

A10 p

PREVALENCE AND EPIDEMIOLOGY OF CANINE PARASITIC ZOONOSSES IN ASSAM.

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Background: Canine parasitic zoonoses are a well - recognised public health problem worldwide. However, as well as acting as definitive hosts for a number of parasite zoonoses, the role dogs play as mechanical vectors of human parasites (via coprophagy) has been largely ignored.

Method: Faecal samples were collected from dogs and humans from two distinct socioeconomic groups from three Tea estates in Assam. Each participant was interviewed with regards to risk factors for infection for themselves as well as their pet dogs.

Results: Virtually all dogs (99%) were found to be positive for at least one zoonotic parasite. Hookworm spp. were found to be the most common parasite in dogs (95%). Apart from parasites that use dogs as their definitive host, parasites presumed to be host specific for humans were also encountered in dog faeces in high intensities. Over 30% of dogs were found to harbour "Ascaris - like" eggs, 25% *Trichuris trichura* and 2% *Isoospora belli*. Molecular tools are currently being utilised to characterise the species of *Ascaris* and hookworm recovered from dogs. Over 60% of the human population were found to be positive for at least one enteric parasite. Hookworm spp and *Ascaris lumbricoides* were the most common infections (33%). Risk factor analysis was conducted on parasite prevalence in both dogs and humans.

Conclusion: The high prevalence of zoonotic gastrointestinal parasites of dogs place the residents of this tea growing community of Assam in extreme risk of contracting parasitic diseases from the local dogs. Moreover, dogs are acting as potential mechanical "sinks" and vectors for human parasites via coprophagy of human faeces.

A11 p

TOXOCARA CANIS IN PIGS . PRELIMINARY RECOVERY OF LARVAE AND TISSUES LESION.

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Background: Pigs were infected with embryonated eggs of *T. canis* to study the migratory pattern and the histopathological lesion in different organs.

Methods: At pigs slaughter, liver, lungs, lymph nodes around the small and large intestines, brain, eyes and a portion of muscle were removed. The presence of larvae in the tissues were determined by Pepsin-HCL digestion method. Lesion in tissues were studied by histopathological serial cut and stained with routine and special method.

Results: Larvae were recovered and histopathological lesions were found in: liver, lungs and lymph nodes around the small intestine between day 7 p.i. and 21 p.i.. The highest recovery was in liver at day 7 p.i. and in lungs at day 14 p.i.

Conclusions: A coincidence were found in the recovery of larvae and lesions in tissues. These results have similarities with the biology cycle of *T. canis* in paratenic hosts. **Acknowledgement:** this research was supported by Universidad de Buenos Aires. Sec. de Ciencia y Técnica. Subsidio TV37.

A12 p

Study of alimentary tract zoonotic Trichostrongylus SPP between human and domestic ruminant in eastern Azarbaijan IRAN.

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In order to investigate alimentary tract Trichostrongylus species in domestic ruminants (sheep, goat and cow) in east Azarbaijan province, IRAN 320 complete sets of alimentary tracts from esophagus to rectum were studied. Samples were taken randomly from slaughterhouses in different cities of the province during March 1998 to February 1999. The rate of Trichostrongylus was 24.45%.

Identified species were 10 as following T. capricola, T. axei, T. orientalis, T. probolorus, T. skerjabini, T. colubriformis, T. vitrinus, T. longispicularis, T. drepanoformis and one new species. First 7 species reported before from human and animal in IRAN. T. orientalis and T. skerjabini before had been reported in human, in present study were seen in animals. Two new recorded species from IRAN were T. longispicularis and T. drepanoformis and the new species was similar to T. drepanoformis but, both of its two spicules were 90 angle. Characteristics of these species along with pictures drawing camera lucida all are shown in the poster.

A13 p

SUSCEPTIBILITY OF DIFFERENT CYPRINID SPECIES TO OPISTHORCHIS FELINEUS

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Background: Recent investigation demonstrated that opisthorchiid flukes are more frequent in Germany than expected. Contrasting information regarding the involvement of the different species of cyprinid fish in the life cycle of *Opisthorchis felineus* is common in the literature.

Method: Five one to two years old specimens of each of *Leuciscus idus*, *Scardinius erythrophthalmus*, *Tinca tinca*, *Carassius carassius*, *Carassius gibelio*, *Cyprinus carpio*, *Hypophthalmichthys molitrix* and *Ctenopharyngodon idella* were kept together in one aquarium. They were exposed to cercariae of *O. felineus*. 6 weeks after the last exposure, fish was killed and examined for metacercariae by artificial digestion. Prior to investigation, 3 control specimens of each of the 8 fish species were checked for spontaneous obtained metacercarial infections.

Results: *Opisthorchis* metacercariae were found only in all *L. idus*, *S. erythrophthalmus* and *T. tinca*. The average number per g body weight in these hosts was 80, 4 and 48 respectively. Apart from their presence in muscle flesh, metacercarial cysts were detected in the fins, the skin and the head of infected fish.

Conclusions: The spectrum of susceptible to *O. felineus* fish is not only restricted to the family cyprinidae in general. Different susceptibility exists within this family. Thus, *C. carassius*, *C. gibelio*, *C. carpio* and the non indigenous species *H. molitrix* and *C. idella* don't play a role in the epidemiology of opisthorchiosis.

A14 p

DEVELOPMENT OF AN IN VITRO VIABILITY ASSAY FOR CRYPTOSPORIDIUM PARVUM OOCYSTS.

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Cryptosporidium parvum is an important cause of diarrhoea in humans and animals. Various methods have been suggested to assess the infectivity of oocysts after treatment with disinfectant. The aim of this study was to develop an in vitro assay for the determination of oocyst viability in order to estimate the effectiveness of anti-cryptosporidial chemicals. *C. parvum* oocysts (IOWA strain) from an experimentally infected calf were used to infect HCT-8 cells grown in vitro. After 48 h cells were harvested, DNA was extracted and PCR was carried out using *Cryptosporidium*-specific primers. To estimate the effect of the disinfectant Neopredisan® on parasite viability, oocysts were treated with varying concentrations (4, 2, 0.2, and 0.02 %) for different incubation times (0, 30, 60, and 120 min), then the disinfectant was removed and the oocysts were seeded into cell cultures for infection. DNA was prepared from duplicate wells and was amplified to a single band of 646 bp when parasite DNA was present. The lowest concentration of untreated oocysts still detectable with PCR was 100 oocysts/well. Heat-inactivated oocysts could not be detected. The effect of Neopredisan® on oocysts was estimated comparing the amplification levels of DNA from cultures with treated or untreated oocysts. The presented data suggest that the diagnosis of parasite viability using in vitro cultivation and PCR is a quick and reliable alternative to animal testing of infectivity.

A15 p

THE GLOBAL IMPORTANCE OF DIFFUSE AMOEBIC HEPATITIS-A PARASITIC ZOONOSES IN INDIA

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BACKGROUND : Diffuse Amoebic Hepatitis is an Amoebiasis Syndrome of Liver, Spleen, Kidneys, Lungs, Skin, Genital Organs and other tissues caused by Ingestion of active trophozoite and mature cysts of *Entamoeba histolytica* with food, water & raw vegetables has been reported in the local tribal community foreigners, wild & domestic animals in the Sylvatic Environment of India.

METHOD : A total Number of 547 Faecal and biomaterials samples were collected from men (both local tribal & foreigners Travel from Temperate & arctic Zone to this subtropical zone and a stay under primitive hygienic conditions of Eco Tourism Projects), wild and domestic animals & tested in the various laboratories by immuno diagnostic test for amoebiasis like indirect haemagglutination, indirect immuno fluorescent and counter-current-electrophoresis techniques as well as microscopic demonstration of Trophozoites & cysts in the faeces.

RESULT : *Entamoeba histolytica* were found positive 34.425% of 61 men, 53.857% of 13 wild boar, 25.925% of 27 spotted Deer, 68% of common Langur, 8.695% of 23 Panther (*Panthera pardus*), 9.090% of 11 Tiger (*Panthera tigris-tigris*), 41.167% of 85 Domestic Pigs, 38.461% of 65 Cattle, 27.271% of 55 Buffaloes, 27% of 25 Dogs, 61.747% of 107 Sheep & Goats.

CONCLUSION : For Disease Surveillance of this Pathogenic food & water borne parasitic zoonoses at Global level this study suggest high techno diagnostic Advance research strategies at Cellular, Molecular, Genetic, Hygienic, therapeutic prophylactic, vector control measures & Eco-developmental levels in the cooperation with developed nations, WHO & FAO for the bright future of International Ecotourism Projects & world Veterinary Public Health in the New Millennium.

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B

Neospora *and other coccidial infections*

B1 THE TAXONOMY AND PHYLOGENY OF TISSUE CYST-FORMING COCCIDIA

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Background: The subclass Coccidia of the phylum Apicomplexa contains a number of genera, the members of which are very important parasites of humans and animals. However, the taxonomic relationships among them are still controversial. This has arisen because their taxonomy and phylogeny has, until the last 10 years, been based on morphology and life cycle characteristics, which are often still not completely known, or are open to interpretation on subjective or technical grounds.

Method: Comparison of 18S rDNA and recently 28S rDNA sequences has provided objective data on which to base phylogeny and taxonomy.

Results: In some cases, the new molecular data definitively suggest that previous placements were incorrect and call for revisions. However, in most of these cases, an analysis of the literature confirms that at least some authors have previously hypothesised relationships found with the molecular analyses, using only phenotypic data. This adds significant weight to the molecular data, and vice versa. The new data confirm that: (1) The genus *Frenkelia* is a synonym of *Sarcocystis*; (2) *Isospora* species with Stieda bodies are clearly a separate lineage from those without Stieda bodies; (3) the genus *Eimeria* is paraphyletic if the genus *Cyclospora* is valid. What are more controversial, are the relationships within and among the genera *Toxoplasma*, *Hammondia* and *Neospora*.

Conclusion: Molecular sequencing techniques have provided new data with which to assess our understanding of the taxonomic relationships of the tissue cyst-forming coccidia.

B2 ANALYSIS OF NEOSPORA ANTIGENS BY TWO-DIMENSIONAL WESTERN BLOTTING

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Background: The tissue cyst-forming coccidium *Neospora caninum* may infect a broad range of intermediate hosts. However, *Neospora*-induced abortion and congenital disease only appear to be of major importance in cattle and dogs, respectively. Little is known on the interaction of *N. caninum* with different host species or on the recognition of *Neospora* antigens by different host sera.

Method: Here we have used two-dimensional polyacrylamide gel electrophoresis to resolve tachyzoite proteins of the NC-1 strain of *N. caninum*. Highly resolved protein spots were transferred to nitrocellulose by Western Blotting and probed with homologous sera from a range of intermediate hosts (dogs, cattle, sheep, rabbits, mice) infected either experimentally or naturally with *N. caninum*, and heterologous sera from hosts infected with other, closely related tissue cyst-forming coccidia.

Results: The homologous sera consistently recognised a pattern of three major antigens of *N. caninum* tachyzoites, within the pI range of 5-6 and the molecular weight range of 11,000 to 18,000. These antigens were not recognised by heterologous sera. Several other antigens were recognised only by homologous, *N. caninum*-specific sera, but this recognition varied among sera obtained from different host species. Recognition of *N. caninum* antigens also varied between sera obtained from dogs with congenital neosporosis and dogs that showed no symptoms of disease.

Conclusion: The results obtained in this study provide valuable information on antigens that may be important in the interaction between *N. caninum* and different intermediate host species as well as antigens that may be associated with disease.

B3 VERTICAL TRANSMISSION OF NEOSPORA IN A NEW ZEALAND DAIRY HERD

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Aim: To determine the rate of vertical transmission in a New Zealand dairy herd with a history of *Neospora*-induced abortion.

Method: A herd which had an outbreak of *Neospora*-induced abortion in May 1997 was subsequently bled 3-4 times per year to determine *Neospora* titres. In addition, precolostral *Neospora* titres from calves were compared to their dam's titres at calving in 1998 (n=47) and 2000 (n=49). Cows sampled were those that calved in a set period of time and were not the same cows on each occasion. Only 4 cows were represented in both years. For the comparison of titres at calving the IDEXX ELISA *Neospora* assay system was used and for earlier testing an in-house ELISA was used.

Results: When comparing only sera collected at calving, the rate of vertical infection was 43% in 1998 (3 from 7 cows) and 71% in 2000 (5 from 7 cows) but when including cows that had been seropositive on at least one occasion from earlier samples, the rate of vertical transmission was only 9% in 1998 (3 from 35 cows) and 14% in 2000 (5 from 35 cows). Vertical transmission mainly occurred in cows which had been seropositive on a majority of sampling occasions. No calves were seropositive if they were born to cows that had never tested seropositive.

Conclusions: The risk of vertical transmission in this herd is lower than that generally reported elsewhere.

B4 RISK FACTORS FOR N. CANINUM INFECTION IN ONTARIO DAIRY CATTLE

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Background: Since 1996 *Neospora caninum* has been the most commonly diagnosed cause of abortion in Ontario dairy cattle. However, risk factors for infection and associated abortion in this province of Canada have not been investigated.

Method: This case control study examined sera from 5080 cattle on 88 Holstein dairy herds for *N. caninum* antibody. At the same time a comprehensive management survey was administered on each farm and milk production data were collected from Ontario Dairy Herd Improvement records.

Results: Risk factors for higher herd seroprevalence included inadequate herd vaccination; and the presence, number and duration that dogs were present on a farm. Risk factors for *N. caninum*-associated abortion included the herd seroprevalence; presence of dogs; and exposure of cattle to a stream or pond. 9 herds exhibited serological evidence of horizontal infection. Milk production in seropositive cows was 192 kg less than seronegative cattle over a 305-day lactation.

Conclusions: While *N. caninum* transmission is primarily vertical, these data indicate the need for additional control measures aimed at preventing horizontal transmission in Ontario dairy herds.

Acknowledgement: Dairy Farmers of Ontario, the Ontario Cattlemen's Association, and the Ontario Ministry of Agriculture, Food and Rural Affairs are thanked for financial support.

*Neospora
a notifiable disease
in Switzerland*

B5

Induction of protective immunity against vertical transmission in bovine neosporosis.

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Background: The main objective of the study was to determine whether infection of cattle with *Neospora caninum* prior to pregnancy would afford protection to the foetus if the dams were challenged with *N. caninum* at mid-gestation.

Method: the experiment comprised four groups of six cattle: group 1 uninfected controls; group 2 inoculated with *N. caninum* 6 weeks prior to mating and challenged with *N. caninum* at mid-gestation; group 3 naive cattle challenged with *N. caninum* at mid-gestation; group 4 infected with *N. caninum* prior to mating and left unchallenged throughout pregnancy. The clinical and immune responses of dams and resultant calves were monitored regularly throughout the experiment.

Results: Following challenge at mid-gestation there was a significant increase in rectal temperature response in animals in group 3 compared to group 2. There were no other clinical symptoms of disease and all cattle proceeded to calving. At calving pre-colostral serum samples were negative for antibodies to *N. caninum* in all calves born to dams in groups 1, 2 and 4. In contrast all the calves born to dams in group 3 had high levels of specific antibody indicating in vivo exposure to the parasite.

Conclusions: The results indicate that the immune response generated in the dams in group 2 prior to pregnancy had protected against vertical transmission of the parasite following challenge at mid-gestation.

B6

SEROLOGY AND PCR TO ASSESS THE EPIDEMIOLOGY OF NEOSPORIN-INDUCED ABORTIONS IN CATTLE

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The protozoan parasite *Neospora caninum* is one of the most important infectious agents causing abortion in cattle in Europe and worldwide. Although the dog was found to be a definitive host, the major transmission of this parasite occurs vertically during pregnancy via the transplacental route. The infection of the fetus mostly results either in birth of a persistently infected - but clinically healthy - calf or in abortion.

In the present study brain material from 242 abortions was investigated by PCR and histological methods for detection of *N. caninum* and respectively caused lesions. Sera were collected from 210 of the corresponding mother cows and were tested for antibodies against *N. caninum*.

In 21% of the aborted fetuses *N. caninum* was detected by PCR in the brain material. In 84% of these cases necrotic lesions were found in histological sections of the brain. The correlation between the two methods was 80%.

The serological examination of the aborting mother cows revealed a low specificity of this method, as only 42 of the 92 seropositive animals aborted a *N. caninum*-PCR positive fetus. In 8 cases *N. caninum*-PCR positive fetuses were aborted, while the respective mother cows remained persistently seronegative for up to 15 months. According to these data, a combination of PCR from the fetal brain and serology of the respective mother cow is strongly recommended for the diagnostic assessment of bovine neosporosis and abortion.

B7

FIELD PERFORMANCE OF THE FIRST COMMERCIAL INACTIVATED NEOSPORIN CANINUM VACCINE IN DAIRY HERD

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Neosporosis is the leading diagnosed cause of abortion among dairy and beef cattle. Abortion and stillbirth due to *Neospora* has been reported worldwide and causes significant economic loss to dairy and beef industries. This study was conducted to determine the field performance and safety of inactivated *Neospora* vaccine commercially available in the USA. Cattle utilized for this study had a history of numerous abortions. Extensive diagnostic testing confirmed *N. caninum* in aborted fetuses and ELISA showed 51 of the 107 animals positive for *Neospora* antibody. Cows recently fresh received three doses, while those 30 to 180 days pregnant received two doses, and those in the last trimester of pregnancy received one dose of the *Neospora* vaccine. The cows were bled for final ELISA testing after *Neospora* vaccination, and results showed that all animals sero-converted and tested positive for *Neospora* antibody. This field trial showed that the 27 abortions recorded on the farm in 1998 were reduced to 4 abortions in 1999 after implementation of the *Neospora* vaccination. Seven heifers that were retained for use as replacement were ELISA tested at six month of age. All of these heifers were negative for *Neospora* antibodies. Out of these seven heifers, six had been born to dams that were *Neospora*-positive before the herd vaccination program began. The results of the field evaluation demonstrate that the *Neospora* vaccine is safe and effective for use in healthy pregnant cattle as an aid in the reduction of abortions caused by *Neospora caninum*.

B8

EVALUATION OF THE EFFICACY OF HERB EXTRACTS AGAINST ACUTE MURINE NEOSPORIN

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Background: *Neospora caninum* is a major cause of abortion in dairy cattle in the United States and other many countries. Abortions and neonatal mortality also occur in other ruminant species.

Methods: The protective effect of herb extracts such as *Sophora flavescens*, *Simoneum acutum*, *Ulmus macrocarpa* and *Torilis japonica* against *Neospora caninum* infection was examined in experimental murine neosporosis. All mice challenged with *N. caninum* were orally administered with the each herb extract.

Results: Survival rate in the group treated with *S. acutum* (75%) or *U. macrocarpa* (75%) was higher than that of the infected control group (20%). Mice in the group treated with *S. acutum* or *U. macrocarpa* that survived a period of 6 weeks p.i. after challenge were more healthy than those of other groups. Mean antibody titre of mice in group treated with *U. macrocarpa* was higher than that of other groups. Brain lesions of mice in group treated with each herb extract were absent or less severe than those seen in mice in infected control group. Any other differences in size of brain lesion by groups were not found, but the lesions in the group treated with each herb extract, especially the group treated with *U. macrocarpa* (brain lesion score=5.00 ±2.31), were fewer in number and had fewer inflammatory cells than those seen in mice in infected control group (brain lesion score=8.03±1.52). All mice treated with each herb extract had lower brain lesion scores than mice in infected control group, significantly ($p<0.05$).

Conclusion: These results demonstrate that *S. acutum* or *U. macrocarpa* induces resistance to *Neospora caninum* infection in mice.

B9

TOLTRAZURIL AND PONAZURIL FOR TREATMENT OF EXPERIMENTAL MURINE AND BOVINE NEOSPOROSIS.

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Neosporosis is a disease affecting predominantly the fetal development in bovine and canine hosts, and it may cause neuromuscular dysfunction in infected new born calves and pups. Predispositions - including e.g. transient immunosuppression during pregnancy - may result in an increased dissemination of the parasite within the host or its offspring. Chemotherapy may be an issue to abrogate infection, provided that an appropriate drug and application strategy is developed. We describe the use of a mouse model for the evaluation of toltrazuril and ponazuril medication as a means of preventing parasite dissemination and subsequent cerebral lesion formation. Experimentally infected mice (i.p. with 2×10^6 *N. caninum* tachyzoites) were subsequently treated with toltrazuril and ponazuril. The infection was monitored at three levels, including symptomatology; histology/ immunohistochemistry for cerebral lesions and cerebral PCR. Chemotherapy using toltrazuril and ponazuril, respectively, both applied in a drinking-water formulation (20 mg toltrazuril or ponazuril per kg body weight / day) completely prevented cerebral lesion formation in all treated animals, as assessed by immunohistochemistry. PCR analyses of these treated animals showed that DNA-detectability was reduced by 91% and 90% upon toltrazuril and ponazuril medication, respectively. Calves were experimentally infected with 2×10^8 *N. caninum* tachyzoites. Ponazuril chemotherapy of infected calves reduced the symptoms (e.g. fever), diminished quantitatively the humoral immune response, and abrogated DNA-detectability and lesion formation in the brain of treated calves. 1

B10

PROTECTIVE IMMUNITY IN BOVINE NEOSPOROSIS

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Background: In a previous cattle experiment we have shown that exogenous challenge with *Neospora caninum* tachyzoite early in pregnancy induces foetopathy. We have used this system to determine whether or not protective immunity exists in neosporosis.

Method & Results: Five naturally-infected, chronically-infected cows were challenged at 10 weeks of gestation. No foetopathy occurred and all five delivered live full term calves, whereas in four concurrently challenged, infection-naïve, pregnant cows all four foetuses died within 3-5 weeks of challenge. Of the live calves born, three were transplacentally infected with *N. caninum*. The kinetics of the maternal anti-*N. caninum* antibody responses during gestation suggested that these transplacental infections were a result of the recrudescence of maternal chronic infection - which also occurred concurrently in non-challenged, chronically infected pregnant controls - rather than the super-imposed challenge.

Conclusion: These data provide the first experimental evidence that protective immunity occurs in neosporosis; and they also suggest that whilst pre-existing infection will protect against an exogenous challenge, this protective immunity will not prevent transplacental infection. This suggests an extremely subtle form of concomitant immunity and has important implications for vaccine development.

Acknowledgements: We thank the Milk Development Council for funding.

B11 OCCURRENCE AND DIVERSITY OF BOVINE COCCIDIA AT THREE LOCALITIES IN SOUTH AFRICA

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Background: Information on the occurrence and diversity of coccidia affecting cattle in different farming systems in South Africa is lacking. The objective of the study was to determine the levels of infection of coccidia in cattle at three localities and to determine the *Eimeria* species occurring at each locality.

Method: Faecal specimens (n=1936) were collected over a 13-month period from a dairy farm (Malleons), a Bonsmara breeding farm (Kaalplaas) and a Nguni stud farm (Pienaars River). Samples were collected from adults >24 months and calves < 12 months old. The modified McMaster technique was used to determine OPG (oocysts per gram of faeces). Samples with OPG's ≥ 2000 were allowed to sporulate in potassium dichromate solution at room temperature. Species identification was done by measurement and morphological characteristic of each species.

Results: At Malleons, 28.6% of specimens were positive, at Kaalplaas 49.6 % and at Pienaars River 52.4%. The most prevalent species were *E. zuernii* and *E. ellipsoidalis* at Malleons, *E. zuernii* and *E. bovis* at Kaalplaas and *E. bovis* and *E. zuernii* at Pienaars River, respectively.

Conclusion: Adults had very low OPG's. There was a pattern at the three localities in that high OPG's were only recorded in calves, which were also shedding the most pathogenic species.

B12

DURATION OF NATURALLY ACQUIRED CRYPTOSPORIDIUM INFECTIONS IN PIGS

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Background: *Cryptosporidium parvum* commonly infects both beef and dairy cattle at a young age. However, little is known about the duration of *Cryptosporidium* infections in pigs.

Method: Prior to weaning, fecal samples were collected once from Yorkshire-Landrace piglets (n=33). After weaning, fecal samples were collected 2 times/wk until *Cryptosporidium* oocysts were detected. Fecal samples were then collected 3 times/wk until oocysts were no longer detected. Piglets were monitored throughout the study for diarrhea that could be associated with *Cryptosporidium* infections. *Cryptosporidium* isolates from these pigs were compared with those obtained from human and pigs using rDNA sequence analysis.

Results: All 33 pigs shed oocysts some time during the study. The initial mean age of oocyst detection was 43.7 days. The mean number of days that oocysts shed by the piglets was 25.4 days, with the number of oocysts remaining relatively low throughout the study. Episodes of diarrhea were not associated with oocyst shedding. *Cryptosporidium* found in these pigs was different than *Cryptosporidium* from humans.

Conclusions: The prevalence of cryptosporidiosis was high in these pigs but was not associated with diarrhea. *Cryptosporidium* infections in these pigs were cleared within 4 weeks. Genetic analysis of oocysts recovered from this herd revealed that these pigs harbour a strain of *Cryptosporidium* different to that previously obtained from humans and unique to pigs.

Acknowledgements: Alberta Pork, NSERC

B13 CONTROL OF COCCIDIOSIS WITH DICLAZURIL IN CALVES

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Background: The efficacy of diclazuril against coccidiosis in calves at the dosage authorized for lambs was validated in the field.

Method: A preliminary trial was conducted in 140 calves on 7 farms as an approach to the curative efficacy of diclazuril at the very beginning of clinical coccidiosis. One group of infected calves, one group of subclinical treated calves and one group of untreated calves were constituted on each farm. A second trial (27 calves) related to the prophylactic value of diclazuril by comparing two groups of calves on one farm. Treatment (therapeutic or prophylactic) with a suspension of 0.25 % diclazuril was given at a dosage of 1 mg/kg of live weight in a single oral dose : 4 ml/10 kg. The analysis criteria related to the state of diarrhoea, the general status of the calves and parasite excretion.

Results: In the first trial, there was a marked reduction in the excretion of oocysts of *E. zuernii*, *E. bovis* and *E. alabamensis* in the group with clear clinical symptoms : more than 99 % on D7 and D14. Likewise, the number of excreting calves was reduced to 86 % on D7 and 82 % on D14. In the group with subclinical coccidiosis similar reductions were noticed. The number of animals with diarrhoea in the first group diminished with 62 % after 7 days and with 86 % after 14 days. No relapse of coccidiosis has been seen. In the second trial where diclazuril has only been used prophylactically the excretion of oocysts was diminished with 98 % and 99 % on D 7 and D 14 respectively.

Conclusion: These results appear to indicate that it is possible to extrapolate the data on diclazuril in sheep to calves. Diclazuril administered at a dose of 1 mg/kg on the appearance of symptoms exhibits good efficacy against coccidiosis.

B14 EVALUATION OF ANTICOCCIDIAL IMMUNITY CONFERRED BY TOLTRAZURIL TREATMENT AFTER COCCIDIA VACCINE APPLICATION

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Coccidiosis is one of the most important poultry diseases. Life vaccines (Immucox®, Coccivac®) based on virulent oocysts show significant protective immune response in the field. However the exposure of birds to stress factors may limit the immune response to vaccination. In these cases, strategies that involve both the use of live vaccines in combination with intermittent medication might lead to an improved coccidiosis control. Our study included seven experimental groups (21 cages with 10 birds each). A high, mixed challenge infection took place on day 24. Groups I, II, III and IV were vaccinated at day 2 of age with Coccivac B®. Groups I, II, III and V were treated with Toltrazuril (Baycox®, dose of 7-mg/kg-body weight continuously via the drinking water) on two successive days post vaccination: 7 & 8, 11 & 12 and 16&17 respectively. Strategic administration of Toltrazuril after vaccination with Coccivac B® lead to a higher antibody titer as measured by IFAT, Immunoblot and ELISA. High antibody titers in the immune sera correlated with higher reduction in oocyst excretion, reduction of lesion scores and increased weight gains in Toltrazuril treated animals after challenge infection. Thus, the combination of vaccination and strategic, intermittent Toltrazuril treatment might be an alternative approach to an improved Coccidiosis control.

B1 p NEOSPORA CANINUM IN BRAIN TISSUES FROM RURAL FOXES IN CATALONIA (SPAIN).

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Background: The epidemiological role of carnivorous wildlife, such as foxes, in *N. caninum* infection has become increasingly important once dogs were described as definitive hosts. Serological studies have shown that foxes can become infected by *N. caninum* and could be implicated in abortion outbreaks in cattle. The present study shows data about prevalence of infection in rural foxes from Catalonia.

Method: Foxes in the study were trapped as part of ongoing official programme for fox control in 20 different rural areas of Catalonia. DNA samples were obtained from brain tissues of dead animals. The presence of *N. caninum* in brain tissues was analysed using a competitive PCR (C-PCR) assay. The assay used a competitor to the target *Neospora*-specific Nc5 genomic sequence. Incorporation of the competitor molecule in the detection assay provided a control for PCR failure and facilitated identification of truly negative samples.

Results: A total of 120 brains were collected, of those 89 (74.2%) were from adult animals (age over 6 months), 24 (20.0%) were from young animals (under 6 months of age) and 7 where age was not recorded. Fifty-five were males (45.8%) and 62 (51.7%) were female, in 3 sex was not recorded. Out of 79 brain samples analysed, 12 have been found positive to *N. caninum* C-PCR, showing a prevalence of 15.2%.

Conclusion: The prevalence shown in the present study indicates that foxes might play an important role in transmission of *N. caninum* infection in Catalonia.

Acknowledgement: To Dr. Jenkins and Dr. Liddell, Departament de Medi Ambient of Catalonia and AGL2000-0904 CICYT (Spain).

B2 p PREVALENCE OF NEOSPORA CANINUM IN DAIRY CATTLE IN POLAND

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Neospora caninum, a cyst-forming coccidian parasite, is recognized as a major cause of bovine abortion in many parts of the world. In Poland, Cabaj et al. 2000, reported the first serological evidence of neosporosis in aborted dairy cows. Here we report sero-prevalence of *N. caninum* in cattle from one dairy farm located in central part of Poland.

Sera samples were obtained from 182 cattle of different age, confined on one farm located in Jastrzębiec near Warsaw. Antibodies to *N. caninum* in the sera were determined by a enzyme linked immunoassay (ELISA), according to the manufacturer's instruction (IDEXX Laboratories, Inc., Westbrook, Maine, USA), using an automated plate reader EL*800, Biotek, Instruments Inc. Serum samples with S/P ratios of less than 0.50 were classified as negative for *Neospora* antibodies, if the S/P ratio was greater than or equal to 0.50, the sample was classified positive for *Neospora* antibodies. Antibodies to *N. caninum* were found in 40 of 182 (22 %) dairy cows. Positive test serum antibodies S/P ratios were ranged from 0.68 to 3.21 while negative test serum S/P ratios ranged from 0 to 0.44. This study indicates that serological screening may be an effective and economical means of identifying *N. caninum*-infected cows. The potential benefits of establishing and maintaining a herd free of *N. caninum* enhances the economical value of cattle industry, and diminishes risks of potential international trade restrictions.

B3 p SEROLOGICAL EVIDENCE OF *NEOSPORA* *CANINUM* IN *BISON BONASUS* L. IN POLAND

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Neospora caninum is a parasite of livestock and companion animals. Neosporosis is a major cause of abortion in cattle. Antibodies to *N. caninum* have been reported in cattle, sheep, goats, dogs, coyotes, cats, horses, camels, buffaloes and deer. The role of wildlife in the natural epidemiology of neosporosis is unknown. It is not known if wild herbivores can act as intermediate hosts. Here we report prevalence of *N. caninum* antibodies data for European bison (*Bison bonasus* L.) located in two ecological regions in Poland. Sera samples were obtained from 33 European bison, of different ages and both sexes, from two natural preserves in Białowieża and Bieszczady regions during 2000-2001. Antibodies to *N. caninum* in the sera were determined by ELISA according to the manufacturer's instruction (IDEXX Laboratories, Inc., Westbrook, Maine, USA), using an automated plate reader EL*800, Biotek, Instruments Inc. Serum samples with S/P ratios of less than 0.50 were classified as negative for *Neospora* antibodies, if the S/P ratio was greater than or equal to 0.50, the sample was classified positive for *Neospora* antibodies. Antibodies to *N. caninum* were found in 4 of 33 (12.1 %) European bison. Positive test serum antibodies S/P ratios were: 3.25, 2.98, 2.49 and 0.63, whereas negative test serum S/P ratios ranged from 0.01 to 0.41. To our knowledge, this is the first attempt to perform sero-prevalence for *N. caninum* in European bison. The findings reported here provide serological evidence that supports the hypothesis of inclusion of European bison as a new intermediate host for *N. caninum*.

B5 p PREVALENCE OF ANTIBODIES TO *NEOSPORA* *CANINUM* IN A DAIRY CATTLE FARM IN MALAYSIA

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Background : *Neospora caninum*, an apicomplexan protozoan of animals, is known to cause abortion or stillbirth in cattle, sheep, goats, horses and deer and has been reported in many countries. However, the prevalence of the parasite is unknown in Malaysia and an investigation into the seroprevalence of *N. caninum* in dairy cattle was therefore conducted in a farm which has history of abortions, stillbirths and infertility problem.

Method : Random serum samples were obtained from 100 adult Sahiwal Friesian cross dairy cattle in a farm which had a population of 550-650 adult animals. The sera were screened at 1:200 for IgG antibodies to *N. caninum* using indirect fluorescence antibody test. The test employed teflon-masked slides which contained Vero cell cultured *N. caninum* tachyzoites as antigen (VMRD, Pullman, USA). Control positive and negative sera were included in the assay.

Results : 9 out of the 100 serum samples had IgG antibodies to *N. caninum*. In the seropositive cattle 1 had history of abortion while another had infertility problem. Among the seronegative animals there were 2 cases of abortion, 1 case of stillbirth and 2 animals had infertility problem.

Conclusion : This paper reports for the first time the serological evidence of *N. caninum* infection in dairy cattle in Malaysia and would be useful for further epidemiological studies of the parasite.

B4 p CATTLE NEOSPOROSIS: EPIDEMIOLOGICAL SURVEY IN THE PROVINCE OF PADUA

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Background: *Neospora caninum* has a world-wide distribution and it's emerging as a major cause of infectious abortion in cattle of many countries. Since no data are available about neosporosis prevalence in Veneto region (North-Eastern Italy), a serological and risk factor survey was carried out in the area of ULSS 15 of the province of Padua.

Method: 368 blood samples were collected from 33 dairy cattle farms in June 2000. Epidemiological data were gathered by a brief questionnaire on the farm management (total number of animal, cow origin: purchased or raised, feeding practice, reproductive problems, presence and number of dogs, other animals in the farm), in order to assess risk factor associated with neosporosis positivity. For serological examination a commercial ELISA kit (HerdChek[®], IDEXX) was used. Epidemiological data were analysed by logistic regression model.

Results: Seroprevalence was 16% in the examined animals and at least a positive was found in 54.5% of the farms. Seroprevalence within the herds ranged from 6% to 61%. The cattle origin (raised instead of purchased), the total number of farm animals and farm location were strongly associated ($p < 0.01$) with increasing seroprevalence in dairy cattle.

Conclusions: Seroprevalence in dairy cattle farms and animals is consistent with the Italian literature. *Neospora* infection is widespread in this area too. The strong association between the practice of raising the animals and the seroprevalence seems to stress the importance of vertical transmission. On the other hand, a hypothesis that could explain why large herd was a major risk factor for neosporosis seroprevalence is that high cow density may have increased the probability of horizontal transmission of infective oocysts.

B6 p *N. CANINUM* FOETAL INFECTION IN BELGIAN CATTLE

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Background: *Neospora caninum* is a protozoan parasite which caused fetal and neonatal mortality in livestock and companion animals. In the present study, diagnostic methods for the demonstration of infection and occurrence of *N. caninum* in a series of abortions in Belgian cattle were used.

Method: A total of 224 aborted beef and dairy foetuses were analysed. An Indirect Fluorescent Antibody Test (IFAT) was used to analyse foetal and maternal sera. A specific antibody titer $\geq 1/25$ or $1/200$ was considered as indicative of *N. caninum* foetal or maternal infections respectively. Histopathological examination of the brain, heart and liver was performed. Immunohistochemistry (IHC) was used when lesions compatible with neosporosis were observed.

Results: 20 dairy cattle sera out of 70 (28.6%) were positive by IFAT whereas 13 beef cattle sera out of 93 (14.0%) were positive using the same technique. A positive titer to *N. caninum* was found in 7 and 3 foetuses from beef and dairy cows respectively. Lesions consistent with *N. caninum* infection were observed in 17 foetuses. Out of 9 beef foetuses, 5 were confirmed by IHC whereas all but one dairy foetuses were confirmed with the same technique.

Conclusion: *N. caninum* both in beef and dairy cattle should be considered as a major cause of abortion in Belgium.

Acknowledgement: This work was supported by a grant N° 5774A from the Federal Ministry of Agriculture, Belgium.

B7 p**SEROEPIDEMIOLOGICAL STUDIES ON
NEOSPORA CANINUM IN AUSTRIAN DOGS**

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Background: In a serological survey comprising 5061 cattle, originating from all federal states of Austria, we detected 2.5% animals serologically positive against *Neospora caninum* antibodies. Cattle may be regarded as a potential reservoir of infection for dogs. To obtain more informations about the mode of *Neospora* infection in Austria, blood samples from dogs were collected.

Method: Blood was drawn from 1899 dogs according to age, breed and sex. The sera were tested by an indirect fluorescence antibody test (IFAT) for the presence of IgG antibodies to *N. caninum*. An anti-dog IgG (heavy and light chains) fluorescein-conjugated IgG fraction from sheep (The Binding Site, Birmingham, UK) in a phosphate-buffered saline (PBS) dilution of 1:30 was used as conjugate. Serum obtained from a dog after a natural *Neospora* infection with clinical symptoms was used as a positive reference, a titer of 1:50 and higher was considered to be positive.

Results: The results of the study conducted in 2000 and 2001 showed that in Austria 3,2 % of dogs had antibodies against *N. caninum*.

Conclusion: Although the seroprevalence rates of cattle and dogs in Austria are low compared to the data published in other countries, it would be of great interest if farm dogs are of a higher risk of exposure to *N. caninum* than in urban dogs.

Acknowledgement: We wish to thank the Practitioners Dr. Leidinger, Dr. Schwarz, Doz. Dr. Kopf and the Vets of our I. Medical Clinic for Ungulates and Small Animals for helping to collect the dog sera.

B8 p**COMPARISON OF *N. CANINUM* ANTIBODY-
ELISAS AVAILABLE IN NORTH AMERICA**Peregrine^{1a}, A., Hobson^{1b}, J., Duffield^{1b}, T., Lissemore^{1b}, K., Martin^{1b}, S.W., Edge^{1b}, V. & Kelton^{1b}, D.

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Background: Since the discovery of *Neospora caninum* in 1984 a number of ELISAs have become commercially available for detection of antibody to *N. caninum* in cattle sera. The objective of this project was to compare the performance of 3 ELISAs that are available in 5 North American diagnostic laboratories.

Method: 12 primiparous Holstein animals in an Ontario dairy herd were blood sampled weekly over 13 weeks. The sera were submitted simultaneously to 4 diagnostic laboratories in Canada and 1 laboratory in the U.S.A. for blinded detection of *N. caninum* antibodies. The resultant data were compared to IFAT results for the same sera as the IFAT is considered an acceptable Gold Standard.

Results: Good kappa values, sensitivities and specificities were observed for 4 ELISAs with estimates in excess of 0.80, 87% and 80%, respectively. However, the results of one ELISA, analyzed at the stated cut-off, were acceptable but not optimal with values of 0.55, 64% and 100%, respectively. Further analysis using a revised cut-off produced results comparable to those of the other ELISAs.

Conclusions: These results indicate excellent performance for 4 of the examined ELISAs and illustrate the effect of cut-off selection on test performance.

Acknowledgement: Dairy Farmers of Ontario, the Ontario Cattlemen's Association, and the Ontario Ministry of Agriculture, Food and Rural Affairs are thanked for financial support.

B9 p**ISOLATION OF *Neospora* sp. FROM THE BRAIN OF A
DAIRY CALF IN ARGENTINA**

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Background *Neospora caninum* causes abortion, premature birth and stillbirth in cattle in many countries, including Argentina. The purpose of this study was to attempt to isolate *N. caninum* from calf in Argentina.

Methods: Brain and pre-colostral serum of a premature dairy calf were tested for *Neospora*. Antibodies to *N. caninum* (titer 800) were detected in serum using the indirect fluorescent antibody test (IFAT). Brain was homogenized and inoculated subcutaneously into two gerbils and one BALBc mouse. One gerbil and the mouse were euthanized one month post-infection. Cell cultures of bovine monocytes (BM) were inoculated with trypsinized brain homogenate. Gerbils and mouse sera were tested for *N. caninum* antibodies with the agglutination test (Vetoquinol) and organs were evaluated immunohistochemically.

Results: Antibodies to *N. caninum* were detected in gerbils and mouse sera one month post-inoculation. Tissue cysts were observed in fresh brain smears of the mouse and one gerbil and were demonstrated by immunohistochemistry in the brain of one gerbil. Cell cultures inoculated with a half of brain of the same gerbil are still under observation. The other gerbil is alive.

Conclusion: Results confirm the presence of *Neospora* species in cattle in Argentina.

B10 p**DEVELOPMENT OF A MURINE MODEL OF
CONGENITAL NEOSPOROSIS**

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Background: In chronically infected cows vertical transmission of *Neospora caninum* occurs without evidence of reinfection. A mouse model would be useful to study the mechanisms responsible for reactivation. The present work aimed at the selection of a mouse strain and inoculum dose leading to cyst production, vertical transmission and resistance of the dam.

Method: Two strains of mice (Swiss-White and CBA/Ca) and 3 inoculum doses (5×10^6 , 1×10^6 and 2×10^5 tachyzoites NCI strain) were investigated in 3 different experiments. Cyst production was studied in mice treated with methyl prednisolone acetate. Lymphoproliferative response, cytokine production, humoral response and histological lesions were evaluated 8 weeks after infection while vertical transmission was investigated by immunohistochemistry in dams inoculated 5-9 days after mating and sacrificed on day 18-19 of gestation.

Results: Cysts were only detected in CBA mice 10 weeks after infection with 5 and 1×10^6 tachyzoites. This strain showed the highest lymphoproliferative response to NCI antigen. In both strains a Th1-like cytokine response with elevated IFN γ and very low IL4 and IL10 secretion was recorded. No histological lesions were found. No parasite were seen in foetuses but presence of tachyzoites was observed in uterus of a CBA and a Swiss dam. Encephalitis and light necrosis were seen in some dam's brain.

Conclusion: CBA females infected with 5×10^6 NCI tachyzoites could be regarded as a useful model for congenital neosporosis.

B11 p CRYPTOSPORIDIUM PARVUM IN THE EUROPEAN RED SQUIRREL *SCIURUS VULGARIS*

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Background: Cryptosporidia are coccidian parasites which cause acute and chronic infections of the gastrointestinal tract of wild and domestic animals. Among the genus *Sciurus*, *Cryptosporidium parvum* was reported in gray squirrel (*S. carolinensis*) and fox squirrel (*S. niger*). We researched its presence during a study on the ecology of the European red squirrel (*Sciurus vulgaris*). **Method:** Two study areas were located in coniferous woodlands in the Gran Paradiso National Park (Northwestern Italy). Fecal samples, collected during live-trapping in August and October 2000, were examined for *Cryptosporidium* oocysts. **Results:** Totally, 25 red squirrels (16 males and 9 females) were examined. *Cryptosporidium* oocysts were found in 17 of the examined animals (68%); they were 11 males (68.7%) and 6 females (66.7%). Within this qualitative study all detected *Cryptosporidium* parasites were identified as *C. parvum* according to morphological characteristics of the oocysts.

Conclusion: As far as we know, this is the first report of *Cryptosporidium* in *S. vulgaris* and its prevalence appears notable with respect to what is generally observed in other wild and domestic animals.

Acknowledgement: This study was supported by a grant to BS by the Gran Paradiso National Park.

B12 p ANTIBODIES FOR *Neospora caninum* AND *Toxoplasma gondii* IN CATTLE, BRAZIL

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599 samples of bovine sera, taken from cows from four counties in the São Paulo State and from two counties in the Minas Gerais State, Brazil, were tested using an immunofluorescent antibody technique (RIFI-IgG) for the presence of *Neospora caninum* and *Toxoplasma gondii* antibodies. A total of 101 (16,86%) of the tested samples were reactive and 498 (83,14%) non-reactive to *N. caninum*, while 295 (49,25%) of the samples were reactive and 304 (50,75%) were non-reactive to *T. gondii*. 54 (9,02%) of the animals tested showed detectable antibody levels for both coccidia. Cows with serum that reacted positively to both these protozoan parasites were seen in all counties assessed. Statistical differences ($P < 0,05$) between counties were observed when comparing the results of the sera evaluation. It can be concluded from the obtained results that the protozoan parasites *N. caninum* and *T. gondii* are largely widespread in herds of dairy as well as beef cattle from the assessed counties in Brazil and that the simultaneous occurrence of antibodies for both parasites can be relatively high (9,02%) in bovine hosts in the studied geographical regions.

B13 p IgG TO *Neospora caninum* IN *Toxoplasma gondii* POSITIVE AND NEGATIVE DOGS

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A total of 163 dogs with neuromuscular, respiratory and/or gastrointestinal disorders, was admitted at the Veterinary Hospital, Federal University of Uberlândia, Brazil, and submitted to serology for *Toxoplasma gondii* (Tg) and *Neospora caninum* (Nc). Assays for *T. gondii* IHA, IFAT-Tg, ELISA, and IB-Tg. Assays for *N. caninum* included IFAT-Nc and immunoprecipitation (IP-Nc). Based on concordant results by the three serological tests for *T. gondii*, and divergent results further confirmed by IB-Tg, the 163 sera were divided into two groups: 59 (36%) Tg-seropositive and 104 (64%) Tg-seronegative samples. Antibodies to *Neospora* were detected in 11 (6.7%) out of 163 analyzed dog sera, with 5 (3.1%) samples reactive to both parasites (Tg+/Nc+), and 6 (3.7%) reactive only to *Neospora* (Tg-/Nc+). Antibodies only to *T. gondii* were found in 54 (33%) samples. Among the 11 *Neospora*-positive sera analyzed by IB-Tg, the 5 sera Tg+/Nc+ showed strong reactivity to TgSAG1 (p30). No reactivity was observed to TgSAG1 in the 6 samples Tg-/Nc+. By IP-Nc, 29 kDa and 35kDa proteins were recognized by all 11 IFAT-Nc positive sera. Our results suggest that the infection by *N. caninum* can be concomitantly present in dogs from this area, although less common, and therefore should be considered in the differential clinical diagnosis with *T. gondii* in dogs with such disorders.

B14 p ANTIBODIES FOR *Neospora caninum* AND *Toxoplasma gondii* IN WATER BUFFALOES

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Water buffaloes (*Bubalus bubalis*), are capable of becoming infected with *Neospora caninum* and *Toxoplasma gondii*, contributing to disease dissemination. The incidence of these antibodies was assessed in sera of adult female water buffaloes, using an immunofluorescent antibody technique (IFAT). Any possible influence of breed or age of the buffaloes was investigated, besides assessment of sera antibody titres of any dogs and humans that were in close contact with the tested animals. Serum samples were taken from water buffaloes (n=411), dogs (n=8) and humans (n=14), from buffalo dairy farms in the São Paulo State, and tested. Of the samples of buffalo sera tested, 56.0% were reactive ($\geq 1:200$) to *N. caninum* and 49.9% were reactive ($\geq 1:64$) to *T. gondii*. Of all, 33.9% of the buffaloes presented antibody titres for both protozoan parasites. Of the canine serum samples, 25.0% presented titres ($\geq 1:50$) for *N. caninum* and 62.5% for *T. gondii* ($\geq 1:16$). The human serum samples did not shown detectable antibodies for *N. caninum* when using a ≥ 100 limit titre for positive findings. The obtained data allows the conclusion that the protozoan parasite *N. caninum*, as well as *T. gondii*, are widely disseminated in the water buffalo herds assessed and, apparently, the factors breed and age do not interfere with frequency of *N. caninum* positives among adult female buffaloes. When concerning *T. gondii*, female buffaloes of 5 to 6 years of age showed more frequent presence of specific antibodies.

Acknowledgment: FAPESP for financial support

B15 p **VENEREAL TRANSMISSION VIABILITY OF *Toxoplasma gondii* IN BOVINES**

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This study was done in an attempt to verify the venereal transmission of bovine toxoplasmosis. Twelve male bovine hosts, in reproductive age, were inoculated with oocysts (1.0×10^6) or tachyzoites (5×10^6) of *Toxoplasma gondii*. Semen samples from these bulls, and from six uninfected controls, were screened for the presence of infective forms of *T.gondii*. The infected bulls showed hyperthermia, cardiac and respiratory alterations from the third to seventh days post-inoculation (dpi), especially those animals inoculated with *T.gondii* oocysts. Parasitemia in the inoculated bulls was detected up to the 84th dpi in both experimental groups. No haematological alterations were observed. *T.gondii* was isolated from semen collected from days 14 to 84 dpi. The main pathological alterations observed were meningeal congestion, pulmonary oedema and enlarged mesenteric lymph nodes. Parasitism was detected in the testis, vesicular glands, epididymides, liver, lungs, retinae, cerebrium, spleen and lymph nodes. These results (tissue and semen) are being confirmed by Polymerase Chain Reaction (PCR). The isolation of *T.gondii* from bovine semen suggests that venereal transmission could be an important mode of dissemination of this zoonosis in this animal specie.

Acknowledgments: FAPESP for financial support

B17 p **EFFICACY OF ARTEMISININ AGAINST *Neospora caninum* TACHYZOITES IN IMMUNOSUPPRESSED MICE**

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Background: *Neospora caninum* is a major cause of abortion in dairy cattle in the United States and other many countries. Abortions and neonatal mortality also occur in other ruminant species. Artemisinin is a effective antimalarial compound derived from a traditional Chinese herbal remedy, Qinghao or *Artemisia annua* L.

Methods: The protective effect of artemisinin against *N. caninum* infection was examined in experimental murine acute neosporosis.

Results: Four mice of nine mice challenged with *N. caninum* and administered intraperitoneally with artemisinin at 1 mg per mouse survived until the end of experiment (21 days post challenge) and two mice of nine mice administered orally with artemisinin at 5 mg per mouse survived. In contrast, all mice administered intraperitoneally with artemisinin at 0.1 mg per mouse died of acute neosporosis within 20 days post challenge and all untreated mice died of acute neosporosis within 19 days post challenge. Brain lesions of mice administered intraperitoneally with artemisinin at 1 mg were absent or less severe than those seen in mice in infected control group. No other differences in brain lesion size by groups were not found, but the lesions of mice administered intraperitoneally with artemisinin at 1 mg were fewer in number and had fewer inflammatory cells than those seen in mice of infected control group.

Conclusion: These results demonstrate that artemisinin inhibited *N. caninum* tachyzoites invasion into host cells or intracellular growth.

B16 p **EFFICACY OF ARTEMISININ AGAINST *Neospora caninum* TACHYZOITES IN CULTURED CELLS**

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Background: *Neospora caninum* is an intracellular apicomplexan parasite that infects a wide range of mammals and has been associated with abortion in cattle worldwide. Artemisinin is an effective antimalarial compound derived from a traditional Chinese herbal remedy, Qinghao or *Artemisia annua* L.

Methods: In present study, the cultured host cells (vero cells or mouse peritoneal macrophages) infected with *N. caninum* tachyzoites were incubated with a -MEM-10%HS supplemented with various concentration of artemisinin (20, 10, 1, 0.1 and 0.01 µg/ml) to examine the efficacy of artemisinin against *N. caninum* tachyzoites invasion into host cells or intracellular growth.

Results: In long-term studies, at 20 or 10 µg/ml for 11 days, artemisinin reduced *N. caninum* and completely eliminated all microscopic foci of *N. caninum*. At 1 µg/ml for 14 days, artemisinin reduced *N. caninum* and completely achieved elimination all microscopic foci of *N. caninum*. There was no apparent toxicity to host cells in long-term studies. In short-term studies, at ≥ 0.1 µg/ml, artemisinin reduced *N. caninum* tachyzoites invasion or intracellular growth, significantly ($p < 0.05$) and appeared to depend on the artemisinin concentrations. Pretreatment of host cells or *N. caninum* tachyzoites with artemisinin had no effect on *N. caninum* tachyzoites invasion or intracellular growth.

Conclusion: These results demonstrate that artemisinin inhibited *N. caninum* tachyzoites invasion into host cells or intracellular growth.

B18 p **OBSERVATION ON THE ENDOGENOUS DEVELOPMENTAL STAGES OF *CRYPTOSPORIDIUM BAILEYI* IN POULTRY BY SEM**

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Background: One isolate of *Cryptosporidium* was gotten from ducks. The ducks didn't show any clinical signs. We were going to identify the isolate and revealed the characteristics of endogenous developmental stages.

Methods: Oocysts of *Cryptosporidium* sp. from ducks were orally inoculated into chickens for multiplication and then were orally inoculated into 30 ducks, 30 chickens and 30 silkiefowls black bone respectively. For electron microscopy, small pieces of tissues from the bursa of Fabricius (BF), intestine, caecum and trachea were fixed in 2.5% glutaraldehyde in 0.1M PBS (pH 7.4). The fixed tissues were washed, dehydrated, critical point dried and then gold-coated. Observed in a S-570 HITACHI scanning electron microscopy.

Results: All endogenous developmental stages of the pathogen embedded in microvillus of the trachea and BF. Ducks were less susceptible to infection with it than chickens and silkiefowls black bone. The pathogen was identified as *Cryptosporidium baileyi*. There were granular residual bodies between merozoites and one spherical residual body was surrounded by 4 or 8 merozoites. The escaping pattern of microgamete from maternal body was discovered. There were two types of parasitophorous vacuole (PV): one had spherical residual body in it and the other not.

Conclusion: Silkiefowl Black Bone is a new host of *C. baileyi*. There were 4 types of escaping pattern of mature merozoite from schizont. It was concluded that comblike lamella of PV base didn't possess the function of feeder organelle, and bottom of PV was feeder organelle according to the observation result by SEM. Microgamete escaping from maternal body and endogenous development stages in goblet cell were observed.

B19 p CHEMOTHERAPY TO PREVENT ABORTION IN BOVINE NEOSPOROSIS

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Background: One of the major economic impacts of bovine neosporosis is abortion, which usually occurs during the mid-term of the pregnancy in infected cattle. Although culling of infected cattle had been advocated, we think that there should exist a chemotherapeutic option for the dairy farmers to control abortion to reduce economic loss. We attempted to use chemotherapy to try to prevent neosporosis-associated abortion.

Methods: A cattle farm in central Taiwan, with 140 adult cows and 110 heifers that had a history of abortion was selected for this study in July 2000. Sera of pregnant cattle were tested for the presence of IgG and IgM antibody against *Neospora caninum* tachyzoite using the indirect immunofluorescent antibody method. A total of 23 cattle, 3-6 month pregnant, that showed high antibody titer as well as those with previous record of abortion, were injected subcutaneously with trimethoprim and sulfadiazine (1:5) at 0.06-0.1mg/kg body weight for the 1st day and then four-fifth of the dosage for the following 4 consecutive days. Untreated seropositive cattle in the same farm served as untreated control.

Results: Among the 23 cattle being treated with the drugs, one 5-month pregnant cattle aborted on the second day after the initiation of the treatment while 22 pregnancies were carried to term. One calf died during a difficult parturition and 21 apparently healthy calves were born. From July 2000 to May 2001, 43 untreated cattle in that farm had aborted.

Conclusion: Thus, it is possible to prevent abortion in *N. caninum* infected cattle by injecting with trimethoprim and sulfadiazine.

B20 p PREVALENCE OF ISOSPORA SUIIS IN SUCKLING PIGLETS WITH DIARRHOEA IN BELGIUM

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INTRODUCTION: Infections of suckling piglets with *Iso spor a suis* are widespread but its prevalence varies considerably between countries. No data are published for Belgium. In this study, the presence of *I. suis* oocysts in faecal samples of litters with diarrhoea was investigated.

METHODS: Between August 2000 and January 2001, faecal samples from 79 litters with diarrhoea in the age from 5 to 28 days were collected from 15 farms with a history of diarrhoea. The samples were taken from the rectum or collected from the pen floor and pooled per litter. Samples were examined by means of the Telemann method. The number of farms and the total number of litters from which *I. suis* was recovered was counted. Furthermore, the sampled litters were arbitrarily divided into 4 different age classes i.e. litters aged 5-7 days, 8-14 days, 15-21 days and 22-28 days respectively. The prevalence of *I. suis* was calculated for each individual age class. The number of litters sampled per age class was different.

RESULTS: *I. suis* was present on 11 of the 15 farms (73%). The parasite was found in 24 out of 79 litters (30%). A prevalence of 13% was found in litters aged 5-7 days, 44% in litters aged 8-14 days, 18% in litters aged 15-21 days and 13% in litters aged 22-28 days. The earliest oocyst output was detected at 7 days of age. The oldest positive litter was 24 days old.

CONCLUSION: *I. suis* is a common parasite on Belgian pig farms with a history of diarrhoea. *I. suis* is often present in litters with diarrhoea with a highest prevalence in the 2nd week of life. Despite the high prevalence of *I. suis*, its actual role in piglet production is still under discussion.

B21 p STUDIES ON THE EFFICACY OF TOLTRAZURIL, DICLAZURIL AND SULPHADIMIDINE AGAINST ARTIFICIAL INFECTIONS WITH ISOSPORA SUIIS IN SUCKLING PIGS

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Background: *Iso spor a suis*, the causal agent of piglet coccidiosis, is a prevalent and economically important parasite. The present study was undertaken to examine the therapeutic potential of various substances.

Method: In the infection model (infection at 3 days of age) 8-12 piglets per group (randomised) were treated with toltrazuril (Baycox® 5%; 20 mg/kg on day 2 p.i.), diclazuril (2 mg/kg on day 2 and 3 p.i.) or sulphadimidine (200 mg/kg on day 2, 3 and 4 p.i.) and compared with an untreated control group. The following parameters were assessed: clinical picture, faecal consistency, oocyst excretion, liveweight development.

Results: The untreated control animals developed the typical picture of severe isosporosis as a result of the infection. In the toltrazuril group oocyst excretion was substantially reduced by the treatment and remained low, faecal consistency was unchanged (normal) and weight development was not impaired. The diclazuril and sulphadimidine groups did not differ significantly from the untreated control animals with regard to the parameters oocyst excretion, faecal consistency and liveweight development.

Conclusion: A single treatment with toltrazuril (Baycox® 5%; 20 mg/kg; 2 days p.i.) controlled a massive artificial infection with *I. suis* in suckling pigs.

B22 p PATHOGENESIS OF ISOSPORA SUIIS INFECTIONS AND ITS MANAGEMENT WITH SYMMETRICAL TRIAZINETRIONES

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Background: *Iso spor a suis* is a primary pathogenic parasite which causes scouring in suckling pigs worldwide. The pathogenesis of the disease and the effect of treatments with a representative of the symmetrical triazinetrones was studied in an infection model.

Method: Piglets were artificially infected at three days old with 10⁴ sporulated oocysts of *Iso spor a suis* and each given one oral treatment with Bay Vi 9143 on day 2, 3 or 4 p.i. The infectious process was assessed with reference to clinical, parasitological (opg; faecal consistency) and pathomorphological parameters (histology and electron microscopy of the jejunum and ileum) and compared with an infected, untreated control group.

Results: The infection resulted in a manifest isosporosis with pathomorphological lesions which included villous atrophy and fibrinoid necroses. The treatments at different time points p.i. controlled the respective developmental stages completely. The clinical and pathomorphological picture improved accordingly.

Conclusion: Bay Vi 9143 is effective during the schizogony and gametogony cycles of *I. suis*. Its therapeutic use at the beginning of the parasite's life cycle completely prevents the symptoms associated with the infection.

B23 p EFFECT OF *LACTOBACILLUS* ON *EIMERIA*
TENELLA INVASION *IN-VITRO*.
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The potential control of infectious diseases of livestock by using probiotic bacteria as agents of "competitive exclusion" is important, given current concerns on use of antimicrobial agents in food-producing animals. *Eimeria* infection, both in poultry and mammals, is often controlled by the use of prophylactic drugs. However, the composition of the gut flora may be one factor which influences the outcome of this and other enteric protozoal infections. Moreover, gut pathogens may also inhibit colonisation of epithelia by beneficial bacteria.

We undertook to examine the interactions between *Lactobacilli* and *E. tenella* using MDBK cell monolayers *in-vitro*, as a model system. Interactions between live, but not killed, *Lactobacilli* and *Eimeria* infection were found.

B25 p THE EFFICACY OF AMPROLIUM AGAINST COCCIDIOSIS
IN CAPTIVE BUFFALO (*SYNCERUS CAFFER*) IN THE
KRUGER NATIONAL PARK, SOUTH AFRICA

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African buffalo (*Syncerus caffer*) are captured in Kruger National Park, South Africa, for a disease-free breeding program. Pregnant females are captured and tested for tuberculosis and brucellosis; negative females are brought in at the Skukuza Research area and are kept in captivity in bomas until calves are born. Calves are then, reared free of tuberculosis, brucellosis, foot and mouth and corridor disease. During this time the animals are kept in bomas; the confinement and increased level of stress are the perfect conditions for coccidiosis to develop. Frequent cases of coccidiosis were found and some young calves subsequently died. The difficulties of developing a non-intrusive anti-coccidial programme where the dosage of medication could be controlled, resulted in the recommendation of using amprolium on the drinking water at the bomas. Faecal samples from the bomas were collected and coccidial counts performed daily and weekly on individual and pooled samples collected from the bomas' floor early in the morning since the buffalo arrived after capture to determine the base level of coccidial infection. Identification of some of the species was attempted and some species were found to be more abundant than others. Water containers were calibrated to determine the daily water consumption of the buffalos and the dosage was decided accordingly. Monitoring of the coccidial infections was done on a daily basis and the consistency of the faeces and presence of clinical signs was also observed.

Three groups of buffalo were selected and randomly allocated to a negative control group, a preventive group (amprolium 30% 5 mg/kg/day for 21 days) and a curative treatment group (amprolium 30% 10mg/kg/day for 5 days). We conclude that both preventive and curative seems to control coccidiosis on captive buffalo. More trials will be conducted

B24 p PROTECTION OF CHICKENS AGAINST *EIMERIA*
TENELLA BY THREE EXPRESSED PROTEINS

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Background: Chicken coccidiosis is caused by *Eimeria* species. The incidence and mortality is high. Weight gain and feed conversion is much influenced. The past ten years research aimed at developing subunit vaccines against these parasites, has showed that developing a subunit vaccine based on a single recombinant antigen from one developmental stage of *Eimeria* was an overly optimistic approach.

Method: Chickens were vaccinated by two or three of expressed proteins (EtMIC2, TA4 and SO7,) per os with living recombinant bacteria and lysis recombinant bacteria or via intra-muscular injection with lysis recombinant bacteria at 7, 14 days old, then challenged with sporulated oocysts of *Eimeria tenella* at 21 days old individually.

Results: The result indicated that the weight gain of vaccinated chickens increased along with the content of expressed protein compared with the unvaccinated chickens, the best one group is 87.5%, orally given 200 µg three proteins with living recombinant bacteria individually. At 7 days post challenge, oocyst production of *E. tenella* decreased along with the content of expressed protein compared with the unvaccinated chickens, the best group is 14.8%, orally given 200 µg three living recombinant bacteria.

Conclusion: TA4, EtMIC2, and SO7 could induce partial protection against *E. tenella*, the best dose is used 200 µg three recombinant proteins with living bacteria.

B26 p COCCIDIOSIS CONTROL WITH BAYCOX®
(TOLTRAZURIL) AS THE SOLE ANTICOCIDIAL
ADMINISTERED FOR TWO CONSECUTIVE DAYS
IN THE DRINKING WATER

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A 56-day (d) floor pen study (70 birds per each of 40 pens) was conducted to determine the appropriate time to administer Toltrazuril (BAY) at 7 mg/ kg BW for control of coccidiosis. The feeding program was starter feed d 0 to 14, grower feed d 15 to 35 and withdrawal feed (nonmedicated (NM)) d 36 to 56. The treatments (TRT) were 125ppm Nicarb (NIC)/noBAY/66ppm Salinomycin(SAL), SAL/noBAY/SAL, BAY d 2-3, BAY d 6-7, BAY d 10-11, BAY d 14-15, BAY d 18-19, and NM/noBAY/NM (NM). Litter was seeded for the one-day olds with field strains of *E. acervulina*, *E. maxima*, and *E. tenella* causing a significant infection. The average live weight and feed conversions on all weigh periods for the SAL/SAL program were significantly poorer compared to all BAY and NIC/SAL TRT birds. Large numbers of oocysts were found from day 21 (12,980) to day 35 (4,416) as well as lesion scores in the intestine indicating subclinical coccidiosis during this time period. BAY provided very good control of the coccidiosis: all treated birds showed good weights and feed conversions at all weigh periods. Very little live weight differences among the BAY TRT were observed. The BAY d 2-3 pens did have a large number of oocysts per gram litter on d 35 (15,581), however even after this early treatment no relapse at day 56 was discovered. All other BAY TRT had low numbers of oocysts after TRT, low lesions (<1) and no reoccurrence of coccidiosis. BAY 10-11, 14-15 and 18-19 had good performance at all weigh periods, in spite of a very high challenge at TRT. Very little performance or control differences were observed between these 3 TRT. It can be inferred from the overall results that the use of BAY as the sole anticoccidial for two consecutive days in the drinking water between day 10 and 18 would be the best time for good coccidiosis control allowing full performance.

B27 p COCCIDIOSIS CONTROL WITH BAYCOX® (TOLTRAZURIL) IN CONJUNCTION WITH ANTI-COCCIDIAL MEDICATED OR NON-MED. FEED

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A 42-day floor pen study (70 chicks per each of 40 pens) was conducted with broiler chickens to compare the anticoccidial (AC) efficacy of Toltrazuril (BAY) given on Days 18 and 19 in the drinking water at 7 mg/kg BW in combination with and without in-feed ACs. The treatments (TRT) were: 125 ppm nicarbazin (NIC) in starter feed (S, Days 0-14) and 66 ppm salinomycin (SAL) in grower (G, Days 16-35) feed, or SAL in S and G, or nonmedicated (NM) S and SAL-G, or NM-S and -G (InfectedNM): all with or without additional Toltrazuril (BAY); and uninfected NM (UINM) control. On Day 14 birds in each treatment were exposed to litter seeded with field strains of *E. acervulina*, *E. maxima*, and *E. tenella*. The coccidial infection in the INM group caused a significant ($P < 0.05$) weight reduction, increased feed conversion, and moderate lesions in all intestinal areas scored. Coccidial infection was moderately controlled in the NIC/SAL/no BAY and SAL/SAL/no BAY TRT. On each measurement day, means for bird weight and feed conversion in the NM/NM/BAY TRT were equal to or better ($P < 0.05$) than treatments that did not include BAY. TRT with BAY were equal to the UINM in performance and lesion control. The TRT with BAY almost completely eliminated all coccidial lesions and oocyst shedding. The performance data, lesion scores and oocyst counts showed that a 2-day TRT with Toltrazuril completely controlled these three major species of coccidia. No relapse of infection was seen on Day 42. Toltrazuril can be used for supplemental control with various in-feed AC programs or, depending on infection pressure, as a primary AC with nonmedicated feed.

B28 p EXPERIENCES ON THE EFFICACY OF TOLTRAZURIL AND DICLAZURIL AGAINST NATURAL INFECTIONS WITH EIMERIA SPP. IN LAMBS

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Background: The purpose of this study was to gather experiences on the therapeutic potential of toltrazuril and diclazuril for the treatment of coccidiosis in lambs.

Method: Groups of ten naturally infected lambs were treated once with either toltrazuril (20 mg/kg LW) or diclazuril (1 mg/kg LW) and compared with an untreated control group. The efficacy of the treatments was assessed by counting the number of oocysts excreted per gram of faeces (opg) over the 3-week duration of the trial. The faecal consistency was also determined.

Results: Coccidial differentiation revealed a mixed infection with seven *Eimeria* species which occurred in all treatment groups and at all time points. In both groups the oocyst excretion declined after the treatment. This effect was more pronounced and sustained in the lambs treated with toltrazuril (20 mg/kg LW). Diclazuril (1 mg/kg LW) reduced oocyst excretion for a period of 12 days, after which levels rose again massively. **Conclusion:** Under the conditions of the trial (late treatment), toltrazuril (20 mg/kg LW) controlled a natural mixed infection in lambs completely and oocyst excretion remained low.

B29 p ISOLATION OF TOXOPLASMA GONDII IN A PIG FROM A LOCAL ABATTOIR IN ZIMBABWE.

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Inoculation of pepsin digested muscle (heart and diaphragm) from a slaughter pig (*Sus scrofa*) into Balb C mice resulted in the isolation of *Toxoplasma gondii*. The *T. gondii* isolate was pathogenic to mice. The six infected mice were retarded in growth when compared to the uninfected controls. Mouse No. 3 and Mouse No. 6 died on days 14 and 16 post inoculation (PI), respectively. Parasites were observed in lung smears of both mice. However, no parasite cysts were observed in the brain tissue of the two mice. Serology at day 15 PI revealed anti-*T. gondii* antibody titres of 1/500 on the modified agglutination test (MAT) in 4/5 of the surviving mice. The surviving mice were sacrificed 13 weeks post infection and multiple *Toxoplasma gondii* cysts were seen in the cerebrum.

B30 p NEOSPOA CANINUM SEROPREVALENCE IN DOGS FROM PARMA PROVINCE OF ITALY.

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Background: *Neospora caninum* has been reported in Italy previously, both in cattle and dogs. There is however very little information regarding the prevalence of exposure in the canine population. Here, we report seroprevalence values for dogs in the province of Parma.

Method: Sera (n=228) were collected from dogs during routine visits at the Clinical Surgery Department at the University of Parma and examined for antibodies to *N. caninum* with an indirect fluorescent antibody test (IFAT), employing the NC-1 strain of the protozoan, isolated by Dubey in 1987. A questionnaire was completed for each dog, including sex, age, breed, contact with cattle and feeding habits.

Results: Of the 228 dogs tested, 49% were males, 51% were females. 16% of dogs were reported as eating raw meat (bovine) regularly and 6% of dogs were reported as having contact with cattle farms. Antibodies to *N. caninum* at titres over 1:50 were detected in 17% of tested dogs (38/228). Of these, 59% were males, 41% females. 6.4% of positive dogs ate raw meat regularly while 13% had contact with cattle farms.

Conclusion: The findings reported here indicate that *N. caninum* is present in the Parma province of Italy and that contact with cattle farms may represent a risk factor for exposure to the parasite.

C

Immunology and parasite pathology

C1

IMMUNE RESPONSES IN AFFERENT LYMPH OF NEMATODE RESISTANT AND SUSCEPTIBLE SHEEP DURING INFECTION WITH *TRICHOSTRONGYLUS COLUBRIFORMIS*

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Background: In order to obtain insight into localized immune responses of lambs to the gastro-intestinal nematode parasite *T. colubriformis*, a pseudo-afferent lymph duct was fitted with a cannula and the lymph draining the intestinal mucosa tested for humoral and cellular constituents. 12 lambs that differed in genotype (nematode resistant or -susceptible) or phenotype (immune or naïve) were used.

Method: Real-Time PCR was used to test for the abundance of cytokine gene transcripts (IL-4, IL-5, IFN- γ , IL-10, IL-13 and TNF- α). Parasite-specific IgM, IgG1, IgG2, IgA and total IgE levels were assayed by ELISA.

Results: The most consistent changes detected after nematode challenge were large increases in parasite specific IgA and total IgE levels. In immune lambs, increased transcription of the IL-5 and IL-13 genes was seen in the third week after challenge. There was no consistent pattern to the mRNA profiles for the other cytokines examined.

Conclusion: In immune lambs regardless of the genotype, temporally increased transcription of the IL-5 and IL-13 genes appears to correlate with elevated IgE secretion into lymph.

C2

CROSS PROTECTION STUDIES WITH GUT MEMBRANE GLYCOPROTEIN ANTIGENS FROM *HAEMONCHUS CONTORTUS* AND *TELADORSAGIA CIRCUMCINCTA*

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Background: Gut proteases of *Teladorsagia* and *Haemonchus contortus* were compared with a view to determining whether they could be cross-protective antigens.

Method: Glycoproteins extracted from each species were compared by SDS-PAGE and immunoblotting as well as in vaccine protection trials in sheep

Results: *Haemonchus* gut proteases were extremely effective against homologous challenge, reducing egg and worm counts by more than 99% and 92%, respectively, but did not provide any useful cross-protection against either *T. circumcincta*, *T. axei* or *C. oncophora*. In a reciprocal experiment, sheep immunised with equivalent glycoproteins from *T. circumcincta*, were not protected against homologous challenge, but were partially cross protected against *Haemonchus*.

Conclusions: *Teladorsagia* does not ingest sufficient immunoglobulin for its gut antigens to be protective.

C3

IMMUNISATION OF LAMBS AGAINST *TELADORSAGIA CIRCUMCINCTA*

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Background: Membrane-bound extracts enriched for cysteine protease activity from adults of the blood-feeding abomasal nematode *Haemonchus contortus*, confer strong protection in lambs against homologous challenge. This study was initiated to define if equivalent extracts from another abomasal nematode, the non-blood feeder, *Teladorsagia circumcincta*, could protect against homologous challenge.

Method: Adult *T. circumcincta* were extracted successively with PBS, PBS+Tween 20 and PBS + 2% Triton X-100. The latter extract was passed over Thiol-Sepharose and the proteins which bound to this medium (TSBP) were used as immunogen in two separate protection trials. Lambs between 7 and 10 months of age were used. A lambda ZAP cDNA expression library is being screened with sera from vaccinated/protected lambs and the outcome of this analysis will be reported.

Results: Final worm burdens and faecal egg counts were reduced by 63% and 71% respectively in the first trial and by 25% and 21% respectively in the second trial. Analysis of the antibody responses is proceeding and these data will be reported.

Conclusion: TSBP did confer a degree of protection but this varied between trials, variation which may reflect the worm's feeding habits. Alternatively, this variation may reflect differential antibody responses.

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C4

EVALUATION OF PROTECTIVITY OF ACEY-1 CYSTEINE PROTEINASE

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Background: There have been many attempts to immunise various hosts against hookworm infections. In the present study we compare results of protein and DNA vaccination of hamsters against *Ancylostoma ceylanicum* infection based on ACEY-1 cysteine proteinase and its cDNA (GenBank accession number AF 120501).

Method: In order to inactivate the recombinant protein, acey-1 cDNA was mutated using PCR method: Cys - 123 was replaced with Ala amino-acid. Recombinant ACEY-1 protein was obtained from *E.coli* strain containing prokaryotic expression vector pGEX-acey-1-M. Bacterial lysate was separated using SDS-PAGE and the ACEY-1 protein was isolated by electroelution. Groups of hamsters were injected intradermally with 50 μ g of the recombinant protein or intramuscularly with 50 μ g of acey-1 - pcDNA plasmid twice in two week interval. The third group of hamsters was injected with the same amount of the empty plasmid. All experimental groups were challenged with 60 L3 larvae of *A. ceylanicum* three weeks after the second vaccination. On day 21 after the challenge hamsters were euthanised and worm burdens estimated.

Results: Parasitological results obtained during both DNA and protein vaccination experiments in hamsters revealed reduced effectiveness of the challenge infection. However, in DNA vaccinated group the reduction was more pronounced and reached up to 60%.

Conclusion: Vaccination of hamsters with ACEY-1 cDNA resulted in reduced effectiveness of the challenge infection of *A. ceylanicum* larvae.

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C5

BODY PROTEIN AFFECTS EXPRESSION OF IMMUNITY TO NEMATODES IN EWES

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Background: Our overall hypothesis is that periparturient breakdown of immunity has a nutritional basis. Here, we tested the hypothesis that breakdown of immunity to abomasal nematodes and its relationship with dietary protein supply in ewes depend on body protein- but not on body fat reserves.

Method: We trickle infected three groups of 20 twin-bearing ewes each, with *Teladorsagia circumcincta* from day₄₃ to day₄₂, relative to parturition, and measured faecal egg counts (FEC) and plasma IgE against L₃ antigen. Until day₂₁, the ewes were fed either to maintain body reserves (HH), to maintain body protein- and reduce body fat reserves (HL), or to reduce body reserves (LL). We then iso-energetically fed each ewe daily either a scarce (LP) or adequate (HP) amount of metabolizable protein until day₄₂ (n = 10).

Results: By day₂₁, HL- and LL-ewes had smaller back fat depths (~body fat reserves) than HH-ewes, and LL-ewes had smaller muscle depths (~body protein reserves) than HH- and HL-ewes (P<0.001). Plasma IgE levels increased from day₄₃ onwards, peaked around day₀ and rapidly decreased until day₄₂. During lactation, and across treatments, LL-ewes had higher FEC and plasma IgE levels than HH- and HL-ewes (P<0.01), and LP-ewes had higher FEC than HP-ewes (P<0.05), but similar IgE levels. LL-ewes offered the LP food had higher FEC than any of the other ewes (P<0.05).

Conclusion: The results support the view that body protein- but not body fat reserves affect expression of immunity to abomasal nematodes. Body protein may overcome at least some of the consequences of dietary protein scarcity on periparturient breakdown of immunity to abomasal nematodes.

Acknowledgement: This work was financially supported by an EU grant (FAIR3 CT96 1485) and SERAD.

C6

KINETICS OF LOCAL CHANGES DURING A PRIMARY COOPERIA ONCOPHORA INFECTION IN CALVES

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Purpose: Primary infection with 100,000 L3 of the intestinal nematode *C. oncophora* in 3 months old calves leads to expulsion of the parasite around 35-42 days post infection. We aimed to characterise the events in the infected gut to get a better understanding of the mechanisms underlying the development of parasite specific immunity.

Methods: Twenty-four three months old calves were orally infected with 100,000 L3 *C. oncophora*, 12 calves served as non-infected controls. The immunological and parasitological changes occurring in the infected gut were investigated by sequential necropsy of 8 infected and 4 non-infected calves at 14, 28 and 42 DPI. The small intestine was divided in six segments starting from the pylorus to the ileocaecal junction. All segments were processed separately.

Results: Worm counts showed that during the patent infection the parasite preferentially homes in the proximal gut. By the time of necropsy a shift from the proximal to the distal gut was observed. Histological examination revealed an increased number of eosinophils in the infected gut by 42 DPI. No changes occurred in the mast cell population. The antibody response in the intestinal mucus was monitored by *Cooperia* specific IgG1, IgG2, IgA as well as total IgE ELISA. *Cooperia* specific IgG1 and IgA, but not IgG2 were significantly correlated with parameters expressing the exposure to the parasite, as well as their effect on the fecundity and the expulsion of the worm population.

Conclusion: Our data suggest the development of a Th2 biased immune response in the infected animals, which is involved in the regulation of the expulsion of *C. oncophora* from the intestine of primary infected calves.

C7

SERUM IGE IN CATTLE INFECTED WITH THE LUNGWORM DICTYOCAULUS VIVIPARUS

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Background: In cattle there is a rapid development of immunity against lungworm which does not correlate with serum IgA, IgG1, IgG2 or IgM levels. We used a mAb against sheep IgE (IE7) to measure total and parasite specific IgE in serum of calves with primary and challenge lungworm infection.

Method: Specificity of mAb IE7 for bovine IgE was checked by affinity chromatography and western blotting. In 2 independent experiments, calves were primary infected at day 0 with 30 L3 (exp.1) or with different doses of 30 to 960 L3 (exp. 2) of lungworm. At day 35 they were challenged together with challenge control calves with 2000 L3. Blood was collected for parasite specific IgA, IgE, IgG1, IgG2 and IgM (Western blot and ELISA) and for total IgE (ELISA). All calves were necropsied at day 70 (exp. 1) or at day 46 (exp. 2) and worm counts were performed to determine the protection against reinfection.

Results: mAb IE7 was demonstrated to be specific for bovine IgE. In both experiments the primary infection resulted in partial protection, ranging from 0 to > 99 % protection of the individual calves compared with the control group. Serum total IgE levels increased after infection and were significantly correlated with protection. Parasite specific IgE in serum depleted of IgG and IgM was detected (western blots) only in protected calves. Parasite specific IgA, IgG1, IgG2 and IgM in serum increased after infection, but were not correlated with protection.

Conclusions: Serum IgE levels, but not IgA, IgG1, IgG2 or IgM correlate with protection. Further research will concentrate on the specificity and the effector site of IgE in lungworm infections

C8

THE ROLE OF INFLAMMATION IN INFECTIONS WITH OSTERTAGIA CIRCUMCINCTA

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Background: Infection of ruminants with *Ostertagia* spp. results in increased abomasal pH. It is not certain whether the loss of secretory ability stems from direct effects of the worms on host epithelial cells or is a by-product of the host's response to the presence of the worms.

Method: Extracts of adult *O. circumcincta* or live L3 were injected into tissue chambers implanted sub-cutaneously in parasite-naïve lambs. After 24 hours, fluid was extracted from the chambers and added to the culture fluid bathing dispersed rabbit gastric glands. Acid secretion was assessed indirectly by accumulation of the weak base, ¹⁴C-Aminopyrine.

Results: The injection of adult extract or live larvae into chambers consistently induced inflammation, with large numbers of neutrophils and small numbers of eosinophils migrating into affected chambers. Chamber fluid obtained following injection of adult extract resulted in significant inhibition of aminopyrine accumulation in 3 out of 4 experiments (P<0.01). The degrees of inhibition obtained were 57, 49 and 41%. Fluid from chambers receiving larvae were also inhibitory in 2 out of 2 experiments (P<0.01), giving inhibition of 28 and 51%. The addition of the crude adult extract directly to the gastric glands, did not result in inhibition, but in one experiment caused a 25% potentiation of the response to histamine (P<0.05).

Conclusion: This series of experiments raises the possibility that the host's acute inflammatory response to parasites may itself be responsible for the failure to maintain abomasal acidity in ostertagiosis.

C9

ANOREXIA IN ABOMASAL PARASITISM IS NOT MEDIATED BY GASTRIC NERVES

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Aim: The involvement of abomasal sensory nerves, which travel in the vagal and sympathetic trunks, in mediating reduced food intake was assessed in lambs infected with a single dose of *O. circumcincta*.

Experimental design: Fifteen, 6-month-old parasite-naïve lambs were fitted surgically with abomasal cannulae and divided equally between the following groups: Group C - gastric nerves left intact; Group V - bilateral abomasal vagotomy; and Group VS - bilateral vagotomy and sympathectomy (celiac ganglia removed). Following a 15-day recovery period, each sheep was given 100,000 L3 *O. circumcincta* per os on Day 0. Appetite was measured daily and serum gastrin, pepsinogen and leptin and abomasal pH monitored regularly until necropsy on Day 37.

Results: Lambs in the VS group had an overall reduced feed intake after surgery compared with the C and V groups. Even complete sensory denervation of the abomasum did not prevent a fall in food intake beginning about 8 days after infection. Food intake in individual animals did not follow changes in serum gastrin or leptin closely, although hypergastrinaemia was apparent in all lambs and serum leptin increased overall during the third week of infection.

Conclusions: Stimulation of sensory or pain fibres in the abomasal wall did not appear to contribute significantly to anorexia in parasitised animals. In individual animals, no single factor was identified as the mediator of appetite suppression during the course of infection.

C10

HORMONES AND IMMUNITY IN THE ENDO-PARASITE PERIPARTURIENT RISE OF SHEEP

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Background: Genetic, endocrine, immunological and environmental factors have been shown to influence the periparturient rise (PPR) of gastrointestinal strongyle (GIS) by several Authors, even though the results are often contradictory. Moreover, few studies dealt with the factor interactions in the PPR dynamics. In May-September 2000, the hormonal and the aspecific immunity profile of 15 sheep in relation to their fisiological and parasitological status was studied.

Method: sheep were divided in 3 groups: a) 4 pregnant sheep GIS negative, b) 9 pregnant and c) 2 non-pregnant sheep both GIS positive. Blood and faecal samples were taken once a week or 3 times per week in the periparturient period. Faecal egg counts were performed. All the animals were tested for progesterone (P4), estradiol (E2), cortisol (C) and for the serum lysozyme, haemolytic complement and bactericidal activity and total serum protein.

Results: 14 sheep out of 15 showed a PPR of strongyle eggs, with individual differences in the emission intensity. On average, PPR began before parturition and stopped within one week following parturition, lasting 2-4 weeks. The "lactation rise" was not evident. Hormonal profile (P4, E2) in all the sheep was consistent with the literature. PPR followed the maximum P4 concentration in almost all the sheep, whereas E2 seemed not to be related to PPR. The C levels and the aspecific immunity profile did not change significantly in relation to the fisiological and parasitological status, except for the level of haemolytic complement activity, highest in negative sheep.

Conclusions: Among the parameters tested, only P4 showed a temporal relationship with PPR. Further studies are needed to assess if the influence of P4 on the gastrointestinal parasite PPR could be a direct (presence of P4 receptors in parasites) or an indirect mechanism (possible interaction of P4 with specific immunity).

C11

PASTURES ENRICHED WITH *Lotus pedunculatus* DO NOT ENHANCE RESISTANCE AND RESILIENCE TO HELMINTH INFECTION

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Background: Forages containing condensed tannins (CT) have been shown to support high levels of animal production during helminth infection (ie. resilience) and condensed tannin extracts have been shown to enhance both resistance and resilience to helminth infection. This experiment was designed to test the hypothesis that lambs grazing pastures enriched with *Lotus pedunculatus* (CT) would have greater resistance and resilience to infection than those which grazed pastures enriched with *Trifolium repens* (non-CT).

Method: Replicate plots (0.4ha) were oversown with *L. pedunculatus* or *T. repens* and 10 Merino weaners (6-7 mo.) were grazed in each plot for 10 weeks. Within each plot, animals received an ivermectin capsule (n=4) on day 0 or a single infection of 15,000 *T. colubriformis* L3 (n=6) on day 7.

Results: Faecal egg counts^{0.33} (FEC) were lower in animals which grazed plots enriched with *T. repens*. At 10 weeks p.i. worm burdens^{0.33} of animals which grazed in *T. repens* plots (13,652) were significantly lower than those which grazed in *L. pedunculatus* plots (19,249). Live weight of capsuled animals was unaffected by legume species. Infection did not reduce live weight of animals which grazed in *T. repens* plots but from week 6 p.i. live weight of animals in *L. pedunculatus* plots was significantly reduced by infection.

Conclusion: When compared with *T. repens*, inclusion of the CT legume, *L. pedunculatus*, in grass-based pastures, did not improve growth and resistance of lambs infected with *T. colubriformis*.

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C12

DOWN REGULATION OF TH2 CYTOKINES DOES NOT ALTER PROTECTION TO *STRONGYLUS VULGARIS*

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Immunization of naïve ponies with radiation-attenuated third stage larvae (IrrL3) provides nearly 100% protection from *Strongylus vulgaris* infection. Protective immunity is associated with a dominant Th2 cytokine response and anamnestic eosinophilia. In contrast, vaccination with soluble adult worm antigen (SAWA) in Ribi adjuvant provides no protection and is characterized by increased Th1 cytokines and diminished eosinophilia. In the current study, cytokine, humoral, and histologic responses were characterized to determine whether prior immunization with Ribi-SAWA would alter the IrrL3 induced protective immune response to *S. vulgaris*. Interestingly, ponies immunized with Ribi-SAWA prior to IrrL3 vaccination were still protected against challenge even in the face of significant alterations in the Th2 driven immune response. These ponies produced higher INF- γ levels and reduced IL-5 levels following challenge. Corresponding with the reduced IL-5 levels, the eosinophilia response was also decreased and delayed one week behind animals vaccinated only with IrrL3. In contrast, IL-4 levels were elevated in naïve and protected ponies indicating that IL-4 was not required for protection against *S. vulgaris*. Also, the humoral immune response in ponies pre-immunized with Ribi-SAWA was reduced in response to SAWA, L4 somatic antigens, and L3 surface antigens when compared to ponies vaccinated solely with IrrL3. These data indicate that Th1 priming by Ribi-SAWA modifies the IrrL3 induced humoral and Th2 cytokine response but this alteration is insufficient to alter the protective response.

C13

ANTIBODY RESPONSE TO L3 ANTIGENS OF EQUINE CYATHOSTOMES

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Previous immunologic studies of equine cyathostome infections have described antibody responses against adult somatic extracts in resistant and non-resistant groups of ponies. Remarkable findings were that antibody was passively transferred to foals and increases in IgG(T) titers were significant in immune animals. The present study focused on antibody response to L3 antigens in these same groups. Results show a transfer of maternal antibodies to somatic extracts of early L3 and to surface of developing DL3 (IgG(T)) but not against surface of early L3s (EL3s). Antibody to *Strongylus vulgaris*, *S. edentatus*, but not *Strongyloides westeri*, or *Parascaris equorum* reacted to surface of cyathostome EL3. However all of these sera reacted to surface DL3. Maternal transfer of antibody was demonstrated in foals to somatic extracts of EL3 (IgG), and surface of DL (IgG, IgG(T)) but not to surface of EL3(IgG, IgG(T)). IgG(T) titers to somatic and L3 surface antigens increased following challenge of parasite free ponies. However, significant differences between the antibody responses of immune and nonimmune groups of ponies were not detected, suggesting that these antibodies may not play a significant role in protective immunity.

C15

ALTERNATIVE MIGRATION ROUTES OF ASCARIS SUUM IN THE PIG

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Background: Experiments were conducted to investigate possible alternative routes of extra-intestinal migration of *Ascaris suum* larvae in the pig.

Method: Pigs were infected either via artificial injection of newly hatched larvae into caecal veins (IV), caecal lymph nodes (LN) or intraperitoneally (IP), while control animals were inoculated orally with infective eggs (PO). Two pigs per inoculation route were necropsied on days 1, 4 and 13 post inoculation, respectively.

Results: The percentage of larvae recovered was considerably greater in pigs inoculated IV or PO on each necropsy day. However, irrespective of inoculation route *A. suum* larvae were able to complete migration to the small intestine by day 13. The number of liver lesions was higher in IV and PO inoculated pigs on days 1 and 4 p.i. while on day 13 considerable numbers of liver spots were observed in all pigs.

Conclusion: The results demonstrate that migrating *A. suum* larvae reach the liver irrespective of infection route in primary infected pigs, but larvae were delayed following injection into caecal lymph nodes of into the peritoneal cavity.

C14

INFLUENCE OF MATERNAL IMMUNITY UPON THE ESTABLISHMENT *ASCARIS SUUM* IN ONE WEEK OLD PIGLETS

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Background: The aim of the present study was to investigate the influence of maternal immunity on the establishment of an experimental *Ascaris suum* infection in the piglets first week of life.

Method: Five immunized gilts, trickle inoculated twice weekly in the feed with 10,000 *A. suum* eggs for 6 months and 4 parasite naive gilts were used. All piglets were inoculated orally with 50 *A. suum* eggs on day 4 and 7 after birth. Post examination was performed at 10 weeks of age.

Results: Median worm count in both groups were significantly different ($p < 0.05$). Worm counts in both groups were heavily overdispersed and the prevalence of infection in piglets from immunized sows (89.4%) was significantly higher ($p < 0.001$) than in piglets from control sows (51.6%).

Conclusion: Gilts long term immunized with *A. suum* eggs unexpectedly didn't transfer passive *A. suum* resistance to their one week old piglets compared to piglets from parasite naive sows. The immunological explanation of this phenomenon remains to be solved, but development of tolerance following long term *A. suum* exposure may have been transferred to the offspring from immune mothers.

C16

ENHANCED FAECAL EXCRETION OF SALMONELLA IN PIGS INFECTED WITH *OESOPHAGOSTOMUM*

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Background: To determine the possible interaction between the nematode *Oesophagostomum* spp. and *Salmonella* Typhimurium in pigs.

Method: Three groups of 10 pigs were infected with: A- a mixture of *Oesophagostomum dentatum* and *O. quadrispinulatum*; B- *O. dentatum*, *O. quadrispinulatum* and *S. Typhimurium*, and C- *S. Typhimurium*. Pigs in groups A and B were trickle infected with *Oesophagostomum* three times weekly throughout the experiment, and 19 days after, groups B and C were challenged once with *S. Typhimurium*. The pigs were euthanised on days 16 and 17 post *Salmonella* infection

Results: Pigs with dual infection of worms and bacteria excreted significantly higher levels of *S. Typhimurium* in faeces than worm free pigs. *Salmonella* was detected both in the caecum and colon with conventional bacteriology in the majority of pigs with worms, whereas, the majority of pigs without worms had *Salmonella* only in the colon. Immunohistochemical examination detected *S. Typhimurium* in seven out of 10 pigs with worms and only in two out of 10 pigs without. *S. Typhimurium*, did not appear to influence worm burdens.

Conclusion: As many pigs are infected with worms and particularly with *Oesophagostomum* the possibility exists that interaction between intestinal worms and bacteria may play a part in *Salmonella* outbreaks.

Acknowledgement: This study was supported by grants from The Danish Research Foundation and the Danish Veterinary Laboratory.

C17 EFFECTS OF COLOSTRUM ON EARLY SCHISTOSOMA MATTHEEI INFECTION IN CALVES

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Background: Cattle living in endemic areas develop protective immunity against *Schistosoma mattheei* infection. The objective of the present study is to determine whether the presence of chronic *S. mattheei* infection in the cow affects the susceptibility of the calf to an early infection.

Methods: A total of 18 calves were purchased at birth. 6 born from mothers not infected with *S. mattheei*, fed colostrum from their mothers (group1). 6 born from non infected mothers, fed colostrum from infected mothers (group2) and 6 born from infected mothers, fed colostrum from their mothers (group3). All were exposed to an experimental challenge of 1000 cercariae 1 month after birth, and perfused 12 weeks later for worm and tissue egg counts. From 6 weeks after the challenge faecal egg counts, miracidial counts and immunological parameters were also monitored.

Results: In group 1 following results were observed: average total worm counts 384; average number of females 120; total tissue egg counts (TEC): 1.121.799. Group 2 showed a reduction of 42.4%, 28% and 42%, respectively. Group 3 had a reduction in total worm counts of 30% but showed no difference in female worms nor in TEC. Overall FEC were significantly higher in group 3, compared to group 1 & 2

Conclusions: Results suggest that infection of cows and/or colostrum may influence the establishment of an early infection in calves. Immunological analyses of the colostrum and the serum samples collected from cows and calves are under progress.

C18 GIARDIA IMMUNOPROPHYLAXIS AND IMMUNOTHERAPY IN DOGS AND CATS

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Background: *Giardia duodenalis* is an enteric protozoan parasite of mammals, birds, amphibians, and reptiles. The immune status of the host influences both the susceptibility of the host to infection and the severity of clinical signs. A vaccine was evaluated for prophylaxis and treatment.

Method: A commercial *Giardia* vaccine (GiardiaVax™) was administered to both dogs and cats (n= 60) while controls received placebo vaccine (n=40). Animals were challenged with human isolates of *Giardia* and examined for the presence of clinical signs of intestinal disease, fecal cysts or intestinal trophozoites. In a separate study, dogs (n=14) with chronic *Giardia* infections where chemotherapeutic treatment had failed to eliminate the parasite, were treated by two or three injections of GiardiaVax™.

Results: *Giardia* vaccination resulted in the rapid elimination of *Giardia* from the challenged animals. It also prevented clinical signs. *Giardia* vaccine was also able to eliminate the parasite in dogs which chronic giardiasis.

Conclusion: Immunoprophylaxis (vaccination) offers a method to control the infection in high-risk populations whether they are symptomatic or asymptomatic. The *Giardia* vaccine helps to break the fecal-oral and waterborne transmission by reducing environmental contamination. Immunostimulation using a *Giardia* vaccine appears also to be beneficial for parasite clearance or elimination of clinical signs in dogs with *Giardia* strains with chronic unresponsive infections.

C19 HUMORAL RESPONSE OF RATS IMMUNISED WITH CYSTEINE PROTEINASE OF FASCIOLA HEPATICA

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Background: Our previous experiments have shown that intramuscular injection of Sprague-Dowley rats with cDNA encoding for a cysteine proteinase (CP) of *F. hepatica* induce a high level of protection against subsequent fluke infection. The aim of the present study was to compare humoral immune responses induced by cDNA or protein form of CP.

Method: Group I of 6 rats was vaccinated intranasally twice, in three week intervals, with CPcDNA. Groups II and III were immunised with CP protein injected intramuscularly or intraperitoneally. All vaccinated rats and 6 control animals were challenged orally with 25 metacercariae. On day 0, 21, 42 and 63 after the challenge, blood samples were collected for evaluation of PC specific antibody responses.

Results: cDNA vaccination induced the highest of all vaccinated groups IgG1 isotype response and also a clear IgG2a response. The challenge infection evoked in Group I an anamnestic IgA antibody response. Rats vaccinated with CP-protein showed clear IgG2a, IgG2b and IgA antibody responses following vaccination (on day of challenge) and no significant increase in the antibody level was observed after the challenge. Neither protein or cDNA vaccination induced clear IgE antibody response.

Conclusions: Intranasal immunisation of rats with CPcDNA favours a TH2 regulated antibody response. Intramuscular or intraperitoneal injections of CP protein stimulate both Th1 and Th2 dependent antibodies.

Acknowledgement: The work reported here was supported through a grant of Polish Committee for Scientific Research No. Z-KBN 004/PO4/98.

C1 p EICOSANOIDS OF PARASITIC ORIGIN- DO THEY PLAY A ROLE IN HOST-PARASITE INTERACTION?

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Eicosanoids, lipid mediators produced by a wide variety of cells and organisms, have multiple functions in mammals, including immunomodulation. Macrophages are a major source of prostaglandins (PG), while other cells of the immune system produce mainly leukotrienes (LT). The release of PGE2, a key eicosanoid in inflammatory and immune reactions, supports enhanced blood flow and chemotaxis of cells but also leads to downregulation of lymphocyte activity. LT, especially LTB4, induce the liberation of pro-inflammatory PG and regulate blood flow. Parasites from different phyla have been shown to produce and release eicosanoids which are supposed to influence the host tissue, especially immune cells, in their immediate surroundings. Eicosanoids are known to be involved in schistosomal egg granuloma formation and in inhibition of immune cell function in African trypanosomiasis. However, it appears that parasite-derived eicosanoids are continuously produced and only partially released. Appreciable changes in serum eicosanoid levels after infection with subsequent generalised immunomodulation cannot be attributed to parasite-derived eicosanoids since these mediators are short-lived and therefore of limited distribution in the host's organism. However, the parasite are obviously able to locally modulate defense mechanisms in favour of its own establishment and survival. Infection models can elicit enzyme expression and eicosanoid production and release upon stimulation, direct effect of parasite lipids on selected host cell populations and influence of eicosanoid depletion on parasite performance.

C2 p

EVALUATION OF GUT MEMBRANE PROTEINS OF HAEMONCHUS CONTORTUS AS CANDIDATE VACCINE ANTIGENS IN SHEEP GRAZING NATURALLY INFECTED PASTURE.

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Background: Numerous trials have demonstrated that gut membrane proteins from *Haemonchus* can protect against an artificial challenge, but the potential against natural challenge in a commercial sheep rearing area has not been investigated.

Method: In a trial lasting eleven months in South Africa, faecal egg counts and haematocrits of sheep vaccinated with gut membrane proteins of adult *Haemonchus contortus* were compared with unvaccinated controls grazing pasture contaminated with the parasite.

Results: Vaccination reduced egg output by more than 82% on average during one four month period of the trial and simultaneously significantly reduced the degree of anaemia and deaths due to haemonchosis. Although vaccine immunity was not sufficiently long lasting to prevent a surge in egg output which occurred after the onset of a period of irrigation, re-vaccinating the sheep at this point cleared their newly acquired infection and rapidly restored protection to approximately the level observed beforehand.

Conclusions: It was clear that a vaccine based on parasite gut membrane proteins could offer substantial benefits in the control of natural haemonchosis.

C3 p

HUMORAL IMMUNE RESPONSE OF CALVES TO SOMATIC ANTIGENS AND EXCRETION/SECRETION PRODUCTS OF THE IMMATURE PHASES OF *Haemonchus placei* (Place, 1833) Ransom, 1911

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Background: *Haemonchus placei* is one of the most important nematodes in Brazil, because of its haematophagous habits and high pathogenicity. The aim of this work was to study the humoral immune response against antigens of immature phases of *Haemonchus placei* in calves using different schemes of infection. **Method:** Calves were divided into groups of 5 and 9 months: A and E were the non infected controls. Group B (5 months) was infected with two doses of 50,000 and na additional dose of 100,000 infective larvae (L3) of *H. placei*, group C (5months) and group D (9months) were prime infected with 100,000L3. **Results:** It was observed a 4 weeks pre patent period. The average of adult worm burden was 13,025 for group B, 27,650 for group C and 21,133 for group D. The larval antigens were obtained through in vitro culture of the nematode and were recovered from somatic antigens and from excretion/secretion products of third and fourth stages. The humoral immune response to *H. placei* infection stimulates the production of specific antibodies against different antigens and phases of development of the parasite, the response is predominantly IgG, sub class IgG1. It was possible to observe an age and number of infections effect in the build up of the humoral immune response. The immune reaction of IgG occurs against proteins of many different molecular weights, including: a) L3 soluble somatic antigens: 40, 44, 56 and 80 kDa for animals of 5 months; 24, 44, 50 54, 60 and 70 kDa for the 9-month animals. b) L4 soluble somatic antigens: proteins of 40, 50 and 35 kDa in the animals of 5 months age, 24, 44, 50, 54, 60 and 70 kDa for the 9-month animals. c) L3 excretion/secretion antigens: proteins of 19, 30, 50 and 75 kDa for the younger animals and 50 and 75 kDa for the animals of 9 months. d) L4 excretion/secretion antigens: proteins of 14, 19, 27, 37, 40, 50, 54 kDa for the younger animals and 14, 35 40 and 50 kDa for the older ones. **Conclusions:** The outcome of infection by *H. placei* is influenced by the age of the host and the number of infections, which affect the build up of the humoral immune response against antigens of the various developmental stages of the parasite.

C4 p

PRELIMINARY CHROMATOGRAPHIC STUDY OF DICROCOELIUM DENDRITICUM ANTIGEN

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Background: The aim of this research was to try to separate different fractions of the *D. dendriticum* antigen to determine their specificity and protection capacity against the parasite.

Method: Somatic antigen samples were studied by ion exchange chromatography (Column: Scharlau Science Nucleosil 300-5 C-18) in reverse phase working in isocratic and in gradient elution comparing water, acetonitrile and methanol as solvents.

Results: Working in isocratic elution the chromatogram shows only one peak at 5 minutes. Fraction separation was not possible. However, in gradient elution using water as solvent A and acetonitrile gradient as solvent B, the appearance of different bands was observed at different times, mainly for low concentrations (10-30%) of solvent B. Nevertheless the main peak belonging to the non-retained fraction appeared at 5 minutes. Other bands appeared earlier, which must correspond to different components presents in the somatic antigen. The appearance of a wide band is also observed at 20 minutes. This band must be related to the presence of different components that are eluted slowly under these conditions.

Conclusion: It is possible to separate different components in the somatic antigen of *D. dendriticum* using HPLC technique.

C5 p

HUMORAL RESPONSE TO TRICHOSTRONGYLID INFECTION IN EWES WITH LOW OR HIGH FECs Moskwa¹, B., Cabaj¹, W., Doligalska², M.

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One option of nematode control in sheep is selective breeding for a reduction in FECs following natural infection. The study was carried out on two divergent groups of naturally-infected Polish Wrzosówka young ewes exhibiting low or high FECs. The aim of the study was to estimate the association between serum IgG, IgA and IgM level against somatic antigens derived from a mixed population of trichostrongylid nematodes using ELISA test. FECs were very low at the beginning of season, then peaked in the second half of season. In both groups, FECs decreased in the last month of the study. IgG and IgM values were lower at the beginning of season (April-May), then increased. IgG levels were stable from July to November, but IgM fluctuated. Contrary to that, IgA values were highest at the beginning of season (April-June), then decreased but achieved the higher level in the end of season.

General Linear Model procedure for FECs, IgG and IgM levels revealed significant differences between groups ($0.001 \leq P \leq 0.032$). Statistical analysis revealed a significant influence of month on all measured parameters ($P \leq 0.001$). In both selected groups, IgA level correlated negatively with IgM and IgG ($P \leq 0.001$) but IgM and IgG level correlated positively ($P \leq 0.001$). Additionally, significant correlations between humoral response and FECs were observed.

C6 p

SYNTHETIC PEPTIDE VACCINE AGAINST *Taenia solium* PIG CYSTICERCOSIS: SUCCESSFUL VACCINATION IN A CONTROLLED FIELD TRIAL IN RURAL MEXICO

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Background: *Taenia solium* cysticercosis seriously affects human health when localised in the central nervous system (CNS) and causes great economic loss in pig husbandry in rural areas of endemic countries. Increasing the resistance to the parasite in the obligatory host the pig may help in curbing transmission. Three synthetic peptides based on protein sequences of the murine parasite *Taenia crassiceps*, which had previously been shown to induce protection in mice against homologous challenge, were tested as a vaccine against *T. solium* cysticercosis in pigs.

Method: Vaccinated and unvaccinated piglets (240 in all) were distributed in pairs among the peasants' households of two rural villages in Mexico in which 14% of the native pigs were cysticercotic. Ten to twelve months later, the effect of vaccination was evaluated at necropsy.

Results: Vaccination decreased the total number of *T. solium* cysticerci (98.7%) and reduced the prevalence (52.6%).

Conclusions: The encouraging results obtained in this field trial bring us closer to the goal of successfully controlling the life cycle of *T. solium*.

The rather simple protective peptides are potentially more cost-effective to produce and less variable in results than antigens that are more complex.

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C7 p

ORAL VACCINATION AGAINST LIVER FLUKE USING RECOMBINATED CYSTEINE PROTEINASE
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Background: Previous experiments have shown that parenteral vaccination of Sprague-Dowley rats with a recombinated cysteine proteinase (CP) from adult *Fasciola hepatica* may induce some protection against subsequent infection with metacercariae of the fluke. Many results suggested that induction of protective immunity against gastrointestinal parasite infection requires delivery of vaccine to mucosal surfaces. The aim of the present study was to evaluate a protectivity of oral immunisation with the CP

Method: A group of 12 female S-D rats was immunised orally with two doses of CP protein in 4 week interval. A month after the second antigen dosing, the vaccinated rats as well as 12 non-vaccinated controls were challenged with 30 metacercariae of *Fasciola hepatica*.

Results: Mean number of flukes found in livers of vaccinated rats on day 35 after infection (1.7) was significantly lower ($p < 0.001$) than in challenge controls (8.25), a degree of protection was as high as 79%. In vaccinated rats peripheral blood lymphopenia was observed. No significant differences occurred between vaccinated and challenge control rats as far as response of CD4+ and CD8+ lymphocytes in blood and mesenteric lymph nodes was concerned.

Conclusions: Results of our experiment suggest that oral vaccination of mammalian host against *Fasciola hepatica* infection using recombinated protein antigen may be feasible.

Acknowledgement: The work reported here was supported through a grant of Polish Committee for Scientific Research No. Z-KBN 004/PO4/98.

C8 p

Immunization of rabbits with midgut antigens against *Hyalomma anatolicum anatolicum* tick

Rajender Kumar and D.P. Banerjee

The gut supernate antigen (GS Ag), gut membrane antigen (GM Ag), triton extracted gut membrane antigen (TX-GM Ag) and SDS treated triton extracted gut membrane antigen (SDS-TX-GM) were prepared from the midgut of partially fed female tick *Hyalomma anatolicum anatolicum*. The New Zealand White rabbits were immunized with GS Ag, GM Ag, TX-GM Ag and SDS-TX-GM Ag after emulsification with Freund's complete (first inoculation) and incomplete adjuvants (second inoculation) and then challenged with 200 larvae and 10 pairs (male and female) of unfed adult *H. a. anatolicum* tick on each ear 10 days apart. A significant reduction ($P < 0.01$) in per cent engorgement and per cent moulting of *Hyalomma anatolicum anatolicum* larvae was observed when fed on rabbits immunized with the GS Ag. Per cent larval rejection was also maximum in response to GS Ag i.e. 30 per cent. A significant increase ($P < 0.05$) in the engorgement and pre-oviposition periods and significant decrease ($P < 0.05$) in engorgement weight, oviposition period, egg mass weight and reproductive index was observed in adult female ticks when fed on rabbits. Maximum protection in rabbits against *H. a. anatolicum* was induced with GS Ag. The GS Ag also induced significant cross-protection against *H. -dromedarii* ticks in immunized rabbits

C9 p

KINETIC OF DIFFERENT IMMUNOGLOBULIN ISOTYPES IN CATTLE WITH HYPODERMOSIS

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The evolution of IgM and IgG immunoglobulins and the IgG₁ and IgG₂ subclasses has been studied, by means of an indirect ELISA test, in two groups of cattle naturally infested by *Hypoderma* spp. using recombinant hypodermin C as antigen Group 1 (G-1): 10 cows sampled throughout the cycle of the parasite maintained as untreated controls, and G-2: 9 animals treated with a subcutaneous injection of ivermectin (0.2 mg kg⁻¹) when first instars are supposed to be at the beginning of their migration. In control animals, the profile of IgG, IgG₁ and IgG₂, at the beginning of the infestation remained relatively constant, and they increased considerably when first instars started their migration towards the back, this increment was two weeks in delay for specific IgG₂, coinciding with the apparition of first warbles (December). The levels decreased significantly when third instars began to leave the host. At the end of the study (My-Jn), antibody levels increased significantly because of a new infestation. In this group IgM levels hardly oscillated throughout the life cycle of the parasite. In cows treated with ivermectin IgG, IgG₁ and IgG₂ levels increased significantly 4 wk post treatment, after that, they decreased significantly until 12 wk p.t. IgM levels did not changed with treatment. At the end of the study all the immunoglobulins increased significantly because a new infestation had occurred.

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C10 p**IMMUNOMODULATION OF SHEEP LYMPHOCYTE SUBSETS BY *FASCIOLA HEPATICA* EXCRETORY-SECRETORY PRODUCTS**Moreau E.¹, Sebbag H.¹, Zhang WY.^{1,2}, Chauvin A.¹¹: UMR ENVN/INRA 1034, Ecole Nationale Vétérinaire de Nantes, BP 40706, 44307 Nantes Cedex 03, France²: College of Animal Science & Technology, Guangxi University, 530005 Nanning, China

Fasciola hepatica excretory-secretory products (FhESP) effects were studied on concanavalinA (ConA)-induced-proliferation of sheep peripheral blood mononuclear cells (PBMC) and lymphocytes subsets (PBLs) (CD2+, CD4+, CD8+, $\gamma\delta$ TCR+ or CD21+ cells).

PBMC (2.10^5 cells/well) were incubated with ConA at optimal ($1\mu\text{g}/\text{well}$) or suboptimal ($0.25\mu\text{g}/\text{well}$) doses and with FhESP at several doses (1.25 to $20\mu\text{g}/\text{well}$). PBLs (10^5 cells/well) were incubated with ConA at suboptimal dose and with FhESP at $5\mu\text{g}/\text{well}$. These cells were incubated with or without monocytes (CD14+ cell at 2.10^4 cells / well).

10 and $20\mu\text{g}/\text{ml}$ of FhESP inhibited the PBMC stimulated with an optimal dose of ConA. FhESP dose-dependent inhibition was observed on PBMC stimulated with suboptimal dose of ConA. CD21+ lymphocyte (B lymphocyte) and CD14+ cell (monocyte) were not stimulated by ConA. All T lymphocyte subsets (CD2+, CD4+, CD8+ or $\gamma\delta$ TCR+ cell) proliferations were decreased by FhESP at $5\mu\text{g}/\text{well}$.

FhESP inhibits the conA-induced stimulation of sheep PBMC and sheep T lymphocyte subsets. Further studies should be done to investigate the FhESP modulation of cytokines secretions.

C12 p**FIRST EXPOSURE OF "RESILIENT" LAMBS TO ABOMASAL PARASITISM**Przemek¹, S.M.C., Simpson¹, H.V., Scott², I., Thomas², D.G., Green³, R.S., Khalaf¹, S. & Reynolds¹, G.W.

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Aim: Romney sheep selected for high greasy fleece-weight (FW) had higher FEC and worm burdens in the field than genetically similar unselected sheep (C) and thus fit the criteria of "resilience". Do parasite-naïve "resilient" lambs show different physiological responses when challenged under controlled experimental conditions?

Methods: A single dose of 50,000 *Ostertagia circumcincta* (O.c.) L₃ was followed 5 weeks p.i. by a 6 week-period of weekly dosing with 10,000 L₃. Blood and abomasal contents were collected daily until Day 35 and less frequently to necropsy. FEC was monitored from Day 6. Wool follicles were examined in skin samples taken weekly.

Results: In FW lambs, serum pepsinogen and FEC, but not worm burdens, were higher, whereas blood eosinophils were lower and increased later in both primary and trickle infections. Abomasal pH rose 2 days later and was substantially lower from Days 8 to 10. 74% (C) and 20% (FW) of the wool follicles contained paracortex 4 weeks p.i. Serum IgA, IgG1, IgG2 and IgM against O.c. were very low in both groups.

Conclusions: Parasite-naïve FW lambs at initial challenge with O.c. already appeared to express "resilience". Abomasal acid inhibition was delayed, FEC and serum pepsinogen were higher with fewer circulating eosinophils. Circulating antibodies did not appear to be involved.

C11 p**THE PATHOLOGY OF AMPHISTOME INFECTION IN CATTLE**Mavenyengwa M.¹, Mukaratirwa S.¹, Obwolo M.¹ and Monrad J.²¹Department of Paraclinical Veterinary Studies, University of Zimbabwe, Mt Pleasant, Harare, Zimbabwe.²Danish Centre for Experimental Parasitology, Royal Veterinary and Agricultural University, Frederiksberg, Denmark.

Background: The pathology of amphistome infection is well documented in sheep while little is known in cattle. The purpose of this study was therefore to understand the pathology of amphistomosis in cattle and use the information obtained as a baseline for future immunological studies.

Method: Different doses of amphistome metacercariae (species to be determined) were used to experimentally infect 16 weaner calves divided into 4 groups, group I (high dose), group II (medium dose), group III (low dose). Group IV was left as a control. Both groups were allowed to graze and one animal per group was sacrificed for necropsy at day 28, 42, 56 and 84. At necropsy the gross pathological and microscopical changes were noted. Samples for histopathology were removed from the anterior ruminal pillar, the fundic part of the abomasum, the pylorus and from the small intestines at one metre intervals for the length of the small intestines.

Results: Significant pathological lesions were observed in the first metre of the duodenum and the abomasum. The duodenal wall was thickened and the mucosa was thrown into convolutions like the brain gyri. The surface was overlaid by a catarrhal exudate. On microscopy there was desruption of the villi structure. The villi were fused, stumpy and with massive infiltration of eosinophils, lymphocytes and other mononuclear cells in the lamina propria. The absorptive enterocytes were replaced by low cuboidal epithelial cells. These changes tended to decrease in severity as the infestation aged.

Conclusion: Amphistome infection in cattle is characterised by severe pathology in the first metre of the duodenum. The infection appears to induce the accumulation of eosinophils, lymphocytes, globule leukocytes, mast cells and basophils among other cells into the intestinal parenchyma, cells which are involved in immunological reactions against gut parasitic infections in ruminants.

C13 p**IMMUNE RESPONSE OF CALVES IMMUNIZED WITH EXTRACTS OF ADULT *HAEMONCHUS PLACEI*.**Jensen, J.R.¹, Vieira-Bressan, M.C.R.¹

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Background: *Haemonchus placei*, is one of the most important nematodes in Brazil, because of its haematophagous habits and high pathogenicity. The usual control method involves the use of anthelmintics, however, there is a need for integrated control, in which immunological control may be included. The objective of this work was to assess the humoral immune response of calves immunized with extracts of *H. placei*, and to determine their protective potential against a challenge-infection.

Method: Holstein calves aged 8-10 months were immunized four times with crude intestinal extracts (Group E) or with a Triton X-100-soluble fraction of adult *H. placei* (Group A), challenge-infected with 120,000 infective larvae and sacrificed 40 days later.

Results: Immunized animals had higher IgG titers than the controls against the tested fractions after the 2nd immunization, peaking after the 4th. Sera from both immunized groups recognized bands of similar apparent mass in both antigenic preparations, in particular one with a 118kDa band, suggesting the presence of common antigens. Egg counts in Groups A and E were 49% and 57% lower than in controls, respectively. No differences were found in worm burden, although clear evidence of protection was shown by two animals of Group E. Female fecundity rates were reduced, although not significantly, in immunized groups. Eosinophil counts were elevated in intestine-immunized animals and haemoglobin concentration was reduced in infected controls.

Conclusions: Half of the intestine-immunized animals were highly protected by immunization.

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C14 p

ULTRASTRUCTURAL CHANGES FOLLOWING A SINGLE INFECTION OF THE NASAL MUCOSAE WITH OESTRUS OVIS IN SHEEP.

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The nasal bot fly, *Oestrus ovis*, is responsible for clinical signs and losses of production. Previous pathological studies have described the histological lesions related to the parasites. However, no ultrastructural observations were available on the changes induced to the epithelial cells. This point represented the objective of the current study in order to improve the understanding of pathogenic mechanisms associated with *O. ovis*. Six lambs were infected with *O. ovis* larvae; 6 non-infected lambs composed a control group. Infection persisted for 8 weeks before lambs were necropsied. Immediately after death, the heads were split opened and the number and stages of the larvae in the nasal cavities were recorded. Tissue samples for transmission electron microscopy (TEM) were taken from the septum, turbinates, ethmoid and sinuses in infected and control lambs. After routine TEM processing for fixation and staining, the tissues were examined. First to third instar larvae were recovered at necropsy. The ultrastructural changes presented a gradation depending on the anatomical sites. Less epithelial damage was found in the septum whereas the most severe lesions occurred in the sinus. The general organization of epithelium was conserved in the septum where the main lesions were limited to cell vacuolations and a swelling of mitochondria. In contrast, the epithelial cells were nearly dissociated in the sinus, presenting rounded shape, enlarged intercellular spaces and showing signs of degeneration.

C15 p

CROSS-REACTIVITY BETWEEN *Fasciola hepatica* AND *Toxocara canis* ANTIGENS IN EXPERIMENTAL RAT FASCIOSIS

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The evolution of IgG immunoglobulins in rats with fasciolosis was evaluated by means of an indirect-ELISA test and excretory/secretory antigens from adults of *F. hepatica* and *T. canis*. One group of 24 rats (G-I) was infected by gastric tubing with 25 metacercariae of *F. hepatica*, and another group of 12 rats remained uninfected as control (G-C). Faecal and serum samples were obtained weekly, from two weeks prior to 14 weeks after infection (w.a.i.).

Antibodies against *F. hepatica* increased from the 2nd w.a.i. in the infected animals, and non-specific response was observed in the control group. Absorbances rose to the 7th w.a.i. and significant differences between G-I and G-C were proved ($U = -9.570$, $z = 0.000$). The IgG response against *T. canis* excretory/secretory antigens was lower than that against *F. hepatica* antigens. The IgG values increased significantly in the rats of G-I at the 1st, 2nd w.a.i., and from the 4th w.a.i. again. By means of the Mann-Whitney U test, significant differences were established ($U = -7.482$, $z = 0.000$). Significant correlation was also observed between the *F. hepatica* and *T. canis* antibodies curves in G-I ($r = 0.363$, $p = 0.000$).

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C16 p

IMMUNOHISTOCHEMICAL STUDY IN EXPERIMENTAL OVINE DICROCOELIOSIS

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Purpose: To characterize the inflammatory cells in liver and hepatic lymph nodes (HLN) of lambs experimentally infected with *Dicrocoelium dendriticum*.

Methods: Sixteen 4-month-old male lambs of the Churra breed were used. Six were infected with 1000 *D. dendriticum* metacercariae each, six with 3000 and four were not infected. Paraffin-wax and fresh frozen sections of liver and HLN from lambs slaughtered at 6 months p.i. were immunolabelled for the anti-ovine macrophages (VPM-32), anti-MHC class II β , anti-WC1, anti-CD8, anti-CD3, anti-CD45R, anti-CD79 α cy and anti-IgG antibodies.

Results: Numerous VPM-32 positive macrophages around septal bile ducts (SBD) were immunostained by the anti-MHC class II β . Lymphoid follicles surrounding SBD were reactive for CD79 α cy and CD45R B lymphocytes whereas diffuse CD3⁺ and CD8⁺ T lymphocytes were present. $\gamma\delta$ T cells and plasma cells expressing cytoplasmic IgG were scattered near SBD. Increased positive immunostaining for all anti-leucocyte antibodies were observed in HLN of infected lambs.

Conclusions: Local immune response observed, probably due to continuous stimulation by antigens of adult worms, was qualitatively similar, suggesting a dose-independent effect. Likewise, this response did not seem to confer a protective effect.

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C17 p

ACQUIRED IMMUNITY TO A LOCAL STRAIN OF *HAEMONCHUS CONTORTUS* BY DWARF GOATS

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Background: The capacity of young Nigerian West African Dwarf (WAD) goats to develop good acquired immunity to their native geographical strain of *Haemonchus contortus* and the correlates of this responsiveness were studied in a laboratory experiment.

Method: 42 7-8 month old kids divided into 6 groups were either not given or given a primary immunising infection of 2000 L3 with or without challenge of 2000 L3 on day 42 (D42). The immunising infection of a group was however truncated with fenbedazole on D35 before the challenge infection.

Result: Infections resulted in a mild chronic infection with a prepatent period of 18-20 days and little or no reduction in worm burden between D14 and D56. However, the group of kids with truncated infection, developed good protection against challenge. Of the measures of infection namely, faecal egg count (FEC), eosinophil responses, packed cell volume (PCV) and body weight, FEC and PCV showed strong correlation with worm burden ($r_s = 0.965$, $P < 0.001$ and $r_s = 0.577$, $P = 0.008$ respectively). The kids exhibited good tolerance to the size of the inoculum used.

Conclusion: The wide individual variability observed in FEC and its strong positive correlation with worm burden are pointers to its genetic origin. There are therefore good prospects for its use for the identification of parasite resistant genotypes among the WAD goat population.

C18 p HISTOPATHOLOGICAL OBSERVATIONS ON PULMONARY FLUKE INFESTATION IN GOATS

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In present study a total of 2217 goat lungs were examined and out of these, 517 specimens showing frank lesions were collected for further histopathological examination. The infestation of flukes recorded in 1.58 per cent cases of the total affected lungs. Grossly, the lesions found on the surface of lungs were pea sized, soft and raised from the surface and were reddish purple in colour. Microscopically, the sections of flukes present in the lung parenchyma showed spiny cuticle and presence of suckers. They were of various size and shapes. Some were immature while the others were mature. One fluke was large in size having a tail like structure and could not be seen completely in one photographic field. In most cases, the surrounding tissue showed haemorrhages and the macrophages in that area were loaded with haemosiderin pigment. Certain amount of cellular infiltration was present in the affected areas predominantly of lymphocytes, eosinophils and macrophages. In some cases the flukes migrated from the site of haemorrhages and were seen among the damaged alveoli. These flukes could not be identified and still open for discussion.

C20 p TRICHURIS SUIS SECRETIONS DAMAGE CELLS AND ENHANCE ENDOTOXIC SHOCK

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Background: *Campylobacter jejuni* (Cj), the leading cause of diarrhea in humans worldwide, colonizes pigs without disease, but becomes invasive during swine whipworm (*T. suis*) infection. We examined the hypotheses that *T. suis* secretions (ESP) 1) disrupts the integrity of artificial intestinal epithelial barriers, and 2) causes lipopolysaccharide-induced (LPS) endotoxic shock and death in mice.

Methods: 1) Spectrophotometric staining and tight junction integrity assays were used to measure the cytotoxicity of ESP on intestinal epithelial cells (IECs). 2) The effect of dose and time of WESP administration on a sublethal LPS challenge was examined in BALB/c mice. Mice were given low (25 µg), medium (50 µg), and high (100 µg) doses of WESP at 7, 2 and 0 days before challenge with LPS. All mice received a sublethal challenge dose (LD30) of LPS on day 0.

Results: 1) Assays revealed a dose-dependent cytotoxic response in ESP-treated IECs. 2) Mice given medium and high doses of WESP concomitantly with LPS died. Medium and high WESP doses given on or daily from Day 7 protected the mice from death. All mice given 25 µg of WESP daily for 7 days died. All control mice survived.

Conclusions: 1) Soluble products released by *T. suis* contribute to IEC damage, and 2) WESP and LPS given concomitantly enhances the ability of LPS to induce endotoxic shock and death in mice.

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C19 p PATHOGENICITY OF AN ARGENTINEAN ISOLATE OF *Toxoplasma gondii* IN PREGNANT EWES.

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Background: In Argentina there are not reports of *Toxoplasma gondii*-induced abortion in sheep. The objectives of this study were to evaluate diagnostic methods for *T. gondii* abortion in sheep infected with an Argentinean isolate.

Methods: *Toxoplasma gondii* oocysts were obtained from feces of a cat fed tissue cysts of an Argentinean isolate originally obtained from one pig. Sporulated oocysts (1000) were fed to 5 pregnant ewes. Four pregnant ewes were not infected served as control group. Pregnancy in all 9 ewes had been ascertained by ultrasound. All ewes were serologically negative for *T. gondii* antibodies. Sera of the ewes and fetal fluids were examined by the indirect immunofluorescence technique (IFAT) and by modified agglutination technique (MAT) using commercially available formalin-fixed whole tachyzoites. For IFAT, RH strain tachyzoites and anti-sheep-IgM and anti-sheep-IgG antibodies were used. Ewes were observed clinically and their corporal temperature was recorded. Tissues from aborted fetuses were bioassayed in mice.

Results: Ewes fed oocysts developed fever ($\geq 41.5^\circ\text{C}$) and diarrhea during the second week. The control ewes remained clinically normal. Two ewes aborted (at 135 and 107 gestational days) autolyzed foetuses, 1 ewe had 1 resorbed fetus and 1 normal lamb. Two inoculated ewes and the 4 control ewes lambed normally. *Toxoplasma gondii* was not isolated from foetuses. Antibodies to *T. gondii* were found in fluids of 2 fetuses. The inoculated ewes developed IgM and IgG antibodies. IgM antibodies peaked 14 dpi and IgG antibodies peaked at 14-42 dpi. At 35 dpi all sera were negative for IgM. Ewes of the control group did not develop antibodies. **Conclusion:** Results indicate that the Argentinean isolate is capable of causing abortion in sheep and that fetal serology is an aid to diagnosis of abortion in sheep.

C21 p IMMUNOPATHOLOGICAL STUDIES IN ORGANS OF PIGS INFECTED AND IMMUNIZED WITH *Ascaris suum*

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Background: The migration of *Ascaris suum* larvae through the host can produce the deposit of some antigenic products which could determinate some pathological alterations in different organs. For this reason, we planned an immunohistochemical study to know the distribution and localisation of different *A. suum* antigens in the liver of infected and immunized animals.

Methods: Fifty-six pigs were divided in 7 groups. Groups 1 and 2 were uninfected and control, respectively. Groups 3 and 4 were weekly infected with increasing doses of *A. suum* eggs and group 4 was also treated with pyrantel. Groups 5, 6 and 7 were weekly immunized with 1 µg/Kg of 14, 42 and 97 kDa proteins, respectively. Groups 2-7 were challenged with 10000 infective eggs and sacrificed 7 days after challenge. Livers were fixed in 10% buffered formalin, embedded in paraffin and analysed by peroxidase-anti-peroxidase method. Immune sera were obtained in rabbits immunized with body fluid (BF) and body wall (BW) extracts from the adult worms; somatic (SL) and excretory-secretory (ES) extracts from the larvae and 14, 42 and 97 kDa proteins from adults.

Results: Evidence of BW, ES, 14 and 42 kDa proteins was patent in all infected and immunized pigs, mainly in the connective tissue. SL was only found in the challenged and immunized animals. BF was found in bile ducts and macrophages in all pigs, while the ES was also evident in cholangioli. The most abundant antigenic deposits was seen in the immunized groups, above all in the group 6. The 42 kDa protein was the most recognized antigen in the animals, with a strong immunohistochemical detection in the fibroblastic areas.

Conclusions: The migration of *A. suum* produces very strong antigenic deposits of different antigens in liver, which could determinate some functional and allergenic problems.

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C22 p THE EFFECT OF *OESOPHAGOSTOMUM DENTATUM* AND DIET ON MORPHOLOGICAL CHARACTERISTICS OF PIGS LARGE INTESTINE

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Background: Previous studies revealed that diets with different levels of digestibility had significant effects on *O. dentatum* population biology. The knowledge concerning the influence of infection with *O. dentatum* on the morphology of the large intestine is particularly scarce.

Objectives: The aims of the present investigation were to study the impact of *O. dentatum* infection and diets with contrasting digestibilities on morphological characteristics and epithelial cell proliferation of the large intestine of pigs.

Results: Infection with *O. dentatum* significantly increased the crypt volume and height, volumes of mucins and caused hypertrophy of muscularis externa at proximal and middle colon. There was no effect on the epithelial cell proliferation. The content of dietary carbohydrates significantly influenced the tissue weight of caecum and colon.

Conclusion: This study shows that infection with *O. dentatum* and dietary carbohydrate composition affects the mucosal architecture and mucin secretion of the large intestine of growing pigs.

C23 p

THE PATHOLOGY OF *TRYPANOSOMA SUI* INFECTION IN PIGLETS

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Background: There is conflicting information on the nomenclature of the rarest Salivarian trypanosome invariably referred to as *Trypanosoma suis* or *T. congolense* (Tsavo) whose pathology has not been documented. The only laboratory isolate of the species is maintained at the Kenya Trypanosomiasis Research Institute, near Nairobi Kenya.

Methods: At varying ages, Large White piglets were experimentally infected by intravenous injection with approximately 10⁵ trypanosomes. The piglets were monitored for clinical disease, and at pre-determined stages euthanised serially. A detailed post mortem was carried out and tissues processed for histopathology.

Results: The prepatent period was 5-7 days. Clinically, the piglets had fever, skin erythema, facial and eyelid oedema. The disease was fatal to piglets under 5 weeks old but older piglets continued to gain weight despite intermittent parasitaemia. The pathology observed was dependent on the age and disease stage at euthanasia. Grossly, haemorrhages were found in the gut, lungs, kidneys and heart. At microscopy, there was generalised cellular infiltration of various organs including hepatitis, pneumonia with hepatisation of the lungs, and gastroenteritis. The brain blood vessels were congested with instances of thrombosis formation consisting of erythrocytes, inflammatory cells and trypanosomes.

Conclusion: *Trypanosoma suis* causes disease in piglets. The severity is dependent on the age of piglets. Older animals exhibit a mild infection.

Acknowledgement: Wellcome Trust (UK) and GOK for funding.

C24 p

CLINICO-PATHOLOGICAL RESPONSE IN *TRYPANOSOMA EVANSI* INFECTED AND IMMUNOSUPPRESSED BUFFALO CALVES

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Background: The present study was planned to examine the role of Dexamethasone and its effect on clinical response in *Trypanosoma evansi* infected buffalo-calves treated with and without Diminazene diaceturate (Trypan, Atarost, Germany).

Methods: Fourteen male buffalo calves aged nine months to one year of age were randomly divided into five groups. Group I (infected & Diminazene treated), group II (infected, Dexamethasone immunomodulated & Diminazene treated), group III (infected & Dexamethasone immunomodulated) and group IV (*T. evansi* infected alone) constituted three animals each and in group V two uninfected control animals were utilized. Experimentally, infection was established by s/c inoculation of 1x10⁷ tryps.calf⁻¹. Mice were used for biological test. Clinical signs were recorded daily. Tissues of buffalo calves and mice died of *T. evansi* infection were fixed in neutral buffered formalin saline, stained with Giemsa and Haematoxylin & Eosin stain for interpretation.

Results: Dexamethasone immunomodulation in *T. evansi* infected buffalo calves slightly decreased the prepatent period, time for the 1st peak of parasitaemia and duration of intermission while the duration of paroxysm and parasitaemia were prolonged. Clinical signs were more pronounced and per cent death rate was high in infected and dexamethasone immunosuppressed buffalo calves. Relapse of parasitaemia post treatment with diminazene was observed in both infected, and infected & immunomodulated buffalo calves. Tissue sections from heart, brain, liver, spleen, lymph nodes and kidney revealed trypanosomes.

Conclusion: Dexamethasone in *T. evansi* infected buffalo calves has increased parasitaemia and is responsible for aggravation of the disease. Further, *T. evansi* parasites sequestered into central parenchyma and cerebrospinal fluid may be the cause of the relapse of the infection.

C25 p

THE ROLE OF SEROLOGICAL TESTS IN EVALUATION AND EFFICACY OF AUTOCLAVED LEISHMANIA VACCINES IN MESHKINSHAHR AREA OF IRAN

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Background: The blood serum of 499 children under 12 years of old from Meshkinshahr district, before and after the vaccination of dogs were studied. 18(7.8%) and 16(6%) of these subjects, who showed antibody titer >1:3200 from serological point of view before and after vaccination (autoclaved leishmania vaccines) dogs, respectively, were considered as being positive. 49(9.8%) of the total subjects, had specific antibody against leishmania in both phases.

Method: Before vaccination 152 domestic dogs were tested by two methods: Elisa and immunofluorescent antibody test, in order to search for antileishmania antibodies. 21.7 percent of dogs were serologically positive. Therefore 33 dogs were excluded from the study. Five ml blood was obtained from these selected dogs (119) a year after the second vaccination. Further studies were performed by using Elisa and immunofluorescent antibody test in order to determine efficacy of the vaccines.

Results: With immunofluorescent antibody test and in control group, 42 dogs (62.7%) from 67 dogs were positive but in vaccinated group with Leishmania major+B.C.G from 23 dogs 43.5%

and in vaccinated group with Leishmania infantum+B.C.G, 27.3% were positive and this data with Elisa were 55.2%, 30.4% and 31.8% respectively.

Conclusion: This research showed: 1. there was a significant difference of antibody titer between control and vaccinated dogs (P<0.05) and 2. it further demonstrates that by using these serological tests we can evaluate and study the efficacy of the autoclaved Leishmania major and Leishmania infantum vaccines, in order to control the zoonotic visceral leishmaniasis, in field.

C26 p **CHARACTERISTICS OF HISTOPATHOLOGY OF TICK BITE IN SPONTANEOUSLY INFESTED DOGS**
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Introduction : Host relationship in the tick bite process is of major importance to determine resistance or sensitivity of the host and may have implications in the transmission of pathogens. Few studies have been conducted in spontaneously infested dogs in comparison to experimental models in other species. We propose here the analysis of the histopathological pattern of tick-bite in dogs.

Material and methods : Biopsies including the attached ticks were performed in infested dogs. Sections were made so as to include the rostrum in order to study the inflammatory reaction and stained (Hematein-eosin and periodic Acid Schiff). Modifications of epidermis and dermis and cellular types were scored from 0 (absent) to 3 (severe).

Results : 52 biopsies from 32 dogs were analyzed. Cement and underlying necrosis were observed in all cases. Hyperkeratosis and epidermal hyperplasia were constant, sometimes in association with neutrophils or eosinophils. Four major patterns were observed in the dermis: two suggestive of sensitivity: silent (no reaction) in 3, necrosis (neutrophilic infiltrate) in 17; two suggestive of hypersensitivity: eosinophilic reaction in 5, or granulomatous (eosinophils and mononuclear cells) in 7.

Conclusions : These reactions were not linked to the species of tick, stage of the feeding or the macroscopic cutaneous lesions. These results suggest that in many (most ?) cases the tick bite in dogs is not associated to an active hypersensitivity reaction that could reflect resistance.

C28 p **IMMUNE RESPONSE AGAINST *Toxocara canis* AND *Ascaris suum* IN EXPERIMENTAL CANINE TOXOCARIOSIS**

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The IgG humoral immune response against *T. canis* and *A. suum* was established in two groups of 2.5 months old Beagle experimentally infected with *T. canis*. One group (G-E) of 8 dogs received an immunostimulant treatment (ESTIMULOCEL[®], 50 mg Procudazol daily, V.O.) from 2 weeks prior to 2 weeks after experimental infection with 3000 embryonated *T. canis* eggs. Other group remained infected without treatment (G-I) and another one was left uninfected as control (G-C). IgG values were assessed by means of an indirect-ELISA and excretory/secretory antigens from *T. canis* and *A. suum*. Piperazine was administered at the 14th week after infection (w.a.i.) to G-E and G-I.

T. canis-specific antibodies were detected from the 2nd week after infection in both infected groups (G-E and G-I). The curves of IgG were very similar in G-I and in G-E, and the highest values were achieved at the end of the study. Significant differences between G-I, G-E and G-P were proved ($\chi^2=5.209$, $p=0.022$). Administration of estimulocel increased the IgG response against *A. suum*, and greater values than that of G-I were achieved. Significant differences between G-I, G-E and G-C were established ($\chi^2=50.516$, $p=0.000$).

C27 p **HEMATOLOGY AND BLOOD BIOCHEMISTRY IN PIGS INFECTED WITH *TOXOCARA CANIS***
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Background: Evaluate the hematology and blood biochemistry changes in pigs infected with eggs of *Toxocara canis*.

Method: 15 pigs were infected with a dose of 100.000 *T. canis* embryonated eggs and 3 pigs were used as control group. Blood was collected from each pig once a week during three months.

Results: The hematology result showed an eosinophil count significantly higher for the inoculated group, between days 7 and 21 post-inoculation (p.i.). The highest count was at day 14 p.i.. The absolute count of eosinophils (RAE mm³) were significantly higher at day 14 p.i. and at day 35 p.i.. Not significant changes were observed in the blood biochemistry analysis in both groups during all the experience period.

Conclusion: Our results suggest that the haematological response is important at the beginning of the infection, the eosinophil count is increasing at the acute phase of the disease and then it becomes normal. The blood biochemistry analysis seems not to be a good parameter to evaluate the process in pigs' illness.

Acknowledgement: This research was supported by Universidad de Buenos Aires. Sec. de Ciencia y Técnica. Subsidio TV 37.

C29 p **LIVER CONDEMNATION DUE TO LIVER FLUKE DISEASE IN IRELAND**
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Background: Estimation of the incidence of liver fluke disease in Ireland is based primarily on liver condemnation rates, which are also used to validate fluke forecasts. However, the accuracy of these data has never been assessed and this study was undertaken in an attempt to obtain detailed information on the levels of liver condemnation in different classes of stock, as well as the specific reasons for their condemnation.

Method: Three abattoirs a month were visited over a period of a year and observations made on numbers of livers condemned to fluke for each class of stock, reason for condemnation and nature of fluke damage.

Results: The study showed that liver condemnation rates were higher than official figures for the same period. The assumption that almost all liver condemnations are due to fluke infection was confirmed for all classes of cattle, but not for lambs, in which less than half of the condemned livers were due to fluke and nearly a third were due to *Cysticercus tenuicollis*. Seasonal damage reflecting fluke epidemiology was seen in lamb livers and in cattle livers damaged by immature fluke.

Conclusion: The methods for collection of these data in the meat factories require review. At present caution should be exercised in the use of such figures for forecasting purposes or for the validation of forecasts.

Acknowledgement: Funded by Janssen Ltd, Merial Ltd and Novartis Ltd

C30 p RELATIONSHIP BETWEEN LUNGWORMS AND MAEDI/VISNA VIRUS (MVV) IN THE SHEEP.

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Background: In our country the presence of lungworms and MVV is wide diffused in sheep flocks. The role of elminthes parasites in the transmission of viruses is often discussed. The aim of this study is to investigate the relationship between lungworms and MVV in producing severe chronic lung pathology in sheep and to verify the possible role of parasites as carriers of virus.

Method: Faeces of 48 sheep belonging to a tuscan flock were examined for lungworms by Baermann test. The same animals were serologically tested to MVV (Innotest-MVV, Innogenetics N.V., Belgium). Lungs of 10 lungworms and MVV positive sheep were submitted to histological examination. Sections were stained with H & E and examined by immunohistochemical stain for evaluation of MVV antigen either free or in association with sheep macrophages inside parasite body.

Results: 25/48 samples of faeces result positive to small lungworms (*P. rufescens*, *N. linearis*, *M. capillaris*, and *C. ocreatus*). The serological positivity of the flock to MVV is 43,75%. Sections of lungs, belonging to lungworms and MVV positive sheep, show a severe verminous bronchopneumonia, extensive follicular proliferation which occur predominantly in perivascular area and interstitial network. Immunohistochemical findings show the presence, inside parasite body, of some well distinguishable cells stained either with moAb-anti sheep macrophage (VPM32, Edinburgh) or with moAb-anti p28 MVV core polypeptide (VMRD, Inc., Pullman, WA, USA).

Conclusion: The chronic presence of lungworms could activate latent MVV infection. Consequently this maybe increased the serological positivity and made the anatomopathological aspects of chronic parasitic pneumonia worse. On the other hand the MVV infection could decrease resistance to parasites. To find the MVV inside the parasite body could be the first step to suppose an hypothetical role of lungworms in MVV epidemiology.

D

Parasitism and aquaculture

D1

APPLICATION OF PARASITE NUMBERS AS AN INDICATION OF WATER QUALITY IN THE VAAL RIVER SYSTEM

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Background: South Africa is an arid country and deterioration of the water quality necessitated development of biological indexes to complement and enhance chemical analysis. Previously an index evaluating the general health of fishes and a parasite index was employed in the Olifants River system and it was found that the intensity of parasites give an accurate indication of the water quality of the environment. This index performed well in river and dam sample sites in drought as well as flood conditions.

Method: Specimens from a variety of fish species were collected with seine nets in two localities in the Vaal River system during 6 surveys in 1999-2000. The localities represented polluted and unpolluted water. The number and parasite group to which it belonged was noted. The parasites were separated into two larger groups, endoparasites and ectoparasites. The data were correlated to water quality and the most indicative parameters were indicated by means of logistic regression analysis.

Results: Fishes from the polluted site had a higher prevalence and mean intensity in parasite numbers for endoparasites in the more polluted sample site in all species studied. Logistic regression indicated endoparasite numbers to be the most reliable indicator, with an accuracy of approximately 75%.

Conclusion: The index is applicable in other river systems provided that the river system is compared to itself over a period of time, at similar times of the year.

D2

PREVALENCE OF PARASITES ASSOCIATED WITH FRESH WATER ORNAMENTAL FISH IN SRI LANKA

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Background: Ornamental fish farming has emerged as a valuable foreign exchange earner for Sri Lanka and it is a rapidly growing industry. Diseases particularly parasitism has become a threat limiting the development of the industry due to over expansion.

Method: One thousand five hundred and twenty ornamental fishes of 13 species namely, Guppy (*Poecilia reticulata*), Goldfish (*Carassius auratus*), Platy (*Xiphophorus maculatus*), Molly (*Poecilia sphenops*), Angel (*Pterophyllum scalare*), Swardtail (*Xiphophorus helleri*), Tetras (*Hyphessobrycon* species), Barbs (*Capaeta* and *Puntius* species), Gurami (*Colisa* species), Carp (*Cyprinus carpio*), Fighters (*Betta splendens*) and others (*Brachydanio* and *Astronotus* species) were examined randomly from the 26 export farms in Sri Lanka during the period from October, 1999 to March, 2000.

Results: Of the 26 fish farms examined 23 farms were positive for one or more parasites. The prevalence of monogenian trematodes were 28.3% (*Dactylogyrus*, 18.4%; *Gyrodactylus*, 9.9%), ciliated protozoa were 18.3% (*Trichodina*, 9.3%; *Tetrahymena*, 4.1%; *Ichthyophthirius*, 1.9%; *Ichthyobodo*, 1.7%; *Oodinium*, 1.2%), copepod crustaceans were 4.8% (*Lernaea*, 2.2%; *Ergasilus*, 1.3% and *Argulus*, 1.3%) and *Capillaria* was 0.4%. In addition, metacercarial stage of *Centrocyclus* was found in gills of 12 fishes at the prevalence of <1%.

Conclusion: The overall prevalence of parasites was 4.38% with monogenian flukes were the commonest.

Acknowledgement: The authors acknowledge the cooperation of all the ornamental fish export farmers for providing samples for the study.

D3

REACTIVITIES OF RABBIT AND TROUT ANTISERA AGAINST CILIATE ANTIGENS

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Background: The immunity of fish against the pathogenic ciliate *Ichthyophthirius multifiliis* has been intensively studied. In order to understand the reactions against this parasite, it is important to identify immunogenic antigens. Antibodies from mice and rabbits have been important tools in this identification, although these antibodies might recognize other antigens than those recognized by fish antisera.

Method: Reactivities of antisera raised in rabbits and trout against *I. multifiliis* has been investigated using immobilization/agglutination assays, ELISA and Western blotting. Three species of *Tetrahymena*, which are closely related to *I. multifiliis*, were examined for cross reactivity. The carbohydrate residues of the ciliates were determined using lectin blotting.

Results: The rabbit antisera possess higher antibody titres (1:8192) than the rainbow trout (1:512) determined by ELISA but the rainbow trout antisera showed stronger agglutinating activity against *I. multifiliis*. Rabbit antiserum recognized four different antigens of *I. multifiliis* with molecular weights between 35 and 60 kDa whereas the rainbow trout recognized two antigens of approximately 40 and 115 kDa. No cross-reactivity of rabbit antiserum against the three species of *Tetrahymena* was found. In contrast, rainbow trout recognized a *T. pyriformis* antigen in Western blotting and immobilized all three species.

D1 p

Parasite-induced changes in the distribution and abundance of intestinal endocrine cells responsible for peptide hormone production in brown trout (*Salmo trutta* (L.)).

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Background: In the vertebrate gut, epithelial cells belonging to the Diffuse Endocrine System (DES) interact with the enteric nervous system to regulate digestive functions such as enzyme secretion, nutrient uptake, and the progression of food through the digestive canal. Intestinal parasites can seriously disrupt structures of the gut wall and interrupt communication between the nervous and endocrine systems. Our previous work have already evidenced noticeable differences in the neuroendocrine system of healthy in comparison with parasitized fishes. In the current investigation we present quantitative data comparing the distribution of cells secreting peptide hormones in the neuroendocrine system of healthy *Salmo trutta* and *S. trutta* parasitized by acanthocephalans and/or cestodes.

Methods: From November 1998 to December 1999, a total of 167 specimens of brown trout (total length ranging from 10 to 48 cm) were sampled with electrofishing from different channels in Province of Padua (North Italy). Immunohistochemical tests with anti-glucagon, -secretin, -CCK-8, -gastrin, and -bombesin sera were applied on sections of the intestinal tissue of healthy and parasitized trout.

Results: Among 167 fish, 119 (71.3%) were infected with intestinal helminths. The dominant parasites were *P. laevis* and *C. truncatus*. The anterior (near the pyloric caeca region) and the central regions of the host alimentary canal were the most heavily parasitized. The number of endocrine cell that showed immunoreactivity to tested antisera (i.e., CCK-8, glucagon and bombesin) decreased in parasitized trout as compared to uninfected individuals. This phenomenon is indicative of the depth of the helminth penetration through the host's intestinal wall and reflects the extensive damage these parasite inflict. The low number of enteroendocrine cells may also be due to an increase in goblet cells that are involved in the excessive mucus secretion that is characteristic of infected trout.

Conclusion: The presence of intestinal helminths in the alimentary tract of brown trout can alter the number of cells that synthesize neurohormones. This finding suggests that these parasites have the potential to substantially modify digestive function in these fish through a direct as well as an indirect way.

D2 p

IN VITRO ACTIVITY OF SOME ESSENTIAL OILS AGAINST SAPROLEGNIACEAE

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Background: Saprolegniosis is a common disease of freshwater organisms, which therapeutic control was always attained with malachite green (banned) or formalin. The environmental impact connected with their use stimulates the research of new products of natural origins. The aim of this study was to test a large number of essential oils against Saprolegniaceae (Heterokonta: Oomycota).

Method: Three strains, *Saprolegnia parasica*, *Saprolegnia* sp. and *Aphanomyces astaci*, were tested with 18 essential oils (Flora srl, Lorenzana, Pisa). The oils were added at different concentrations (from 0.005% to 1.5%) to Glucose Yeast Extract agar, which was inoculated with the tested strains (Alderman D.J., Journal of Fish Diseases, 1982, 5, 113-123). After incubation at 18°C for 24 hours, the colony diameters were determined as the mean of two axes measured at 90° to each other. To verify the total mycelium growth inhibition, the plates were examined also at 7 days of incubation.

Results: With this method, suitable as screening test, the MIC for Saprolegniaceae after continue exposure was 0.005% for oregano essential oil and 0.01% for thyme and savory, while eucalyptus, lemon, camphor and rosemary essential oils was not effective at the higher tested concentration. Some oils curbed the growth of mycelium, compared to untreated control after 24 hours, at lowest concentration as to their MIC.

Conclusion: The composition of plant oils is known to vary according to local climatic and environmental conditions; however, the oils well-known as containing carvacrol (oregano, thyme and savory) seems to be more effective against Saprolegniaceae.

Acknowledgement: The work was supported by a MURST ex 60% grant.

D3 p

MONOGENEAN PARASITES FROM TROPICAL FISH COLLECTED IN AQUARIUM OF GENOVA (Italy)

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Background: Infectious diseases, easily introduced with fish from natural habitats, may flourish in artificial environments because of increased effectiveness of transmission from fish to fish in restricted body of water, because of inadequate diet and space, and consequent reduction in resistance of the fish.

Method: The tropical fish examined in this study were obtained by Aquarium of Genova, in particular have been analysed 56 tropical fish subdivided in 16 different genera, collected from Indopacific-area from 1998 to 1999.

Results: Two monogenean were classified: *Haliotrema hannulocirrus* and *Pseudempleurosoma* (Yamaguti, 1965) sp., Syn. *Metadiplectanotrema* Gerashev, Gayevskaya and Kovalena, 1987.

H. hannulocirrus has been found in *Cahetodon madagascariensis*, *Chaetodon ephippium* and *Chaetodon auriga*.

Pseudempleurosoma sp. has been found in *Acanthurus leucosternon*, *Centropyge bicolor*, *C. auriga*, *C. ephippium*, *C. madagascariensis*, *Chelmon rostratus*, *Coris gaimard*, *Gnathanodon speciosus*, *Pomacanthus semicirculatus*.

Conclusion: Studies on *Pseudempleurosoma* are now in progress to verify the species.

Acknowledgement: The authors are grateful to dr. Pavel Gerashev and Claudia Santos, for information concerning specimens.

D4 p

ENDOPARASITIC HELMINTHS OF THE MARINE MARKET FISH IN THESSALONIKI REGION

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Background: The aim of this study was to find helminth parasites in commercial marine fish species from Thessaloniki region in Greece.

Method: More than 100 complete parasitological dissection of 12 commercial fish species were performed - *Scomber japonicus*, *Engraulis engrasicholus*, *Clupea pilchardus*, *Trachurus trachurus*, *Boops boops*, *Boops salpa*, *Belone belone*, *Mugil* sp., *Spicara vulgaris*, *Pagellus mormurus*, *Diplodus* sp., *Morone labrax*.

Results: Intestinal nematodes were found only in 2 species of examined fish. Intestine of *Boops boops* and *Scomber japonicus* were infected by two and four nematodes from *Anisakis* group.

Conclusion: The low intensity of infection by endoparasites of marketable fish have been registered. The *Anisakis* sp. is important fish - zoonotic parasites. These results may have practical application for parasitological control of commercial fish. This project will be continued on marketable fish from Aegean Sea.

Acknowledgement: This work was supported by research Slovak - Greece grant No Gr/S1/A83/1999-2000.

D5 p

CYPRINID FISH AS A SOURCE FOR TREMATODE INFECTIONS

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Background: 58% of the fox population in Berlin were positive for liver flukes. *Metorchis bilis* was the most frequent (56%) fluke identified followed by *Opisthorchis felineus* and *Pseudamphistomum truncatum* in 18 and 1.3% livers respectively. In connection with these findings, it was of interest to examine cyprinid fish to find out their role as source for opisthorchiidosis.

Method: Altogether 506 specimens of 4 cyprinid species: id (*Leuciscus idus*), bleek (*Alburnus alburnus*) silver bream (*Blicca bjoerkna*) and roach (*Rutilus rutilus*) caught in the Teltow canal of Berlin were examined by digesting individual flesh samples in artificial gastric juice. Metacercariae were determined and counted.

Results: 8 types of metacercariae were identified: *O. felineus*/ *M. bilis* (both species are not distinguishable morphologically), *M. xanthosomus*, *P. truncatum*, *Apophallus muehlingi*, *Holostephanus dubnini*, *Posthodiplostomum cuticola*, *Hysteromorpha triloba* and *Bucephalus polymorphus*. Id and bleek had not only the highest prevalences of the relevant species but also had the highest metacercarial densities per g flesh. While *O. felineus* and *P. truncatum* cause infections in mammals and man *M. xanthosomus*, *H. dubnini*, *P. cuticola* and *H. triloba* are parasites of fish eating birds. Adults of *M. bilis* and *A. muehlingi* can be found in both host classes and *B. polymorphus* is a parasite of fish of prey.

Conclusions: Different susceptibilities of various fish to different metacercariae could explain the observed differences in prevalences and metacercarial densities. Id and bleek are the most important sources of mammalian opisthorchiidosis.

D6 p Parasitosis under conditions of toxic pollution and measures for improving health of fish in aquaculture. Davydov, O., Kurowsky, L., Isaeva, N.

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Subject of inquiry – fish cultures of cyprinids, salmonids and sturgeons and pathogenic organisms. Integral measures have been developed to cope with infectious diseases and fish toxicosis, based upon accounting for both modifying environmental factors and the action of prophylactic chemicals used against the pathogenic agents, and the functional condition of the fish-hosts.

Life – time express-methods have been elaborated and tested for distinguishing fish diseases; based on visual and behavioral features, physics and chemistry, physiology and biochemistry, immunological approaches, which allow to accomplish a prospective assessment of the fish quality during cultivation.

D7 p Some tumours of fish from aquaculture Ukraine Davydov, O., Kurowsky, L., Temnikhanov, Y., Bareev, R.

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Background: Among tumoral diseases of fishes in requirements of aquiculture are recorded Epithelioma papulosum cyprini (EPC) carp, Cyprinus carpio and Hepatic neoplasia (Hepatoma) rainbow trout, Salmo gairdneri more often.

Method: For microscopic sections tumours from carp and trout within 50 minutes fixed in 2 % solution glutar of an aldehyde on 0,05 M of phosphatic buffer, and then within 60 minutes in 1 % solution osmium of an acid.

Results: EPC carp clinical was developed as lactic dense scurf on a skin and fin. In drugs from EPC carp found mass effuse of epithelial cells of the polyhedral form and different value. process propagated on a liable muscle tissue, which loses usual elasticity and seepage by fluid. The originator EPC carp - virus family Herpesviridae.

Hepatoma trout at earlier stage of development forms yellowish - white nodules (1-2 mm in diameter) on a surface of a liver. At histological research some phylums Tumour Hepatoma, representing different stage of tumoral process are detected. The cause of originating Hepatoma trout is aflatoxin - products of vital activity of musty funguses Aspergillus flavus and A. Parasiticus.



E

Epidemiology of parasitic diseases

E1

10 YEARS OF CAPACITY STRENGTHENING IN LIVESTOCK HELMINTHS RESEARCH

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Background: For over 10 years the Danida-funded research capacity strengthening Livestock Helminths Research Project involving the veterinary faculties of Kenya, Tanzania, Zambia and Zimbabwe has been supporting Masters and PhD students who conduct research related to parasitological problems of livestock belonging to rural smallholder farmers in the participating countries, i.e. livestock parasitic diseases which are a nutritional and economic burden for these farmers and their families. **Method:** By way of a "sandwich" programme the students usually undertake research methodology and project design training in Denmark, return to their home country to conduct relevant field research and then return to the veterinary faculty and collaborating institutes in Denmark for data analysis and composition of their thesis/dissertation.

Results: Four PhD studies have been completed and 7 more are underway. Ten Masters degrees have been earned and 2 more are anticipated. Research topics receiving support have included important helminthoses of large & small ruminants, pigs, poultry and donkeys as well as anthelmintic resistance. Research findings are presented at an annual project meeting. **Conclusion:** The project has been effective at enhancing helminthological research capacity at the veterinary faculties and government/other research institutes in the targeted countries, promoting south-south and north-south collaboration as well as extending research findings to end users and other stakeholders. The research findings in many instances are relevant for agricultural development, food security/safety and public health.

Acknowledgement: The Danish International Development Agency's Enhancement of Research Capacity (ENRECA) Programme for funding.

E2

PASTURE INFECTIVITY ON COW PASTURES AT THE END OF THE GRAZING SEASON

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Background: Anthelmintic treatment of dairy cows can improve milk yield. However, most dairy cows graze exclusively on cow pastures and faecal egg output in adult cows is low. Therefore, it is difficult to understand why these production losses occur. Because very little is known on the pasture infectivity of cow pastures a survey was carried out between September and November 2000.

Methods: On 25 dairy farms faeces were collected from 15 cows and blood from 5 of these. Random herbage samples and samples from the bushes around faecal pats were collected on two cow pastures. Parameters included EPG (Wisconsin Method); differentiation of pooled larval cultures; Baermann (21 farms only); serum pepsinogen values; ELISA with crude worm antigens of *Ostertagia* and *Cooperia oncophora* and a 14.2 recombinant *Cooperia* protein as antigens and Pasture larval counts.

Results: Mean faecal egg counts were 4 EPG, ranging from 1-11 per farm. *Ostertagia* and *Cooperia oncophora* were the dominating larval types. In 6 out of 21 farms lungworm larvae were detected. Pepsinogen and ELISA values were low. Larvae were found on the pastures of 24 farms. Numbers ranged between 0 and 3437 larvae/kg dry herbage with geometric means of 84 and 136 larvae in random samples and bushes, respectively. Again *Ostertagia* and *C. oncophora* were the dominating larval types. Considering that the random samples will more or less reflect larval intake this implies a mean daily intake of 1000 L3/day/cow, a level that cannot be considered harmless for calves.

Conclusion: Despite the low EPG, pasture infectivity on cow pastures in autumn is high enough in most farms in the Netherlands to explain production losses.

E3

PROTECTION AGAINST *Dictyocaulus viviparus* FOLLOWING PRIMARY INFECTION AT DIFFERENT DOSE LEVELS

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Background: Immunity against lungworm develops rapidly and strongly following high primary infection dose levels. In reality calves usually encounter much lower primary infections than often tested in experiments. Experiments were conducted with a range of primary infection doses to study development of protection against a challenge infection, and to fill in some gaps in a provisional simulation model on the parasitic phase of lungworm infection in calves.

Methods: In 2 experiments pairs of calves were primary infected with one of a series of dose levels, ranging from 5 to 2000 L3. Calves were challenged with 2000 L3 on day 10 (experiment 1) or day 35 (experiment 2). In each experiment challenge control calves were included. Eleven days after the challenge calves were necropsied for worm counts and measurements. Results were compared with predictions from a simulation model.

Results and conclusions: Establishment of the primary infection was dose independent, being on average 20-30%. The ratio female:male worms in the counts from primary or challenge infection was consistently close to 1:1. Level of early protection (10 days after a primary infection) against establishment of the challenge infection depended significantly on primary infection dose level. At 10 days after a primary infection low dose levels did not result in protection against a challenge infection. In contrast, similarly low dose levels did result in partial protection 35 days after the primary infection. Results confirmed that our provisional simulation model satisfactorily predicts primary infection outcomes, but that it doesn't accurately predict levels of protection against re-infections.

E4

GASTROINTESTINAL PARASITES IN TRADITIONAL ZEBU CATTLE IN IRINGA, TANZANIA

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Background: This study aimed to determine the prevalence and intensity of nematode and cestode infestation in traditional Zebu cattle in Iringa region of Tanzania through an abattoir survey.

Method: Gastrointestinal tracts were randomly purchased at the abattoir in Iringa and transported to the laboratory. The abomasum, small intestines and larger intestines were ligated to prevent mixing of intestinal contents. Each portion was opened separately, washed in water and the contents sieved to recover worms.

Results: Results indicated that 100% of animals were infected. All infected animals had more than one parasite specie. Helminth species isolated and their prevalence were *Haemonchus contortus* (100%), *H. similis* (28.5%), *Oesophagostomum radiatum* (71.4%), *Cooperia pectinata* (57.1%), *C. punctata* (42.8%) and *Moniezia benedeni* (14.2%). The average worm burden in animals was 557 (range 4-2533) worms per animal. There was no difference in worm burden between male and female animals. *Haemonchus similis* is reported for the first time in Iringa.

Conclusion: We concluded that there is high prevalence of nematode infestation in Zebu cattle in Iringa. Studies on the precise epidemiology should be carried out in order to design appropriate control measures.

Acknowledgement: This study was wholly sponsored by DANIDA through the ENRECA Livestock Helminth Research Project in Tanzania.

E5

DAIRY FARM CIRCUMSTANCES AND
HELMINTHOSIS IN MBALE DISTRICT, UGANDA

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A cross-sectional study involving coprological examination of animals, stratified according to agro-ecological zone (AEZ), grazing system and age, was carried out to determine the magnitude and spectrum of helminth infections in the smallholder dairy farms in Mbale district. Concurrently, a structured and closed questionnaire was administered to capture information on dairy farm circumstances. The results showed that in the midland and upland AEZs, the common grazing systems were zero-grazing and tethering, while in the lowland AEZ, free range grazing system was the most popular. Most of the dairy farm-related activities were carried out by the household head, assisted by children, other adults and hired labour. Housewives were increasingly involved in these activities in areas bordering the town, while the hired labour was commonly used in rural areas. The prevalence of mixed nematode infection was highest in animals <1 year old, especially under zero-grazing and free range grazing systems in the lowland and upland AEZ's, respectively. However, under tethering system, the prevalence was independent of AEZ. The prevalence of fasciolosis was associated with the grazing systems, but not with AEZ's. The prevalence was higher in animals >1 year old than those <1 year old under the free range and zero-grazing systems. Generally, helminth control practices and intensity varied with AEZ and grazing systems and in many cases, they were haphazard. There is, therefore, a need to develop cost-effective control measures for helminthosis, based on location/production systems-specific investigation that target biological, management and socio-economic parameters of the production systems.

E7

LIVER FLUKES IN SHEEP AND CATTLE IN
SOUTHERN ITALY-PARASITOLOGICAL MAPS
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Background: The most common liver flukes of sheep and cattle in Southern Italy are *Fasciola hepatica* and *Dicrocoelium dendriticum*. The aim of this research was to study the distribution of these parasites in sheep and cattle bred in a mainly hilly and mountainous area comprising part of three regions of Southern Italy: Campania, Basilicata and Apulia.

Methods: The study area cover 92 contiguous municipalities. The sample size, 197 ovine and 81 bovine farms, was calculated using the formula proposed by Thrusfield, considering the total ovine and bovine pasturing farms in the area, the expected prevalence, the desired absolute precision, and the confidence interval. Using *Idrisi*, a G.I.S. software, the farms were selected in the area by the systematic sampling method. Faecal samples collected from 3,940 sheep and 975 cattle were processed by quantitative flotation and sedimentation techniques.

Results: Out of the 197 ovine farms, 8(4.1%) were positive for *F. hepatica* and 133 (67.5%) for *D. dendriticum*. Out of the 81 bovine farms, 9 (11.1%) were positive for *F. hepatica* and 43 (53.1%) for *D. dendriticum*. The results were evaluated statistically and parasitological maps were drawn using *Idrisi*.

Conclusion: *D. dendriticum* was the most spread liver fluke in the ovine and bovine farms of the study area.

Acknowledgement: This research was supported by POM-A13 grants.

E6

PARASITE EGG AND LARVAE OUTPUT TENDENCIES
IN SHEEP DURING ONE YEAR IN A EXTENSIVE
FARM OF MAGALLANES, CHILE.

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Background: To describe the epidemiology of sheep parasitosis in Magallanes, a study was done in a extensive farm from spring 1999 to spring 2000. The tendency of egg and larvae output of gastrointestinal and pulmonary parasites was determined from groups of 9800 Corriedale spring born lambs, 1800 ewe hoggets and 10500 ewes. The animals did not receive anthelmintic treatment.

Method: Every 14 days, faeces were obtained from 25 animals of each age group sampled at random. Epg of nematodes and cestodes and presence of pulmonary nematodes larvae were determined coproscopically.

Results: The lambs started showing egg outputs of *Nematodirus*, *Ostertagia* and *Trichostrongylus* in spring, reaching their maximum in summer; low epg were found during fall and winter; *Nematodirus* was predominant. In the ewe hoggets and ewes the egg output presented similar tendency but with lower epg counts. *Nematodirus* epg output was low in ewe hoggets and absent in ewes; *Trichostrongylus* predominated during fall and winter. All nematode epg values presented a similar tendency as the mean monthly temperature of Magallanes, without peri-parturient spring-rise. *Moniezia expansa* eggs were shown in lambs and ewe hoggets during spring. *Dictyo-caulus filaria*, *Muellerius* and *Protostrongylus* larvae were detected in small percentages of the samples in the groups of ewe hoggets and ewes during winter months. Through necropsy the gastrointestinal nematodes identified were *T. vitrimus*, *T. axei*, *O. circumcincta*, *N. filicollis*, *Oesophagostomum venulosum*, *Chabertia ovina*, *Trichuris* sp. and *Capillaria* sp.

Conclusions: No clinic or acute parasitosis were observed in the animals during the study and no anthelmintic treatments were necessary.

E8

EXPERIMENTAL STUDIES ON CONGENITAL
TRANSMISSION OF SCHISTOSOMA JAPONICUM

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Objective: Experimental observation on Congenital transmission of *Schistosoma japonicum*. **Method:** Mother rabbits in different pregnancy were infected with different dose of *S. japonicum* cercariae. A challenge infection was done for prenatal infected rabbitlets. **Results:** In middle and later pregnancy, 15.0 % and 46.7 % of kittens were infected respectively. The rabbits in late pregnancy were infected with 300, 500 or 700 cercariae. The infection rates of kittens were 46.7 %, 61.9 % and 79.0 % respectively. There was no significant difference between experimental and control group of prenatal infected rabbits with 20 cercariae of challenge infection. **Conclusion:** It was confirmed the early funding of congenital transmission of *S. japonicum* infection in rabbits. The infection rate of rabbit kittens might be related to different pregnancy of mother rabbits, embryo development, infection dose of cercariae, etc. The challenge infection indicated that the prenatal infected rabbitlets has no protective immunity.

E9

EPIDEMIOLOGY AND PATHOLOGY OF AMPHISTOMOSIS IN RUMINANTS OF BANGLADESH

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Background: Amphistomosis is a snail borne trematode parasitic disease of ruminants. A general notion is that the adult parasites are harmless. Further, the epidemiology of the disease is poorly understood in Bangladesh. This paper is an appraisal on the epidemiology, and pathology of amphistomosis due to adult amphistomes in ruminants.

Method: Visceral organs of slaughtered cattle, buffaloes, sheep and goats, and aquatic snails from different water bodies from both hills and floodplains were examined for 2 consecutive years. The gross and microscopic changes caused by the adult amphistomes were recorded.

Results: The animals were found infected with at least 3 or more species of the flukes. The overall burdens and ratio of adult, young and immature flukes varied among the animal species, and were very much age related. Infections both in the ruminants and snail intermediate hosts occurred throughout the year. A high incidence of amphistomes in ruminants during July – October and low during March – June, was consistent with the abundance of snail intermediate hosts in the pasture. Amphistome cercariae in the snails (*Indoplanorbis exustus*, *Bithynia tentaculata* and *Thiara tuberculata*) were marked during September – November than other months of the year. Young and adult amphistomes caused moderate to severe denudations of the rumen papillae leading to chronic inflammatory changes with marked parakeratosis, and hyperkeratosis of the epithelial layers.

Conclusion: This study revealed amphistomosis as a chronic disease of ruminants all over Bangladesh, more or less in all seasons of the year. This also revealed cattle and buffaloes as the most adapted hosts of amphistomes than sheep and goats.

E10

MIXED HELMINTH INFECTIONS IN PIGLETS

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Background: Mixed helminth infections are common in outdoor reared pigs, but only little is known on neonatal infections in piglets.

Method: Three groups of sows were either uninfected (n=12), trickle infected with a low (n=13) or a high level (n=14) of *Ascaris suum*, *Trichuris suis* and *Oesophagostomum dentatum* during pregnancy. Half of each litter was trickle infected with the same 3 helminths from week 1 after farrowing until weaning (week 7) when all piglets and sows were slaughtered to obtain worm counts.

Results: Infected sows initially excreted high levels of *T. suis* eggs until onset of worm expulsion after which excretion of *O. dentatum* eggs rose rapidly, indicating an interaction between the two species. Patent *A. suum* infections were not obtained in the sows and very few *A. suum* and *T. suis* were recovered at slaughter. Average *O. dentatum* burden $\pm$ SD for the low and high dose sows was 3797 $\pm$ 3130 and 31309 $\pm$ 16192, respectively.

Overall, piglets did not excrete eggs of *A. suum* and *T. suis*, and even *O. dentatum* egg counts were scarce despite prepatency is only 3 weeks. Average worm counts for the infected piglets were 131 $\pm$ 122, 151 $\pm$ 132 and 527 $\pm$ 828 for *A. suum*, *T. suis* and *O. dentatum*, respectively.

Conclusion: Piglets seem more susceptible to *A. suum* infections than older pigs and *T. suis* may have a negative effect on *O. dentatum*. The possible influence of the infection status of the sows on the piglets infections is discussed.

Acknowledgement: The Danish National Research Foundation supported the study.

E11

PARASITIC FAUNA OF THE SICILIAN BLACK PIG

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Background: Sicilian black pig is a particular morphotype animal, which lives wild mainly in the Nebrodi park (about 11.000 ha in the province of Messina, western Sicily). Despite of the growing interest registered for this animal, only few parasitological surveys have been carried out. The aim of this survey is to study the parasites enriching the taxonomic data with SEM description and some epidemiological inferences.

Method: During the 1999-2000, 72 pigs were necroscopically examined for parasites, 41 of them were free ranging in the Nebrodi park and 31 were farmed.

Results: The identified parasites in free ranging and farmed pigs were respectively: *Sarcocystis* spp. (85.4% versus 0), *Ascaris suum* (46.5% vs 0), *Ascarops strongylina* (76% vs 3.2 %), *Globocephalus urosulatus* (76% vs 0), *Macracanthorhynchus hirudinaceus* (32% vs 0), *Metastrongylus elongatus* (48.4 % vs 0), *M. pudendotectus* (29.2% vs 6.4%), *Oesophagostomum dentatum* (90% vs 3.2%), *Physicocephalus sexalatus* (5% vs 0), *Trichuris suis* (0 vs 10%), *hydatid cysts* (49% vs 0), *Haematopinus suis* (7% vs 0).

Conclusions: The parasites found are the same of the wild boars in other Italian regions. Some of these are known to have a high pathogenicity and for the recover, in a modern zootechnical system, a strategy for a control plane is strongly needed.

E12

EFFECTS OF NEMATODES ON HEALTH AND PRODUCTIVITY OF OUTDOOR SOWS AND SUCKLING PIGLETS

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Background: The influence of nematode infections on performance of young sows in gestation and lactation and on the health and productivity of suckling piglets was investigated in an out-door environment.

Method: Three groups of young sows were either trickle infected with low or high doses of *A. suum*, *O. dentatum* and *T. suis* (group L (n=13) and H (n=14), respectively) during pregnancy or served as uninfected controls (group C (n=12)). Half of the piglets in each litter were trickle infected with the same nematode species during the 7 week suckling period after which animals were slaughtered for worm counts. The sows were moved outdoor in early gestation.

Results: The litter size and birth weight of piglets were not markedly affected by infections of sows during gestation but the piglet mortality during lactation was higher in group H litters. The infected piglets (half of each litter) had means of 131 $\pm$ 122 *Ascaris*, 527 $\pm$ 828 *Oesophagostomum* spp. and 151 $\pm$ 132 *Trichuris* at slaughter whereas uninfected piglets had negligible worm counts. The weight gains of infected and uninfected piglets (week 0-7) were 397 $\pm$ 59 and 407 $\pm$ 57 g/day, respectively, representing a reduction of 2.5 % due to infection (p<0.05). The slaughter weight of infected piglets was reduced by 4.3 % (p<0.001) and the killing out percentage by 2.0 % (p<0.001).

Conclusions: Concurrent *A. suum*, *O. dentatum* and *T. suis* infections during gestation had no major impact on the reproductive performance of sows. Piglets infected early in life experienced marked growth retardation during lactation but growth rates were not affected by infection history of their dam.

E13

PREVALENCE OF SWINE COCCIDIOSIS IN THE PHILIPPINES

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Background: In the Philippines, diarrhea is an economically important problem in pigs but coccidia have not been reported as pathogens. A previous study showed the presence of *Eimeria* sp. but not *Isospora* sp. The study was aimed to determine the prevalence of coccidiosis in nursing piglets and examine the relationship of some management and housing factors on prevalence.

Method: Faecal samples from 5 to 30-day old piglets and 10 sows of nursing piglets in 14 farms in 5 locations were examined for coccidiosis. Management and housing data obtained were subjected to multiple regression analysis.

Results and Conclusion: *I. suis* was the only coccidian species detected in 12 of 14 farms sampled and only from piglets 6 to 27 days of age. Overall prevalence was 26%, varying between 8% and 67% in different farms. It was found in diarrheic (72%) and formed feces. Management and housing variables were not found correlated with prevalence. The study indicates that *I. suis* is an important pathogen in suckling piglets and should be considered as a cause of preweaning diarrhea. The present study is the first national record of *I. suis* in swine.

E15

THE EFFECT OF AGE AND SEX ON THE FAECAL EGG COUNT (FEC) IN THE DONKEY.

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Background: To establish whether donkeys predisposed to endoparasites fell within certain age or sex categories, a retrospective analysis of faecal egg count data from the Donkey Sanctuary herd was performed.

Method: As part of an anthelmintic control strategy, faecal egg counts were monitored on two Donkey Sanctuary farms. 15% of the resident donkeys were randomly selected at the start of the sampling period. Faecal samples were collected every two weeks from eight weeks after treating the herd with anthelmintic prior to turnout. This continued through the grazing period, until housing. Faeces were submitted to the Donkey Sanctuary laboratory for analysis using the modified McMaster technique. The egg counts were recorded as the number of strongyle eggs per gram (epg) of faeces. If more than 25% of the sample group had FECs greater than 200 epg then the herd was treated with oral anthelmintic. During the two years of this study moxidectin oral gel (Equest; Fort Dodge) was the chosen anthelmintic. Both descriptive and bivariate analyses, were performed controlling for time to explore the effects of age and sex whilst taking into account other significant factors.

Results: Both age and sex were significant in bivariate analyses older animals (>23 years) had significantly higher counts than younger animals ($P=0.02$) and jennies had significantly higher counts than geldings ($P<0.001$).

Conclusion: Aged donkeys and jennies appear to be predisposed to strongyle parasitism as measured using faecal egg counts.

Acknowledgements:

The Donkey Sanctuary, especially the Veterinary Department and farm staff.

E14

TRANSMISSION OF *TOXOPLASMA GONDII* IN CONFINEMENT-REARED PIGS

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Background: *Toxoplasma gondii* has been found at prevalence rates as high as 50% in pigs raised in outdoor production systems in the U.S., raising concerns about the role of pork in transmission of this parasite to humans. In this study, we determined infection rates in pigs raised in confinement systems and identified management factors that contribute to transmission on the farm.

Method: Pigs from 58 pork production sites representing total confinement management systems were tested, by ELISA, for antibodies to *Toxoplasma gondii*. All *Toxoplasma*-positive sites and a cohort group of *Toxoplasma*-negative sites were visited and an audit used to evaluate risk factors for transmission of this parasite.

Results: The overall prevalence of *T. gondii* was 0.09% (33/37,908). Eight production sites produced pigs positive for *Toxoplasma*; infection rates on these sites ranged from 0.1 - 2.0%. Cats were observed on 5/8 *Toxoplasma*-positive sites, but only on 6/16 *Toxoplasma*-negative sites. Cats were never observed inside production facilities and security measures indicated that cat entry was unlikely. In all cases, for *Toxoplasma*-infected sites, producers did not use "barn-only" boots when entering the production facility.

Conclusion: Despite strict biosecurity, limiting entry of cats into pork production sites is not completely effective in eliminating exposure to *Toxoplasma* oocysts which may be in the environment. Pig producers must use barn only boots as a preventative measure.

E16

ISOLATION OF EQUINE *BABESIA* IN MONGOLIA AND ITS GENETIC ANALYSIS

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Background: High prevalence of equine babesiosis was observed in Mongolia. However, isolation or characterization of parasites was not studied in Mongolia. In the present study, in vitro cultivation was used for isolation of *Babesia* parasites from Mongolian horses and genetic analysis of isolated parasites was reported.

Methods: Twenty blood samples were collected from horses and used for in vitro cultivation. DNA was extracted from cultured parasites and used the amplification of EMA-1 gene by PCR, and nucleotide sequences were determined.

Results: One sample was found positive result at the first day of microscopic examination. Fourteen *B. equi* and one *B. caballi* isolates were acquired by culture although samples were positive in IFAT for both *Babesia* parasites. Analysis of EMA-1 gene sequences indicated a 12% and 5.8% difference at nucleotide and amino acid sequence level, respectively, between USDA and Mongolian isolates.

Conclusion: These results would be valuable for the understanding of epidemiology of these parasites.

E17 NON-CHEMICAL APPROACHES TO NEMATODE PARASITE CONTROL OF CATTLE IN SWEDEN

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Background: Nematode parasitic infections and their control in young cattle are becoming increasingly important issues in Sweden. Factors that impact on this are economic, consumer concerns, government policy and maintenance of open landscape.

Method: A grazing trial was carried out over three consecutive years during 1998 - 2000. Each year, first-year grazing cattle were used on improved pasture paddocks with the following 5 treatment comparisons: 1) rotational grazing using pasture grazed by older cattle the previous year as spring pasture and a mid-summer move to aftermath; 2) biological control using the nematophagous fungus *Duddingtonia flagrans*; 3) ultra-low dose copper treatment (COWP bolus); 4) untreated; and 5) Ivermectin bolus treated animals. Apart from the rotational strategy, all treatments were set stocked. Treatments 4 and 5 represented negative and maximum parasite control measurements respectively.

Results: The evasive strategy proved to be very effective as these animals grew as well as the bolus treated cattle. The biological control treatment resulted in an average weight gain advantage of 40 kg in contrast to the untreated set stocked animals. COWP bolus had very limited benefit.

Conclusion: These results clearly illustrate the importance of subclinical nematode parasitism in young grazing cattle in Sweden. In this trial the untreated, set-stocked animals showed no overt signs of parasitism. However, substantial benefits (40 - 60 kg weight gain) can result from effective parasite control. Importantly, this has been shown to be achievable and sustainable without resorting to the use of chemical drugs.

Acknowledgement: The Swedish Council for Forestry and Agricultural Research (SJFR)

E19 EPIDEMIOLOGICAL DATA: CONDUCTING FIELD STUDIES TESTING PARASITICIDES IN PETS

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Background: Since 1995 five studies were conducted testing parasiticides in Germany, France and/or Switzerland according to Good Clinical Practice. Feces of a huge number of dogs and cats were screened in all countries in ~50 veterinary practices. Epidemiological data on the frequency of parasitic diseases occurring simultaneously is short and the gathered data is analysed for this purpose.

Results: In total 2750 fecal samples of dogs and 1600 fecal samples of cats were analysed using a modified McMaster technique. Tapeworms (Echinococcae, Dipylidium and Taeniae), Toxocara/Toxascaris, Ancylostoma/Uncinaria, Trichuris, Isospora and Capillaria were observed. The prevalence, incidence of concurrent parasitic disease as well as differences per countries and the influence of the age of the animals are presented. Potential difficulties to apply to the guidelines are discussed.

Conclusion: The data show that it is getting more challenging to conduct antiparasitic field studies according to GCP. The number of samples collected show the tremendous effort that needs to be taken to get a certain number of positive samples. Although animals < 1 year have a higher prevalence, round- and tapeworms occur in elder animals also. The data show the need for products that are efficacious against both round- and tapeworms.

E18 A SURVEY OF BENZIMIDAZOLE RESISTANCE IN SCOTTISH SHEEP FARMS

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Background: A previous non-random in vitro anthelmintic resistance study conducted in 1991 in Scotland showed that about 24% of the farms had worm populations that were resistant to the benzimidazoles (BZ). Since studies at Moredun Research Institute suggest that there is little possibility of reversion to susceptibility the aim of the survey was to determine the current extent of anthelmintic resistance in Scotland.

Methods: Anaerobic sample collection kits together with a questionnaire on worm control and anthelmintic usage were sent to 227 Moredun Foundation members. Eggs recovered from the submitted faecal material were used in egg hatch and larval development assays.

Results: Over 47% of the kits were returned, 92% of the returned kits contained sufficient parasite material for inclusion in the study. Overall BZ resistance was evident on 60.8% of the farms, with respective prevalences of 81%, 56% and 45% for lowland, upland and hill farms. BZ resistance was equally evident in both ewe and lamb samples. The survey provided no evidence of resistance against drugs within the imidazothiazole/tetrahydropyrimidine or macrocyclic lactone families. Preliminary analysis of the questionnaire data showed that farmers treated their ewes and lambs 2.8 and 3.1 times per annum (TPA) respectively, on farms where BZ resistance was evident lambs were treated on average 3.5 TPA compared to 2.7 TPA on farms where there was no resistance.

Conclusions: There has been a marked increase in the prevalence of BZ resistance in Scotland over the last ten years in some areas the prevalence is over 90%. These findings have clear implications for the farming community which, given the paucity of novel anthelmintic products on the horizon, need to adopt measures to conserve the efficacy of the other two broad spectrum families.

E1 p PHOTOPERIOD EFFECT ON THE HIPOBIOSIS OF O. OSTERTAGI UNDER FIELD CONDITIONS

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Hipobiosis is an important aspect of the biology of some gastrointestinal nematodes of the ruminant, particularly *O. ostertagi*. The occurrence of this phenomenon is predominantly before the unfavorable season for the free living stages, i.e. in the Southern Hemisphere during spring. The aim of the present work was to study the effects of photoperiod upon the induction to hipobiosis in a high proportion of the infective larval stage (L3) of *O. ostertagi*. The experiment was done during the spring of 1997 (September to January). Faeces from donor animals were collected for coprocultures. The obtained L3 were distributed at the beginning of each month in paddocks free of parasites and exposed to the natural conditions of the season. At weekly intervals during the corresponding month, and then monthly until January, 2 tracer animals were introduced to each paddock to graze during 3 days. After grazing, the animals were stabled and fed with lucerne hay, free of parasites. 3 weeks later, the tracers were slaughtered and the parasite burden of their abomasum was analyzed. The percentage of growth inhibition for each treatment was calculated.

There is a strong correlation between the percentages of larval inhibition and the photoperiod (Spearman, $r=0.87$ $p<0.0001$). The highest proportion of inhibited larvae (64-86%) was found during December, when the photoperiod is longer than 14 h, but these values drop abruptly (6-10%) at the end of December and at the beginning of January, when the day-length stabilizes and the photoperiod starts to decrease. Our observations indicate that a day-length greater than 14h 45min would have a negative effect on the induction to hipobiosis.

E2 p

BOVINE NEMATODIASIS IN ARGENTINA: THE DEVELOPMENT OF EGGS TO LARVAE IN FAECES.

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Gastrointestinal trichostrongylid infections are the major cause of production losses in the grazing systems of the world. Cattle are more susceptible in their first grazing season, and the parasite nematode species involved are *Ostertagia*, *Cooperia* and *Trichostrongylus*. Knowledge of the parasite bioecology during the free-living stages becomes important to establish efficient control alternatives. The aim of this study was to determine the effects of temperature upon the rate of larval development. The experiment was done on pasture consisting of grass and legumes, covering 0.96 ha. The area was divided into 16 paddocks using an electric fence, and surrounded by a 20 cm deep ditch. During 4 years, from July 1994 to June 1998, 2 infected calves were introduced to graze monthly. On the 15th day of each month, the faecal pats were removed from the paddock being contaminated and, keeping its original shape, placed outside the electric fence. To determine the time required for the eggs to hatch and develop to infective larvae (L3), a 5 cm² portion of the pats was collected at weekly intervals and the L3 recovered in an aqueous medium. The development period from egg to L3 was inversely proportional to the temperature, being of 3-6 weeks in winter, 1-4 weeks in spring, 1-2 weeks in summer and 2-6 weeks in autumn. With a temperature $\geq 20^{\circ}\text{C}$ and below 5°C , the maturation from egg to L3 took 1 week and 6 weeks, respectively. The highest prevalence and the fastest development was observed during autumn-winter for *Ostertagia* and *Cooperia*, and during summer for *Cooperia* and *Trichostrongylus*.

E4 p

A STUDY OF THE EPIDEMIOLOGY OF INTERNAL PARASITES OF CATTLE IN THE HIGHVELD AND LOWVELD COMMUNAL FARMING AREAS OF ZIMBABWE: AN OVERVIEW

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Between January 1999 and December 2000, 17 500 cattle faecal samples from randomly selected dipping sites in the Lowveld and Highveld communal farming areas were examined for helminths eggs and coccidial oocysts. Strongyle larvae were also identified following the culture of pooled faeces. The monthly presence or absence of metacercariae was noted from grass samples collected around water drinking points and monthly snail densities were also determined.

Strongylid nematodes were the most prevalent parasites, followed by paramphistomes species and *Fasciola gigantica*. Infections with other parasites was less prevalent. Calves had highest the prevalence of strongylid nematodes and coccidia while adults had the highest prevalence of paramphistomes species and *Fasciola gigantica*. The prevalence of most common internal parasite infections was higher during the wet season.

Data from larval culture indicated that the animals carried mixed parasite burdens. *Cooperia*, *Haemonchus* and *Trichostrongylus* were the most prevalent genera found. *Haemonchus* was more prevalent in summer, while *Cooperia* and *Trichostrongylus* were present in significant numbers during winter and the hot dry season.

Metacercariae were qualitatively found to be present on grass samples collected around watering points from January to August and November to December.

Districts with a higher prevalence of *Lymnaea natalensis* had higher fasciola infection in cattle. With respect to paramphistomes, districts with higher prevalence of either *Bulinus tropicus* or *Bulinus forskalis* or *Biomphalaria* also had a higher paramphistome infection in cattle. Schistosomiasis in cattle was found to be higher in districts where the prevalence of *Bulinus globosus* was high.

E3 p

BOVINE NEMATODIASIS IN ARGENTINA: SURVIVAL OF LARVAE 3 IN PASTURE

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The aim of the present study was to determine the seasonal effect on the survival of gastrointestinal parasite nematode infective larvae (L3) in pasture.

The experiment was carried out at the University Campus, in Tandil, on a pasture consisting of grass and legumes, covering 0.96 ha. The area was divided in 16 paddocks, using an electric fence, and surrounded by a 20 cm deep ditch. During 4 years, from July 1994 to June 1998, the paddocks were monthly contaminated by 2 infected calves grazing each paddock. At weekly intervals the contamination was monitored and a contamination curve was drawn using the values from the weekly egg per gram of faeces counts (e.p.g). The infectivity of the pasture and the survival of the L3 was analyzed every second week. The contamination levels were similar to those described for cattle in our area, being the maximum e.p.g. values during autumn-winter, and decreasing by the end of the spring and summer. The transfer of L3 from faecal pats to pastures was associated with rainy periods. Infectivity was prominent during winter and low during summer, indicating a high mortality of L3 during the later period due to adverse climatic conditions. Infective larvae originated in the second half of the year that survived inside the pats during the summer, contributed to the basal contamination for the animals grazing during autumn, particularly after a dry summer. In contrast, the L3 originated during the first half of the year, did not survive the adverse period, but were inhibited in their development until the beginning of the summer. The species with the highest persistence in the pasture (more than a year) were *Ostertagia* and *Cooperia*, particularly those larvae which arose from spring contamination.

E5 p

SEASONAL AVAILABILITY OF STRONGYLATE NEMATODE LARVAE ON PASTURE IN KENYA

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Background: Studies on the seasonal differences in infective nematode larval availability are essential to the understanding of control of strongylate nematode infections.

Methods: The level and type of infective strongylate nematode larvae on pasture were followed using a series of tracer calves from July 1995 to June 1996 in the central highlands of Kenya. Data from faecal nematode egg counts, pasture larval recoveries and worm counts from the tracer calves were used to investigate the infectivity pattern.

Results: The number of infective larvae on pasture was moderate, reaching 1485 kg⁻¹ dry matter of grass during the period of and soon after the rains, and remained low in the absence of rainfall. Larval counts on pasture were directly related to the rainfall pattern, but were also influenced by the number of raindays in the period. *Haemonchus* was the most predominant genus, followed by *Trichostrongylus*, *Cooperia*, *Oesophagostomum* and *Bunostomum* in decreasing order. The mean total adult worm burdens of tracer calves were related to the levels of herbage infective larvae.

Conclusion: These results indicates that a reduction in the contamination of pasture with nematode eggs before the rains could result in pastures carrying fewer larvae and thus form the basis of effective worm control programmes for cattle.

E6 p

PARASITOLOGICAL SURVEY ABOUT CATTLE BREEDED IN GALLURA (SARDINIA)

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Background: The pasture bovine breeding represents, lacking an environmental and animal control, a risk factor for parasitoses. The aim of this survey is to study the parasitological situation in cattle free living in Gallura (Sardinia) where this kind of breeding represents the main livestock resource.

Method: From June 1999 to May 2000, 1029 cattle coming from 52 breeding units was coprologically examined. On the faecal samples was carried out a quali-quantitative analysis. Results were processed stratifying the sample according to age, sex, kind of pasture, anthelmintic treatment.

Results: 90,4% (42) of examined herds and 32,6% of cattle resulted positive. The parasites identified were: gastrointestinal strongles (GIS) (+109; 10,6%); *Eimeria* spp. (+63; 6,1%); *Toxocara vitulorum* (+13; 1,3%); Paramphistominae (+181; 7,6%); *Fasciola hepatica* (+16; 1,6%); Anoplocephalides (+11; 1,1%). A cattle resulted positive for *Dictyocaulus viviparus*. The infestation levels for GIS resulted of mean gravity (70,6%), while the Paramphistominae ones reached an high level of clinic risk (23,2%).

Conclusion: The low level of GIS showed a good control of this parasitoses, while paramphistomosis appears to represent a problem that need a different approach to reach a good level of control.

E7 p

EXPERIMENTAL ELAPHOSTRONGYLOSIS OF CATTLE

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Background: *Elaphostrongylus cervi* is a common in Eurasia parasite of intramuscular connective tissue and central nervous system of Cervides. The aim of this work was the examination of susceptibility on infection with this parasite of cattle.

Methods: Four calves 4 months old were infected per os with the rising doses from 10000 to 50000 L_{III} *E. cervi*, and one calf was the control.

Results: 6 dpi in calves infected with 30000 and 50000 L_{III} *E. cervi* were observed lack of appetite, weakness, cough, and mucous outflow from nostrils. There were also present rales in bronchi and exacerbated murmur in lungs. Calf infected with 30000 L_{III} 15 dpi completely lost appetite and on 20 dpi showed significant wasting. Still was present very strong paroxysmal cough and purulent outflow from nostrils. The calf was anaesthetised and necropsied 30 dpi. In the calf infected with 50000 L_{III} from 18 dpi the cough was less and disappeared murmur in lungs, but during 8 days have been observed decrease of motility of gastrointestinal tract and a little flatulence in the region of left iliac fossa. The calf was anaesthetised and necropsied 60 dpi. Necropsies of both calves showed presence of liquid in pleural and peritoneal cavities, enlargement of mesenteric and mediastinal lymphonodes, inflammatory changes in lungs, foci of infarct in kidneys and hyperaemia in central nervous system. Spinal cord was surrounded by fibrinous exudate in which were found fragments of dead larvae of *E. cervi*. In animals infected with lower doses none symptoms of invasion have been seen.

Conclusion: In cattle only very high invasions with nematodes *E. cervi* cause clinical symptoms in respiratory and digestive systems.

E8 p

HELMINTHOSES AND COCCIDIOSIS OF RUMINANTS IN THE NORTHERN GUINEA SAVANNA ZONE OF NIGERIA.

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BACKGROUND: Ruminants are the major source of protein for a large majority of the Nigerian populace. A large percentage of ruminants are found in the Northern states where they are predisposed to malnutrition, environmental stress and most importantly, parasitic infections. The effective control of gastrointestinal parasites requires knowledge of their epidemiology.

METHOD: Faecal samples of ruminants obtained by ambulatory clinicians from various herds throughout the zone were subjected to flotation and sedimentation techniques. Infection rates as well as monthly and yearly distributions of the infections were calculated and statistical differences tested by the use of the analysis of variance (ANOVA).

RESULT: A total of 7828 samples were analyzed between January 1985 and December 1994. The overall prevalences of various parasites recorded were: *Strongyle* spp (54.2%), *Coccidia* (63.8%), *Strongyloides* spp (15.2%), *Fasciola gigantica* (5.8%), *Paramphistomum* spp (4.98%), *Monilezia* spp (8.2%), *Trichouris* spp (4.1%), Hookworm (3.4%), *Neosascaris* spp (1.6%), *Dicrocoelium* spp (0.10%), *Avitellina* spp (0.01%), and *Thysanites* spp (0.01%). The highest percentage of infection was recorded during the rainy season (July-October) and the lowest percentage during the dry season (January - May). The yearly prevalence of infection fluctuated irregularly with no discernible pattern.

CONCLUSION: At any point in time, a high population of ruminants in this zone harbour gastrointestinal parasites. This has obvious implications for their productivity.

E9 p

THE PREVALENCE OF *ONCHOCERCA OCHENGII* IN CATTLE IN UGANDA

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Background: *O. ochengi*, a parasite of cattle, was first described in Uganda. Its epidemiology is not known but would be expected to be highest in the west (Fort Portal) where there are many swift streams. A survey was undertaken to determine its prevalence in cattle at three abattoirs.

Method: The two halves of the hides were palpated for nodules. These were differentiated from tick bites. Skin snips were incubated in phosphate buffered saline at 37°C and microfilariae counted. Nodules were digested and worms identified.

Results: 5/115 cattle in Kampala had nodules and 36 had microfilariae. Percentages were similar in cattle from the central area. By contrast 3/25 from Fort Portal had microfilariae and none had nodules.

Discussion: Prevalence of *O. ochengi* is low in the parts of Uganda examined and very low in the areas with swift streams, home for black flies (*Simulium* sp.). This is probably due to both an onchocerciasis control programme and widespread use of pyrethroids to control ticks on cattle. It is concluded that *O. ochengi* is not a practical problem in Uganda.

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E10 p

THE EPIDEMIOLOGY OF FASCIOLOSIS IN MINAS GERAIS STATE, SOUTHEAST OF BRAZIL

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The epidemiology of *Fasciola hepatica* infection has been studied since 1998 in the region of Itajubá, located at Minas Gerais state in the southeast of Brazil. In this region, the prevalence of *Fasciola hepatica* in cattle is 10.59% based on the examination of fecal samples. The calves begin to be infected at the age of two months which is when they begin to graze. The highest level of contamination occurs in the dry season (March-September). *Lymnaea columella* is the intermediate host, which lives in the drainage furrows of the pastures, in association with the aquatic plants *Eichornia azurea* and *Heteranthera reniformis*. The highest populations of *Lymnaea columella* were observed in the month of November, beginning of the rainy season. The removal of the aquatic plants from the drainage furrows in March and October caused a reduction of the snail population in these months. The rate of natural infection of the *Lymnaea columella* varied from 0 to 5%.

E12 p

FREQUENCY OF LARVAE I OF *Hypoderma lineatum* IN CATTLE FROM MEXICO

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Background: The aim of the present work is to communicate the presence of Larvae I (L-I) of *Hypoderma lineatum* from cattle sacrificed in Chihuahua, Mexico.

Method: The material consisted of oesophagous collected from two slaughterhouses from Chihuahua. Monthly samples were obtained during July, September, October and December, 2000. Longitudinal cuts of the oesophagous were carried out and L-I were isolated, and counted.

Results: In July: 21(38.8%) out of 54, and 1(1.8%) out of 53 oesophagous were found positive, at the slaughterhouses (SLH) No. 1 and 2, respectively. In September: 17(51.5%) out of 33 were positive. at SLH No. 1 and all organs collected at SLH No. 2 were found negative. In October: 4 (20.0%) out of 20 and 9 (45.0%) out of 20 oesophagous were positive at SLH No. 1 and 2, respectively. In December: 1(4.54%) out of 22 oesophagous were found positive at SLH No. 1 and all organs (31) collected at SLH No. 2 were found negative.

Conclusion: The presence of L-I of *Hypoderma lineatum* was observed in the four samplings. The highest number of larvae were collected between July and October. Further investigations should be undertaken for at least one more year of sampling, to corroborate the presence of L-I larvae during the months previously observed to suggest future measures of early diagnostic and control of *Hypoderma lineatum* at Chihuahua, Mexico.

E11 p

PREVALENCE OF INFESTATION BY *PARAMPHISTOMUM daubneyi* AND *FASCIOLA hepatica* IN LIMOUSIN

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Background : The two trematodes *FASCIOLA hepatica* and *PARAMPHISTOMUM daubneyi* use the same intermediate guest for the biological cycle, the *Lymnaea truncatula* ; to complete the development of their larval stages. The fasciolosis is very well known in the world and has been studied in many fundamental and clinical research programmes. It is different from the paramphistomose whose emergence is more recent.

Method : The prevalence of *PARAMPHISTOMUM daubneyi* and *FASCIOLA hepatica* was studied in 13 000 coproscopies tests on cattle and carried out for eight years in the Corrèze department. The analysis was also completed by parasitology post mortem on 45 young beef cattle after their first year in pasture. The zootechnical development evaluated of the young cattle in growth.

Results : For ten years *PARAMPHISTOMUM daubneyi* infestation of cattle was observed in 17.4 per cent of animals and *FASCIOLA hepatica* in 19.53 per cent. The prevalence of infestation by *PARAMPHISTOMUM daubneyi* increased from 5.2 per cent in 1990 to 44.7 per cent in 1999, while infestation by *FASCIOLA hepatica* increased by 25.25 per cent in 1993 and decreased by 18.6 per cent in 1999. Both infestations more frequently appeared during November at March. The paramphistomes infestation affected 28.8 per cent of young cattle after their first year in pasture.

Conclusion : In the breeding conditions of Limousin cattle, the animals infestation by both *FASCIOLA hepatica* and *PARAMPHISTOMUM daubneyi* occurred in the same pasture zones. The therapeutical control of the *FASCIOLA hepatica* favoured the *PARAMPHISTOMUM daubneyi* development.

E13 p

CATTLE TREMATODOSIS IN THE SENEGAL RIVER BASIN AFTER THE CONSTRUCTION OF DIAMA AND MANANTALI DAMS

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Ecological changes in the Senegal River Basin resulting from the construction of a barrage at Diama to prevent the intrusion of sea water into the river and a dam at Manantali to control the flow of water have been responsible for changes in epidemiology of cattle fasciolosis, paramphistomosis and schistosomosis on the mauritanian side of the river. Necropsies data from slaughterhouse, cattle herds surveys (egg excretion examination), serological examination of calves cohorts, malacological studies (diversity, population dynamics, natural and experimental infections with Trematodes) were performed between 1995 and 1998. Negligible before the Diama dam construction, fasciolosis prevalence reaches now 20% and 60% in some particular areas. Paramphistomosis and Schistosomosis are also highly prevalent (60 to 70%). Transmission occurs mainly during the late dry season during the peak of *Lymnaea natalensis* and *Bulinus truncatus* populations. The maximum of egg excretions is reported at the end of rainy season, when animals grazed on sahelian pastures and not in the Senegal Valley. Favourable and unfavourable factors to transmission of cattle Trematodes are discussed as well as control strategies.

E14 p

MICROFILARIAL PREVALENCE IN CATTLE IN NORTH WESTERN NIGERIA.

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BACKGROUND: Bovine filarial parasites play an important role as models for chemotherapy, diagnosis and vaccine development for human onchocerciasis in addition to possibly causing unexplained neurological disturbances and modulating infection with *Onchocerca volvulus* in man.

METHOD: Blood samples were collected from 621 cattle in 6 herds in the zone and processed using the modified Knott's technique. Positive smears were stained with either Giemsa, Mayers haemalum or brilliant cresyl blue and isolated microfilariae identified using morphological criteria.

RESULT: The overall prevalence rate in all the herds was 8.21%. Herd prevalences ranged from 11.48% to 0%. More males (8.61%) than females (8.09%) were infected even though the difference in infection rates was not statistically significant. The highest infected breeds were the Crosses (18.18%) and Ndama (14.20%) followed by the White Fulani (9.66%) and Sokoto Gudali (0.99%). No infections were recorded in the Red Bororo breed, possibly due to lack of exposure to infection. Isolated microfilariae were mostly *Setaria labiotopapillosa* with only one case of *Onchocerca armillata*.

CONCLUSION: The presence of blood dwelling microfilariae in cattle in an area endemic for human filarioses has obvious Public Health significance.

E15 p

TRANSFER OF *BOVICOLA BOVIS* AND *LINOGNATHUS VITULI* FROM DONOR TO PRINCIPAL CATTLE

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OBJECTIVE: A study was conducted to test a method of transfer of *B. bovis* and *L. vituli* from donor to principal cattle.

METHODS: Thirty-six male Fleckvieh cattle (donors) were ranked according to Day -1 *B. bovis* (predominant species) counts into 2 strata and then by Day -1 b. w. (128-188 kg) to 3 replicates of 6 animals. Twenty-four male Fleckvieh cattle (louse-free principals) were randomly allocated on Day -1 b. w. (130-197 kg) to 4 replicates and were then assigned by block, 1 to a pen (size: 7.5 m²). Donors were randomly assigned to 1 of 4 exposure groups: G1 - 1 donor to each principal for 3 days; G2 - 2 donors to each principal for 3 days; G3 - 1 donor to each principal for 6 days; G4 - 2 donors to each principal for 6 days. Lice were counted on the principals on Days 0, 7, 15, 21, 28, 35, 42, 49 and 56 and on the donors on Days -1, 3/6, 13, 27 and 34 from 12 predilection sites.

RESULTS: A significant ($p < 0.05$) overall effect of sampling day was noted for *L. vituli* and *B. bovis* counts. *L. vituli* transferred to 100% of principals in all groups. For *L. vituli*, principals exposed to 2 donors had higher louse counts (26.6-50.2) than those exposed to one donor (17.8-36.6) ($p = 0.0506$). For *B. bovis*, principals exposed to donors for 6 days had higher counts (2.3-3.1) than those exposed for 3 days (1.5-1.0) ($p = 0.0527$).

CONCLUSION: The results indicate that this method may serve as an alternative technique to the induced infestation in cattle louse trials.

E16 p

EPIDEMIOLOGY OF STRONGYLE INFECTIONS OF SHEEP IN THE MARMARA REGION, TURKEY

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Background: This study was performed to investigate the epidemiology of strongyle infections in lambs in the Marmara region, western Turkey.

Methods: On each of four governmental farms (A-D) groups of 50 lambs of local breed, 3-4 months old at the trial's start, were examined for nematode egg counts and haematocrit values and were weighed in monthly intervals during the grazing season (April or May to November) of 1998. No anthelmintic treatments were given. In addition five lambs per month were necropsied for worm counts on farm B.

Results: All lambs started to shed strongyle eggs in May to July. On farm A, high egg counts (mean: 2200-2700 EPG) were found in June and July, and larval cultures showed *Haemonchus* as the predominant species. Low haematocrit values were measured in July to September. However, the farm A lambs showed the highest mean weight gain (28 kg) at the end of the trial. In contrast, the strongyle egg output remained generally low on farms B-D, and maximum egg counts (mean: 115-330 EPG) were found in October or November. The predominant species on these farms were *Ostertagia* and *Trichostrongylus*. Lambs necropsied on farm B harboured only low worm burdens of *Ostertagia*, *Trichostrongylus*, *Nematodirus*, *Chabertia* and *Oesophagostomum*; mucosal larvae were not detected using the pepsin-HCl digestion technique in any animal. Farm B-D lambs gained only 4-11 kg bodyweight in average at the end of the trial.

E17 p

Some Studies on Helminthes of Sheep

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Examination of 173 sheep carcasses from January to the end of December 1999 showed that 45.1% of examined sheep had been infested with different helminth parasites.

It is found that 5.2% infested with nematodes, 25.4% infested with cestodes and 3.5% infested with trematodes.

Nematode species were; *Haemonchus contortus* (3.5%), *H. placei* (1.7%), *Trichostrongylus axei* (5.8%), *Parabronema skrjabini* (2.9%), *Ostertagia trifurcata* (1.2%), *Chabertia ovina* (0.6%) & *Strongyloides papillosus* (0.6%) and *Graphidiopsis species* (2.9%). Trematode species were *Camyrtus gregarius* (5.8%), *Paramphistomum species* (3.5%), *Fasciola gigantica* (2.9%) and *Cotylophoron cotylophorum* (1.7%). Cestode worms were *Moniezia expansa* (19.7%), *Avitellina centripunctata* (14.5%), *Stilezia globipunctata* (10.4%), *Hydatid cyst* (1.2%) and *Cysticercus tenuicollis* (11.6%).

It was found *Graphidiopsis species* infesting abomasum as well as duodenum of sheep and considered to be recorded for the first time in Egypt.

Regarding to seasonal prevalence infection with nematode parasites was high during autumn (15.2%) followed by summer 11.1%, winter (9.4%) and spring (5.6%). Infection with trematode worms was high during summer (22.2%) followed by winter (9.4%), autumn (9.1%) and spring (7.4%). Infection with cestode worms was high during autumn (42.4%), followed by spring (38.9%), summer (18.5%) and winter (11.2%). Also infection with cestode larvae among sheep was high during summer (40%) followed by autumn 27.3%, winter (31%) and spring (0%).

E18 p *Raillietia caprae*: ISOLATION OF *Mycoplasma* spp. IN MITES FROM MEXICO

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Background: Previous investigations in Brasil, Australia and U.S.A. have communicated the presence of several species of *Mycoplasma* in mites of the genus *Raillietia*.

Objective: The aim of the present work is to communicate the presence of *Mycoplasma* spp. in *Raillietia caprae* mites of the ear canal of goats.

Method: The material consisted of isolated mites employing commercial cotton wool swab of goats sacrificed at two different slaughterhouses in Mexico. The mites were crushed out using Petri dish covers, capillary tubes and Friss liquid medium. Then anaerobic cultures were produced in the before mentioned media. The growth of *Mycoplasma* colonies was observed in places where solid liquid media was sown.

Results: The number of positive cases to mites were 20. The number of isolated mites varied from 1 to 12. The growth of *Mycoplasma* spp. was observed in 15 (75.0%) out of 20 cases.

Conclusion: This is the first report on the presence of *Mycoplasma* spp. isolated from mites *Raillietia caprae* in Mexico.

E19 p DETECTION OF ANTIBODIES AGAINST *Sarcoptes* MITES IN RED FOXES (*VULPES VULPES*) IN THE CITY OF BERLIN, GERMANY

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Background: Apart from human specific *Sarcoptes* mites other representatives of this genus are also able to infect man causing pseudo-scabies. Complaints of hunters about the increase of scabies in the fox population in Berlin were the reason to analyse this problem.

Method: 1025 sera from Berlin foxes sent for necropsy between 1996 and 1999 were sampled and analysed by an indirect ELISA for antibodies against *Sarcoptes* mites. The cut off was established comparing the extinction of serum samples of 110 negative farm foxes with those of 198 wild foxes showing clinical scabies. Clinical and serological findings in foxes of different age groups, bagging time and origin were compared. Prevalence and C.I.'s were generated with EPI-INFO 6.04.

Results: 16.4 and 21.7% of young and adult foxes respectively showed scabies lesions while antibodies against *Sarcoptes* mites were found in 37.9 and 48.1% of the examined hosts in both age groups respectively. The percentage of foxes with scabies lesions rose from 13.8% in 1996 to 22.2% in 1999. There was also an increase in serologically positive foxes being highest in 1998 (50.8%). Serological prevalence in adult foxes throughout the year varied between 46 and 52%, while in juvenile foxes it rose from 26% in the second quarter to 49% at the end of the year.

Conclusions: Compared to rural areas the scabies prevalence under the conditions of the city of Berlin is much higher. The reason for increasing clinical cases may be seen in connection with rising fox densities. Initially, mites are transmitted from bitches to their puppies. Clinical cases are connected with stress factors.

E20 p OCCURRENCE OF SARCOPTIC MANGE IN RED FOX POPULATION IN EAST SLOVAKIAN REGION

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Background: One of the most common diseases of red foxes is mange caused by *Sarcoptes scabiei*. Therefore sarcoptic mange is considered one of limiting factors furthering the decline in red fox population density.

Method: Gross examination of the predilection sites by the mites located on the rump, tail, flanks and back. For the demonstration of the parasite and its developmental stages deep skin scrapings were taken and examined under the microscope after maceration in 10% KOH.

Results: 42 individuals (15.5%) from 271 wild red foxes (*Vulpes vulpes*) hunted in East Slovakian region during years 1989-2001 were infected with *Sarcoptes scabiei*. According to age structure from 189 adult foxes 36 individuals were affected (20.1%), and from 82 juvenile foxes 6 ex. (7.3%) were affected. There were no significant differences of affected animals between sexes. 13 ex. (14.1%) from 92 females and 29 ex. (16.2%) from 179 males was mange positive.

Conclusion: We suppose that real prevalence of sarcoptic mange in wild red fox population is higher than data obtained in our study.

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E21 p STUDY ON SMALL RODENT HELMINTHOCENOSIS IN CONNECTION WITH THEIR COMMUNITY STRUCTURE IN BELARUS

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Background: Ecological and morpho-physiological similarity of small rodents determines a joint base of their helminthocenosis, but difference between the rodent species leads to a species-specific helminth infestation. Therefore, the revealed pronounced habitat-related variation in the rodent community should form very flexible rodent helminthocenosis in relation to difference in landscape structure. The basic question in that is to analyse small rodent helminthocenosis in connection with their community structure

Method: 692 small rodents of eleven species captured in the whole variety of habitats were helminthologically dissected. Small rodent community structure was investigated by snap-trapping during 17000 trap-nights.

Results: Helminth species composition of eleven small rodent species was revealed. Frequency of occurrence of each helminth species in population of each small rodent species and number of helminth individuals in the small rodents infested were estimated. Habitat-related specificity of small rodent community was studied.

Conclusion: Structure of helminthocenosis of small rodent community is mostly determined by helminthocenosis of dominant species due to both higher invest of its own helminths and adequate trend in the helminthocenoses of other small rodent species.

Acknowledgment: The Institute of Zoology NAS of Belarus financially supported the study.

E22 p**HELMINTH FAUNA OF THE FAROESE PUFFINS (*FRATERCULA ARCTICA*)**Saeed¹, J., Nolsø², A. & Mouritsen², K. N.

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Background: The helminth parasites has not yet received attention in Faroese puffins. We were very fortunate, therefore, in being able to examine atlantic puffins helminthes on this islands

Method: A total of 173 puffins collected at their summer breeding area in a colony in the Faroe Islands during July and August 2000. They were dissected and examined for helminthes.

Results: helminthologic examinations revealed the total of 5 helminth species were found at autopsy: three nematodes- *Streptocara crassicauda* in the esophagus and gizzard, *Seuratia shipleyi* in the crop and *Contracaecum oscutatum* in the small intestine; one trematode- *Gymnophallus deliciosus* in the gall bladder; one cestode- *Alcataenia* sp. In the small intestine. Moreover, a tongue worm (*Reighardia* sp.) was found in the different organs. The variations in the infection of different ages and sexes are discussed. The average number of each helminth is tabulated.

Conclusion: All these species are first records in Faroe islands.

E23 p**PARASITES OF CAMELIDS IN THE UK**

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Background: Small American camelids (SAC) are becoming popular animals in the UK, but there is very little information about their parasitic problems and the optimal methods of parasite control.

Method: A questionnaire was sent to members of the British Llama and Alpaca Association. Faecal samples collected before and after treatment by owners were counted using the modified McMaster technique. Larvae were identified from faecal cultures and nematodes were examined from gut samples collected at post-mortem. Wool combings were taken from post-mortem animals.

Results: Sixty five questionnaires were completed. The most commonly reported parasitic problem was 'mange' (23%) respondents, but the species involved are not known. *Psoroptes ovis* were collected from the ears of one SAC under clinical investigation. All faecal samples from adults were low (<450 epg) and half the herds where there were pre- and post treatment samples had < 50 epg before treatment. 89% of respondents were using ivermectin for nematode and ectoparasite control. The two most common genera from faecal cultures were *Ostertagia* (*Teladorsagia*) and *Trichostrongylus* and from post-mortem animals, *Nematodirus* and *Trichuris*.

Discussion: Samples collected suggest that in the UK SACs suffer from similar parasites to sheep, but that adult animals do not normally carry heavy nematode burdens. Further research is required into the problem of 'mange' and what percentage is caused by *P. ovis*.

Acknowledgement: Supported by Merial Animal Health.

E24 p**CAPILLARIA SP IN FARMED LESSER RHEA (*PTEROCNEMIA PENNATA*), FIRST REPORT**

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Background: *Capillaria* spp are known to parasitize the intestinal tract of domesticated and wild birds. In this paper we present the finding of *Capillaria* eggs in faeces from lesser rhea (*Pterocnemia pennata*), a flightless bird naturally distributed in Argentina and Chile.

Method: The helminth levels in farmed lesser rheas were examined on 10 farms of Northern Patagonia, Argentina, over 1-year period. 310 faecal samples were collected from birds of various ages on 3 occasions and analysed by sedimentation and flotation methods. On positive samples, faecal egg counts (epg) were performed. All the birds of these farms were raised from eggs artificially incubated. Neither clinical nor subclinical helminthoses were observed on any of the farms.

Results: 9 (41 %) out of 22 samples from only one farm were positive to *Capillaria* eggs, and 5 epg counts were performed ($\bar{x} = 740 \pm 239$). The eggs had the characteristic bipolar plugs and 16 of them were measured showing $44-56$ ($\bar{x} = 48,5 \pm 3,5$) by $20-28$ ($\bar{x} = 21,5 \pm 2,9$) μm . The infestation by *Capillaria* sp detected could have been produced by cross-contamination with other avian species, such as chickens and turkeys, present on this farm. Also, the careless management, the high animal density, as well as the poor hygiene observed inside of the pen, must have contributed to the parasitic infection.

To the best of our knowledge, this is the first report of *Capillaria* in lesser rhea. Further studies concerning taxonomic classification and epidemiology of *Capillaria* sp in this specie are needed.

Acknowledgement: This study has been funded by INTA and FONCYT (PICT No. 8-3568).

E25 p**MTDNA DIVERSITY IN *HAEMONCHUS CONTORTUS* FROM SYMPATRIC CHAMOIS AND GOATS**

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Background: *Haemonchus contortus* is a major constraint for sheep and goats worldwide, causing production loss and in some cases clinical parasitic gastroenteritis. Moreover, it is considered a generalist nematode, easily adaptable to several different host species and important from a sanitary point of view because of its high pathogenic potential. In recent years, *H. contortus* has been correlated to body condition loss and severe abomasitis in wild chamois and roe deer on Italian Alps, mainly in areas where dramatic increase of sheep and goat flocks occurred on the grazing pastures. Therefore, domestic ruminants have been considered as the true reservoir of this parasite. The aim of this work is to investigate by molecular techniques the possible existence of different populations of *H. contortus*, specific of wild or domestic ruminants.

Method: Partial mitochondrial ND4 gene was amplified and sequenced in 30 *H. contortus* males from sympatric chamois and goats. Sequences have been then compared in order to assess genetic variability. Sequences from *H. placei* and *Teladorsagia circumcincta* have been used as outgroups.

Results: Preliminary data seem to indicate that no variation exists in ND4 sequences between *H. contortus* from chamois and goat. Nevertheless, it is interesting that phylogenetic analysis on ND4 splits the examined samples in two groups, with quite high bootstrap support, each one including sequences from chamois and goat parasites.

Conclusion: Further investigations are needed to define molecular epidemiology of *H. contortus* on alpine pastures. Analyses on ND4 gene are still in progress, and examination of other genes (e.g. ribosomal genes) should be considered.

E26 p THE MEGATRYPANUM TRYPANOSOMES FROM CERVIDAE IN POLAND

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Background: The trypanosomes of the subgenus *Megatrypanum* are recovered from wild ruminants in Poland. However, they are not very well known, and their relativity is also unclear.

Method: Seven (6 females, 1 male) of 46 (13%) fallow deer (*Dama dama*) in age from 1,5 up to 4 years and 3 (1 male, 2 females) of 13 (23%) moose (*Alces alces*) in age from 2 up to 6,5 years from Mazurian Lakeland (north-east Poland) were positive for trypanosome infection by direct microscopic examination of blood centrifuged in microhematocrit tubes and on Giemsa stained blood films. Bloodstream trypanosomes were identified on morphological, mensural and statistical grounds.

Results: Trypanosomal forms seen on slides were typical *Megatrypanum* trypomastigote and epimastigote stages. Trypanosomes from moose were bigger than from fallow deer (mean BL 44.18 and 36.03 µm, respectively). However, the proportions of body were similar (mean NI 0.89 and 0.80, KI 2.12 and 2.25, FF:BL 5.24 and 7.12, respectively). They were compared with similarly handled trypanosomal material from red deer (*Cervus elaphus*) from Poland. No significant differences appear to exist between trypanosomes from *Alces alces*, *Dama dama* and *Cervus elaphus* and they were identified as *Trypanosoma cervi* Kingston and Morton 1975.

Conclusion: Our studies indicate, that trypanosomes infection are quite common in wild ruminant in Poland (red deer, moose, fallow deer). They show a low level intensity of infection and weak pathogenicity in their vertebrate hosts.

E27 p *Giardia duodenalis* INFECTION IN DOMESTIC ANIMALS IN ARGENTINA

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Background *Giardia duodenalis* is a protozoon distributed worldwide that causes an important enteric parasitic infection that affects animals and humans. In Argentina there is little data about giardiasis in domestic animals. The objective was to study the prevalence of *G. duodenalis* infection in dogs under veterinary supervision and to detect it in other domestic species from Buenos Aires Province.

Methods An examination of 1260 faeces samples from several species of domestic animals from Buenos Aires Province was performed at the Laboratory of Parasitology and Parasitarian Diseases, using the formalin-ether concentration method. Data concerning age and sex were registered for all animals and the presence or absence of diarrhea was also recorded for 200 dogs.

Results *G. duodenalis* was found in 70 (7.7 %) of 907 dog samples; the prevalence was 10% for dogs aged 2 years or less and 1.5 % for dogs older than 2 years. Diarrhea was reported for 10 (50 %) of 20 giardia infected dog samples. The parasite was detected in 7 (3.2%) of 218 cat samples. The parasite was detected in diarrheic faeces as follows: in 28 (40 %) of 70 bovine samples (aged 15 days to 3 months, obtained from 9 dairy and 2 beef farms); in 7 (23.3 %) of 30 sheep samples (aged 6 months); in 2 (13.3%) of the 15 adult equine samples; in guinea pigs and in chinchillas.

Conclusion The results obtained indicate that giardiasis could be widely distributed in the province of Buenos Aires. More studies would be necessary to determine the economic and public health impact of the disease.

E28 p CLINICO-THERAPEUTIC STUDIES ON THEILERIOSIS IN CALVES OF THAR OF INDIA

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Theileriosis is a tick borne protozoan disease caused by *Theileria annulata* in cattle causing high mortality in young calves and susceptible adult cattle with heavy economic losses. Out of some 450 clinical cases of theileriosis in calves observed during 1999-2000, thirty calves of either sex, different age groups (3 days to 6 month old) and breeds (native Rath and its crosses with HF/Jersey/reddane) were selected for detailed clinico-pathological and therapeutic trial in this study. Clinical manifestation were pyrexia (up to 106°F), pallor of mucosa, anorexia, dyspnoea, diarrhoea, weight loss and enlargement of superficial lymph nodes. In some cases, convulsions with paddling movements were also recorded. The cases were confirmed with blood smear and lymphnode biopsy examination. Haematology and biochemistry revealed leukopenia, decreased haemoglobin and packed cell volume and reduction in serum glucose levels. The cases were treated with different regimen by using injections of oxytetracyclines, Berenil, chloroquine phosphate and buparvaquone. A mortality rate of 25-30% was recorded. Effect of injection buparvaquone with oxytetracycline showed better results. To reduce the economic losses some newer vaccines and control of drug resistant ticks was necessary.

E29 p CHRONIC SURRA AMONG CAMELS IN NORTH-WEST DESERT TERRAIN OF INDIA

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The major constraints of augmenting productivity and draft efficiency among camels in the desert terrain of North-Western parts of India are trypanosomiasis (Surra) caused by *Trypanosoma evansi* due to the abundance of vectors viz., *Tabanid* flies, persistent shortage of water resources with attendant scarcity of green foliage and widespread prevalence of ectoparasitic infestation viz., mange as well as insidious helminthosis.

Camels play a pivotal role as important component of transportation to the public as well as paramilitary patrolling agencies like the Border Security Force (BSF) in addition to providing means of livelihood to thousands of nomadic desert dwellers in this part of the country.

With a view to assess the current status of health 46 samples of blood were screened for haematological changes and parasitological examination by way of microscopic examination by wet blood and thin stained blood smears and antibody detection was conducted by card agglutination test for *T. evansi* (CATT). The above camels belonged to three BSF battalions stationed at various parts of Rann of Kutch extending over Gandhidham, Bhuj and Banaskantha districts of North Gujarat.

Of the 46 samples screened 8 (17.39) were positive for Surra by blood examination and 12 (26.08) camels sera was positive for antibodies by CATT. Barring unthriftiness associated with low grade haemolytic anaemia, they did not exhibit any other untoward clinical signs. Chemotherapy with Quinapyrimine proslits lent appreciable improvement.

The authors are highly thankful to the Commandants of different BSF battalions, India for providing camels for the research work.

E30 p *Trypanosoma evansi* IN ROYAL BENGAL TIGERS (*Panthera tigris*) OF DHAKA ZOO, MIRPUR, DHAKA, BANGLADESH
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Three Royal Bengal Tigers (*Panthera tigris*) in Mirpur Zoo, Dhaka, Bangladesh suddenly became ill in August 1999. One male cub (1yr 21 days old) and his mother (7 Yrs. 2 months old) and the father (8 Yrs. 7 months) showed sudden onset of symptoms like recumbency, tremor, and dyspnea with high temperature 104.5°F. Despite treatment with antitrypanosomal drugs the cub died within two hours and the parents within 4-5 hours of the onset of symptoms. Another two cubs (O + O) showed similar symptoms in mid September '99 the female cub died within three days whereas the male cub survived; they were treated with different antitrypanosomal drugs combined with vitamins.

Blood samples examined from the affected tigers were found positive to *Trypanosoma evansi* except one cub. Smears stained with Leishman's stain showed heavy infection with *T. evansi*. A large number of the parasites were found devoid of flagellum and were in multinucleate and binucleate forms apparently in the process of division.

The suspected mechanical vectors such as *Tabanus* sp and *Stomoxys* sp. could not be detected in the zoo area. Therefore, it was assumed that after ingestion of beef from infected cattle, *T. evansi* might have entered the circulation through injured oral mucous membrane of the tigers and caused the disease. After detection of *T. evansi* on 25-8-1999 from affected tigers all the other apparently healthy 14 tigers and 18 lions were treated with Triquin (Quinapyramine sulphate+ Quinapyramine chloride) as a precautionary measure. Blood samples from all the tigers and lions were again examined on 21-9-99 and the samples were found negative to *T. evansi*. Thereafter, blood smears of all the cattle slaughtered for beef for Zoo animals were checked occasionally to ensure free from *T. evansi*. Then the beef were cooked and supplied to the tigers/lions and no deaths occurred since then. *T. evansi* is prevalent among pony and cattle in Bangladesh; it has also been recorded in dogs. But this is first recorded case in Bangladesh causing deaths in Royal Bengal Tigers.

E31 p THE IMPACT OF SURRA AMONGST VARIOUS CAMEL BREEDS: A PILOT STUDY IN LAIKIPIA DISTRICT OF KENYA.

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Background: Kenya has a camel population of approximately 1 million. Nomadic pastoralists keep most of these animals under traditional management system. Trypanosomosis caused by *Trypanosoma evansi* is the major disease affecting camel production. In the present study, camel trypanosomosis was investigated to determine its impact on the productivity of various camel breeds. **Methods:** Data was collected on herd production parameters and health variables for a period of 16 months. Four herds (2 male and 2 female) consisting of Somali, Turkana, Pakistan breeds and Pakistan-Somali cross were monitored in Laikipia District.

Results: Mean *T. evansi* prevalence ranged from 11.1% by microhaematocrit centrifugation technique to 37.9% using CATT/*T. evansi* test. Trypanosomosis reduced the daily milk off-take by 57.1%, 42% and 35.3% in Pakistan, Somali and Turkana breeds respectively. Overall mortality rate due to Surra was 5.5%. Young stock mortality and calving percentage varied with seasons and breeds. The cost of veterinary care was on average US\$ 2.36 per camel per year. Veterinary intervention reduced the overall mortality rate to 2.3% per year.

Conclusion: This study indicated the potential of improving camel productivity through effective trypanosomosis control coupled with professional advice.

Acknowledgement: The authors would like to thank staff of Biochemistry Division at KETRI for participating in this work. This study received financial support from Kenya Government and FARM-Africa.

E32 p CHRONIC WASTING DISEASES IN CATTLE: A PARTICIPATORY STUDY IN TONJ COUNTY, SOUTHERN SUDAN.

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Background: Participatory research involves active participation of the researcher and the target groups in planning, implementation and evaluation. This makes participatory research an important tool in diagnosis of animal diseases.

Method: A survey was conducted in Southern Sudan using participatory appraisal tools and parasitological techniques to monitor the diseases that cause chronic wasting in cattle.

A total of 637 animals were sampled from 4 cattle camps in Thiet region of Southern Sudan. Information was collected through review of secondary data, matrix scoring, haematological test, post-mortem and faecal sampling.

Results: Results indicate that trypanosomosis is prevalent in this zone as shown by the Buffy coat method (5.08%) and Ab. Elisa (45.5%). All infecting trypanosomes were identified as *Trypanosoma congolense* through giemsa stained thin smears. Post-mortem findings in adult cattle with chronic weight loss showed heavy infestation with schistosomes and flukes. Analysis of disease matrices demonstrated significant agreement between informant groups.

Conclusion: From this study, it is evident that trypanosomosis, schistosomiasis and fascioliasis may infect in combination and may be associated with chronic wasting in cattle. To boost animal production, these diseases must be included in the control programmes.

Acknowledgement: We thank Participatory Approaches to Veterinary Epidemiology (PAVE) project for sponsoring this work and Director KETRI for allowing us to participate in this work.

E33 p OBSERVATIONS ON THE OLD WORLD SCREW WORM FLY, *CHRY SOMYA BEZZIANA* VILLENEUVE (DIPTERA: CALLIPHORIDAE), IN SAUDI ARABIA

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The occurrence of the Old World screw worm fly (OWS) in the Gulf area is a threat to public health, livestock and wildlife in the Middle East. However, little is known about cutaneous myiasis in general and OWS infestations in particular in Saudi Arabia. Data on traumatic myiasis were recorded from 1993-1998 at Jeddah and Bureida Veterinary Diagnostic Laboratories. Maggot samples and their accompanying data were collected from myiasis cases seen at the laboratories or sent from various parts of the country. Some larvae were allowed to pupate to obtain adult flies.

Five species of flies were identified in one or more of the 33 cases of cutaneous myiasis recorded. Of these cases, 13 were caused by OWS, 10 by *Wohlfahrtia nuba* and 5 by *W. magnifica*. Cases due to OWS and *W. magnifica* were recorded in the central and northern regions, respectively, whereas cases due to *W. nuba* were found in the most regions of the country. Sheep and goats were the most common hosts, with the anus and udder being the most common sites of infestation. Most cases occurred in winter and spring (December-May).

It seems certain that more OWS myiasis cases occurred but were not reported, owing to the failure of veterinarians to submit samples and the lack of awareness of owners of the need to bring their animals for treatment. It is expected that OWS infestation will spread and increase only slowly in such a generally harsh desert environment, with widely dispersed flocks of sheep and goats. Further intensive studies are needed to provide more detailed data on the geographical distribution and pest status of OWS in Saudi Arabia. Successful control should be based on identification of infested areas and a complete involvement of owners and veterinarians in a well organized campaign of prevention and treatment. This is the first record of *W. magnifica* in Saudi Arabia and our reports indicate that *W. nuba* could be an important agent of cutaneous myiasis there.

Acknowledgement: We thank Dr. Martin Hall, of the Natural History Museum, London, for identification of some flies and revising the abstract.

E34 p

GASTRO-INTESTINAL HELMINTHOSES OF PIGS IN ZARIA, NORTHERN NIGERIA.

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BACKGROUND: Nigeria has a pig population of 7 million with the lowest distribution in the largely Moslem Northern part of the country. Very little information is available on porcine helminthoses in this zone to assist the few piggeries that exist.

METHOD: Twenty pigs semi-intensively raised were used for the study. After slaughter and evisceration, the gastrointestinal tract was ligated and post mortem worm count carried out. Isolated worms were identified using morphological characteristics.

RESULTS: Ninety percent of all pigs examined were infected with gastro intestinal helminths. All the worms encountered were nematodes and included *Oesophagostomum dentatum* (65%), *Ascaris suum* (30%), *Ascarops strongylina* (30%) and *Physicocephalus sexalatus* (30%). Mixed infections were encountered in 50% of the sampled animals. There was no significant difference in infection rates for male and female animals. All age groups were infected with the highest infection rate (100%) in the pigs below 9 months of age.

CONCLUSION: Gastrointestinal helminthoses are highly prevalent among pigs in Northern Nigeria and should be controlled to improve productivity.

E36 p

PREVALENCE OF TAENIA HYDATIGENA CYSTICERCOSIS IN PIGS IN BALI, INDONESIA

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Background: A slaughterhouse survey was carried out to determine the prevalence of *Taenia hydatigena* cysticercosis in pigs in Bali.

Methods: The peritoneal and pleural cavities of 185 porcine carcasses were examined by meat inspection in local slaughterhouses of six districts in Bali between December 1999 and March 2000. The localisations of cysticerci found were recorded.

Results: *T. hydatigena* cysticerci were found in 67 (36%) of all pigs examined. Cysticerci were located in the omentum (60%) or under the serosa of liver (46%), rectum (3%), diaphragm (2%), urinary bladder (2%) and lungs (2%).

Conclusion: The present results indicate that *T. hydatigena* cysticercosis is common in pigs in Bali, and the prevalence is higher than previously reported (11-18%).

E35 p

FAECAL AND SEROLOGICAL SURVEY ON ENDOPARASITE INFECTIONS OF SOWS IN BALI, INDONESIA

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Background: The pig population in Bali consisted of 970,000 animals including 124,000 sows in 1998. The aim of this cross-sectional study was to obtain first substantial data on endoparasite infections of pigs in Bali.

Methods: Faecal (n=464) and plasma samples (n=520) were collected from breeding sows of 138 owners from rural communities in two districts (Gianyar, Tabanan) between March and May, 1999. Faecal samples were examined by the McMaster technique and the formalin-ether sedimentation method. Plasma samples were analysed by ELISA techniques for antibodies to *Toxoplasma gondii*, *Sarcocystis* spp. and *Trichinella* spp. Data on the sanitary conditions, husbandry and control measures were collected by a questionnaire.

Results: Faecal stages of the following parasites were found: (i) helminths: strongylids (48% of the sows), *Ascaris* (11%), *Trichuris* (4%), *Metastrongylus* spp. (4%), *Strongyloides* (3%) and *Physicocephalus* (0.4%); (ii) protozoa: *Balantidium coli* (92%), *Eimeria* spp. (60%), *Jodamoeba* (37%), *Entamoeba* (36%), *Chilomastix* (11%) and *Giardia* (0.4%). Feeding with weeds and plant residues was positively ($p < 0.05$) associated with the prevalence of strongylids, *Ascaris* and *Eimeria* infections. No other risk factors were found. Antibodies to *T. gondii*, *Sarcocystis* spp. and *Trichinella* spp. were present in 32%, 18% and 0.4% of the sows, respectively.

E37 p

Ctenocephalides felis felis BOUCHÉ, 1835 (Arthropoda: Siphonaptera), INFESTING *Didelphis albiventris* (Marsupialia: Didelphidae) IN THE PROVINCE OF CORRIENTES, ARGENTINA.

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Background: *Ctenocephalides felis felis* Bouché, (1835) is the common cat flea which also is found on several other species of wild and domestic mammals. The aim of this study is to report the findings of *Didelphis albiventris* (white-eared opossum) infested by *Ctenocephalides felis felis* (Arthropoda: Siphonaptera) and to describe some external morphological features of the fleas using a scanning electronic microscope (SEM).

Method: The A.A. studied n=31 *Didelphis albiventris* hunted by traps in their native environments near Corrientes City, Argentina (59° W. longitude, 27° S. latitude). The fleas, collected on the skin, were washed into a physiological solution and then classified using a conventional optic microscope (COM). Later these specimens were put into a 3% glutaraldehyde solution, dehydrated, dried by critical point, assembled, metalised, observed and photographed using a scanning electronic microscope (SEM).

Results: 29 *Didelphis albiventris* (93.5 %) were infested by fleas. Using a conventional optic microscope (COM) and according to Bicho & Ribeiro's key, all the specimens were classified as *C. felis felis*.

Conclusions: This study is the first report in Argentina on *Ctenocephalides felis felis* infesting *Didelphis albiventris* (white-eared opossum) and the A.A. considered it was caused by a secondary infestation from cats

E38 p

MORPHOLOGICAL ASPECTS OF *Brachylaima* sp. DUJARDIN, 1843 (Digenea: Brachylaimidae) INFESTING *Didelphis albiventris* (Marsupialia: Didelphidae) IN THE PROVINCE OF CORRIENTES, ARGENTINA.
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Background: *Didelphis albiventris* (white-eared opossum) is one of the native marsupials very common in Argentina

Brachylaima sp. is a cosmopolite fluke for *Didelphis albiventris*, as well as for other mammals and birds. In this study the A.A. describe, for the first time in Argentina, some external morphological features of *Brachylaima* sp. Dujardin, 1843 (Digenea: Brachylaimidae) using images obtained by conventional optic microscope (COM) and by scanning electron microscope (SEM).

Method: The A.A. studied n=31 *Didelphis albiventris* hunted by traps in its native environment near Corrientes City (59° W longitude, 27° S latitude), Argentina. The parasites were collected by necropsy from the small intestine, caecum and large intestine, and then were washed into physiological solution in order to be classified using a conventional optic microscope (COM). Later the specimens were put into a 3% glutaraldehyde solution, dehydrated, dried by critical point, assembled, metalised, observed, and photographed using a SEM.

Results: 13 (42 %) *Didelphis albiventris* were parasited; 18 animals (58%) were found free. Using a conventional optic microscope and according to Yamaguti's key all the specimens were classified as *Brachylaima* sp. The annexed photos explain the different morphological features of this fluke.

Conclusions: This is the first descriptions of some external morphological features of *Brachylaima* sp., carried out by SEM in Argentina.

E39 p

HYPOPODES OF A NEW MITE : *SCIUROPSIS* N. SP. AGENT OF SKIN LESIONS IN *TAMIA STRIATUS*)

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Background : The development of clinics of "exotic pets" is the occasion to identify new pathogenic parasites. We describe here a new parasite in rodents responsible of original clinical signs.

Case report : A chipmunk (*Tamias striatus*), was presented for the apparition 2 months ago of erythematous and mildly pruritic skin lesions located on the nose, tail, hindlegs and scrotum. Deep skin scrapings revealed numerous active mites. The mites were identified as hypopodes (deutonymphs) of Glycyphagidae belonging to a new *Sciurosis* species. An intrafollicular location associated to an interface folliculitis was demonstrated by Histopathology. Characteristics of this new subspecies are presented. A second animal was also infected. The chipmunks received weekly injections of ivermectin and the cage was disinfected by carbaryl powder. At week 4 one animal responded and the condition was slightly improved on the second one. The treatment was followed by weekly application of a 10% containing pyriproxyfen spot on. After two months only one of the chipmunks was completely clinically cured.

Conclusion : Hypopodes of Glycyphagid mites are known to develop in wild rodents and many species remain to be described. This case demonstrate the very long asymptomatic carriage of mites and the difficult control by traditional parasiticides.

E40 p

HELMINTH FAUNA OF MARTENS *Martes foina* AND *M. martes* IN THE SOUTHERN PART OF BELARUS

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Background: Wild animals including of martens may be hosts many species of helminths dangerous for humans, farm and domestic animals.

Method: 11 *M.foina* and 31 *M.martes* killed by the hunters between 1980 and 2000 in the southern part of Belarus (Brest and Gomel regions) were subjected to complete helminthological dissection by K.I.Skrjabin.

Results: The total rate of infection of martens by helminths was 76.2%. 7 *M.foina* and 25 *M.martes* were infected. Both marten species were hosts for 18 species of helminths: 3 Trematoda (*Alaria alata* larvae, *Apophallus donicus*, *Isthmiophora melis*), 3 Cestoda (*Mesocoeloides lineatus*, *Spirometra erinacei* larvae, *Taenia mustelae*), 11 Nematoda (*Aonchotoca putorii*, *Baylisascaris devosi*, *Crenosoma vulpis*, *Eucoleus aerophilus*, *Filaroides martens*, *Molineus patens*, *Pearsonema mucronata*, *Skrjabingylus petrowi*, *Spirocercus lupi* larvae, *Strongyloides martis*, *Trichinella* spp. larvae), 1 Acanthocephala (*Centrorhynchus ninni*) species. All trematode species, two cestode species (*M.lineatus*, *S.erinacei*) and three nematode species (*E.aerophilus*, *S.lupi*, *Trichinella* spp.) are very important for medical and veterinary science. These helminths was caused by zoonotic diseases that registered in humans and domestic animals (cats, dogs). Larvae of *A.alata* are localized in marten muscles and lungs, *A.donicus*, *I.melis* and *M.lineatus* in intestines, larvae of *S.erinacei* and larvae of *Trichinella* spp. in muscles, *E.aerophilus* in trachea, larvae of *S.lupi* in walls of intestines.

Conclusion: Martens promote to support of natural helminthoses centers in Belarus which may be dangerous for humans and domestic animals as well causes of zoonotic diseases.

E41 p

A MULTICENTRIC STUDY ON DIGESTIVE TRACT NEMATODES INFESTATIONS IN DOGS.

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Objective : to determine the comparative frequency of infestation by *Toxocara/Toxascaris*, *Ankylostoma/Uncinaria* and *Trichuris* in dogs of various signalments and living conditions.

Materials and methods : The study was made on during a 4 month period in 14 veterinary practices. Dogs of different categories were included : age (under 3 months, 3 to 12 months, greater than 1 year old), breed, sex, urban, rural or semi-rural, clinical signs and previous anthelmintic treatment. A fecal sample was then submitted for the detection of digestive tract nematodes.

Results : 392 dogs were included in the study (81.4% above 1 year old, 55.8% urban, 53.8% whelped within the previous year) and 10.2% were found to be infested. An overall significant decrease of infestation was observed with age (30%, 24.5% and 6.6%). Rural dogs were significantly more frequently parasitized. Relative frequency in decreasing order were : *Trichuris vulpis* 40.5%, *Toxocara canis* 32.5%, *Uncinaria stenocephala* 17.5%, *Ankylostoma caninum* 10% and *Toxascaris leonina* 5%. Frequency of parasitism increased with age for *Trichuris* and hookworms and decreased for ascarids.

Discussion : These results underline that taking in account epidemiological factors of risks is of importance for an effective control of parasites. In this study the infestation was not influenced by an anthelmintic treatment within the previous year.

SPECIES OF FLEAS FOUND ON CATS IN FRANCE

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A survey has been performed in 1999 on 403 cats infected by fleas, coming from 75 of the 95 départements distributed over french territory. Among the 1337 fleas collected, 8 species have been identified : *Ctenocephalides felis felis* (97.9%), *Spilopsyllus cuniculi* (0.9%), *Ctenocephalides canis* (0.5%), *Ceratophyllus sp.* (0.3%), *Xenopsylla cheopis* (0.07%), *Pulex irritans* (0.07%), *Archeopsylla erinacei* (0.07%) et *Leptopsylla segnis* (0.07%). 97.8% of cats were infected by *C. felis* and only 0.74% by *C. canis* or *Spilopsyllus cuniculi*. *C. felis* has been identified on all over the country, on animals living inside or outside. All animals living inside were only infected by *C. felis*. On the other hand, other species were all collected on cats which had access outside, most of them had predatory habits.

PARASITE INFECTIONS IN SEMI-DOMESTICATED DOGS IN BALI, INDONESIA

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Background: Knowledge about parasite infections of dogs in Bali is very scanty. Therefore the present study was performed to examine semi-domesticated dogs for parasite infections.

Methods: A total of 18 young (>3 months old) and 32 adult (>1 year old) dogs were purchased from owners of different villages and necropsied in August 2000. The gastrointestinal tract, liver, urinary bladder, lungs and heart of 45 animals were examined for helminth infections. Tongue and muscle samples were examined for *Trichinella* larvae using the pepsin-HCl digestion technique. Serum samples were collected from 38 dogs for serological examinations.

Results: The following gastrointestinal helminths were found: *Ancylostoma* spp. (89% of all dogs), *Taxocara canis* (51%), *Dipylidium* spp. (18%) and *Taenia* spp. (7%). *Dirofilaria immitis* adults were present in the heart of one animal but circulating heartworm antigen (Idexx Ltd.) was found in another dog. No helminth parasites were detected in other organs. Two dogs (5%) were seropositive for *Trichinella* spp. (ELISA); however, muscle larvae were not isolated from any animal. Antibodies to *Babesia canis* (IFAT), *Ehrlichia canis* (IFAT) and *Sarcoptes canis* (ELISA) were found in serum of 3%, 5% and 32% of the dogs, respectively. All dogs reacted seronegatively for *Leishmania* spp. (ELISA) and *Neospora caninum* (ELISA).

EPIDEMIOLOGICAL SURVEY OF ANGIOSTRONGYLOSIS IN DOGS IN FRANCE : PRELIMINARY RESULTS.

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Introduction : *Angiostrongylus vasorum*, sometimes called the " French heartworm " had been widely studied in France fifty years ago. The purpose of this study is to evaluate the actual incidence of the disease in the experience of veterinary practitioners.

Material and methods : During the spring of year 2000 a survey was mailed to 2000 veterinary clinics to evaluate the knowledge, clinical aspects, diagnosis and frequency of canine angiostrongylosis. Clinics that positively replied were then contacted by mail and call phones to precise informations for each case.

Results: Survey results were obtained from 222 clinics (64% small animal clinics 37 % mixed (small and large animal). Canine Angiostrongylosis was diagnosed in 25 (44% from mixed clinics). Diagnosis was averaged from 1 to 5 cases per clinic. A total of 54 cases was collected primarily in veterinary clinics where a high percentage of hunting dogs were seen (above 5%). Cases were observed and diagnosed throughout the country and 66% were diagnosed during the last ten years.

Conclusions : These results suggest that canine Angiostrongylosis is a widely distributed parasitosis in dogs in France. The frequency of cases diagnosed during the last decade may both reflect an improvement of diagnostic methods and the active circulation of the parasite in exposed populations of dogs.

A COPROLOGICAL SURVEY OF RED FOXES (*VULPES VULPES*) IN SLOVAKIA

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A survey was conducted to determine the prevalence of intestinal helminths and trichinelosis of red foxes (*Vulpes vulpes*) from Slovakia. In the course of an epidemiological survey concerning the prevalence of *Echinococcus multilocularis*, the autopsies of 613 foxes from the whole area of Slovakia have been carried out. These foxes have been shot by hunters in spring and autumn. For a coprological examination, 4 g of faecal samples were examined by standard faecal flotation. In addition, 10 g of muscle from each of foxes were digested and examined for the presence of *Trichinella* larvae.

Of the 311 coprological samples examined during spring, 8 different species of helminth eggs have been found: Ascarid eggs (62.4 %), *Trichuris vulpis* (57.9 %), Strongyle eggs (56.9 %), Coccidia oocysts (28.9 %), *Taenia* eggs (16.7 %), *Capillaria* spp. (1.6 %), *Alaria alata* (1.0 %) and *Dipylidium caninum* (0.6 %). Of the 302 samples investigated during autumn, 12 species of helminth eggs have been found: Ascarid eggs (60.1 %), *Trichuris vulpis* (36.8 %), Coccidia oocysts (33.1 %), Strongyle eggs (19.2 %), *Mesocostoides* sp. (12.9 %), *Taenia* eggs (7.0 %), *Alaria alata* (3.0 %), *Spirocerca lupi* (2.3 %), *Hymenolepis* spp. (2.3 %), *Opisthorchis* spp. (1.3 %), *Capillaria* spp. (0.7 %), and *Dipylidium caninum*. (0.3 %). The positivity of 5.8 % in the spring and 5.5 % in the autumn of *Trichinella* in the muscle samples has been recorded. A more detailed information is given about the geographical distribution of the helminth eggs and *Trichinella* larvae found in the survey.

E46 p THE HELMINTH FAUNA OF *VULPES VULPES* AND *NYCTEREUTES PROCYNOIDES* IN THE UCKERMARK DISTRICT, GERMANY

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Background: According to official hunter statistics the racoon dog bag has increased considerably in the federal states Mecklenburg-Pomerania and Brandenburg over the last few years. Since both racoon dogs and red foxes share the same habitats it was of interest to compare the helminth fauna of both carnivores.

Method: Internal organs of 101 racoon dogs and 144 red foxes killed between March and September 1998 in the north eastern part of the Uckermark District of the Federal State Brandenburg were subjected to complete helminthological examination after K.I. SKRJABIN. Stomach contents were used for food spectrum analyses.

Results: The helminth fauna of both racoon dog and red fox in the study area consisted of *Isthmiophora melis* (30.7%/ 3.5%), *Opisthorchis felinus* (2.0%/ 5.6%), *Metorchis bilis* (17.8%/ 18.8%), *Alaria alata* (61.4%/ 29.9%), *Mesocostoides* spp. (60.4%/ 56.3%), *Taenia polyacantha* (12.9%/ 37.5%), *T. crassiceps* (2.0%/ 3.5%), *Capillaria aerophila* (18.8%/ 28.5%), *Molineus patens* (4.0%/ 0.7%), *Crenosoma vulpis* (16.8%/ 6.3%) and *Toxocara canis* 32.7%/ 43.1%). In addition, foxes harboured *Pseudamphistomum truncatum* (0.7%), *Angiostrongylus vasorum* (2.1%) and *Toxascaris leonina* (4.9%). *C. plica* was present in both hosts but in most samples urinary bladders were missing. Significant differences were obtained between prevalences of *I. melis*, *Alaria alata* and *T. polyacantha*.

Conclusions: In principal, both carnivores occur in the same habitats and use the same diet. However the proportions of different foods must be different since frog transmitted trematodes were more frequent in racoon dogs but the vole transmitted *T. polyacantha* was more often found in foxes

E47 p Epidemiological and clinical study of the canine spirocercosis in the Réunion Island.

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Background: Canine spirocercosis is one of the major dog diseases in Réunion Island (Indian Ocean). It is often diagnosed in veterinary clinics but no epidemiological data are available.

Methods: An epidemiological and clinical survey has been conducted in vet clinics during 3 years, including 2498 dogs from pet owners. Endoscopy, radiography and coproscopy were made to diagnose the infestation on symptomatic dogs. The results of the treatment were followed by the same techniques. 51 asymptomatic dogs from vet clinics and 96 stray dogs were assessed in 1999 to estimate the prevalence of asymptomatic infestation.

Results: **Clinical study:** 120 symptomatic dogs were infested by *Spiloverca lupi* which indicates a clinical frequency of 4.8%. Four clinical types have been identified: moderate infection (20.8%), acute and haemorrhagic infection (3.8% of cases), classical type (71.20 %) and tumoral evolution (4.16% of cases) with in some cases an hypertrophic osteoarthropathy (1.67%). The coproscopy allowed to diagnose 80% of infestations. 32% of dogs had a blood numeration with eosinophilia up to 8%. Radiographic signs were seen in 74% of cases. The parasitic cysts were seen by endoscopy in the stomach in 5.5% of infested dogs, the others were localized in the oesophagus. No sex or season predispositions were identified. The majority of infested dogs were less than 3 years old. In contrary, the tumoral evolution were diagnosed in older dogs (mean of 7 years old). An association of ivermectin and nitroxinil induced a cure in 81.6% of cases.

Epidemiological survey: 59 of the 96 stray dogs (61.5%) and 14 of the 51 asymptomatic dogs from vet clinics were infested (27.5%). These results indicate the role of stray dog population as a reservoir, and the importance of asymptomatic infestation compared to the symptomatic dogs suspected on clinical basis (only 4.8%).

E48 p PREVALENCE OF DIROFILARIASIS IN DOGS OF BUENOS AIRES, ARGENTINA

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Background: This work was conducted to study the prevalence of canine dirofilariasis in animals living in Buenos Aires (Bs.As.), from 1997 to 2001.

Method: Blood samples were extracted from the city of Bs. As., the south, the north and the west area of Bs. As. suburbs. Samples were analyzed for *Dirofilaria immitis* with a circulating antigen detection test.

Results: Of the 782 blood samples, 72 dogs were found to be positive (9.2 %), and lived in the north and south zones. Samples extracted from the city of Bs.As and the west zone resulted negative. The prevalence in females (37.5%) was less than in males (62.5%) ($p < 0.05$). Positive animals were between 5 and 8 years old.

Conclusions: It is remarkable the geographic distribution of the disease, showing the existence of specific ecological niches for the development of the biological cycle of

Dirofilaria immitis.

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E49 p CANINE DIROFILARIASIS: CURRENT SITUATION IN BRAZIL

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Canine heartworm disease is presented as an important cause of acquired cardiopathy in the small animal medicine. However, clinical presentation sometimes it's being occult. The importance of this disease is not only because the damage in ten heart and pulmonary arteries on infected animals, but also for being recognized as zoonosis since 1979 for World Organization of Health. The goal of this research was to know the occurrence of canine heartworm, from the first report until the current days. The study was carried through the prevalence related by parasitological and serological surveys. The results showed an average of 10.17% of the positive animals to the parasitological examination, while 9.07% of the same canine population displayed a circulating antigen. When the prevalence was evaluated by region, the result found in north region was given only by Knott method, with average of 5.24% infected animals. In the other regions, as Northeast, Southeastern, Center-west and South, parasitological and serological surveys had been carried with the prevalence of 10.64%, 17.20%, 5.89% and 12% respectively for research of circulating micro filariae and 13.89%, 13.46%, 6.90% and 2.04% for circulating antigen respectively.

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E50 p

PREVALENCE OF *Dirofilaria repens* AND *Dirofilaria immitis* AMONG DOGS IN GREECE

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Introduction: *Dirofilaria immitis* is a rather uncommon zoonosis. It is of significant importance, as misdiagnosis may frequently occur. To date, in Greece 25 case of human *dirofilariasis* have been described. In these cases the worm had subcutaneous, ophthalmic or pulmonary localization. So far *dirofilariasis* in dogs, the main host of the nematode, has not been studied in much extend. In the present study we aimed to estimate the prevalence of *Dirofilaria immitis* and *D.repens* in dogs from Western and Northern Greece, using classical methods and polymerase chain reaction (PCR).

Materials and Methods: Blood samples from 178 dogs were collected from two District Areas. Samples were examined for the presence of *D.immitis* and *D.repens* by Knott and by PCR with special primers.

Results: Forty out of the 178 samples analyzed were positive for Knott and/ or PCR to *D.immitis* or to *D.repens*. Fifteen samples were positive by PCR, and negative by Knott (positive to *D.repens* 8, to mixed infections 7).

Discussion: The prevalence of *D.repens* (14,1%) among dogs is rather high. The coexistence of dogs, vectors and humans in the same domestic areas explain the incidence of human *dirofilariasis*. Compared to the conventional microscopic approach, PCR has proved to be a powerful tool either for epidemiological studies, especially in cases of low parasitaemia.

E51 p

OCCURENCE OF CANINE SPIROCERCOSIS IN ISRAEL

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Background: Spirocercosis has become a serious problem in the recent few years in Israel. In the years 1996 - 1998 we studied *Spirocerca lupi*, its distribution, epizootology, prevalence, treatment, and control.

Method: We examined 206 fecal samples twice a year from 21 public parks and gardens in 9 towns in central Israel, and dissected 2582 dung beetles. Fecal samples were examined by a routine concentration Mc Master flotation method using a sugar solution (1.27 specific gravity). Thoracic radiographs, endoscopy and autopsy were used for diagnosis of spirocercosis. Life cycle of *S.lupi* in intermediate host (*Onthophagus sellatus* - dung beetle) by an artificial infection was studied as well as efficacy of Ivermect at 0.2 mg/kg given in different intervals.

Results: We found 9.7% prevalence of *S. lupi* in examined dogs and 5.5% dung beetles (*Onthophagus sellatus*) were naturally infected. Only *Onthophagus* species of collected dung beetles were found to harbor infective larvae of *S.lupi*. Treated dogs showed improvement in clinical condition, total disappearance of *S. lupi* eggs in fecal samples 2 - 4 weeks after treatment.

Conclusion: The results of the present study demonstrate that *S.lupi* infection became established during last years in several new towns in Israel. These towns are located at a distance of 20 km from Ramat Gan, where the infection was first established in the early 90's.

E52 p

Serological Identification of Plague Wild Small Mammals Reservoir in Bondowoso District, East Java Province, Indonesia

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A wild small mammals (rodents and insectivores) trappings to identify the possible reservoir of plague were conducted in the period of 12 days in October 1999 in 5 villages located in 4 Sub-Districts in Bondowoso District, East Java Province, Indonesia. A number of 146 blood sera were collected from 4 species of rodents (rats) and 1 species insectivore (shrew). Species identification of the trapped animals revealed 3 rats species consist of 1 *Rattus argentiventer*, 3 *Rattus exulans*, 116 *Rattus rattus diardii*, and 1 *Rattus tiomanicus* and 1 shrews species (26 *Suncus murinus*). The sera were tested for plague antibody by competitive enzyme-linked immunosorbent assay (ELISA) technique that uses F1 antigen of *Yersinia pestis*. The anti-*Yersinia pestis* antibody prevalence rates among the five animal species were 0,0 %, 33,3 %, 4,3 %, 0,0 %, 3,8 % respectively. This data indicate that *R. exulans*, *R. rattus diardii* and *S. murinus* are reservoir of plague in Bondowoso District, East Java Province, Indonesia and that *R. exulans* is a predominant reservoir. It is suggested to intensified surveillance of the disease and control of rodents and insectivores as well as their ectoparasites in Indonesia.

Key words: plague, rat, shrew, serology, ecology

E53 p

Study of prevalence of fungal mastitis in cows husbandry in Tabriz-Iran

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Back ground: Mastitis in cows and other cattle is one of the most important factors in the reduction of their milk. Which is caused by bacterial and fungal agents. And this not only leads to harm for animal husbandry but also can spread in dairy products and cause their decay.

Method: In this study 300 milk samples (100 healthy cows and 200 acute or chronic mastitis) were collected. About 1 ml of milk was collected in a steril disposable tubes and transferred to the lab. Then direct exams and cultures were performed on the sediment of samples.

Results: In this study in 18.5% of infected cows and in 3% of healthy cows fungal agents were isolated. 78% of them were yeasts (*Candida* = 24, *Tricho-sporon* = 3, *Rodotrolla* = 2) and the remaining 22% were molds (*Aspergillus* = 3, *Penicillium* = 1, *Alternaria* = 1, *Rhizopus* = 1 and *Geothricum* = 2). All of the agents isolated in milk samples of healthy cows were *Candida*.

Conclusion: In this study fungal agents accounted for 18.5% and was similar to other studies in some countries such as Germany, Sudan and Brazil recently indicating that fungi are important factors in cow mastitis.

All these agents caused a reduction in milk and through milk they found their way into milk products such as cheese produced by traditional methods in some areas of Iran. By this manner they have been able to destroy dairy products and cause Mycotoxicosis in users.

E54 p ABOMASAL NEMATODES IN RED DEER FROM THE STELVIO NATIONAL PARK (ITALY)

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Background: In the last years an increase of number of red deer (*Cervus elaphus*) from the Stelvio National Park (north-eastern Italy) and a lightly increase of deer mortality were observed. To verify the diffusion of the abomasal Trichostrongylidae in red deer a survey was carried out.

Method: Abomasa from 211 shot or found dead red deer (n=188 and n=23 respectively) throughout the years 1997,1998 and 1999 were collected. The deer were classified into the following age classes: fawns (n=67), juveniles (n=71) and adults (n=67). The age classe of 6 deer was not determined. Body features and Kidney Fat Index (KFI) were also determined. For each parasite species abundances (A) and prevalences (P) were estimated.

Results and conclusions: The overall prevalence of infection was >90%. *Spiculopteria spiculoptera* and *Ostertagia leptospicularis* were the most frequent species (P=75%, A=78.6 and P=39.3% A=13.7 respectively). *Rinadia mathevossiani* and *Ostertagia kolchida* showed intermediate values (P=26.5%, A=6.3 and P=18%, A=3.9 respectively) *Marshallagia marshalli*, *Trichostrongylus axei*, *Haemonchus contortus* and *Teladorsagia circumcincta* had low prevalences and abundances. The nematode abundance difference related to sex and host age was evaluated. Further, the impact of infection on red deer body conditions was estimated by some host fitness parameters (KFI, foot and mandible length).

F

Genetics and parasitism

F1

SELECTED AND UNSELECTED LINE KID
RESPONSES AGAINST NEMATODE INFECTION.
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Background: In 1992 a selection programme was initiated to increase the responsiveness of Scottish Cashmere Goats.

Method: Eight randomly chosen fifth generation selected line (SL) and unselected (USL) kids that had been born and maintained indoors under worm-free conditions were used in the pen study. The kids were challenged on 5 occasions each week with 2000 L₃ of *Teladorsagia circumcincta* and *Trichostrongylus vitrinus* over a 35 week period and were bled and weighed weekly, worm burden and histological data were collected at post mortem at week 36.

Results: SL kid faecal egg counts were 23.6% lower than USL kids during the study and SL kids carried 49.3% fewer adult worms at post mortem and had 23.3% more immature worms than USL kids. There were no significant differences in plasma pepsinogen or circulating IgG, IgA or IgE values in the SL and USL kids. During the second phase (weeks 13-36) SL kid peripheral eosinophil counts were more than 1.5 times higher than those of USL kids.

Conclusions: SL kids had a superior ability to regulate worm numbers and rate of growth of intestinal worms and an ability to reduce worm fecundity. There was no evidence that selection had resulted in increased circulating antibody levels. Artificially challenged SL kids performed better than their USL counterparts

F2

NATURAL HAEMONCHUS CONTORTUS
INFECTIONS IN BACK-CROSS AND PUREBRED
DORPER AND RED MAASAI LAMBS

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Gastro-intestinal (GI) roundworms are the most important disease constraint to small ruminant productivity in the tropics and anthelmintic control is too costly for smallholder farmers. A potential option to drugs is the breeding of genetically resistant animals. The Red Maasai (RM) sheep of East Africa have developed genetic resistance due to intense worm challenge over time. This work aims to identify molecular markers linked to regions of the ovine genome that are responsible for resistance to GI parasites in this breed.

Mating 6 RM-Dorper F1 rams to RM (resistant) and Dorper (susceptible) ewes will generate six double backcross families. The aim is to phenotype about 200 backcross lambs per F1 sire family by exposing the lambs to endoparasites (predominantly *Haemonchus contortus*) at pasture, then to an experimental trickle challenge with *H. contortus*. Resistance/ susceptibility is assessed by faecal egg counts (FEC) and blood packed cell volume (PCV), followed by worm counts after necropsy. Subsequently, the most resistant/ susceptible lambs will be genotyped using microsatellite markers evenly spread over the genome. Quantitative trait loci (QTL) controlling resistance will then be located by integrating the phenotypic and genotypic data through linkage analysis.

After weaning at 3 months of age, 1291 lambs (532 3/4 Dorper, 569 3/4 RM, 110 RM and 80 Dorper) were moved to a contaminated pasture and monitored for 6-8 weeks. The RM and 3/4 RM lambs maintained a higher PCV and a lower FEC than the Dorper or 3/4 Dorper lambs. In addition 13.8% of the Dorper, 5.3% of the 3/4 Dorper, 0.7% of the 3/4 RM and 0% of the RM were drenched because of low PCV (<15%). Assuming that the treated animals would have died if left untreated, 68.8% of mortality would

F3

THE STATE OF DNA IN SPERMATOZOONS AT
EXPERIMENTAL NEMATODIASIS

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Purpose: The aim of this study is the investigation of the influence of migrative *Ascaris suum*, *Trichinella spiralis*, *Toxocara canis* larvas metabolites on state of a molecule DNA in spermatozoons of mice.

Methods: The experiences are performed on 280 mice-male of CBA line. Mice are infected in dosage of 5 and 20 invasive eggs or larvas on 1g of body mass. The alterations are taken into consideration on the 21st, 28th, 35th and 60th days from the invasion beginning. Mutagenic influence of nematodes migrative larvas metabolites is taken into consideration in the case of using the spermaries chromatingeterogenic test elaborated by us.

Results: It is established that nematodes migrative larvas metabolites in infective dosage of 5 eggs or larvas on 1g of mice body mass has led to the growth of the number of spermatozoons with one-chain denaturated molecule DNA on 28th and 35th days of observation in comparison with the data of intact control. In increasing of infected dosage till 20 eggs or larvas the number of spermatozoons with denaturated DNA on the 21st, 28th and 35th days from the invasion onset has been established. The level of spermatozoons with denaturated DNA in the mice groups with infected dosage of 20 eggs or larvas in 1,5-2 folds has been higher on the 21st and 28th days than in the animal group with infected dosage of 5 eggs or larvas.

Conclusion: The *Ascaris suum*, *Trichinella spiralis*, *Toxocara canis* migrative larvas metabolites posses by the strong mutagenic influence on mice spermaries cells which is characterised by the growth of spermatozoons with one-chain denaturated molecule DNA and it depends on the invasive material dosage. The violations in spermatozoons of animals caused by nematodes metabolites can result to the growth of generative mutations and lowering of the genesial function of the host.

F4

IGE ANTIBODY AS A MARKER FOR
RESPONSIVENESS AGAINST TELADORSAGIA
INFECTIONS IN SHEEP

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Background: Immunological markers associated with responsiveness against GI nematodes have the potential to be used as supplementary phenotypic markers for the selection of responder line animals. Recent studies on the mechanisms of immunity to nematodes in sheep have demonstrated that IgE response is directed predominantly against the L₃ stage of *Teladorsagia circumcincta* larvae. This has led to the identification of a specific *T. circumcincta* L₃ antigen, which appears to play a major role in the stimulation of local IgE antibody production.

Methods: 100 Grey Suffolk Crosses that were grazing on pasture infected with *T. circumcincta* were ranked using their cumulative faecal egg count scores from material taken fortnightly from May to September. Ten lambs that had the lowest egg count rankings termed "responsive" and 10 lambs that had the highest egg count rankings termed "non-responsive" were selected. Serum samples collected from these lambs during the grazing season were tested on ELISA, using the specific *T. circumcincta* L₃ antigen to detect concentrations of both IgE and IgA antibodies.

Results: Statistical analysis using ELISA data covering three time points (weeks 0-2, 2-10 and 10-20) showed that responsive animals had significantly higher levels of IgE during the middle period at a time when they had lower FEC's. There was no evidence during the middle period of any similar relationship as far as IgA was concerned. There was no correlation between faecal egg counts and IgE or IgA at the other time points.

Conclusions: The results from this study suggest that an IgE ELISA may provide a rapid and sensitive early phenotypic marker of responsiveness, further studies required to confirm this are currently

F5

PARASITE RESISTANCE IN AN IMPROVED WEST INDIAN HAIR SHEEP BREED

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Background: Sheep producers in the southeastern U.S. are increasingly interested in hair sheep breeds that appear to demonstrate increased parasite resistance. The Katahdin sheep was developed by crossing white Virgin Island sheep with wool breeds. Katahdin sheep retain the hair characteristic but are larger and more suitable for the US lamb market. Katahdin producers also claim that the sheep have retained the resistance to trichostrongylid parasites shown by Virgin Island sheep in the U.S. This study investigated whether Katahdin sheep show similar levels of parasite resistance during grazing of infected pastures compared to a West Indian breed.

Methods: Katahdin and Barbados Blackbelly (BB) lambs were grazed together on naturally contaminated pasture during the first half of the grazing season. Fecal egg counts (FEC) and packed cell volumes (PCV) were determined monthly. Four Katahdin and 5 BB lambs were euthanized and total worm burdens evaluated. Abomasal tissue samples were also evaluated for globule leucocytes numbers. During the same grazing season, FEC and PCV of Katahdin and BB ewes were also monitored.

Results: Katahdin lambs had higher FEC and lower PCV at the time of euthanasia and also significantly higher total worm burdens than BB lambs. Globule leucocyte counts did not differ. FEC and PCV of BB and Katahdin ewes on pasture were similar.

Conclusion: Katahdin sheep may show some of the parasite resistance of Virgin Island hair sheep, but full development of resistance may be delayed in Katahdin sheep, leaving lambs as susceptible as non-resistant breeds.

F1 p

POPULATION GENETICS AND ANTHELMINTIC RESISTANCE IN *T. CIRCUMCINTA* OF SHEEP AND GOATS

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Background: Anthelmintic resistance in *Teladorsagia circumcincta* is an increasing problem world-wide. However, little is understood regarding how resistance develops and spreads in parasitic nematode populations. Indeed, there is limited information on the population genetics of this organism and few tools available for such studies. Consequently, we are developing population genetics tools for *T. circumcincta* to address several questions. In particular, the relationship between the sheep and goat parasite populations, and the extent of gene flow between parasites of the two hosts, including whether or not there may be "cryptic species" with different host specificities. This has important implications for the development and spread of anthelmintic resistance since the extent to which goats are as source of anthelmintic resistant parasites in sheep is unclear.

Methods: We have PCR amplified the ribosomal RNA non-transcribed spacer region (rRNA NTS) from individual *T. circumcincta* and various other trichostrongylid parasite species, using primers designed from *C. elegans* sequence. We are also isolating microsatellite sequences to allow multilocus genotypes of single parasites to be compared.

Results: Our preliminary results show, in addition to the main NTS PCR product, a number of less abundant products were amplified which appear to be species specific. This may provide a quick and simple method of speciating trichostrongylid eggs and L3s. We have also sequenced the NTS region from *T. circumcincta* and are currently comparing parasite populations isolated from sheep and goats.

F6

THE INFLUENCE OF HOST GENOTYPE ON TRYPANOSOME-INDUCED MODULATION ON AN INTESTINAL TRICHOSTRONGYLID INFECTION IN MICE.

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One of the best known effects of African trypanosomiasis on its hosts is the suppression of their immunological responsiveness to concomitant infections. In concurrent trypanosome-nematode infections this invariably results in significantly enhanced survival, fecundity and pathogenicity of the nematode. In mice, these effects are mediated largely through down-regulation of Th2-type responses of the host. We have used *Trypanosoma brucei* (Tb)-*Nippostrongylus braziliensis* (Nb) interaction in C57BL/6, NIH and BALB/c mice to test the hypothesis that trypanosome-induced modulation of intestinal nematode infection is influenced by host genotype. Concurrent Tb-Nb infections in 6-8 week-old mice of the three strains dramatically altered their characteristic rapid expulsion phenotype to a slow one, with corresponding increase in fecundity, in a host strain-dependent manner. These effects were associated with significant suppression of ConA-driven in-vitro T lymphocyte proliferation, down-regulation of serum IgG and IL-5 responses and increase in IFN- γ production. The magnitude of the effects on host immune responsiveness was also influenced by the strain of the host. Our data shows that the capacity of the three inbred strains of mice to control a primary infection with Nb was markedly compromised by concomitant Tb infection, probably through suppression of Th2-dependent mechanisms, and that this effect was strongly influenced by host genotype.

F2 p

RESISTANCE TO EXPERIMENTAL INFECTION WITH *HAEMONCHUS CONTORTUS* IN SHEEPS.

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Resistance to experimental infection with *Haemonchus contortus* was compared in Barbados Blackbelly, St. Croix and a Domestic Crossbred lambs by means of a repeated infection with *Haemonchus contortus* L3 (10,000 y 15,000 L3; respectively). The variables measured were eggs per gram of feces, packed cell volumes and adult worm count. Shedding of eggs per gram of feces was similar ($P > 0.10$) between the three racial groups during second week post reinfection, but from week three until week seven in that experiment conclude, Barbados Blackbelly lamb consistently shed more eggs per gram of feces (11.8%, $P < 0.01$) than St. Croix and domestic crossbred lambs present 22% higher values ($P < 0.05$), compared against St. Croix and Barbados Blackbelly breeds in all meditions from 2-7 week post reinfection. For all racial groups the values of packed cell volumes were diminished a 12% ($P > 0.01$) by parasitism effect. The number of adult worm encountered in abomasum was not modified ($P > 0.82$) by the racial group of the animals (St. Croix, 486; Barbados Blackbelly, 619; Domestic Crossbred, 745).

It conclude that Barbados Blackbelly breed is less resistant to the experimental infection, compared with St. Croix and Domestic Crossbred composed by 5/8 Barbados Blackbelly and 3/8 Dorset-Suffolk.

F3 p

Phytohaemagglutinin (PHA) affect upon rats' non-specific resistance against Trichinellosis Zanginyan, H., Harutunyan, M., Asatrian, A.

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Background: Non-specific prophylaxis is one of the ways for struggle with animals' helminthosis.

Methods: The experiments were carried out on 15 male-rats of wistar line. The animals were separated into 3 groups, 5 heads per each. The first group included intact animals, the second one – infected with *T. spiralis* – served as a control. The third group animals had been gaining PHA in 70 mg/kg of body weight during 5 days long before the infection. The infection was carried out orally – per 20 larvae of *T. spiralis*.

Results: It was observed general resistance factors suppression of the control infected animals upon rats lancing on the 35-th infected day. Blood serum lizozymic activity as compared with intact ones was coming down for 70%, germicidal activity – 90%, T- lymphacids quantity – 75%. The cozinophyls quantity increased 23 times, compliment titre – 15%. PHA application reduced to a considerable extent the invasion pathogen affect on the owner organism. By comparison with the control infected animals the blood serum lizozymic activity turned out to be higher for 29%, bactericidal activity – 54%, leukocyte phagocyte activity – 26%, T- lymphacids quantity – 43%, compliment titre – 41%, cozinophyls quantity – 4.6 times, trichinella larvae have been discovered less than 23.6%.

Conclusion: So then, PHA application during 5 days before the infection, in 70mg/kg dose of alive mass, increases rats' non-specific resistance against trichinellosis.

F4 p

THE EFFECTS OF HELMINTH INFECTIONS IN FREE RANGE CHICKENS Magwisha¹, H. B., Kassuku¹, A. A., Kyvsgaard², N. C. and Permin², A.

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Background: Helminth parasites are ubiquitous in free range chickens. However, their economic impact is not well understood. This study was carried out to determine the effect of helminth infections on weight gain and mortality in free range chickens. **Method:** A total of 213 six-week old chicks of local breed were distributed to 12 randomly selected farmers in Kibwaya village, in the Morogoro Region of Tanzania. The chicks were sent to the village in the rainy season and kept together with farmer's own chickens and left for two weeks to acclimatize before start of the experiment. The chicks were divided into two groups. Group one chicks received suppressive treatment against helminth parasites every fortnight using a single dose (50 mg/kg body weight) of mebendazole (Kukuzole®, Interchem Pharma) whereas group two chicks were left as untreated controls. Mortality and weight gains were recorded every two weeks from age of 8 - 34 weeks when the experiment was terminated. **Results:** Results showed consistent higher weight gain in treated group than controls in 11 flocks. The mean cumulative weight gain was statistically higher ($p < 0.05$) in the treated group compared to the untreated controls from the weeks. The treated group had gained average weight of 1136.2 grams versus 931.7 grams of untreated controls giving an increment of 22% due to treatment. Overall mortality was 51.2%, the main cause being predators (34.4%), diseases (14.8%), and other causes (2%). Chi square test showed no association between mortality and helminth infections ($p > 0.05$). At autopsy 13 species of nematodes and 7 of cestodes were isolated in the controls with relatively low worm burdens when compared to previous studies. The treatment was effective against most of the parasite species (>80%) with the exception of *Raillietina tetragona* (39.3%), *Strongyloides avium* (67%), and *Choanotaenia infundibulum* (71%)

Conclusion: This study demonstrates a 22% increase in weight gain due to the treatment. The relatively low worm burdens observed in this study could be explained by the effect of mebendazole to nematode eggs. It is therefore concluded that helminth parasites need to be controlled in order to improve the health and productivity of the rural poultry.

Acknowledgement: We wish to thank DANIDA-Enreca for the financial support.

F5 p

HERITABILITIES OF ASCARIDIA GALLI EGG OUTPUT IN CHICKENS

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Background: The aim of this study was to estimate genetic differences of *A. galli* egg output in laying hens of two lines following a single infection.

Method: Twenty week old helminth-naive Lohmann LSL (n=46) and Lohmann Brown (n=40) laying hens, kept in battery-cages, were orally infected with 250 embryonated *A. galli* eggs. During the following 12-month laying period individual faecal samples were collected in monthly intervals and examined for faecal egg counts (FEC) using a modified McMaster technique. Two uninfected control groups of 15 LSL and 20 Brown hens, kept under same conditions, were sampled in parallel.

Results: All infected hens excreted *A. galli* eggs, whereas the control hens remained uninfected. There were no significant differences in laying performance and egg weights between infected and control groups in both lines. The percentage of positive faecal samples (FEC ≥ 50) was 69.1 and 33.7 in LSL and Brown hens, respectively. LSL hens had significant ($p < 0.01$) higher mean $\log_{10}$ FEC (2.38) as compared to Brown hens (1.89). The estimated heritabilities for mean $\log_{10}$ FEC in months 1–6 p. i. were 0.19 and 0.10 in LSL and Brown hens, respectively.

Conclusion: The estimated heritabilities open a way of genetic selection for *A. galli* resistance in chickens, which will be of importance for birds kept in alternative and organic farming systems.

G

Ticks and tick-borne diseases

G1

A MODEL FOR TICK-BORNE INFECTIONS WITH VIRAEMIC AND NON-VIRAEMIC TRANSMISSION

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Background: In several tick vector virus systems workers have demonstrated that tick hosts which do not produce a viraemic response, will permit non-viraemic transmission between co-feeding ticks. A general model framework was developed and the consequences of non-viraemic routes of infections examined. Non-viraemic transmission was examined by considering transmission between all tick stages that could co-feed on non-viraemic hosts.

Method: The model was analysed through local stability analysis using the Perron-Frobenius theory and Loop Analysis. Of particular interest was the stability of both the tick-free equilibrium and the pathogen-free equilibrium.

Results: The threshold conditions for the persistence of the tick population and the pathogen in the system were analytically derived with respect to the transmission process and the effects on the basic reproductive ratio of the pathogen. The most important finding was that the non-viraemic transmission removes the possibility of a 'dilution effect' caused by the presence of too many non-viraemic hosts.

Conclusion: The results of this general model could be applied to any tick borne pathogen system in which both viraemic and non-viraemic transmission occur. Some special, but important, cases, where co-feeding occurs only within nymphs, or between nymphs and larvae, have been analysed in detail.

G2

A PCR-BASED SURVEY OF TICK-BORNE INFECTIONS IN DANISH CATS AND DOGS

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Background: There is increasing interest in ticks and tick-borne pathogens affecting companion animals as a result of pet travel and climate change. Infection rates have traditionally been assessed using serological surveys, which may lack both sensitivity and specificity. In this study we employed the polymerase chain reaction (PCR) to detect micro-parasites in the blood of cats and dogs from Denmark.

Method: Blood samples (1ml EDTA) were collected from 572 dogs and 163 cats from 52 Danish veterinary clinics. Habitat exposure, travel history and clinical signs of illness were recorded for each animal. DNA was extracted by a rapid silica-binding method and samples analysed for DNA sequences specific for genera *Bartonella* and *Borrelia* and for *Ehrlichia phagocytophila*. The presence of PCR products was demonstrated by ethidium bromide staining of agarose gel electrophoretic gels.

Results: Bacterial DNA was detected in both sick and healthy animals. *Borrelia burgdorferi sensu lato* was detected in 14/572 dogs (2.4%) and 2/163 cats (1.2%). *Ehrlichia phagocytophila* was detected in 4/572 dogs (0.7%) and 2/163 cats (1.2%). *Bartonella* sp. was detected in 6/572 dogs (1.0%) and 5/162 cats (3.1%).

Conclusions: *Bartonella*, *Borrelia* and *Ehrlichia phagocytophila* infections, all potential human pathogens, are present in normal and sick companion Danish dogs and cats and the prevalence reflects considerable exposure to infected ticks.

G3

THE IMPORTANCE OF SMALL MAMMALS IN THE LIFE CYCLES OF SOUTHERN AFRICAN TICKS

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Background: Several African ixodid tick species are unable to complete their life cycles without small mammals acting as hosts for their immature stages. The purpose of this communication is to elucidate the role of small mammals in the life cycles of some economically important ticks.

Method: Small mammals at various localities in South Africa were examined at regular intervals for the immature stages of ticks and these were counted and identified.

Results: Rock elephant shrews, *Elephantulus myurus* are preferred hosts of the immature stages of *Ixodes rubicundus*, *Rhipicephor nuttalli* and *Rhipicephalus warburtoni*, all ticks that as adults can cause paralysis. Scrub hares, *Lepus saxatilis* are preferred hosts of the immature stages of *Hyalomma marginatum rufipes*, vector of *Anaplasma marginale* and *Babesia occultans* in cattle, and of *Hyalomma truncatum*, vector of *Babesia caballi* in horses. Both ticks can also transmit the virus causing Crimean-Congo haemorrhagic fever. Murid rodents are preferred hosts of the immature stages of *Haemaphysalis leachi*, vector of *Babesia canis* in domestic dogs, and of *Rhipicephalus simus*, a vector of *A. marginale*.

Larvae of the three-host ticks *I. rubicundus*, *R. simus* and *R. warburtoni* are generally present in the largest numbers on their small mammal hosts from autumn to winter and nymphs from winter to spring. Larvae and nymphs of the two-host *Hyalomma* species are present from autumn to spring.

Conclusion: Because the immature stages of these ticks feed on small mammals only the adults can be controlled by the application of acaricides.

G4

RESISTANCE OF BOOPHILUS SPP TICKS TO ACARACIDES FROM A COMMUNAL FARM IN THE TRANSKEL, EASTERN PROVINCE, SOUTH AFRICA

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Background: The use of acaricides in plunge dipping tanks remain the main method of tick control at communal farms in South Africa. These acaricides are supplied to farmers at no cost by the Directorate of Veterinary Services. A policy of 'using the same product until resistance is evident' is followed by the Directorate. The presence of *Boophilus spp.* resistance to acaricides was investigated at a dipping tank from a communal farm in the Transkei, Eastern Province, using a combination product in excess of 8 years. **Method:** In July 1999 engorged *Boophilus spp.* ticks were collected from cattle at Kei Farms in the Transkei. Resistance of the ticks to Amitraz, Chlorfenvinphos and Alphamethrin was tested by means of the Engorged Adult Female Immersion Test as described by Drummond *et al.* Approximately one year later in June 2000 *Boophilus spp.* ticks were again collected from the same farm. Resistance of the ticks to Amitraz (250ppm) and a combination product of Chlorfenvinphos (300ppm) and Alphamethrin (30ppm) was tested by means of the Stall Test (SABS Test Method 5441/E140H). **Results:** In July 1999, based on the Reproductive Estimate, 99.34% control of *Boophilus spp.* collected from the farm was obtained with Amitraz (250ppm), while Chlorfenvinphos (500ppm) gave 19.02% control and Alphamethrin (70ppm) gave 11.25% control. In June 2000, the % Control in Drop of ticks after using the combination product was negative, for the first 2 weeks after treatment. The % Control in Drop obtained with Amitraz at 250ppm was 91.49% two days after treatment which improved to 100% five days after treatment. **Discussion:** The incidence of *Boophilus spp.* resistance can be tested with both the Engorged Adult Female Immersion Test as described by Drummond *et al.* and The Stall Test (SABS Method 5441/E140H). Ticks treated with a combination of an organophosphate and a pyrethroid will develop resistance to both actives after a period of time to an extent where control of the ticks with any of the two actives will be impossible.

G5

CONTROL OF IXODES HOLOCYCLUS ON DOGS WITH FLUMETHRIN

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Aim: To confirm the efficacy of a commercial, plastic dog collar containing 2.25% w/w flumethrin plus 10% w/w propoxur to kill *Ixodes holocyclus* ticks infesting treated dogs. Fipronil, 10% w/v topical, Frontline Topspot® was used as a positive control with a single treatment at recommended label rates.

Method: Seven dogs were fitted with collars, 7 treated with fipronil and 6 untreated controls. Dogs were allocated to the groups at random after ranking for body weight. All dogs were exposed to 8 unfed, adult, female *Ixodes* ticks on the head and neck (site of predilection) at 7, 14, 21, 28 days after treatment and the site of attachment was recorded. Four days after each infestation the viability of any attached ticks was assessed.

Result: Flumethrin plus propoxur collars were 99.5% effective. Only 1 viable tick from a total of 223 was found 4 days after infestation at day 28. Fipronil was 64% effective with 71/215 ticks alive with live ticks at all assessments. Untreated dogs had 168 live from 185 ticks.

Conclusion: Flumethrin plus propoxur collars provided control over 4 weeks. Fipronil did not provide effective control at 7, 14, 21, or 28 days after treatment.

G6

TESTING FOR RESISTANCE TO COMMONLY USED ACARACIDES ON COMMERCIAL FARMS.

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Background: The development of ectoparasite resistance particularly the acaricide resistance to parasites is of major importance to the livestock industry. Ectoparasiticides have been the cornerstone of tick control for the past 70 years. Use of these chemicals has resulted in the development of resistance by selection. The presence of resistance was investigated on commercial farms in South Africa.

Method: Engorged adult female ticks (predominantly *Boophilus decoloratus*) were collected on farms where acaricides appeared to be failing. At the same time farmers were asked to complete a questionnaire on their dipping practices. The modified Shaw Larval packet test was conducted on the larvae bred from these ticks.

Results: 46% of the farms tested showed resistance to one of the three groups of acaricides (Synthetic pyrethroids, Amidines, Organophosphates) tested for, 34.86% showed resistance to at least two of the three groups tested for and 0.08% of the ticks were resistant to all three groups of actives tested for. Resistance to cypermethrin was 47.22% of all samples (n36) in 1998, 80% (n59) in 1999 and 59.65% (n57) in 2000. Amitraz resistance increased from 41.67% to 50% and 56.14% for all samples tested during the same period. Chlorfenvinphos resistance was initially 19.44%, this increased to 28.33% in 1999 and then decreased to 15.7% in 2000.

Discussion: The increase in the number of samples submitted for testing and the increased numbers testing positive for resistance indicate that resistance is on the increase and that acaricide failure and it's management is becoming an increasing problem in parts of South Africa.

G7

STATUS OF TICKS AND TICK-BORNE DISEASES IN MBALE DISTRICT, UGANDA

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A cross-sectional study was conducted in smallholder dairy farms in Mbale district, Uganda to understand the farmer's circumstances and the epidemiological status of ticks and tick-borne diseases (TBDs) under 4 different grazing systems and 3 agro-ecological zones (AEZs). A structured and closed questionnaire was administered to capture information on dairy farm circumstances. A three-stage stratified sampling methodology was performed at district, farm and animal levels. A total of 102 farms were visited and 478 animals sampled. Thin and thick blood and lymph node biopsy smears were made to determine the rates of haemoparasites and *Theileria* schizonts, respectively. Serum samples were collected for the determination of seroprevalence of TBDs, using ELISA technique. Tick counts were made from one side of animal body. The results showed that the standards tick control practices differed across the grazing systems. The farmers in fenced and zero-grazing systems were better in the choice and application of acaricides than those in tethering and free range grazing systems. Synthetic pyrethroids were the acaricides of choice in the fenced and zero-grazing systems. Pour-on was the most effective method of tick control when compared with spraying and hand dressing. The prevalence of ticks and serum antibodies to tick-borne infections differed across the grazing systems and AEZs and increased with lower elevation and unrestricted grazing. The lowest tick challenge and prevalence of serum antibodies were recorded in zero-grazing system and the upland AEZ. Based on these results, there is a need to identify and develop cost-effective and sustainable tick and TBDs control measures tailored to cattle populations at risk and production systems.

G8

TICK-BORNE DISEASES IN CATTLE: AN EPIDEMIOLOGICAL STUDY IN SOUTHERN ITALY

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Background: Although tick-borne diseases (TBD) have been reported in Italy since the beginning of this century, there is scanty information about their epidemiology, geographical distribution, impact on livestock production, and the tick species involved. This paper presents the preliminary results of a clinical and epidemiological study on TBDs in Southern Italy.

Methods: 150 cow farms were randomly selected in three regions (Apulia, Calabria and Basilicata). On each farm 10 female calves were chosen after the first delivery; thus a total of 1500 underwent clinical examination and blood sampling for hematological and serological assays. In samples with PCV $\leq$ 24% a blood smear and buffy coat was also performed. *Anaplasma marginale* and *Babesia bigemina* antibodies were assayed in the sera by an ELISA test. Any ticks present on the animals were also collected and identified.

Results: A high level of infection was found on the 150 farms: 94.7% for *A. marginale* and 98% for *B. bigemina*. Out of the 1500 calves tested, 55.6% were serologically positive for *A. marginale* and 59.4% for *B. bigemina*. Assessment of haemograms showed that 365 (24.3%) animals had differing degrees of anemia and examination of the blood films revealed the presence of haemoparasites. All the data were processed by a GIS (Geographical Information System).

Conclusions: These results show that TBDs are widely spread in the regions considered. Their endemic diffusion and likely pathogenic effect point to the need for integrated management and control plans.

Acknowledgements: This work was supported by a grant for UE and Ministero per le Politiche Agricole, Italy. P.O.M. - Misura 2.

G9

EVALUATION OF A RAPID DIAGNOSTIC METHOD FOR CANINE MONOCYTTIC EHRLICHIOSIS

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Canine monocytic ehrlichiosis (CME) is a rickettsial disease transmitted by the tick *Rhipicephalus sanguineus*. The causative agent, *Ehrlichia canis*, infects monocytes forming morulae in the cytoplasm. In French military kennels, located in the Bouches-du-Rhône, Corsica and Africa, there were many cases of ehrlichiosis in the early eighties, which were responsible for haemorrhagic syndrome of variable intensity. Ehrlichiosis may be asymptomatic, in which case dogs are only carrier of *E. canis*. The healthy carrier dogs have a decisive epidemiological role.

Methods: A panel including 207 serum and 37 plasma samples collected from various endemic areas (Africa, French Guyana, Corsica, Reunion Island) was selected to evaluate a rapid immunomigration (RIM) commercial kit. The test results were compared with those obtained by indirect immunofluorescent assay (IFA) which was used as a reference method. Comparison were also made with symptoms data from 14 diseased dogs. A kinetic study was also performed on 5 experimentally infected dogs.

Results: The RIM test is reliable tool to detect anti-*E. canis* antibodies (sensitivity : 95% ; specificity : 100%). The test was implemented and validated in the context of a clinical cases and field conditions. In the kinetic study, the RIM assay has provided a positive result from Day 15, while the mean time of onset the disease (fever) was 12 days. RIM test performance was similar to that obtained with ELISAs.

Conclusion: The RIM test method can be successfully used either to identify asymptomatic ehrlichiosis (when coming back from an enzootic area), to perform field epidemiological surveys, or to confirm a clinical diagnosis

G10

Seroprevalence's study of the canine ehrlichiosis infection in the Réunion island

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Background : In the Réunion island (French Territory, Indian Ocean), dog ehrlichiosis (*Ehrlichia canis*), transmitted by *Rhipicephalus sanguineus*, appears very common, but no data are available. The canine population includes about 400,000 animals : 80,000 owners' dogs, which are given medical care; 120,000 dogs having an owner but living in the wild and having rare medical care; and 200,000 stray dogs. Those last two groups may constitute an important reservoir of pathogens. The objective of this study was to precise the prevalence of ehrlichiosis and to identify characteristic symptoms.

Method : 45 dogs from vet clinics, which developed suspect symptoms (fever syndrome, coagulation troubles, ear haematoma...) were studied in comparison with 52 asymptomatic dogs, which had come for vaccination or to undergo surgery. 79 stray dogs have been sampled in the Island. Blood samples and data (age, sex, breed...) were collected. The prevalence was studied by serology (indirect immuno-fluorescence).

Results : Dog ehrlichiosis is highly endemic in Reunion Island. Significant differences are shown between dog populations. The rate of positivity between the group of dogs from vet clinics (22,7%) and that of the stray dogs (59,5%) is radically different ($p < 0.001$). This result stresses the reservoir role of stray dogs. There is no influence concerning the breed, nevertheless large dogs are more often infected. There is no significant difference concerning the sex. The risk of contamination increase significantly with the age. Only 8% of the dogs before 2 are positive whereas 34% of them are positive after they are 2 ($p < 0.01$). The comparative study between symptomatic and asymptomatic dogs indicates that the association between hyperthermia and coagulation troubles is the first sign of ehrlichiosis. Trombocytopaenia and ear haematoma are significantly observed in infected dogs ($p < 0.01$) and are important signs for the diagnosis.

G11

BABESIOSIS, ANAPLASMOSIS AND ASSOCIATED TICKS ON CATTLE IN SOUTHERN ITALY

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Background: The aim of this paper is to provide further information on the distribution of *Babesia bovis*, *B. bigemina* and *Anaplasma marginale* in Southern Italy and identification of the associated tick species.

Methods: The survey was carried out in an Appennino area covering part of three southern Italian regions: Campania, Basilicata and Apulia. The sample size, 81 farms (over a total of 420 farms with more than 15 animals kept in semi-confined conditions), was determined using the formula of Thrusfield and the farms were equally distributed over the whole area using a G.I.S. software. From May to June 1999, blood samples were collected once from a total of 506 animals, older than 18 months, and sera were tested using ELISA kits. Ticks were collected from each sampled animal and identified.

Results: 8 farms (9.8%) out of the 81 examined were positive only for *B. bigemina*, 3 (3.7%) only for *A. marginale* and 70 (86.4%) for both. None of the farms were found to be positive for *B. bovis*. Ticks were found on animals in 62 of the 81 farms. 1410 adult ticks were collected and 11 species were identified. The most frequently found species was *Rhipicephalus bursa*, followed by *R. sanguineus* and *Haemaphysalis punctata*.

Conclusion: *B. bigemina* and *A. marginale* and their potential vectors are extremely common in the examined area and represent a likely risk for cattle imported from TBD-free areas. *Haemaphysalis punctata* and *H. inermis* are reported here for the first time in Basilicata and *Hyalomma marginatum*, in Campania.

Acknowledgements The authors are grateful to Prof. Gerrit Uilenberg and to Dr. Dermot O'Brien for their suggestions. This research was supported by Programma Operativo Multiregionale (Research Project: A13).

G12

FELINE BABESIOSIS IN SOUTH AFRICA: AN UPDATE

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Babesia felis, originally described from wild cats *Felis sylvestris*, was subsequently incriminated as causing clinical disease in domestic cats. Babesiosis appears to be a significant feline disease only in South Africa. Recent findings are reported upon.

Feline babesiosis is assumed to be a tick-borne infection, but the vector has not been identified. Apart from a single inland focus, the disease is reported regularly only from coastal regions.

Typical disease-causing *Babesia felis*, as well as piroplasm isolates from lions *Panthera leo* have recently been sequenced. The lion isolate was distinct from true *B. felis* and has been described as a new species. A conservative approach suggests that the clade that includes the felid Babesias is ancestral to the other piroplasms.

Feline babesiosis is not associated with pyrexia and tends to be a chronic, low-grade disease. The most frequently reported complaints by owners are anorexia and lethargy. The main clinical findings are anaemia, depression and occasionally icterus. Concurrent infections (e.g. *Haemobartonella felis*, FeLV, FIV) are potential complications and may contribute to the clinical picture. Laboratory findings commonly include regenerative anaemia, elevation of alanine transaminase (but not alkaline phosphatase) and total bilirubin concentrations and a variety of electrolyte disturbances. Secondary immune-mediated haemolytic anaemia can be seen occasionally.

Various drugs and drug combinations have been screened for activity against *B. felis*. Drugs effective against other *Babesia* species give variable and questionable results. The drug of choice is primaquine phosphate, which effects clinical cure but does not sterilise the infection. Repeated or chronic therapy may be required. Doxycycline may add potential benefits in treatment of this disease.

G13 TRAP HOMOLOGUES IN *BABESIA DIVERGENS*? Skerrett¹, H., Zintl¹, A., Westbrook¹, C., Gray², J., Mulcahy¹, G.

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Thrombospondin-related parasite adhesive proteins (TRAP) play a vital role in the locomotion and invasion strategies of parasites of the phylum Apicomplexa. TRAP, which are stored in the micronemes of the apical complex, bind to host ligands and are involved in substrate-dependent locomotion of the parasites. TRAP homologues have been identified in *Eimeria tenella*, *E. maxima*, *Toxoplasma gondii*, *Cryptosporidium parvum*, *Neospora caninum* and all *Plasmodium* species so far investigated. These proteins are characterised by one or more copies of the type 1 repeat of human platelet thrombospondin (TSP1), a motif with the amino acid sequence EWSPCSVTCGXGXRXR.

In order to determine if TRAP homologues are present in the tick-borne, intra-erythrocytic apicomplexan *Babesia divergens*, *B. divergens* DNA from infected cows was subjected to a polymerase chain reaction (PCR) using degenerate primers based on the TSP1 motif.

The PCR products were cloned into the pGEM-T vector and sequenced.

G14 INVASION, SHORT- AND LONG TERM SURVIVAL OF *BABESIA DIVERGENS* (PHYLUM APICOMPLEXA) IN NON-BOVINE ERYTHROCYTES

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Bovine babesiosis caused by the intra-erythrocytic protozoan parasite, *Babesia divergens*, is widespread in Europe. Disease signs include fever, anaemia, haemoglobinuria, haemoglobinaemia and jaundice. In splenectomised humans *B. divergens* infections may be life threatening. Attempts to develop potential vaccines have focused attention on *in vitro* culture systems for the parasite. *B. divergens*, maintained in bovine erythrocytes overlaid with serum supplemented HL-1 medium in MASP (microaerophilic stationary phase) cultures were transferred into equine, human and ovine erythrocytes. According to Giemsa-stained and FITC labelled blood smears, *B. divergens* invaded all erythrocyte types. Proliferation following invasion, monitored by measuring the uptake of tritiated hypoxanthine, was reduced compared to parasites maintained in bovine RBC's. Continuous cultures of *B. divergens* in human erythrocytes were characterised by slow growth and low peak parasitaemias. Attempts to establish long-term cultures in horse RBC's failed. *B. divergens* cultures in bovine RBC's overlaid with sheep serum supplemented medium grew at a similar rate to parasites in bovine serum supplemented medium. Horse and human sera, on the other hand, caused a reduction in parasitaemia and hypoxanthine uptake. We wish to thank Pat Holman, Texas A&M University for her invaluable advice on *Babesia in vitro* culture and the personnel on Lyons Research Farm, Ireland for supplying us with material.

G15 CURRENT STATUS OF BABESIOSIS IN INDIAN RURAL AND SYLVATIC ENVIRONMENT - A CASE STUDY

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BACKGROUND: Babesiosis caused by *Babesia bigemina* have been reported in the form of morbidity, mortality and Immuno Suppressive-factor in Domestic and Free-ranging Ruminants in & Adjacent area of Ranthambhore Tiger Project in India.

METHOD: Clinical case history, Necropsy reports, Serum samples & Blood Smears were collected from Ailing & Carcass of 21 Buffaloes, 11 Cattle, 7 Sambher, and 17 Spotted Deer. Autopsy were also conducted. All the samples were analysed by ELISA and Stein Blood smears examination (SBSSE) of all species.

RESULT: *Babesia bigemina* infection were found positive in Domestic and Free ranging ruminants in 11.00%, 5.70% by ELISA and in 10.03%, 5.70% by SBSSE. Watery blood, Brownish Red-Urin, Haemosiderin in the cells of vital organs observed microscopically.

CONCLUSION: This is the first field report of the existence of *Babesia bigemina* infection in free ranging ruminants in the Study area. Pathogenesis in Adult (78%), in young (19%) and calves (3%) in all species of both sex. Suggest a high-techno veterinary based research and control strategies at the Cellular, Molecular, Genetic, therapeutic prophylactic, Immuno - prophylactic, Ticks controls and Eco-developmental levels in the scientific Cooperations with development, WHO, FAO and OIE for the bright future of Indian Livestock industry & wild life in the new Millennium.

ACKNOWLEDGEMENT: I am thankful to Prof. K.M.L. Pathek Parasitologists, Prof. R.S. Chauhan, Pathologists, Prof. J. Igarasi, Parasitologists Obihiro University, Japan and Mrs. Sarvesh Singh for their excellent technical support, advice and supervision.

G1 p DYNAMICS OF TICK POPULATIONS IN DOGS IN FRANCE.

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Introduction: Ticks are major parasites in dogs and important vectors of diseases in this species. We present here an epidemiologic survey on the populations of ticks on dogs in two areas in France.

Material and methods: During a ten months period (september to june) dogs presented in 2 veterinary clinics were systematically examined for the presence of ticks. One (A) was located in brittany and the second (B) in the center of France. For each dog the systematic research allowed to precise the location and the number of ticks. Ticks were then evaluated: identification of species, stage, feeding (unfed, partially fed or fully engorged).

Results: A total of 3445 dogs were examined and 207 (6%) harboured ticks. The percentage of parasitized dogs varied from 1.9% (june) to 7.7% (nov) in A and from 0.7% (dec) to 15% (nov) in B. The average number of ticks/dog/month varied from 1.1 to 5.7 in A and from 1 to 4.1 in B.

From 610 ticks identified the populations were different in the two locations: *Dermacentor reticulatus* 96% and *Ixodes ricinus* 4% in A and *D.retic.* 43.5%, *I. ricinus* 55.2% and *Rhipicephalus sanguineus* 1.3% in B. In both areas the percentage of engorged ticks was the same: unfed (53.3 and 56%), partially fed (27 and 25.7%) and engorged (19.7 and 18.4%). The number of males and females was similar (42.6 and 57.3%) for *D. reticulatus* whereas 97.7% of *I. ricinus* were female.

Conclusion: As a result of this study, parasitism by ticks in dogs may vary amongst the different parts of France. The variation of frequency or intensity of infestation were not correlated to season and climatic variations.

G2 p

CLIMATE CHANGES AND SPREAD OF *Ixodes scapularis* IN THE UNITED STATES WITH DOUBLE CO₂ LEVELS.

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Background: Global climate warming is being recorded in the last years, as a combined result of some natural episodes, together with anthropogenic activities. This global warming is affecting the distribution of several vectors and vector-borne diseases. General atmospheric circulation models have been developed to predict the rate of change in temperatures. Most of these models predict atmospheric data for CO₂ levels with double concentrations as those of today. Furthermore, models based on habitat similarity are being developed for some arthropod, included ticks. In this study I present data from a logistic regression model developed to map the expected distribution of *Ixodes scapularis* (IS) in Continental USA with double CO₂ levels (approximately in 100 years).

Method: Data for this model have been assembled from several already existing database about evapotranspiration, surface temperature, rainfall and water vapour pressure over the Continental USA and resampled to 10 sq. km. per pixel. A logistic regression model has been developed using the point locations of IS records as well as negative sites. The model includes yearly average, maximum, and minimum climate data from database above, seasonal evolution of these parameters, as well as data from amplitude and phase of Fourier-transformed monthly climate data.

RESULTS: Compared with current data, the model has a sensitivity of 0.91 and a specificity of 0.84. When predicted climate conditions at double CO₂ levels are used to model the distribution of IS, most of central and eastern USA are expected to be adequate habitat for the tick, except for some areas in Apalachians. Changes in main climate parameters are argued and the expected dispersal of the tick in the area discussed.

G4 p

A SURVEY OF TICK SPECIES FOUND ON PET DOGS AND CATS IN DENMARK

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Background: Although *Ixodes ricinus* is considered the major tick vector of disease in northern Europe, both *Rhipicephalus sanguineus* and *Dermacentor reticulatus* are extending their range northwards as a result of animal movement and climate change.

Method: The species of ixodid ticks found on 278 pet dogs and 81 cats from 52 Danish veterinary clinics was determined. For each animal, information on exposure to different habitats, travel history, clinical signs of illness and site of tick attachment was recorded. Ticks were collected from each animal into glycerol alcohol and an EDTA blood sample was taken for polymerase chain reaction analysis for tick-transmitted pathogens.

Results: Of the 764 ticks were collected, 4 tick species were identified with *I. ricinus* dominating in both dogs (269/278 dogs and 529/600 ticks) and cats (76/81 cats and 141/164 ticks). *I. canisuga* was found on 7 dogs and 2 cats while *I. hexagonus* was found on 1 dog and 3 cats. *R. sanguineus* was identified on 2 dogs, neither of which had travelled outside Denmark. The average number of ticks found on dogs and cats was 2.02 and 1.7 respectively with occasional heavy infestations of *I. ricinus* adults and *I. canisuga* nymphs. Only two dual infestations were recorded; one dog had *I. ricinus* and *I. hexagonus* and another had *I. ricinus* and *R. sanguineus*.

Conclusions: This study confirms the predominance of *I. ricinus*. However, there is a potential for *Rhipicephalus*-transmitted diseases to establish in Danish dogs.

G3 p

ESTIMATING THE PHYSIOLOGICAL AGE OF *Rhipicephalus appendiculatus*Madder, M.¹, Chaka, G.², Speybroeck, N.¹, Berkvens, D.¹ and Brandt, J.¹

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Background: Most of the methods for determining the physiological age (PA) of ixodid ticks are based on histological and anatomical characteristics, reflecting the state of their nutritional resources. One of the major criticisms is that these methods are based on visual, subjective estimation of qualitative characteristics, which may lead to interpretation errors by individual observers. An easy and reliable method would be useful to determine the PA of field captured or laboratory reared unfed adult ticks to develop fine-tuned models for the ecology and epidemiology of tick-borne diseases.

Method: Laboratory reared and field captured *Rhipicephalus appendiculatus* adults were used to estimate the PA, calculated as:

$$\text{physiological age} = \frac{\sqrt{\text{weight (g)}}}{\text{scutal length (mm)}}$$

The laboratory ticks were exposed to two different humidities: 87%RH and 72%RH, both at 22°C for 414 days.

Results: Analyses of the data using non-linear modelling revealed a decreasing trend of the ratio in time in both males and females. The ticks exposed to the two humidity groups showed a significantly different evolution in time: ticks at lower humidity aged significantly faster. The physiological age ratio of the field captured ticks corresponds with a calendar age of about 10 months if the ticks would have been exposed to optimal conditions in the laboratory.

Conclusions: The PA ratio could be used to determine the age grades of living ticks and allows researchers to study the phenology of ticks.

G5 p

ACTUAL STATE OF *BOPHILUS MICROPLUS* (CAN) ERADICATION PROGRAM IN ARGENTINAMattos, C.E.¹ - D'Agostino, Beatriz.¹ - Citroni, D.¹ Signorini, A.¹

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Background: The most important activity in Argentina is cattle production. Since its introduction in Argentina the cattle tick *Boophilus microplus* (Can.) wide-spread up to 34° parallel of latitude S. and yearly losses due to this parasite had been estimated in US\$ 90 millions. In order to prevent cattle tick infestation to southern regions, on 1938 a compulsory *B. microplus* eradication program was established by Federal Laws N° 12.566 and N° 7663 / 54.

Methods: For epidemiological control SENASA divided Argentina into three areas: **Tick infested area** where the farmers are dipping their cattle according to tick infestation. **Eradication area**, where the farmers are obliged to dip their cattle according to a compulsory dip program and all the cattle movements are subordinated to severe measures of quarantine. **Tick free area**, where *B. microplus* (Can.) had been eradicated.

Results: Up to 31st December 2000, *Boophilus microplus* (Can.) had been eradicated from 29.140.000 hectares where 97.000 beef cattle farms are situated and 12,5 millions cattle are grazing.

The meat production increased yearly 300.000 tons.

Conclusions: The progress of the cattle tick eradication program in Argentina suggested to the A.A. that *B. microplus* eradication is feasible by severe measures of quarantine to control cattle movements in tick infested areas and by compulsory plunging dips using Amidine and S. P. as well as using S.P. and IGR tickicides by pour-on treatments and also using a long-acting injectable Ivermectin 1%.

G6 p

IN VIVO ISOLATION OF A RICKETSIA (COWDRIA RUMINANTUM) USING AMBLYOMMA VARIEGATUM TICKS COLLECTED FROM APPARENTLY HEALTHY CATTLE IN NIGERIA.

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Cowdriosis affects cattle, sheep and goats in Nigeria causing high mortalities especially in imported stock. Among the methods of control adopted, immunization of susceptible stock with local isolates seems to be the most appropriate. Most isolates are obtained from tissues of clinically sick animals which can be difficult to preserve. Sixteen adult *A. variegatum* ticks were collected from two apparently healthy White Fulani cows in Zaria Northern Nigeria, surface sterilized with 70% ethanol and homogenized using chilled phosphate buffered saline as diluent. The homogenate was allowed to settle at room temperature and 5ml of the supernatant inoculated into a 5 month old goat kid intravenously. Moderate febrile response of 39.7°C was observed 9 days post inoculation. The kid died on the second day of reaction and the brain was positive for *C. ruminantium* colonies. Blood from the kid taken 24 hours prior to death initiated an intense febrile response of 41.5°C in recipient adult goats inoculated intravenously. Vials of stabulates were prepared at the height of temperature reactions using 10% Dimethyl Sulphoxide (DMSO) and snap frozen in liquid nitrogen as "Basawa" isolate.

G8 p

A STUDY ON TICKS AND TICK-BORNE DISEASES IN BANGLADESH

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ABSTRACT

A study was conducted on Ticks and Ticks-borne disease of domesticated and some wild animals in Modhupur Forest area of Tangail district, Bangladesh between July 1999 and June 2000 with the objectives to determine their incidences and relationship with that of the ecological conditions. 1143 cattle, 558 goats, 106 dogs, 68 pigs, 24 jackals, 11 cats, 3 civets, 7 mongoose, 9 horses and animal habitats were examined for collection of ticks; ticks were also collected from two infested men. Blood samples from 239 cattle, 123 goats, 67 dogs, 27 pigs, 20 jackals, 6 cats, 5 civets, 9 mongoose, 6 horses and 2 man were also examined for the detection of tick-borne protozoa.

Species of ticks recorded were *Boophilus microplus* and *Haemaphysalis bispinosa* in cattle and goats; *H. bispinosa* in pigs, *Rhipicephalus sanguineus* in dogs *R. sanguineus* and *H. kinneari* in jackals. *B. microplus* was also recorded in two men. Overall incidence was 28.2%, 55.2%, 59.4%, 66.6%, 13.2% respectively in cattle, goats, dogs, jackals, pigs and none in monogoose, cats, civets, and horses. In cattle infestation by the major tick *B. microplus* in different months of the year varied from 13.7% in September to 52.8% in June; monthwise mean load varied from 27.2±9.8 in September to 72.7±29.6 in May. Incidence of *H. bispinosa* in goats varied from 28.6% in September to 79.5% in June; monthwise mean load was lowest (17.6±8.8) in September and highest (40.3±11.0) in June. The incidence of *H. bispinosa* was 13.2% in pigs, *R. sanguineus* was 59.4% in dogs and *H. kinneari* was 66.6% in Jackals. Larvae and ovipositives *B. microplus* was detected in habitats mostly in March-May and November-December, whereas that of *H. bispinosa* was detected in September and March-May. Blood protozoa recorded in cattle were *Babesia bigemina*, *Theileria annulata*, *T. mutans*, and *Anaplasma* sp; *T. ovis* in goats and *B. canis* in dogs.

Total rainfall and evaporation rate was found to have positive effect on the incidence of *B. microplus* and only evaporation rate to have positive effect the incidence of *H. bispinosa*. Biotic potential and host specificity were also assumed to have influenced both incidence and parasitic loads.

A direct relationship between the incidence of *Theileria mutans* and *H. bispinosa* was detected. Relationship between incidence of *B. microplus* and *B. bigemina* and *H. bispinosa* and *T. ovis* was insignificant.

G7 p

BLOOD MEAL ANALYSIS IN TICKS FOR HOST IDENTIFICATION.

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Background: A method is described here that determines the host identity of blood meal remnants in unfed questing ticks, so that the host source of any pathogens present may be determined. In a feasibility study (Kirstein & Gray, 1996) PCR primers based on the mammalian *cytochrome b* gene were used in a nested PCR to generate DNA fragments from the blood of various host species. Oligonucleotide probes based on these fragments detected host DNA in unfed *Ixodes ricinus* nymphs. Subsequent studies have shown that universal primers based on the *cytochrome b* gene do not include a sufficient range of tick hosts. To overcome this problem an alternative two step approach has been adopted.

Method: Step 1) identification of vertebrate subgroups (e.g. ruminant, rodent, bird etc) by targeting the highly conserved 18SrDNA. Step 2) identification of host species within the identified subgroup using specific *cytochrome b* primers.

Results: Universal primers for 18SrDNA were designed and shown to generate PCR product from all species tested including species where no sequence data is yet available. Vertebrate groups (e.g. mammal and bird) may then be identified from product length. Studies on ticks fed on rabbits and mice showed that this nuclear DNA is present in sufficient quantities for detection. An evaluation of the persistence of the DNA in tick gut under natural conditions is in progress. Specific primers based on *cytochrome b* were designed for a range of host species and successfully tested on 14 mammal species and three bird species.

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G9 p

BORRELIA SPP. INFECTION IN A NATURAL POPULATION OF APODEMUS MICE: PRELIMINARY RESULTS

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Background: *Apodemus* mice are among the most important reservoir hosts for the persistence of *Borrelia* spp. infection in Europe. In this study we examined in detail the pattern of *Borrelia* spp. infection in live captured and recaptured individuals within an high endemic focus of Lyme disease in Trentino (Northern Italy).

Method: *Apodemus* mice were live trapped and tagged weekly with subcutaneous chips from May to November 1999. Ticks count and serial blood samples were obtained from each individual. PCR and DNA sequencing were used to identify *Borrelia* spp. infection.

Results: 17 individuals in a sample of 87 mice were infected with *Borrelia* spp. with a mean prevalence of infection of 19.8%. Males had a grater intensity of tick infection with *I. ricinus* larvae (mean intensity=10.03±0.73) and an higher prevalence of infection with *Borrelia* spp. (26%) than females (larvae mean intensity=5.4±0.57; prevalence of infection =11%).

Conclusion: These preliminary findings showed that males, which carry greater level of tick infestation, are also more infected than females with *Borrelia* spp. thus generating heterogeneities within the rodent population and *Borrelia* spp. transmission even in natural condition.

G10 p ISOLATION OF Ixodes ricinus TRANSMITTED BORRELIA AFZELII FROM A HORSE IN GERMANY

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High seroprevalence of antibody titers (47.9%) against infection with *Borrelia burgdorferi* s.l. (B.b.) were demonstrated in 1492 horses clinically suspicious for Lyme borreliosis (L.b.). In an area in Germany where cases of Lb in humans occurred *B.burgdorferi* s.l. was demonstrated by PCR in *Ixodes ricinus* collected from the vegetation and in *I. ricinus* from horses after tick bites. Recently we could describe from the same area for the first time clinical cases of LB in 4 horses. Intra vitam *B. burgdorferi* s.l. repeatedly could be cultured in BSK- H medium from biopsy samples of the skin, conjunctiva and synovia of the horses. Post mortem *B. burgdorferi* s.l. was cultured from tissues of the skin, synovial membrans of several joints, the eyes, several lymphnodes, truncus aortae, lungs, liver, spleen, kidney and brain. Specimens of the cultured borrelia strain from the spleen were isolated. A 1.5 kbp DNA fragment was amplified and both strands were sequenced. The sequence analysis showed that the borrelia strain isolated from the horse was identical to *B. afzelii*. Reference strains of *B. burgdorferi* s.s. (B31), *B. garinii* (N34), *B. afzelii* (Pk0), *B. valaisiana* (VS116) and the isolate of the horse were also analysed by Restriction Fragment Length Polymorphism (RFLP). Dra I and Mse I were used for cleavage. Dra I restriction fragment sizes (50/170) and Mse I restriction fragment sizes (75/110) were identical with *B. afzelii*. To our knowledge this is the first isolation and identification of a *B. burgdorferi* s.l. strain (*B. afzelii*) from a horse with symptoms of Lyme borreliosis. The cases described here were considered as late infections of Lyme borreliosis.

G12 p SHEEP AS A NEW MODEL FOR STUDYING BABESIA DIVERGENS BABESIOSIS

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Babesia divergens was cultivated in sheep erythrocytes in RPMI 1640 supplemented with 10% of Fetal Calf Serum (FCS) or sheep serum. *In vitro* cultures were initiated with human erythrocytes infected *in vitro* with *B. divergens* Rouen 1987 or with gerbil blood infected with several isolates from bovine origin. After the first 3 to 5 passages on sheep erythrocytes, a ten-fold multiplication of the parasites was obtained within 48 h. Parasitaemia of 50-60 % could be reached easily. The cultures could be stored frozen in liquid nitrogen and reactivated *in vitro* without any passage in gerbils.

Erythrocytes from 3 splenectomized sheep were infected *in vitro* with *B. divergens*; when parasitaemia reached 10%, sheep were inoculated with homologous parasitized erythrocytes. All sheep expressed hyperthermia with a peak between the 6th and the 9th days post-infection (p-i) and a transitory parasitaemia 10 days p-i. *In vitro* primary cultures were performed on two sheep; parasites could be observed after 10 to 20 days of culture, demonstrating the parasite persistence at very low parasitaemia in the infected animals.

This is the first report of *B. divergens in vitro* adaptation to sheep erythrocytes. Gerbils are commonly used as models for acute babesiosis; splenectomized sheep could be a new model for studying chronic babesiosis.

G11 p IgM, IgG1 AND IgG2a ANTIBODY RESPONSE TO BABESIA MICROTI INFECTION IN BALB/c MICE

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Babesia microti, tick-transmitted, intraerythrocytic protozoan infects rodents and human. The identification of its antigens could be used to develop of effective vaccine against this parasite.

The aim of the study was to investigate IgM and IgG antibody response against specific protein bands of *B. microti* antigen in BALB/c mice infected twice. Primary dose of 5×10^7 infected erythrocytes given intraperitoneally resulted in a high, transitory parasitaemia peaked at 8 days post infection (DPI), (mean 80% infected erythrocytes). Contrary to that, the challenge dose resulted in a very low level of parasitaemia (0-3%).

The next step of the study was to characterise the protein bands of the crude *B. microti* merozoites antigen. SDS-PAGE electrophoresis revealed high heterogeneity of protein bands varied from 6.5 to 205 kDa. Using immunoblotting, only two of detected protein bands (47 and 58 kDa) reacted with IgM, IgG1 and IgG2a antibody.

Additionally, the dynamic of serum antibodies after the primary and the challenge dose were investigated. After the primary dose, IgM response was detected at 8DPI against both protein bands, but IgG response was detected later at 14DPI against 47 kDa only. IgG response against both protein bands was observed at 24DPI. After challenge dose, both IgM and IgG response were detected against 47 and 58 bands from 8DPI.

In conclusion, the appearance of IgG response against 58 kDa protein correlated well with low level of parasitaemia and suggested that this protein could be used to compose an effective vaccine against *B. microti*.

Presented study was done on master degree in Department of Parasitology, Institute of Zoology, University of Warsaw, Warszawa, Poland.

G13 p BOVINE BABESIOSIS IN TANZANIA

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Bovine babesiosis is a tick borne disease (TBD) of major economic importance and hence a constraint to livestock production in many countries. The disease is caused by two pathogens namely *Babesia bovis* and *Babesia bigemina*. Both pathogens are transmitted by the tick *Boophilus microplus*, while a related species *Boophilus decoloratus* transmits *B. bigemina* alone. The two tick species are found in Tanzania, although they exist in different ecological zones. *B. bovis* is the more pathogenic of the two agents and causes more mortality per outbreak than *B. bigemina*. The importance of bovine babesiosis in Tanzania has always been underestimated, due to inadequate diagnosis, which in many cases has led to poor/wrong treatment. It is after introduction of ECF immunisation, and the subsequent reduction of tick control, that the importance of bovine babesiosis has become apparent. Lately, there have been increasing reports of *B. bovis* infection, even in areas where it had never been previously reported. Tick and serological surveys are currently going on, to monitor changes in distribution of tick vectors and to establish infection prevalence in affected areas. Results of the preliminary work carried out in some areas of Tanzania are presented, with suggestions on possible remedial measures to alleviate the problem.

G14 p DETECTION OF ANTIBODIES TO *BABESIA EQUI* IN HORSES BY LATEX AGGLUTINATION TEST USING RECOMBINANT EMA-1

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The gene encoding the entire *Babesia equi* (*B. equi*) merozoite antigen-1 (EMA-1) was inserted into a baculovirus transfer vector, and a recombinant virus expressing EMA-1 was isolated. The expressed EMA-1 was transported to the surface of infected insect cells as judged by an indirect fluorescent antibody test. The expressed EMA-1 was also secreted into the supernatant of a cell culture infected with recombinant baculovirus. Both intracellular and extracellular EMA-1 reacted with a specific antibody in Western blots. The expressed EMA-1 had an apparent molecular mass of 34 kDa that was identical to that of native EMA-1. The secreted EMA-1 was used as an antigen in a latex agglutination test (LAT). The LAT was able to differentiate very clearly between *B. equi*-infected horse sera and *B. caballi*-infected horse sera or normal horse sera. The LAT results were identical to those of a previously developed enzyme-linked immunosorbent assay. These results indicate that LAT using recombinant EMA-1 might be very useful as a routine screening method for the diagnosis of *B. equi* infection.

G15 p CULTURING AND ATTENUATION OF A POTENTIAL LIVE VACCINE AGAINST BOVINE BABESIOSIS

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Vaccination has long been recognised as the best control option for the tick borne disease, bovine babesiosis, which is widespread in Europe and has a high prevalence in Ireland (approximately 20,000-30,000 cases per year). An experimental live vaccine derived from gerbils (*Meriones unguiculatus*) was found to be highly effective in field trials conducted over the last 10 years in Ireland, involving more than 15,000 cattle. The present project seek: i) to transfer production of the live vaccine from the low scale *in vivo* gerbil system to a large scale non-bovine *in vitro* system in order to increase production levels; and ii) to develop attenuated lines of the vaccine parasite strain in order to decrease vaccine virulence and thus increase safety. Two strains of *Babesia divergens* have been adapted to a microaerophilous stationary phase *in vitro* blood culture using bovine erythrocytes and serum supplemented HL-1 medium. Preliminary studies showed that one and possibly both of the isolates will grow in human RBC's. It was observed that long term culture results in almost complete loss of infectivity for the gerbils, but that infectivity and pathogenicity can be fully restored by only 5 serial gerbil passages. A comparison of the virulence in gerbils of culture adapted parasites following serial subpassage in gerbils, suggested that the degree of parasite attenuation could be manipulated by *in vitro* culture. Isolates were also compared to determine the effect of culturing on functional antigens.

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G16 p EHRLICHIA PHAGOCYTOPHILIA INFECTION IN CALVES AND LAMBS DURING THEIR FIRST GRAZING SEASON AT A RIPARIAN MEADOW IN DENMARK

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Background: *Ehrlichia phagocytophilia*, an *Ixodes ricinus* transmitted Rickettsia infection, was monitored during two consecutive grazing seasons in 1999 and 2000 by monthly blood sampling from May to October of first time grazing lambs and calves.

Method: White blood smears were prepared by cytocentrifugation were stained with May-Grünwald-Giemsa and Ehrlichia organisms were identified at 600 x magnification as intracytoplasmic morula or free organisms in neutrophil granulocytes

Results: The cumulative incidence was 90% in 20 lambs and 68% in 40 calves by the end of grazing season in 1999. In 2000 cumulative incidence was 63% in 40 lambs and 47.2% in 40 calves. During the two years Ehrlichia positive samples occurred repeatedly in the same animals and by the end of grazing season free Ehrlichia organisms were demonstrated in neutrophils from both cattle and sheep. In 1999 serological examination of the cattle with indirect immunofluorescence assay showed seroconversion in 13 animals (33%). The Ehrlichia infection was subclinical as no animals showed any signs of illness.

Conclusion: Ehrlichia phagocytophilia infection has in the present study shown to have a high prevalence, and it is possibly more widespread in grazing ruminants in Denmark, than previously anticipated.

G17 p HOST RESISTANCE TO TICKS IN CATTLE AND OTHER INTEGRATED TICK MANAGEMENT (ITM) AS AN ALTERNATIVE APPROACH TO TICK CONTROL IN ZAMBIA

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Ticks and tick-borne diseases of cattle in Zambia was recognised as a major constraint to the development of the cattle industry as early as 1900 following the introduction of exotic breeds of cattle into the country. Ever since chemical control of ticks on cattle by use of acaricides has been the main method used. Current economic trends in the country have however, rendered chemical control of ticks in the traditional cattle sector (non-commercial) ineffective.

Alternative approaches to the problem of tick control need to be considered. Host resistance to ticks and pasture spelling applied in Australia for *Boophilus microplus* control could be utilised in Zambia. Indications so far show that host resistance to *Boophilus decoloratus* exists in some individual cattle. This could be extended to *Amblyomma variegatum* and *Rhipicephalus appendiculatus*.

A combination of host resistance, pasture spelling, bush clearing and fencing could limit the infestation levels of the three tick species.

G18 p

COMPARED EFFICACY OF AN AMITRAZ COLLAR, A DELTAMETHRIN COLLAR, AND SELAMECTIN AGAINST *RHIPICEPHALUS SANGUINEUS*, "IN VITRO".

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Background: *Rhipicephalus sanguineus* ticks are a serious pest of domestic dogs in many parts of the World. Its ability to survive indoor and colonize the kennels and other human constructions is a drawback in the control of tick-borne diseases to pets. A severe ixodic activity is critical to keep the levels of ticks on animals at its minimum. Furthermore, a fast action is also necessary when trying to break the transmission cycle of tick-borne diseases. In this study, data about the compared efficacy of three compounds against *R. sanguineus* in laboratory, controlled infestations, are presented.

Methods: Compared are an Amitraz Collar (Preventic ©) a Deltamethrin collar (Excalibur ©) and Selamectin (Stronghold ©). The protocols included the delivery of the treatment on Day -1 (D-1), the infestation of dogs with 100 females and 100 males of laboratory derived *R. sanguineus* ticks on D0 and the daily count of alive, dead, feeding, and unfed tick females in the following 7 days.

Results: These three compounds are able to control the infestations of *R. sanguineus* in different ways. A specific degree of control is observed for each compound. Selamectin treatment is the most slow in the control, as no effects are observed until D4. Both Amitraz and Deltamethrin performed well in this protocol. However, ixodic activity of the former is faster (90% of control at D1, 94% at D2) as compared with that of Deltamethrin (68% of control at D1, 75% at D2) indicating a greater ability to break the transmission of tick-borne pathogens for the Amitraz collar. At the end of the protocol (seven days) the efficacy of the compounds was 99% (Amitraz) 96% (Deltamethrin) and 52% (Selamectin).

G19 p

FIELD TICK EFFICACY STUDY IN ELAND WITH TWO POUR-ON FORMULATIONS

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Introduction: This study was designed to evaluate the efficacy of two pour-on formulations in the control of natural tick infestations in relocated Eland (*Taurotragus oryx*). The two pour-on formulations were an amitraz/cypermethrin combination and abamectin. The efficacy against natural infestations of *Rhipicephalus appendiculatus* and *Boophilus decoloratus* was determined.

Experimental design: The study animals were allowed to acclimatize for 7 days before commencement of the experimental phase. Eland with visible infestations of external parasites and which appeared healthy were selected for this study. No ectoparasitic treatment should have been applied for at least 3 weeks before the study. The Eland were anaesthetised and then weighed. The animals were treated at random to reduce handling stress. The required dose was applied. Tick counts were conducted while the animals were anaesthetised. The 2 treatment groups (one for each pour-on formulation) consisted of 6 animals each. The untreated control group consisted of 5 animals. The numbers of ticks were determined on Day 0 and on Day 7.

Housing and Management: The eland were housed in a boma consisting of two holding pens. There was a hinged gate between the two pens to allow animals to be removed from the pen once an animal has been sedated. The sides of the boma were covered with plastic sheeting to reduce the stress to the animals inside the pens. At the end of the study the Eland were allowed to enter the game reserve.

Results and Conclusion: The amitraz/cypermethrin combination was 86.11% effective against *R. appendiculatus* on 98.1% against *B. decoloratus*. The abamectin pour-on was 87.48% effective against *R. appendiculatus* and 88.1% effective against *B. decoloratus*.

G20 p

BOOPHILUS MICROPLUS ERRADICATION TESTS USING FLUAZURON POUR ON.

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Introduction: Biological activity of Fluzuron pour on to eradicate *Boophilus microplus* in Argentina was studied. Simultaneously, the possibility to obtain authorization of use same active ingredient in the *Boophilus* eradication zone, was also evaluated. Since 1938, Argentina adopted an official and compulsory fighting program against *Boophilus microplus*, with the intention of parasite eradication. (the target is moving the border of the infested area, from south to north. In highly infested areas, reduce degree of infestation)

Method: Three reinfested farms, placed in Corrientes province (Argentina) were selected. Once the level of infestation was determined, 8 Fluzuron pour on treatments were applied, during one year period. Dosage: 2,5 mg a.i./kg bw and 42 days of treatments interval. 10-20% of treated animals were periodically checked to evaluate infestation levels.

In the last control, presence of viable parasites were registered.

Results: On 1058 treated cattle population (several categories and ages), 84 days after last treatment, total absence of ticks on animals was confirmed. This status was observed for 7 months latter.

Conclusions: Based on these results, Fluzuron pour on was approved, under SENASA supervision, to be used in the compulsory tick eradication areas.

G21 p

REPORT ON THE STUDIES CARRIED OUT FROM 1999 TO 2001 COMPARING EFFICACY OF FLUMETHRIN AND FIPRONIL SPOT-ON TO CONTROL ADULT *RHIPICEPHALUS SANGUINEUS* INFESTATION.

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Background: These studies were carried out over tick seasons, from 1999 to 2001, in two kennels, situated in the district of Hurlingham (Argentina, in order to compare the tickicidal efficacy of Flumethrin and Fipronil spot-on to control adult *Rhipicephalus sanguineus* infestation.

Method: In each kennel dogs were randomly divided into three groups, one for Flumethrin, other for Fipronil and the third was used as control. The tested products were topically applied according label instructions on the nape of the neck. The control dogs were untreated.

Results : Experienced technicians carried out *R.sanguineus* counts on dogs before and after the spot-on treatment up to 45 days and they observed both tested products killed and detached, (72 hs. after treatment) all *R. sanguineus* specimen stationed on treated dogs and prevented tick reinfestation in the following three weeks. After three weeks ticks on dogs treated by Fipronil followed up normally their biological evolution, and from day 28 it was possible to collect fully engorged females. On dogs treated by Flumethrin only from day 33 it was possible to collect fully engorged females. All the collected specimens were observed in a Lab. (T. 28° C and 80% H.R.) during an 8 weeks period. In this period females collected on Fipronil treated dog laid fertile eggs which later hatched living larvae. None of the fully engorged *R. sanguineus* females collected on Flumethrin treated dog up to 45 days after the treatment laid eggs.

Conclusions : The Flumethrin's inhibition of egg laying controls ticks reinfestation because not having eggs no ticks reinfestation is possible.

G22 p

CONTROL BY FLUMETHRIN 1% SPOT-ON OF A *RHIPICEPHALUS SANGUINEUS* INFESTATION IN A STRAY DOGS ASYLUM.

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Background: Controlling a severe *Rhipicephalus sanguineus* infestation in a stray dogs asylum situated in Buenos Aires metropolitan area.

Method: This study was carried out during the summer tick season 1999 - 2000 using Flumethrin 1% spot-on at the dosage rate of 1 ml / 10 kg. b.w. (1 mg / kg.b.w.), in a stray dogs asylum where 171 animals were treated twice in the first year (45 days interval between treatments) and in the second year all them had a prevention treatment at the beginning of the tick season. In order to avoid a new tick infestation very rigorous prophylactic measures were taken so that all the new arrived stray dogs had no contact with the treated animals and they remained in a quarantine kennel over a three months period. In this period they were treated twice by Flumethrin 1% spot-on, (45 days interval between treatments).

Results: Over this study it has been observed that a Flumethrin's 1% spot-on treatment was able to kill and to detach during the first 48 hours, all *R.sanguineus* specimens as larvae, nymphs and adults stationed on dogs and also was able to avoid reinfestation by these specimens for the following three weeks. All adult *R.sanguineus* fully engorged females, collected up to 40 days after the treatment, were kept in a Lab over 8 week period and none of them was able to lay fertile eggs.

Conclusions: This egg inhibition action of Flumethrin 1% spot-on is very important to control *R.sanguineus* reinfestation in a tick cleaned area because the biological cycle is cut off.

G23 p

GENETIC VARIATION AMONG *IXODES RICINUS* TICKS COLLECTED IN ITALY

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Background: *Ixodes ricinus* ticks are widespread throughout Italy in humid, mountain areas. Populations of this tick are present both on the Alps and on the Appennini. However, there is no information on the genetic structure of these populations. We are investigating the population genetics of *I. ricinus* in Italy through analysis of mitochondrial and nuclear genes.

Methods: Ticks were collected from several areas in northern Italy (Trentino and Veneto). Other ticks were collected from different locations in central Italy (Marche and Toscana). Primers were designed for amplification of the AT rich region in the mitochondrial genome and of the ITS2 in the nuclear genome. Sequence data were generated for a total of 25 specimens and subjected to phylogenetic analysis.

Results: Sequence analysis of both genes revealed genetic variation even among ticks collected in the same area. Phylogenetic analysis did not reveal any correlation between collection location and phylogenetic clustering.

Conclusion: Based on our preliminary results, we do not have any evidence for a genetic subdivision among the tick samples examined. Our results thus suggest that gene flow actually occurs among the different tick populations. Future studies to confirm these initial observations will include examination of further specimens and microsatellite analysis.



H

Parasite biology and systematics

H1 PHYLOGENY OF THE ANCYLOSTOMATIDAE (STRONGYLIDA)

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A phylogenetic analysis of the Ancylostomatidae (Strongylida) based on morphological characters is proposed. Ancylostomatidae adults live as parasites in the intestines of eutherian mammals and feed on intestinal epithelia by inserting with their anterior end in the host tissue. The specimen, collected by Prof. R. Sachs from East African mammals, were studied with light microscopy and SEM.

I propose a homology of the stoma architecture in Strongylida with those of rhabditid nematodes. A hypothesis for the transformation of the buccal cavity (cheilostom, gymnostom and stegostom) within the Ancylostomatidae is presented. In the common ancestor of the Ancylostomatidae, the mouth opening has shifted from a terminal position to the dorsal body side and the gymnostom is shortened on the dorsal and elongated on the ventral side. The stem species had gymnostomal teeth surrounding the oral margin (plesiomorphic), however, they assume a special shape (apomorphic). They have been transformed within the Ancylostomatidae to semilunar plates in a subventral position, similar to those in *Necator*. Within the group a dorsal tooth had evolved. I suspect that the monotypic genus *Schulzinema* is the sister taxon of the Ancylostomatidae, as the gymnostom teeth are synapomorphic.

This project is part of the graduate student program „Evolutive Transformationen und Faunenschnitte“, supported by the DFG.

H2 RESEARCH OF WOLBACHIA IN SOME SPECIES OF SPIRURIDS AND MUSCIDS

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Background: *Wolbachia* are cytoplasmic symbiotic α 2-proteobacteria found in arthropods and filarial nematodes. These bacteria are associated with reproductive alterations of their hosts. Five species of biting (*Haematobia irritans* and *H. titillans*) and non-biting Muscidae (*Musca domestica*, *M. larvipara*, *M. autumnalis*) and five species of spirurids (*Habronema muscae*, *H. microstoma*, *Thelazia callipaeda*, *T. gulosa*, *T. lacrymalis*), have been tested by PCR for the presence of *Wolbachia* in order to acquire further evidence on its presence in some insects and in some spirurids of veterinary importance.

Methods: For each species of muscids and nematodes tested genomic DNA was extracted by proteinase K-treatment from 10 pools of 5 ovaries each and from three single adult worms, respectively. In order to amplify the 16S rRNA gene of *Wolbachia*, PCR was performed with universal bacterial and *Wolbachia* primers. PCR amplicons were purified and sequenced directly or after cloning. The sequences obtained have been aligned with those available in databases.

Results: *Wolbachia* was detected in 7 and 2 samples of *H. irritans* and *H. titillans*, respectively. The molecular analysis of 16S rRNA gene showed a similarity of 99%, with one transversion and two insertions in *H. titillans* compared to *H. irritans*. All the samples of *Musca* and nematodes analysed were found to be PCR negative for the presence of *Wolbachia*.

Conclusion: The results provide information on the distribution of *Wolbachia* in some parasites which have never been examined before. The presence of *Wolbachia* in *H. irritans* confirms the findings of Jeyaprakash A., Hoy M.A. (Insect Molecular Biology, 2000, 9:393-405) in USA, based on the *wsp* sequences.

H3 A NEW GENOTYPE OF TRICHINELLA FROM AFRICAN CROCODILES (CROCODYLUS NILOTICUS)

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Background: Since 1995, *Trichinella* larvae have been detected in muscles of up to 40% of commercially reared crocodiles (*Crocodylus niloticus*) on farms in which have been found to be infected in Zimbabwe. Experimental infections have shown that these nematodes can infect mammals (domestic pigs, laboratory rats and baboons). The aim of the present work is the identification of morphological, biological, and molecular characters of this *Trichinella* strain and their relationship with the known species.

Method: The morphology of adults, muscle larvae, and nurse cells, were studied by a light and/or an electron microscope. The reproductive capacity index (RCI) in Swiss mice has been evaluated. Three DNA sequences have been investigated: the large subunit ribosomal LSR-DNA, the expansion segment five (ESV), and the CO 1 gene.

Results: At the isolation in immunosuppressed mice, the RCI was 8.6. The light and electron microscopic studies showed that larvae from crocodiles are non-encapsulated and the changes in the nurse cell are similar to that detected in the other non-encapsulated species. The LSR-DNA and CO 1 sequences were shown to be more similar to that of *T. papuae* (95% and 91% of similarity, respectively) than to those of *T. pseudospiralis* (89% and 88% of similarity, respectively) or to those of the 5 encapsulated species (86-88% and 85-86% of similarity, respectively). Similar results have been obtained for the ESV.

Conclusion: These preliminary results suggest that *Trichinella* larvae detected in crocodiles belongs to a new genotype which seems to be more similar to the non-encapsulated species *T. papuae* than to the other non-encapsulated species *T. pseudospiralis* or to the 5 encapsulated species.

H4 INHIBITED DEVELOPMENT OF Haemonchus contortus FROM BRAZILIAN GOATS

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Goats constitute the majority of livestock in Paraíba backwoods as they can thrive the climatic and topographic conditions prevailing in this state. From January 1998 to December 1999 forty-eight abomasas from goats were examined for the presence of adult and larval of *Haemonchus contortus*. The mucosal abomasas were digested in pepsina solution and hydrochloric acid. Ten (20,8%) goats were infected with *H. contortus* adults and sixteen (33,3%) with larvae. Overall adult *H. contortus* counts and infections rates were lowest in dry season and the larvae were lowest in rainfall season. In Brazil larval inhibition was only observed in cattle and plays an important role in the *H. contortus* life cycle, since this parasite survives the dry season as arrested larvae. The observation of fourth stage larvae of *H. contortus* during the whole year with a significant increase ($p < 0.05$) during the dry period (May to December) in relation to the rainy period (January to April) demonstrated that in the Paraíba backwoods the hipobiosis was the mechanism to survive during the long dry period.

Keywords: Hypobiosis, Goats, Semi arid, *Haemonchus contortus*.

H5

EXPLAINING PREMUNITION WITH KIN SELECTION

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Background: In premunition, current infection protects against challenge infection. Using *Haemonchus contortus* smooth and rough isolates (with and without vulval flaps), we tested whether premunition is a parasite-induced response for kin selection.

Method: Helminth-free lambs (48) were divided into 8 equal groups (S1-S4 and R1-R4), and orally inoculated with 1,000 smooth (s) or 1,000 rough (r) larvae once or twice, 4 weeks apart. S1 received s + s, S2 s + 0, S3 s + r, S4 0 + s; R1 received r + r, R2 r + 0, R3 r + s, R4 0 + r. Four weeks later, lambs were slaughtered, parasites collected, males counted, and females categorized and counted.

Results: There were no differences in the total numbers of female worms recovered between the different groups. However, for each respective isolate, the number of dissimilar worms that succeeded in developing into adult females was only half of the total worms recovered.

Conclusion: Adult *Haemonchus contortus* infections inhibit the development of dissimilar isolates, non-kin, from developing to their maximal capacity and at great expense to self. The phenomenon of kin selection can be used to explain premunition in most relationships where this condition has been described.

H6

ON THE ROLE OF *LUMBRICUS TERRESTRIS* AS A PARATENIC HOST FOR *ASCARIS SUUM*

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Background: Earthworms have been shown to serve as paratenic hosts or even intermediate hosts for *Ascaris suum*. Thus, infective eggs may hatch in *Lumbricus terrestris*, in which the larvae to migrate into the coelom. When earthworms were fed to guinea pigs, *A.suum* larvae could be isolated from the lungs. The present experiment was set up to confirm this role of *L.terrestris*.

Method: *Lumbricus terrestris* from the local anglers' shop were allowed to eat highly infective *A.suum* eggs. The earthworms emptied their intestines thoroughly for any unhatched *A.suum* eggs afterwards and were examined by dissection, the agar-gel technique, and pepsin-HCl digestion. Twenty-five earthworms were administered to each of 3 pigs by stomach tube and the livers, lungs, and small intestine were later examined by the agar-gel method. **Results:** No *A.suum* larvae were recovered from neither earthworms nor livers, lungs or small intestines of earthworm inoculated pigs by days 4, 7, and 11 pi (post inoculation). Instead, few unidentified filariform nematode larvae were found both in the earthworms and in the lungs of the earthworm inoculated pigs by day 4 and 7 pi.. It turned out that the earthworms were imported from Canada.

Conclusion: In contradiction to some previous studies, the present investigation did not indicate that *L.terrestris* may play any role as a paratenic host for *A.suum*. The finding of an unknown nematode indicates that human mediated transport of intermediate/paratenic hosts may introduce parasites to new continents.

Acknowledgement: The study was supported by The Danish National Research Foundation.

H7

MONITORING *ASCARIS SUUM* EGG SURVIVAL USING SENTINEL CHAMBERS™

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Background: The Sentinel Chamber was developed to monitor the inactivation or long-term survival of eggs and protozoan cysts in soil and other matrices under natural conditions or following various inactivation processes. Chambers have been used to examine the survival of pathogens in soil, water, sewage digesters and lagoons, water treatment sludges, manure piles, and aquifer material. *Ascaris suum* eggs are routinely used as an indicator organism because of their highly resistant nature and their presence in swine waste in spite of modern anthelmintics.

Method: The chamber is filled with the matrix, inoculated with eggs from swine feces, and sealed. Egg survival is monitored by embryonation after the chambers are collected and emptied. When comparing survival of eggs in chambers to eggs free in the surrounding media, eggs are added to both.

Results: Eggs in chambers have performed similarly to eggs seeded directly into the soil or biosolids around the eggs.

Conclusion: The chambers are simple and reliable method for looking at the viability of eggs under many different conditions. The chambers have the advantage that they can be processed quickly and the can be used to monitor very large systems where either public health concerns or size would make seeding impossible.

H8

IN VITRO DEVELOPMENT OF *TOXOCARA CANIS* TO THIRD-STAGE LARVAE WITHIN THE EGG COLLECTED FROM DIFFERENT ISOLATES

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Background: The infective stage of *Toxocara canis* within the egg is the second stage larvae according to most textbooks in parasitology. However, several authors have reported that two molts occur within the egg.

Method: *Toxocara canis* eggs were collected from dogs and a fox and cultured *in vitro* in 0.1N H₂SO₄, 0.1% formalin or moist vermiculite at 25°C, then hatched artificially and examined under microscope three times weekly. The infectivity of the eggs was tested by inoculation of mice.

Results: The results showed that two molts take place within the egg, one occurring immediately after the other. The first larval stage (L1) appeared in the egg after 6-8 days of cultivation in 0.1% formalin, the second larval stage (L2) started after 14 days, and the last stage (L3) observed after 21 days and continued during the 35 days. The rate of development and morphogenesis of the eggs from cellular divided until L3 stages were similar in 0.1N H₂SO₄, 0.1% formalin and moist vermiculite. However, development in 0.1N H₂SO₄ was fastest. The same results were observed with the eggs isolated from worm uteri or from the feces of Danish dog, Tanzania dog and Danish fox. It was observed that the cultured eggs in the three different methods from Danish dog or fox were equally infective in mice, but it was significantly different from that in Tanzania dog origin.

Conclusion: It is clearly documented that two molts occur in *T. canis* larva within the egg, so that means the second stage larva is claimed to be the infective one, and there infectivity depending on the worm origin.

H9

FIRST BLOOD MEAL OF *C. FELIS* AND *C. CANIS* : TIME TO INITIATION AND DURATION

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Two experiments were conducted on dogs to evaluate interval to initiation and duration of the first blood meal of *Ctenocephalides canis* (Curtis) on dogs and *Ctenocephalides felis* (Bouché) on cats. Percentage of fed male and female fleas was calculated for fleas held on dogs for 5, 15, 30, 60 min, 6 and 24 hr and fleas held on cats for 5, 15, 30 and 60 min. When *C. canis* were free in the dog hair coat, 21.2% had begun blood feeding within 5 min. After 1 hr, 72.5% of fleas had fed. After 6 hr, 95.2% of males and 100% of females had taken a blood meal and 24 hr after deposition all fleas had fed. The mean delay between deposition and biting for fleas which began feeding within 15 min, was 2 min 52 sec $\pm$ 3 min 2 sec for female fleas and 3 min 8 sec $\pm$ 2 min 45 sec for males. The mean duration of female and male meals was 5 min 3 sec $\pm$ 3 min 41 sec and 6 min 9 sec $\pm$ 6 min 8 sec, respectively. There was no significant difference between the 2 sexes. When *C. felis* were free in the hair coat 24.9% were engorged after 5 minutes. After 1 hour, 97.2% of fleas were engorged. When fleas were confined in a vial maintained on the skin, feeding was quicker : 60% of females had fed within 5 minutes. After 1 hour, near all fleas had taken a blood meal. The mean delay between deposition and biting was 24 $\pm$ 31 seconds for female fleas and 23 $\pm$ 44 seconds for males. The mean duration of female and male meal was 25 min $\pm$ 18 min and 11 min $\pm$ 8 min respectively. The dog flea took its blood meal on dogs more slowly than the cat flea did on cats; this meal was significantly longer for *C. felis* than for *C. canis*.

H10

MEROGONY IN *IN VITRO* CULTURES OF *THEILERIA PARVA*

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Background: *Theileria parva* is the causative agent of East Coast fever, one of the most devastating livestock diseases in Eastern, Central and Southern Africa. The protozoan is transmitted by the tick *Rhipicephalus appendiculatus* and infects bovine lymphocytes which become transformed into lymphoblastoid cells. The aim of this experiment is to evaluate the duration of merogony in newly infected PBL's in order to purify them for further life cycle studies.

Method: Tick stabilates of two different *T. parva* strains were purified using a DEAE-cellulose column to avoid bacterial and fungal contamination. The concentrated sporozoites solution was added to stimulated PBL's of a naïve calf. After infection, daily samples were taken to calculate macroschizont and merogony prevalence.

Attempts were made to re-induce merogony in cryo-preserved cell cultures of *T. parva*.

Results: In both *T. parva in vitro* infections, merogony was first seen from day 10-12 on until day 60 after infection. Re-induction of merogony in the cryo-preserved cultures was ineffectual.

Conclusions: Contrary to *T. annulata*, merogony in *T. parva* is considered to be a one time event in newly infected PBL's, during a well defined period (10-60 days). Most probably still unknown conditions induce merogony in the synchronously dividing schizont infected transformed cell which lead the liberation of the merozoites and ultimately the death of the cell. Only the remaining non-merogony-schizont-lymphoblasts divide and remain in the fast-growing cell culture.

H11

STUDIES ON THE DEVELOPMENT OF *OESOPHAGOSTOMUM DENTATUM* *IN VITRO* AND *IN VIVO*.

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Oesophagostomum dentatum is frequently being used as a model parasite in population dynamic and biochemical studies. *In vitro* cultivation of larvae provides defined, clean material. However, the rates and sizes do not reach those of L4 *in vivo*, and adults cannot be recovered from cultures. Biochemical parameters also differ between L4 grown *in vitro* and *in vivo*. *In vitro* cultivation seems to be suboptimal for parasite development and may therefore have undesirable effects on the biochemical composition of larvae. We investigated the effects of *in vitro* cultivation on growth and survival of different parasite stages, and tested the ability of pre-cultivated L4 to establish *in vivo* after transplantation into the host. L4 and adults recovered from intestinal contents of pigs were cultivated in standard medium and compared to L4 grown *in vitro*. Pre-cultivated L4 were rectally transplanted into the large intestines of parasite-free pigs, recovered after 3 or 14 days and measured. Although growth and development rates of cultivated L4 are lower than those of larvae recovered from intestinal contents after oral infection of pigs, pre-cultivated L4 are able to develop into egg-laying adults without nodule formation within 9-11 days (compared to 17-19 days after oral infection with L3). On the other hand, L4 and adult worms recovered from intestinal contents cannot be maintained in cultivation medium for more than 1 week, although most L4 grow and moult to pre-adult fifth-stage larvae during the first 48 h. Although the standard cultivation conditions for mass production of L4 are not suitable for development or maintenance of preadult and adult stages, L4 recovered from *in vitro* cultivation have the ability to establish *in vivo* like L4 from oral infections, indicating that the basic biological functions are acquired and maintained *in vitro*.

H12

EFFECTS OF TETRACYCLINE ON *WOLBACHIA* AND ON FILARIA MOLTING

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Background: several species of filarial nematodes of veterinary and medical interest have been shown to harbour the bacterial endosymbiont *Wolbachia*. The effective role of *Wolbachia* in the filarial biology is still unknown. Like others Rickettsiales, tetracyclines are effective on *Wolbachia*, and some studies have shown that treatments with these antibiotic are also detrimental to those filariae which harbour *Wolbachia*.

Methods: in our study, we artificially infected jirds (*Meriones unguiculatus*) with the lymphatic filaria *Brugia pahangi*. Jirds were then treated with tetracycline in drinking water. The treatment schedule was optimised on the presumably timing of molting between L₃ to L₄ and L₄ to L₅ of *B. pahangi* in jirds. Worms were recovered from jirds at the end of the treatments.

Results: tetracycline treatments led to the following results: 1) treatments started at day 13 post-infection (PI) had prophylactic effect; 2) treatments started after day 26 PI led to a bias of the sex ratio of the parasites (i.e. a reduction of the number of females).

Conclusion: in *B. pahangi*, the L₃-L₄ molt takes place around day 8-9 PI, while the L₄-L₅ molt occurs around day 23 PI in males and a few days later in females (around days 27-33 PI). Tetracycline treatments started before the molt between L₄ to L₅ (day 13 PI for both males and females, and day 26 PI for females only) seems to affect the development of the worms. Our preliminary results thus indicate that tetracycline interferes with the L₄-L₅ molting in *B. pahangi*. This suggests that the endosymbiont *Wolbachia* is implicated in the molting process.

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p

REPRODUCTION OF DIPHYLLOBOOTHRIUM LATUM AND MORPHOLOGY OF GENITAL DUCTS

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Background: The process of reproduction in various groups of cestodes is not only determined by the biology of each species, but also by the structural and functional features of the different components of the male and female genital system. The purpose of this study is to provide information about some aspects of reproduction of *Diphylobothrium latum*.

Method: *D. latum* were obtained from an experimentally infected cat and plerocercoids were recovered from the body cavity of pike collected from Rybinsk reservoir, Russia. The worms were processed for TEM.

Results: The thick egg shell of *D. latum* is formed by the deposition of vitelline cells in the egg's surface whilst it is in the ootype-uterine duct. The internal surface of the uterus is covered with microtriches which facilitate the movement of eggs within the uterus. The distal region of the uterus has a powerful musculature and contractions of these muscles allow the eggs to be deposited directly into the host's intestine. The wall of the distal section of the uterus is characterized by the presence of numerous vesicles - the secretions from which form envelopes around the eggs, which protect them during transport within the host's intestine. The copulatory organ of *D. latum* is provided with numerous specialized prostate glands, the secretions of which envelop spermatozoons. The lamellar structure of the myocytes of the cirrus pouch facilitates the mobility of one part of the copulatory organ independently of another. A possible function of tangoreceptors surrounding the region of the genital atrium may be related to the neural control of copulation, with the possibility of cross-insemination.

Conclusions: Morphology based differentiation of the genital system constitutes a structural basis for adaptation in the process of reproduction.

p

GENITAL MORPHOLOGY OF PSOROPTES SP.

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Background: Very few is known about the internal morphology of the scab mites *Psoroptes* sp. In this work, ultrastructure of the reproductive system has been investigated at the light and TEM microscopy levels.

Methods: Adult males and females were collected from ears of rabbits. Legs were cut and parasites were fixed in 2,5% glutaraldehyde.

Postfixation in 1% osmium tetroxide was performed before dehydration in increasing ethanol concentrations. Specimens were then embedded in Epon. Each block contained a single mite, transversally orientated. 2 µm sections were dried and stained in Toluidine blue. Ultra-thin sections were transferred onto Formvar grids and routinely processed. A 3-dimensional reconstruction of the genital system was carried out by serial sections recordings.

Results & conclusion: The female genital system consists of a pair of ovaries containing ovogonies, ovocysts, vitellogenesis cells and a nursing cell. Ovogonies join the nursing cell, with plurilobular nucleus, via a "butt" rich in dense material. The male genital system consists of a pair of testis, containing spermatogonies, spermatocysts, spermatozoa and a nursing cell. Observations are similar to those observed in other astigmatic mites.

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p

Sarcocystis (Lankester, 1882) (Apicomplexa : Sarcocystidae) detected in Brazilian swines: morphology, identification of species and hygienic-sanitary importance.

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According to rules and patterns of the Brazilian Ministry of Agriculture for cysticercosis in swine commercialized at stalls of Brazil, material utilized must be constituted by muscular portions of anatomic regions of gammon, pallet, diaphragm, heart, head and tongue. 171 anatomic samples were collected to identify the species of the genus *Sarcocystis*. Based on morphological characteristic of sarcocysts, localized on cells of striated, skeletal and cardiac muscles, three species of this genus should be characterized as *S. miescheriana*, *S. porcifelis*, and *S. suihominis* which have dogs, cats and men as definitive hosts, respectively. These three species were pointed out as first time in some Brazilian regions. Pigs carcasses commercialized for human consumption were examined and could be detected the presence of the three species as follows: the pallet had the highest frequency of *S. miescheriana* with 42,52%; the gammon with 36,90% positive to *S. suihominis*; and heart with 31,95% to *S. porcifelis*. Frequency of the species was independent on the conditions of the studied local breeding, where hygienic-sanitary conditions were not satisfactory which were highest favorable to their development and proliferation. Oftentimes, this condition could be also favorable to other etiological agents, which has had the pig as an intermediate host. As an example, the *Cysticercus cellulosae*.

p

CUTICULAR ARCHITECTURE OF *Hassalstrongylus epsilon* (NEMATODA:TRICHOSTRONGYLIDAE)

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Introduction: *H. epsilon* is a small nematode, whose adults forms are found firmly spiraled around the intestinal microvilli of the water rat *Nectomys squamipes*. The complex architecture of Trichostrongylidae cuticle allows its use in taxonomic and phylogenetic studies (Linchtenfels, J.R. et al; J. Parasitol 86 (5), 2000, p. 1093-1098) (Duret-Desset, et al; Int.J. Parasitol. 24 (6), 1994, p. 887-898).

Method: Nematodes were collected in 0,9% saline, fixed in 2,5% glutaraldehyde in 0,1M Cacodylate buffer, pH 7,2, post-fixed in 1% OsO₄ and 0,8% FCnK, dehydrated in acetone and embedded in Epon. Semi-thin sections were stained with methylene blue. For transmission electron microscopy, ultrathin sections were stained with uranyl acetate and lead citrate.

Results: The cuticle near the posterior end shows 22 ridges, 11 dorsal and 11 ventral, and 23 at mid-body level. Its surface presents longitudinal ridges. Struts that arise from median layer extending outward to the epicuticle internally support these ridges. The morphology, number, and orientation of struts vary along body as the width of the fluid region of the median layer. In cross sections of the posterior end these structures are seen as simple struts that arise from the rich collagen fibrils area of the outer medium layer, while in the middle of the body some of these structures show a solid appearance. In oblique sections the struts appear to be formed by a row of superposed structures electrondense in the outer region and highly irregular in shape.

Conclusions: Our results indicate that the synlophe is formed by a serie of rings of modified cuticle around the body that gives support and at the same time allow the worms movement.

Acknowledgments: CAPES, CNPq, FUJB, and PRONEX.

H5 p

CYTOGENETIC STUDY ON THE SPECIES OF
BOTHRIOCEPHALUS (CESTODA)

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Background: The tapeworms of the cosmopolitan genus *Bothriocephalus* Rudolphi, 1808 are common parasites of marine and freshwater teleosts. The taxonomic status of species within the genus has provided considerable discussion. Cytogenetic studies can supplement morphological, biological, biochemical and other characteristics used for systematic analysis.

Method: Diploid complements of mitotic metaphase plates of *Bothriocephalus gregarius* and *B. claviceps* were studied using conventional Giemsa staining and karyometric analysis. Mature specimens were collected from *Scophthalmus maximus* and *Anquilla anquilla*, respectively.

Results: All specimens of both species showed a karyotype with $2n=14$ chromosomes. The two first pairs of homologues were metacentric and markedly large than the remaining elements. All chromosomes of *B. claviceps* were metacentric or meta-submetacentric, what may be considered characteristic of stable karyotype. Very close karyotype structure of *B. achellognathi* was determined (Nedeva, I., Mutafova, T. Khelminthologiya, 1988, 26, 39-46). The karyotype of *B. gregarius* differs in chromosome morphology of pairs No 5 and 7, which had terminally or subterminally located centromeres.

Conclusion: The similarity in the karyotype structure does not exclude the possibility to discriminate *Bothriocephalus* species by karyotypic characters. The small number of chromosomes ($2n=14$) together with the prevailing biarmed elements indicates that fusion and deletion of chromosomes have played some role in evolution. Cytogenetic data provides evidence to indicate that *Bothriocephalus* is a highly specialised and monophyletic genus.

H7 p

HISTOLOGICAL ASPECTS OF *Echinostoma paraensei*
(PLATYHELMINTHES: TREMATODA) WITH THE
DESCRIPTION OF A NEW STRUCTURE.

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Introduction: *E. paraensei* is largely used in studies of molecular biology, morphology and relationship of parasite-invertebrate and vertebrate hosts, even so lacks information about its histology and internal morphology.

Methods: Worms were collected from experimentally infected vertebrate hosts *Nectomys squamipes* (Rodentia). They were fixed and prepared for histological studies by optical microscopy and for scanning electron microscopy following (Lanfredi et al, 1992 Mem. Inst. Oswaldo Cruz 87 (sup.1) 1-10).

Results: By scanning electron microscopy it was possible to observe a folded protrusion with a pore on its top located just posteriorly the genital pore. When the cirrus is protruded is difficult to see the protrusion. The histological sections of this region evidenced that the cirrus protrudes at the location already know as genital pore. The cirrus pouch is highly muscular and the prostatic glands can be easily seen. The cirrus duct is proximally capped with a columnar ciliated epithelium at the middle region there is a glandular epithelium. The cirrus duct presented only spermatozoa, no eggs could be seen in it. The folded protrusion presents an aperture on its top. This is the opening of another duct that presents eggs inside it.

Conclusions: Our observations allowed us to conclude that *Echinostoma paraensei* presents another ventrogenital opening besides the known genital pore were the cirrus protrudes. This opening is an uterine pore. This is the first record of two genital openings at the ventral region of *E. paraensei*: a male were the cirrus protrudes and probably used for copulation and a female one used for eggs discharge.

Acknowledgements: CNPq, FAPERJ, FUJB and PRONEX.

H6 p

THE STRUCTURE OF RAILLIETINA S.L.
(CESTODA, DAVAINIDAE)

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Prior to our paper (Movsessian, 1966) the structure of Raillietina was known to accommodate 4 subgenera: Raillietina (Raillietina), R. (Fuhrmannetta), R. (Paroniella) and R. (Skrjabinia).

In 1966 the subgenera mentioned were elevated to independent generic taxa and ranged under new subgenera, i.e. Raillietina (Raillietina), R. (Nonarmina), Fuhrmannetta (Fuhrmannetta), F. (Mathevossiannetta); Paroniella (Paroniella), P. (Nonarmiella); Skrjabinia (Skrjabinia) and S. (Armacotabulum).

On the base of long-term studies it was suggested that to classify genera in question as subgeneric taxa was not justified. In this connection the following generic taxa were proposed for Raillietina s.l.: Raillietina (Fuhrmann, 1920) Movsessian et Nikogossian, 1997; Fuhrmannetta (Stiles et Orleman, 1926) Movsessian et Nikogossian, 1997; Paroniella (Fuhrmann, 1920) Movsessian et Nikogossian, 1997; Skrjabinia (Fuhrmann, 1920) Movsessian et Nikogossian, 1997.

Thus all the taxa are recognized as valid and do not fall into subgenus categories. Cestode species corresponding to generic diagnosis 1997, are included to the genus Raillietina (Fuhrmann, 1920) Movsessian et Nikogossian, 1997. Its characteristic features are as follows: proboscis armed with 2 (seldom 1) rows of davainoid hooks, suckers with/without armature, genital pores unilateral, uterus disintegrated into egg capsules each with several eggs.

The Raillietina are parasites of birds and mammals.

Type species: Raillietona tetragona (Molin, 1858) Fuhrmann, 1920.

215 cestode species has been known so far worldwide among which there are causative agents of raietinoses, especially in galliformes and colubiformes birds.

H8 p

Haematobia irritans: EVALUATION OF ITS
POTENTIAL AS A BIOLOGICAL VECTOR
FOR *Dermatobia hominis*, IN ARGENTINA.

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The tropical warble fly *D. hominis* is an important cattle grub in South America, unique for its curious way of using captured diptera as biological vectors. Approximately 50 mosquitoes and flies have been described as biological vectors. The adult female deposits its eggs on their abdomen, preferably under the wings; when the female vector feeds on its cattle hosts, the emerging larvae penetrate the skin. In Argentina, endemic areas in the humid NE and mesopotamia provinces have reported a significant increase in tropical warble fly infestations. *H. irritans* has been described only once as a vector both in Brazil (1998) and in Argentina (1999), where it entered in late 1991. In both reports a single carrier *H. irritans* was found. In order to further determine the importance of *H. irritans* as a vector in Argentina, 7,369 flies on 5 endemic *D. hominis* sites were examined. On 2 in the NE of Formosa, 4,820 *H. irritans* were collected on cattle with a low infestation (<10% of herd); 1 in the NE of Corrientes, and 4 in the SW and NW of Misiones (660, 633 and 1,256 flies, respectively) all from medium to high infested cattle (>20 warbles/animal, 60-75% of herd infested), were screened under a 12X stereoscope. Conclusion: *Haematobia irritans* was not confirmed as a biological vector for *Dermatobia hominis*, but new evaluations are scheduled for spring and early summer. Other factors are considered to explain the increase of warble levels.

H9 p

THE GNATOSOMA OF *Raillietia caprae* (ACARI: GAMASIDA).

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Background: *Raillietia caprae* (Quintero, Bassols and Azevedo, 1980) have been recorded from the ear canals of goats and sheep. It is the most important cause of otitis in caprine in Brazil and, probably, elsewhere, although the economic importance of the parasitism is still unknown. Research carried out in the last decade in Brazil and elsewhere has shown that these mites are pathogenic and can transmit microparasitosis.

Method: In this work, female of *R. caprae* was collected from ear canal of cattle, fixed in glutaraldehyde 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).

Results and conclusion: S.E.M. studies of *Raillietia* mites show an important piercing structure: the chelicerae, that has two horn-like structures distally in its body: the fixed digit and movable digit, both makes contact like a tweezer and 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. The rounded shape of the gnathotectum in *R. caprae* permit distinguish them from *R. flechtmani* (pentagonal shape) and *R. auris* (triangle shape).

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H11 p

A COMPARATIVE KARYOLOGICAL STUDY OF SOME GENERA OF DIPLOSTOMIDAE

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Background: Diplostomidae are fairly abundant and widespread group of trematodes parasitizing birds, mammals. The growing interest in species of Diplostomidae has been related to the particular pathogenic impact of metacercaria on the organism of fish (with economic consequences). In search of good criteria to separate species, cytogenetic methods can offer a new tool for larval-stage identification. In recent years, more than once cytogenetic studies were a good supplement for morphological, biological and other characteristics used for systematic analysis.

Method: A karyological analysis of the 12 species of the genera *Diplostomum*, *Tylodelphys*, *Posthodiplostomum* and *Alaria* was made using an air-drying technique and Giemsa staining. Chromosome number and morphology were studied in somatic cells of parthenites from naturally infected first intermediate hosts - molluscs.

Results: Karyotypes of all investigated species display a typical pattern with 20 chromosomes. The chromosomes of acrocentric and subtelocentric type are dominant in the group of comparatively large 1st - 5th pairs of genome units. 6th - 10th pairs are represented by comparatively small, subtelocentric, submeta- and metacentric chromosomes.

Conclusion: A low symmetrical nature of karyotypes of Diplostomidae and the presence of subtelocentric chromosomes indicate active evolutionary changes in the genome of diplostomes. The interspecific differentiation of chromosome set more actively occurred in the group of smaller genome elements. The fundamental similarity of chromosome sets support the hypothesis, that diplostomes represent a monophyletic group.

H10 p

Haemonchidae phylogeny based on molecular data

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The *Haemonchidae* family is constituted of nematodes, parasite of different hosts such as bovids, camelids, cervids, lagomorphs, sciurids, suids and tragulids. Durette-Desset et al. (1999) proposed a phylogenetic analysis of the *Haemonchidae* based on morphological characters. We intended to use molecular data in order to construct a phylogeny to be compared to the one proposed from morphological data. The ITS2 (Internal Transcribed Spacer) sequence (260bp) was amplified in 3-10 worms/genus for *Teladorsagia*, *Spiculopteragia*, *Graphidium*, *Haemonchus*, *Mecistocirrus* and *Ashworthius*. Consensus sequence was obtained for each genus. *Marshallagia*, *Ostertagia* and *Camelostomylus* ITS2 sequences were found in Genbank. The evolutionary trees were constituted using parsimony, and neighbour joining (absence of evolutionary clock hypothesis) or UPGMA (evolutionary clock hypothesis) construction methods. *Cooperia* and *Trichostrongylus*, who belong to other closely related families were chosen as outgroups. The ITS2 sequence variability indicates that *Haemonchus*, *Mecistocirrus* and *Ashworthius* are close genera and could be grouped in the *Haemonchinae* subfamily, in agreement with the results obtained from morphology. The *Haemonchinae* constitutes a monophyletic group. The grouping into the subfamily *Ostertiaginae* of *Teladorsagia*, *Ostertagia*, *Marshallagia*, *Graphidium* and *Spiculopteragia* based on morphological information could be equally accepted using molecular ITS2 data. The within arrangements of the tested genera into the subfamily were differently organised. Other genes should be analyzed to confirm the tree derived from molecular phylogenetic using ITS2.

H12 p

MORPHOMETRIC STUDY OF

Acuaria (Dispharynx) spiralis FROM CHICKEN

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Different species of *Acuaria (D.) sp* has been found in birds and related the pathogenicity that infection is an important predisposing factor in the mortality of birds (McOrist et al., 1982; *Avian Dis.*, 26:957-960) and in the experimental infection was observed a mortality rate of 29.1% of chicks (Ramaswamy & Sundaram, 1985; *Vet. Parasitol.*, 17:309-317) and presented 8.3% a 19.3% of prevalence in rural scavenging poultry in Tanzania (Permin et al., 1997; *J. Helm.*, 71:233-240). A new species *Acuaria coloradensis* was related of swallows of the mountains of Colorado (Sherwin & Schmidt, 1988; *J. Paras.*, 74:336-338). In Brasil don't have studies about the occurrence of *Acuaria (D.) spiralis* nor pathogenicity. In a lethargic and inappetent chicken was found 286 specimens of *A. (D.) spiralis* in proventriculi, observed extensive fibroplasia in the organ and fibroadenomatoid growths develops in the mucosa obliterating the partial lumen. Fifty males and females were measured. The males presented 33.4 mm (±4.3) total length; spicules right and left measuring 87.2 µm (±10.8) and 238.3 µm (±26.4) respectively and presented nine pairs of anal papillae. The females measured 66.7 mm (±11.2) total length. The comparison of the morphology and the mensurations was diagnosed as *A. (D.) spiralis*.

Keywords: *Acuaria (D.) spiralis*, morphometric study, chicken, Brasil.

H13 p

IN VIVO DEVELOPMENT OF IN VITRO CULTURED HAEMONCHUS CONTORTUS eL4

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Background: *H. contortus* L3 can be exsheathed artificially and will moult *in vitro* to early 4th stage (eL4), the first parasitic stage in sheep, in a variety of culture media. The similarity of these cultured larvae to those that develop from L3 *in vivo* was assessed by injecting eL4 after 6 days in culture into the rumen (ir) or abomasum (ia) of sheep.

Methods: L3 were exsheathed with CO₂, then cultured to eL4 in RPMI 1640 with added nutrients. In Expt A, 3 doses (5,000, 10,000 or 20,000) of eL4 were tested in each of 2 sheep (experimental, ir); controls were given the same doses of L3 orally. In Expt B, these 3 doses were injected ia into single sheep and one control was given 10,000 L3 ir. FEC were determined from experimentals and controls between 18-34 days PI and mature worms, and their sex ratio and length, compared after 34-38 days.

Results: In Expt A, FEC were lower and only 3-5 % of eL4 became established in the experimentals. In Expt B, all sheep had positive FECs at Day 18 and FEC were higher in those sheep given the eL4. 31 % of cultured eL4 became established compared with 49 % in the sheep given 10,000 L3.

Conclusions: Cultured eL4 did not develop when injected ir, but established successfully when injected ia. Their rate of development, size and fecundity were similar to those from sheep injected with L3.

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H14 p

DEVELOPMENTAL EXPRESSION OF HAEMONCHUS

CONTORTUS GUT SURFACE COMPONENTS

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Background: The gut microvilli of sexually mature *H. contortus* are 8-10 µm long; their axial cores terminating in a cytoskeletal entity, the endotube, 0.2 µm thick. The microvillar membrane is the expression site for the isoforms of the highly protective hidden antigen H11 and protein complexes such as H45 and H-gal-GP.

Methods: Parasites were obtained from the abomasa of sheep killed at intervals from 4 days to 32 days post infection and from *in vitro* cultures. Developmental regulation of expression of gut surface proteins was analysed by Northern blotting and compared with measurements of microvilli length and endotube thickness determined from electron micrographs and phase optics light micrographs.

Results: Earlier data were confirmed and extended: the growth curve for the microvilli was sigmoidal with the maximum rate of growth 11 to 18 days post infection. This coincided with the peak of expression of the three isoforms of H11 and components of the H45 complex. Expression rates then declined slowly returning to the level seen in L4s.

Conclusions: The absorptive surface of the parasite intestine with associated membrane ecto-enzymes is fully established prior to sexual maturity, turnover is then minimal as presumably protein synthesis switches to production of eggs and spermatozoa.

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H15 p

INHERITANCE OF GENETIC CHARACTERS IN TRICHINELLA SPIRALIS

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Background: The development of PCR technologies and the recognition of a polymorphism in the GT microsatellite of the expansion segment V (ESV) locus of *Trichinella spiralis* made possible to study the transmission of genetic characters along the generations.

Method: Mating experiments were carried out in mice using single larvae of 2 *T. spiralis* isolates (codes ISS297 and ISS328), which are homozygous for the presence or absence of a GT repeat (GT⁺, GT⁻) in the GT microsatellite of the ESV. The study of the genotypic constitution of the ESV of parents, F1 and F2 generations, in both males and females, was done by sequence and heteroduplex analyses of PCR products. The allelic and genotypic frequencies were evaluated by the Fis index.

Results: The sequence analyses showed that all larvae of the isolate ISS297 and those of the isolate ISS328 were GT⁺ and GT⁻, respectively. The analysis of heteroduplex structures of the F1 and F2 generations showed a percentage of homozygotes and heterozygotes as expected for a character inherited according to Mendel's laws. The expected and observed genotypic frequencies of F2 (Fis= +0.02; P= 0.58) showed no deviation from the Hardy-Weinberg expectations.

Conclusion: The results show that the inheritance of the GT repeat character in *T. spiralis* is in agreement with the Mendel's laws and it seems neither influenced by the reproductive system nor by telogonic gonads. This work opens the possibility to approach the study of the genetic structure of *Trichinella* populations and their microevolutionary mechanisms.

H16 p

TRICHINELLA SPECIES AND THEIR SYSTEMATIC POSITION

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Background: The system of *Trichinella* species according to Pozio et al. (1992) based on biological and other criteria appears to be insufficiently conclusive (Bessonov, 2000). Now Pozio et al. (2000) recommend to use methods of DNA analysis by multiple PCR and PCR-RFLP for the same purpose. It is assumed that five different primers at their simultaneous application provide the unique electrophoretic band of DNA specimens for each species and genotype.

Method: Analysis of references on biology of parasites and formation of species.

Results and conclusion: Although the reliability of PCR-diagnosis of *Trichinella* doesn't cause doubts it is impossible to accept this criterium as practically the sole indication. The comparative examination of amino acid sequences of proteins of different organism groups as well as nucleotide composition of DNA and RNA (genodiagnosis) allow only to supplement the systematic characteristics and to reveal the relations between groups. In order to recognize the species as a valid one it is necessary to have a complex of features: non-crossing with other species, the common morphological signs and types of relations, the common area and others. Only 2 *Trichinella* species remain to be valid: *T. spiralis* and *T. pseudospiralis*. As far as concerned *T. nativa*, *T. nelsoni* and other genotypes they should be considered as the varieties of two mentioned species.

H17 p MORPHOLOGICAL VARIABILITY OF *FASCIOLA HEPATICA* EGGS IN SHEEP

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Background: Variations in size and shape of *Fasciola hepatica* eggs are often discussed according to the host species, but no study investigates the real source of variability: the observed differences may be then artefacts.

Method: The variability is explored within a single host species, the sheep. 1380 *Fasciola* eggs are used, collected from 33 experimentally infected sheep. The influence of different biological or experimental factors is statistically analysed.

Results: The morphological variability of *Fasciola* eggs is considerable, already within a single host species, the sheep. Almost all the variability may be observed from a single fluke. Between flukes, the most influent factor of variability is the infection trial. The age of the flukes is also influent. The season seems to have a little effect. No other influence can be shown with our data.

Conclusion: The high variability of the eggs shed by a single fluke and the great influence of the infection trial seem to overlap the hypothetical influence of the host species. The observed differences between host species can be explained by stochastic effects.

H18 p SURFACE CARBOHYDRATES OF *E. DUODENALIS* IN PHEASANT CHICKS.

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Background: Intestinal lesions in coccidial infections of poultry vary with the *Eimeria* and host species. We hypothesise that the coccidial attachment and penetration to specific mucosal regions is based on the interaction of carbohydrates and lectins on the parasites and intestinal epithelium.

Method: We examined the carbohydrate expression of the colonizing *Eimeria duodenalis* during the developmental stages using lectin probes. We detected the carbohydrate residues on the developing parasitic forms *in situ*. Serial sections of paraffin embedded duodenal epithelium of control and *E. duodenalis* (5k/animal) infected pheasant chicks were examined 1 and 6 days post infection with biotinylated lectins listed with abbreviations and target carbohydrates: Dolichos biflorus (DBA), alpha-D-galNAc; Ulex europeus I (UEA), alpha-L-fuc; Griffonia simplicifolia I.B₄ (GSA) alpha-D-Gal; Arachis hypogaea (PNA) beta-gal-D-galNAc; Lens culinaris (LCA), alpha-man and Maackia amurensis II (MAL), alpha-sialyl. Lectin binding to carbohydrates *in situ*, was detected by Avidin-Biotin, Vector Kit[®].

Results: There was a marked divergence of binding with individual lectins to *E. duodenalis* at days 1 and 6 p.i. Glycocalyx of the intraepithelial stages of the *Eimeria* stained only with, GSA, DBA and UEA lectins.

Conclusions: We conclude that the parasite-host interaction in coccidiosis is associated with qualitatively altered expression of carbohydrates on the *Eimeria* surface. This developmentally defined glycocalyx alteration is characterised by an increase in the expression of terminal, alpha-D-Gal, alpha-D-galNAc and alpha-L-fuc residues. Further quantitative exploration of these findings is being currently undertaken.

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H19 p ANTIGENIC CHARACTERIZATION OF A BRAZILIAN ISOLATE OF *Anaplasma marginale* WITH APPENDAGE

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Background: Bovine anaplasmosis causes progressive anemia and considerable economic losses in tropical and subtropical regions. The presence of appendage in *Anaplasma marginale* isolates has been associated to some biological aspects, particularly to tick transmission and to the host immune response. This paper describes the antigenic characterization of a Brazilian *A. marginale* isolate with appendage.

Method: A panel of monoclonal antibodies was produced and used to characterize two Brazilian isolates (one with appendage) through IFAT, ELISA, Western blotting and immunohistochemistry.

Results: A 66 kDa was present in both isolates, while a 45 kDa band was identified only in the isolate with appendage. Immunolabelling confirmed its specificity to the appendage.

Conclusion: Antigenic variation occurs among Brazilian *A. marginale* isolates and a 45 kDa band is appendage-specific. This antigenic diversity may play an important role in immunodiagnostic and immunoprophylactic programs.

H20 p SEASONAL DYNAMICS OF THE SKIN TREMATODE *COLLYRICLUM FABA* IN WILD BIRDS IN A CARPATHIAN LOCALITY

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Background: The skin trematode *Collyriclum faba* (Bremser in Schmalz 1831) has been recorded in a wide range of bird species in Eurasia and both North and South Americas.

In 1996-2000, we found a few endemic foci of this trematode in wild birds in the Carpathians in the Slovak Republic and one endemic focus in a part of the Carpathians in the Czech Republic. The aim of the present study was to characterize seasonal dynamics of *C. faba* in an endemic locality.

Methods: In 2000, we carried out the non-invasive parasitological study of wild birds in the Bílé Karpaty Mountains (south-eastern part of the Czech Republic). In one-month intervals, we captured birds during three-day period from April to October. We mist-netted and examined 345 birds belonging to 30 species.

Results: The skin capsules with *C. faba* were found in *Erithacus rubecula* (14 positive/75 tested), *Fringilla coelebs* (2/8), and *Turdus merula* (1/25). The prevalences of *C. faba* in individual three-day periods per month were 0 % (0/60), 2 % (1/51), 8 % (4/51), 12 % (7/57), 7 % (5/68), and 0 % (0/58) in the end of April, end of May, end of Juni, end of July, beginning of September (this sampling was done in 1999), and end of September, respectively. When taking into account only bird species infected, the prevalences were 0 % (0/14), 11 % (1/9), 22 % (4/18), 58 % (7/12), 19 % (5/17), and 0 % (0/28), respectively.

Conclusion: The trematode *C. faba* occurred in the study locality from May to September with the highest prevalence in July. The seasonal dynamics of the adult parasite probably coincides with dynamics of the still unknown intermediate host of this trematode.

H21 p DISTRIBUTION OF PARASITIC NEMATODES IN THE INTESTINE OF THE SHEEP

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The problems of distribution and location of parasitic helminths in the alimentary tract of hosts is one of the most interesting subjects in parasitology. That's why studying the epidemiology of the sheep gastrointestinal strongylatosis in different landscapes of Armenia we touched this subject too.

In this work we present the data on distribution of some parasitic nematodes (Nematoda: Strongyloidea) in the intestine of sheep. The small and large intestines were removed from 233 sheep. Duodenum (d.), jejunum (j.), ileum (i.), caecum (c.), sectors of colon - ansa proximalis (a.p.), ansa spiralis (a.s.), ansa distalis - (a.d.), and rectum (r.) were cut off immediately and examined separately. The results of investigations are summarized in the following table. Our results confirmed that the general site location of Chabertia ovina is intestine colon, rarely - in the intestine caecum where only single specimens were found. The jejunum and ileum intestines also proved to be the sites of location for Bunostomum trigonocephalum. Yet our investigation showed that Oesophagostomum venulozum mostly locate in the intestine colon.

Table

Species	Prevalence (%) in the sectors of small and large intestines							
	d.	j.	i.	c.	colon			r.
					a.p.	a.s.	a.d.	
Ch.ovina	-	-	-	19	21	100	21	-
B.trigonocephalum	-	81	100	-	-	-	-	-
O.venulozum	-	-	-	27	73	-	-	-

H23 p THE RELATIONSHIP BETWEEN DEVELOPING EGGS AND UTERINE WALL IN CESTODES

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Background: Little attention has been paid to a fine structure of the relationship between developing eggs and uterine wall in Platyhelminthes.

Method: Adult worms *Proteocephalus thymalli* and *Bothriocephalus acheilognathi* were removed from the intestine of the fish *Coregonus lavaretus* and *Gobio gobio cynocephalus*, were fixed in 2,5% glutaraldehyde in 0,1M sodium cacodylate buffer pH 7,4, postfixed in 1% OsO₄ and routinely processed for TEM examination.

Results: The thin outer envelopes of eggs contact with each other and with an uterine wall and form reticular syncytium in *P. thymalli*. The large cytoplasmic outgrowths and numerous lamellae of uterine epithelium are believed to intimately contact with the egg-shell in *B. acheilognathi*. The developing shell in *B. acheilognathi* and outer envelope in *P. thymalli* get in touch with the uterus forming a contact on any part of the egg surface and not prevail over any pole of an embryo. This interlace takes place until egg-shell becomes sclerotised and hardened and the eggs become unfixed and are loosely located in an uterine lumen. Such relationships are associated with active trophic interaction between the parasite organism and its maturing eggs.

Conclusions: The relationship between oligolecital eggs and uterine epithelium as in *P. thymalli* exist for a long period because in this case a delicate egg envelope doesn't prevent the trophic exchange. The hard egg-shell in polylecital eggs makes difficult a trophic interaction as in *B. acheilognathi*.

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H22 p SETTLING OF HAEMONCHUS CONTORTUS IN THE ABOMASUM OF THE SHEEP

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To study the topographical distribution of *Haemonchus contortus* in abomasum, five 7-months-old male lambs from mountainous -forest landscape zone were killed in September. The abomasums were removed immediately and separated from omasums and duodenums. Then each abomasum was snipped along the curvatura major, the content was emptied in the cup and examined separately. The abomasum was stretched on a special device and worms recovered from the three glandular zones: gl. cardiace (I)- light, pink; gl. gastricae propriae (II) - reddish, with plicae spirales; and gl. pyloricae (III)- yellowish with small plicae spirales and strong muscles. All the lambs were infected by *Haemonchus contortus*. They were not found in the contents of the abomasums. The found worms lay on the mucous membrane of the abomasum and were counted and classified as male (m.) and female (f.). The following table shows the number of *Haemonchus contortus* recovered from the three glandular zones of the abomasum.

Table

No. of lambs	number of H. contortus found in the abomasum			number of H. contortus found in the diverse glandular zones					
	Total	Male	Female	I		II		III	
				m.	f.	m.	f.	m.	f.
1	203	148 (72,9%)	55 (27,1%)	16	6	116	41	16	6
2	173	87 (50,3%)	86 (49,7%)	11	12	73	74	3	-
3	431	169 (39,2%)	262 (60,8%)	-	95	147	154	22	13
4	103	84 (81,6%)	19 (18,4%)	5	5	71	5	8	9
5	126	70 (56,6%)	56 (44,4%)	2	7	62	44	6	5

H24 p MORPHOLOGY OF LARVAL STAGES OF ANCYLOSTOMA TUBAEFORME AND TOXOCARA CATI

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Few reports concerning the morphology of developmental stages of the hookworms of cats are currently available. Most of these reports are based on stages isolated from naturally infected animals. We assessed the morphology and developmental composition of two cat nematode species. Artificial infestations of cats with *A. tubaeforme* (n=16) and with *T. cati* (n=16) were established using ~300 infective stages of each parasite. Eight animals each within the *A. tubaeforme* group were necropsied at 12 and 16 days post infection (d.p.i.) while those in the *T. cati* group were necropsied at 19 and 29 d.p.i. Following washing of the gastrointestinal tracts, small intestines, suspended by wire, were soaked in 0.9% saline at 37oC for approximately three hours to allow migration and subsequent sedimentation of mucosal parasite stages. Additionally, the mucosal membrane from the small intestine of the *A. tubaeforme* - infected cats was subjected to pepsin digestion. At 12 d.p.i., the number of L3, L4 and immature adult *A. tubaeforme* stages recovered per animal was 14.75, 40.13 and 4.00 respectively, while at 16 d.p.i., the number of each stage recovered was 6.38, 51.25 and 7.63 respectively. No adult stages were recovered. Within the *T. cati* group, the numbers of L4 and immature adult stages recovered per animal at 19 and 29 d.p.i. were 7.25 and 0.00 respectively and 9.50 and 5.88 respectively. A description of the morphological characteristics of each stage of *A. tubaeforme* and *T. cati* recovered will be presented.

H25 p A NEW *TOXOCARA* SPECIES OF CATS

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Background: A nematode identified as *Toxocara canis* was reported in 11% of Malaysian domestic cats (Lee *et al.*, Trop.Biomed. 1993, 10:79-80). Further studies were initiated as *T. canis* rarely occurs in cats elsewhere. A comparison of aligned rDNA ITS-2 sequences showed only 89 and 76% homology, respectively, with *T. cati* and *T. canis* whereas within-species variation for collections of these species from different geographical regions was 0.3 and 0%, respectively, suggesting that the Malaysian worm represents a separate species (Zhu *et al.*, Parasitology 1998, 117:155-164).

Method: The material used for molecular studies was augmented with further specimens from Kuala Lumpur and Ipoh and used for detailed morphological comparison with related species.

Results: A number of consistent features were identified which allow this worm to be differentiated from the nearest species occurring in domesticated and wild hosts. Although superficially similar to *T. canis*, the Malaysian worm differs with regard to lip structure, shape of cervical alae in cross-section, and spicule length (Gibbons *et al.*, J. Parasitol. 2001: in press).

Conclusion: Molecular and classical biosystematic studies have converged to confirm the validity of this new species.

H27 p DYNAMIC OF EGG EXCRETION IN ARCTIC FOXES (*ALOPEX LAGOPUS*) AFTER SINGLE INFECTION WITH *TOXOCARA CANIS* EGGS.

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Background: A comparative study on the dynamic of *Toxocara canis* faecal egg counts (EPG) in arctic foxes infected with different single dose was conducted for 4 months.

Method: Six cubs aged 6 weeks were infected with 30, 100, 300, 1,000, 3,000, and 20,000 *T. canis* eggs, respectively. EPG counts were carried out daily until the end of excretion of egg.

Result: The lowest dose (30 eggs) and the highest dose (20,000 eggs) foxes did not develop into patent infections. Initial appearance of eggs in the faeces of the remaining foxes occurred 35 - 38 days post infection (dpi), the patent period varied significantly between 8 and 72 days, and the maximal EPG were ranged from 980 - 5700 (39 - 85 dpi). **Conclusion:** These results showed that *T. canis* adult worms established well in 6 weeks cubs in the infected dose around 300-1000 eggs.

H26 p VERTICAL TRANSMISSION OF *TOXOCARA CATI* IN THE CAT

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Nine queens were used for the investigation of vertical transmission of *T. cati*. In experiment I five queens with a natural acquired and coproscopically confirmed infection were mated in the patency period. The parasitological examination of the litters included pepsin digestion of tissue, half of the kittens of each litter was examined immediately after birth whereas the second half was examined after a suckling period of 21 days maximum. Half of the queens of experiment I was examined including the mammary glands.

In experiment II four pregnant queens were infected experimentally from day 15 to 10 prior to birth with a daily infection dose of 1,000 embryonated eggs until birth. Patency was confirmed coproscopically and the tissue of the offspring was examined for somatic larvae between day 1 and day 22 post partum.

In experiment I one single larva was found in one kitten on day 21 p. p. In experiment II between 1 and 333 L3 were found. Only acute infections during the last phase of pregnancy lead to a lactogenic transmission. Only if migrating larvae during the early phase of infection are met by hormonal condition during the last third of pregnancy larvae migrate to mammary glands and are certainly transmitted via milk. Intrauterin infections do not occur. Apparently there is no reservoir of arrested larvae from chronic infections.

H28 p DEEP-ETCHED VIEW OF THE SHEATH AND CUTICLE OF MICROFILARIAE OF *Ltomosoides chagasfilhoi* (NEMATODA: FILARIOIDEA).

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Introduction: The surface of parasites is of great importance since it interacts directly with body fluids and tissues of the host. The present study of *L. chagasfilhoi* (Moraes Neto, Lanfredi & De Souza, 1997, *Parasitol. Res.*, 83: 137-143), is the first using the deep-etching technique for microfilariae.

Method: The microfilariae were fixed with 2.5% glutaraldehyde + 4% paraformaldehyde in 0.1M cacodylate buffer (pH 7.2) and 5mM CaCl₂. For TEM the samples were prepared following De Souza *et al.*, 1989. (*J. Submicrosc. Cytol. Pathol.*, 21: 121-129). For deep-etching technique, the fixed microfilariae were rinsed in distilled water, slam frozen on a copper block, freeze-fractured and deep-etched in a Balzer's apparatus, and rotatory replicated with platinum and carbon. The replicas and thin sections were observed in a Zeiss 900 electron microscope.

Results: In thin sections the sheath (70 nm thick) presents three layers: one inner, moderately electron-dense that is in contact with the space that surrounds the epicuticle; one intermediate electron-lucent and one outer and homogeneously dense layer that is in contact with the space where other neighbouring microfilariae is observed. The cuticle is narrow (70 nm thick) and composed of two regions: trilaminated epicuticle and inner cuticle, that is homogeneous and is in contact with the hypodermis. In deep-etching, the sheath (68 nm thick) presents two layers: the inner composed of tightly arranged globular material (mean diameter: 15 nm) and the outer that is slightly smooth. The cuticle (64 nm thick) presents two regions: the trilaminated epicuticle (20 nm thick), with particles of 15 nm and the inner cuticle (44 nm thick), composed by two parallel rows of globular structures of 22 nm.

Conclusions: The cuticle is formed by a network of globular elements that give this structure a certain rigidity compatible with the supposition that the cuticle is an extracellular matrix. The empty spaces among the components are filled by an aqueous material *in vivo* which was sublimated during the etching procedure. This aqueous material is probably associated with the diffusion of substances across the cuticle.

Acknowledgments: PRONEX, FINEP, CNPq, FUIB/UFRJ and FAPERJ.

H29 p EFFECTS OF IVERMECTIN AND *DIROFILARIA IMMITIS* ON *AEDES AEGYPTI*

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Background: Ivermectin is widely used in dogs as a preventative for infection with *Dirofilaria immitis*. It is known to have an effect on blood feeding arthropods. A study was conducted to determine if blood from a dog treated with a prophylactic dosage of ivermectin had an effect on *Aedes aegypti* mosquitoes and larval *D. immitis*.

Method: Blood collected from a dog before and 5.5 hours after oral dosing with 10 mcg/kg of ivermectin was fed to *A. aegypti* 7 days after a blood meal with uninfected dog blood or dog blood containing microfilariae of *D. immitis*. Observations were made on mosquito survival and fecundity.

Surviving mosquitoes were dissected two weeks after a blood meal containing *D. immitis* microfilariae and L₃ were recovered and counted.

Results: The survival and fecundity of mosquitoes that were fed blood from a dog treated with ivermectin were similar to mosquitoes fed blood collected prior to treatment from the same dog. In contrast, mosquitoes that were fed an initial blood meal containing microfilariae of *D. immitis* had a lower survival rate and produced 15% fewer eggs than mosquitoes fed uninfected blood. The recovery of *D. immitis* L₃ from mosquitoes fed pre-treatment blood, ivermectin post-treatment blood, or no second blood meal was 7.1, 12.1, and 10.6, respectively.

Conclusion: The survival and fecundity of *A. aegypti* and the development of larval *D. immitis* were apparently unaffected by a second blood meal 7 days after infection with blood from a dog given a standard heartworm preventative dosage of ivermectin. Infection of *A. aegypti* with *D. immitis* did reduce the survival and fecundity of the mosquitoes.

H31 p A NEWLY DISCOVERED MITE IN THE RABBIT: *PSOROBIA LAGOMORPHAEN. SUBSP.*

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Introduction : Although commonly used as a laboratory, food or companion animal since centuries the rabbit is the host of yet unknown parasites.

Case report : A young dwarf rabbit 6 month old was presented for the development of skin lesions of 2 weeks duration, located on the neck. They were characterized by a diffuse alopecia with slight erythema without any visible inflammatory reaction or secondary lesions. There was no human contamination. Multiple adhesive tape tests and trichograms were negative. Multiple skin scrapings allowed the observation of only five original mites. Histopathology was not informative. The general morphology of the parasite allowed the identification of the genus *Psorobia*. Notable differences with the already described but rarely observed *Psorobia lagomorphae* support the proposition of a new subspecies. A first attempt of treatment was the application of Pyriproxyfen 10% spot on. Although mites were no longer found, no clinical improvement was obtained after 3 weeks. Subsequently, an injection of ivermectin was made. Progressive regression and cure was obtained without relapse after 3 months.

Conclusion : Psorergatidae are a group of parasites closely related to Demodex and Psorobiosis is a new ectoparasitosis to take in account in the differential of alopecia and pruritus in rabbit.

H30 p STRAENLENSIOSIS (*STRAELENIA CYNOTIS*) A NEW AND SEVERE PARASITOSIS IN DOGS.

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Introduction : Common parasitic skin diseases in the dog are considered well documented. The increasing use of skin biopsies gives the opportunity to describe new aspects of parasitic disease. We present here an original clinical manifestation associated to a new specific parasite in dogs.

Material and methods : Over a period of 5 years, biopsies were collected from 22 dogs, most of these living in rural areas in the south of France. The dogs suffered from a chronic, painful extensive to generalized dermatitis, with multiple papules crusts and suppurations. Dogs were rapidly prostrated emaciated and anorectic. No parasites were found in skin scrapings and treatments (antibiotics, corticosteroids, antiparasitic) were not efficient. On biopsies a unique histopathological pattern was observed showing a "cystic" reaction of follicular origin centered on parasitic structures. The cavity of the cysts contained systematically one parasitic element not damaged by the host reaction. The parasites had an average size of 213 X 486 µm. and elements for identification in sections conducted to identify a trombiculid mite. Parasites were finally obtained after dissection of biopsies in alcohol. The parasite were identified as the larval stage of a new species : *Straelensia cynotis* (Lecanichneumonidae).

Discussion: These cases of intrafollicular parasitism with cystic reaction differ from the traditional knowledge on trombiculids. The disease appeared from february to september, widely apart from the usual autumnal activity of these acarines. Epidemiological survey suggests that foxes could be the natural reservoir. The histopathological pattern seems specific. This new ectoparasitosis from dogs described in France illustrates an original development of a new parasite in this host.

H32 p INCREASED ATTACK FREQUENCY OF *SIMULIUM SP.* IN SUBURBAN AREA OF WARSAW, POLAND

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Background: Blackflies have world-wide distribution and are vectors of human onchocercosis. Blood sucking females of blackflies attack cattle causing significant economic losses. Although the attacks usually occur near breeding grounds, the flies are thought to be capable of travelling for distances of up to 6 km.

In this report, we describe severe attacks on humans in suburban areas near Warsaw, Poland.

Methods: Eleven patients with dermal changes, 20-58 years old, were assessed clinically, some also histopathologically. Causative organisms were collected from legs of the patients and from grass in the vicinity, and identified as *Simulium sp.*

Results: All patients presented with dermal symptoms, at various intensity. In sites of bites, intense pruritus, swellings, extensive irregular erythematovesicular changes accompanied with pain occurred. Inflammation, haemorrhagic effusions and fever developed in 3 persons, in whom cutaneous lesions remained for more than 2 months. Most of the patients required antiseptic and anti-allergic treatment.

Conclusion: It is likely that environmental factors may be responsible for increased occurrence of blackflies in a vicinity of human habitation resulting in serious medical consequences.

H33 p COMPARISON OF JUMP PERFORMANCES OF *Ctenocephalides canis* AND *C. felis*

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Jump performances of *Ctenocephalides canis* and *Ctenocephalides felis* have been measured and compared on unfed young imagos. Mean length of *C. felis felis* jump was 19.9 ± 9.1 cm, minimal jump was 2 cm, the longest one was 48 cm. *C. canis* jump was significantly longer (30.4 ± 9.1 cm; from 3 to 50 cm). For height jump evaluation, grey plastic cylindric tubes measuring 9 cm in diameter were used. Their height was increasing from 1 to 30 cm by 1 cm. Groups of ten fleas of the same species were deposited on the base of the tube. The number of fleas which succeeded in jumping above the tube was recorded. The mean height jump carried out by 50% of fleas was calculated after linearisation of the curves: it was 15.5 and 13.2 cm for *C. canis* and *C. felis* respectively. The highest jump was 25 cm for *C. canis* and 17 cm for *C. felis*.

H34 p CHARACTERIZATION OF THROMBOSTASIN: A NOVEL ANTITHROMBIN PROTEIN IN THE SALIVA OF HORN FLIES

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Background: Horn flies (*Haematobia irritans*) are blood-sucking ectoparasites of cattle that produce significant economic impact on livestock production. In early studies aimed at understanding the nature of horn fly blood-feeding, we identified anticoagulant activity in fly saliva that was shown to be due to a thrombin-inhibitory protein.

Method: Saliva from field-collected flies was used for HPLC purification of the horn fly anti-thrombin protein and for further characterization of its structural properties, including molecular mass and N-terminal sequence. Degenerate primers, designed from N-terminal amino acid sequence, were used to amplify a corresponding nucleic acid sequence from salivary gland cDNA.

Results: Mass spectroscopy analysis revealed at least 3 major isoforms ranging in mass from 9,213 to 9,274 Da. Conceptual translation of the 243-bp coding sequence amplified from cDNA revealed a novel 81 amino acid peptide (named Thrombostasin, "TS") whose first 30 amino acids matched that of the N-terminal protein sequence and had a mass of 9,213 Da. The full-length TS gene was generated by 5' RACE and sequential PCR amplification. It contained a 5'-end 12-bp segment preceding the putative ATG start codon, followed by a 54-bp sequence corresponding to a secretory signal and an additional 228-bp coding sequence preceding residues revealed by N-terminal sequencing of purified TS. Fidelity of the PCR-generated TS sequence was confirmed in genomic DNA and by biological activity of recombinant TS produced in a baculovirus expression system.

Conclusion: Because of the paucity of other anti-hemostatic factors in horn fly saliva, TS may play a critical role in maintenance of the ectoparasitic lifestyle of horn flies.

Acknowledgement: This work was supported by USDA/NRICGP Award #9601791 and #9802578

H35 p *In vitro* DEVELOPMENT BEHAVIOUR OF UNTREATED *Boophilus microplus* (Can.) IN ARGENTINA.

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Background: The efficacy evaluation of formulations for tick control, requires knowing the normal development behaviour of *B. microplus*, under no product pressure. Compared with the results reached with treated tick, the data will permit an evaluation of the effect and efficacy of the formulation under study.

Method & Results: The total number of adult females detached during 24 days from 80 stabled donor cattle were incubated in quantities of up to 20 per animal/day at 28°C and 80% RH, determining the following parameters: total weight of the aliquot; number of surviving tick on day 10; total weight of egg production to day 18; and from the uncubation of up to 1 g of eggs/animal/day, the total weight of larvae on day 14 post-hatching. Total weight of the aliquot was 11.375,2 g; 33.604 ticks survived on day 10; 1976,53 g of eggs were incubated, producing 1029,9 g of viable larvae.

Conclusion: The analysis of this information permits knowing *in vitro* development behaviour, under climatic conditions in the endemic tick area of north Argentina, of *B. microplus* considered normal and with a known chemo-susceptibility. This is specially important for the correct evaluation of acaricides applied by methods other than in immersion dips.

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I

Molecular and biochemical parasitology

11

A MALE-SPECIFIC SER/THR PHOSPHATASE FROM *OESOPHAGOSTOMUM DENTATUM*

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Background: There is currently little information on the molecular biology of sexual reproduction in parasitic nematodes. We therefore used RAP-PCR to isolate genes expressed in a gender-specific manner in *O. dentatum*, and identified a male-specific Ser/Thr phosphatase (*Od-mpp1*).

Methods: A male *O. dentatum* cDNA library was screened with the partial *Od-mpp1* sequence that was isolated using RAP-PCR. Antiserum raised to baculovirus-expressed recombinant Od-MPP1 was used on Western blots of parasite extracts. To study the function of *C. elegans* homologs, RNA interference (RNAi) experiments and localisation of expression using promoter-GFP constructs were conducted.

Results: The 311 amino acid protein predicted from the full-length cDNA sequence has similarity to the "class one" phosphatases (PP1), and greatest identity (92%) to two predicted *C. elegans* proteins, W09C3.6 and T03F1.5. PP1s are involved in regulation of many cellular functions. Western blots confirmed expression of a 35 kDa protein in adult male *O. dentatum*. RNAi of the *C. elegans* homologues resulted in a reduction in F1 progeny. Expression was localised using the reporter gene GFP, with T03F1.5 expressed only in male *C. elegans*.

Conclusions: The Od-MPP1 protein may be involved in regulation of male reproduction in *O. dentatum*.

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13

THREE ISOFORMS OF THE *HAEMONCHUS CONTORTUS* PROTECTIVE ANTIGEN H11

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Background: H11 is a highly protective integral membrane protein from the gut microvilli of parasitic *H. contortus*, predicted by sequence of a cDNA clone to be a microsomal aminopeptidase. Sequences of partial clones selected by immunoscreening of a cDNA library indicated the presence of more than one isoform of H11.

Methods: Full-length cDNA clones were isolated by a combination of screening and PCR, and the sequences compared with the published H11 sequence (now designated H11-3). Developmental regulation and localisation of expression were analysed by Northern blotting and *in situ* hybridisation respectively.

Results: The predicted amino acid sequences of three isoforms, H11-1, -2 and -3, had ~60% identity. All had a predicted N-terminal signal sequence and a conserved HEXXHXXW zinc-binding site. H11-2 was expressed at the highest levels, followed by H11-1 and H11-3. Specific mRNAs were first detected in early L4 larvae and reached highest levels in young immature adults, before declining with sexual maturity. In all cases, mRNA expression was localised to the intestine of the adult worm.

Conclusions: The presence of at least 3 isoforms of H11 with significant differences in sequence suggests that a recombinant vaccine may need to include some combination of these different proteins.

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12

IS *CAENORHABDITIS ELEGANS* A GOOD MODEL FOR TRICHOSTRONGYLID NEMATODE DEVELOPMENT?

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Caenorhabditis elegans is a free-living nematode used as a model organism to study many aspects of cell and developmental biology. The completed genome sequence and wide range of molecular and genetic tools to study this organism has led to *C. elegans* being one of the best understood of all organisms. However the extent to which the basic biology of this organism is conserved across the nematode phylum, and hence its relevance to parasites, is unknown. We are addressing this question by looking at the degree to which molecules that regulate *C. elegans* hypodermal and gut development are functionally conserved in *H. contortus*. Several members of the *C. elegans* GATA transcription factor family play key roles in hypodermal and gut development. For example, *elt-1* and *elt-2* are necessary for normal hypodermal and gut development respectively and are also sufficient to activate the respective programs of differentiation. We are now attempting to identify *H. contortus* orthologues of these genes using a strategy of PCR with degenerate primers. We have identified a *H. contortus* orthologue of the GATA factor *elt-2*, a gene essential for *C. elegans* gut development. The DNA binding domain is highly conserved, as well as a second "pseudo" zinc finger, but the rest of the polypeptide is very divergent. We are determining whether the *Haemonchus* gene is functionally conserved with *C. elegans* by assaying its ability to activate downstream target genes and rescue the *C. elegans* mutant phenotype in transgenic *C. elegans*.

14

CHANGE OF HOST INDUCES CHANGES IN THE NERVOUS SYSTEM OF PARASITIC WORMS

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Objectives: To study the changes taking place in the neuroendocrine system (NS) of *Diphyllbothrium dendriticum*, during the transfer of the larva from the cyst on the stomach wall of the whitefish to the gut of the final host, the seagull.

Status in fish. The temperature is 5-15°C. The larva is immobile, its attachment organs are inactive and it grows very slowly.

Status in bird. The temperature is 37-38°C. In the seagull the larva is freed from the cyst. It begins to move actively, attaches to the gut wall and begins to grow very actively.

Methods. Spectrofluorometry, NADPH-diaphorase (NADPHd) histochemistry, immunocytochemistry.

Generally. The neuroendocrine system is activated promptly.

The aminergic NS. In larvae, the concentration of 5-HT is about 0.1 µg/g tissue. In adult worms, the concentration of 5-HT is 0.5 µg/g tissue.

The nitrergic NS. In larvae, the NADPH-d reaction is weak. In adult worms, the NADPH-d reaction is strong and associated to muscle fibres.

The peptidergic NS. In larvae, the paraldehyde fuchsin (PAF) reaction is weak. Already one hour after transfer to the final host, the PAF reaction is very strong. In larvae, no epidermal growth factor immunoreactive (EGF-IR) neurones occur. Ten hours after transfer to the final host, many EGF-IR neurones, localised close to the actively growing regions, were detected. In larvae, no small cardiac peptide B (SCPb-IR) nerves occur. Thirty hours after transfer to the final host, SCPb-IR nerves, surrounding the musculature of the attachment organs, were detected.

15

ANALYSIS OF STAGE-SPECIFIC MOLECULES OF *HAEMONCHUS CONTORTUS* BY RAP-PCR

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Background: Parasites undergo marked changes as they pass through their developmental stages, particularly in the transition from the free-living to the parasitic stages. However, little is known about the molecular mechanisms underlying these changes.

Methods: mRNA from L3 and adult parasites was used in random arbitrarily-primed PCR (RAP-PCR). Products which differed between the stages (Differential Display) were recovered from acrylamide gels, cloned and sequenced. A selection of adult-specific products were characterised further by Northern blotting and *in situ* localisation.

Results: 32 and 30% of PCR products were specific to L3 or adult parasites, respectively. Of 15 adult products characterised further, 4 were expressed in adults only and 4 were expressed in both stages but with different transcript sizes. Database searches revealed clones sharing identity with vitellogenins, cysteine proteases, ATP synthase and α -tubulin, as well as with *C. elegans* predicted proteins of unknown function and clones with no homologs in the databases. A number of transcripts were shown (by *in situ* hybridisation) to be expressed in the gut, while others were also detected in other organs.

Conclusions: Differential Display is a useful technique for identifying stage-specific molecules. It has the advantage over EST sequencing that many of the house-keeping genes expressed in common are eliminated.

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17

A ROLE FOR NEMATODE CATALASE IN DEFENCE AGAINST HOST OXIDANTS

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Background: The release of oxidants by the respiratory burst of phagocytes is thought to play a role in expulsion of intestinal parasites. The mechanism by which *Haemonchus contortus* defends itself from hydrogen peroxide (H_2O_2) was examined in this study by determining the impact of inhibiting the nematode's catalase in the presence of H_2O_2 *in vitro*.

Methods: The toxicity of H_2O_2 was measured in concentration-response experiments using uptake of [^3H]inulin as an indicator of nematode viability. The effects of the catalase inhibitor aminotriazole (AT) on nematode catalase activities and on the ability of the nematode to survive in environments containing H_2O_2 were determined.

Results: Nematodes showed a dose-dependent susceptibility to the presence of H_2O_2 . Catalase enzyme was readily detectable in nematode enzyme preparations, however, it did not appear to be secreted into the assay medium. Inhibition of nematode catalase with AT led to a 33-fold decrease in the concentration of H_2O_2 required to inhibit feeding by 50%.

Conclusions: *H. contortus* possess significant levels of catalase enzyme. Inhibition of this enzyme increases the susceptibility of the parasite to the toxic effects of exogenous H_2O_2 , indicating a protective role for the enzyme. This suggests that catalase has the potential to play a significant role in allowing the parasite to withstand the respiratory burst of host phagocytes.

16

SMALL SUBUNIT RIBOSOMAL RNA ANALYSIS OF *Dictyocaulus* LUNGWORMS

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Background: Development of genetic markers has opened the possibility for resolving patterns of evolution between parasitic nematodes. The aim of the present study was to characterise the genetic diversity of lungworms of the genus *Dictyocaulus*.

Method: Total DNA was isolated and the small subunit (SSU) rRNA was amplified by PCR. Products were verified and sequenced. The complete SSU rRNA gene sequences were determined for lungworms isolated from cattle, fallow deer, moose, muskoxen, roe deer, red deer, reindeer and sheep. Sequences were aligned and phylogenetic trees were constructed. In these analyses the SSU rRNA gene sequences of other genera such as *Parafilaroides* sp. and *Haemonchus contortus* were included as well.

Results: The sequence differences within the genus *Dictyocaulus* were ranging between 95 and 99%. Preliminary analysis indicated that the members of *Dictyocaulus* were most closely related with the metastrongylid *Parafilaroides*. Maximum likelihood analysis revealed a topology with 5 branches. *D. filaria* of sheep were the most distantly related species, whereas the worms of moose, muskoxen, red deer and reindeer were identical. This is probably one and the same species and equal with *D. eckerti*. It was distinct both from *D. cervi* nov. sp., *D. viviparus* and a novel *Dictyocaulus* species that was found in fallow deer.

Conclusion: This study demonstrated that the genus *Dictyocaulus* is most closely related to the superfamily Metastrongyloidea. Based on analysis of the SSU rRNA sequences the taxonomic position of *Dictyocaulus* within Trichostrongyloidea can be questioned. The genus *Dictyocaulus* also contains 2 novel taxa that have not been morphologically described. Consequently, based on genetic data the species composition appears to be more complex. Moreover, differences that previously have been observed in the ITS2 were reflected in the SSU rRNA gene.

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18

BACULOVIRUS EXPRESSION OF *HAEMONCHUS CONTORTUS* H11 ANTIGENS

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Background: The membrane aminopeptidase H11 is a highly protective antigen from the gut microvilli of parasitic *H. contortus*. Three isoforms (designated H11-1, -2 and -3) have been described. Recombinant expression of H11 is required to develop a commercial vaccine.

Methods: The N-terminal anchor sequence of each H11 cDNA was removed by PCR, prior to cloning into a customised baculovirus vector designed so that the expressed secreted proteins retain a His-tag for purification. Baculovirus-infected insect cells were grown, the recombinant H11 antigens purified, and characterised by SDS-PAGE, Western blotting and aminopeptidase (Ap) enzymatic activity. Sheep were immunised with 3 doses of purified recombinant H11 in Freund's adjuvants, and their ability to resist a challenge infection assessed.

Results: The H11 isoforms were expressed at high, but varying, levels. All 3 proteins were purified to near homogeneity and were cross-reactive with antibodies to native H11. Activity assays showed that H11-1 was an ApA, while H11-2 and -3 were ApMs. Antibodies obtained by injection with recombinant H11 were capable of inhibiting both native and baculovirus-expressed H11 ApA and ApM activity *in vitro*. However, sheep were not protected against *H. contortus* challenge infection.

Conclusions: Expression in insect cells may not produce H11 which is identical to the native protein, despite being enzymatically active.

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19

IVERMECTIN RESISTANCE AND GLUTAMATE-GATED CHLORIDE CHANNELS IN THE CATTLE NEMATODE, *Cooperia oncophora*

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The endectocide ivermectin is believed to act by binding with a glutamate-gated chloride channel (GluCl). Selection for specific alleles of the GluCl genes has been seen in *Haemonchus contortus* and these genes have been implicated in ivermectin resistance in *Caenorhabditis elegans*. Ivermectin resistance in *C. oncophora* is starting to emerge, and we are interested in studying GluCls in this cattle nematode. To this end, we have cloned two full-length GluCl cDNAs from the cattle nematode *C. oncophora*. The first one is termed *Cogbr2* because of its high sequence similarity with the *gbr2* sequences reported in GenBank. To analyze the genetic variability of this gene, single-strand conformation polymorphism (SSCP) was used. Two field isolates of *C. oncophora* were used: one susceptible to ivermectin, and the other resistant to the same drug. One allele had a significantly higher frequency in the resistant isolate compared with the susceptible isolate, and may have been selected for with ivermectin treatment. These results suggest that *gbr2* may be involved in ivermectin resistance in *C. oncophora*. A second full-length cDNA encoding a putative GluCl- β subunit has also been isolated from *C. oncophora*. The predicted protein is 432 amino acids long, and possesses all the characteristics of subunits of the ligand-gated chloride channel superfamily. It bears 90% identity to *H. contortus* GluCl- β , and 76% identity to *C. elegans* GluCl- β .

110

Beta tubulin geno- and isotyping of benzimidazole susceptible cyathostomes

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Beta tubulin is the target of benzimidazole (BZ) anthelmintics. Resistance against benzimidazoles has been described for small strongyles (Cyathostominae) world-wide. Studies on sheep parasites revealed that a single nucleotide polymorphism in codon 200 of isotype 1 of the beta tubulin gene is correlated with BZ-resistance. The characterisation of the beta tubulin gene of the small strongyle species *Cylicocyclus nassatus* elucidated a point mutation in codon 200 which leads to the change of phenylalanine to tyrosine. According to these results an allele-specific PCR has been developed and has been used for the investigation of 100 adults of an anthelmintic naive cyathostome population. The observed allele frequencies and the distribution of the genotypes in codon 200 enlighten the role of this polymorphism as a genetic marker for BZ-resistance.

To elucidate the existence of different beta tubulin isotypes we have investigated the 3' end of the beta tubulin gene of four cyathostome species. cDNA of *Cyathostomum pateratum*, *Cyathostomum coronatum*, *Cyathostomum catinatum* and *C. nassatus* have been applied in a 3'RACE approach using a degenerate forward primer located in a highly conserved region of exon 7. The expected 400 bp PCR product has been observed in all species and an additional band of about 600 bp occurred in three species. Sequencing of all PCR products revealed that the differences of 400 bp attribute to a length polymorphism in the 3' UTR. The alignment of the deduced amino acid sequences of the carboxy-terminus of the cloned PCR products revealed high identities (99-100 %) to the former reported isotype 1. Therefore we conclude that the isotype 1 is the predominant beta tubulin-isotype in the examined cyathostome species.

111

QUANTITATIVE SPECIES SPECIFIC PCR FOR TRICHOSTRONGYLE SPP. FROM SHEEP

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The trichostrongyle parasites from sheep *Haemonchus contortus*, *Ostertagia* spp. and *Trichostrongylus* spp. cause severe illnesses in infected stock. Moreover, the different species or genera are characterized by a different pathogenicity. However, the differentiation by coproscopy of the trichostrongyle eggs and third stage larvae is not sufficiently reliable.

We therefore developed a PCR method for the differentiation of the species and genera mentioned above, based on specific primers and probes derived from the second internal transcribed spacer (ITS-2) ribosomal DNA region. As for *H. contortus* the number of stages shed with the faeces correlates with the worm burden, we established a quantitative PCR. We used 6-carboxyfluorescein (FAM) labeled TaqMan® probes and molecular beacons for PCR. DNA was prepared from first stage and exsheathed third stage larvae isolated from the faeces of experimentally infected sheep. The method reproducibly allowed to identify single larva equivalents and differentiated one homologous larva out of 1000 heterologous larvae. The primer and probes combinations gave comparable results when applied with core reagents from different suppliers and with both Mx4000® and ABI 7700® instruments. Titration of plasmid DNA revealed a sensitivity of 10 copies.

This technique provides the means for the reliable and quantitative detection and differentiation of the most important sheep parasitic nematodes.

112

CLONING OF A *Haemonchus contortus* P-GLYCOPROTEIN GENE (PGP-A) PROMOTER REGION

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ABSTRACT

The involvement of the P-glycoprotein gene in anthelmintic resistance is no longer in doubt. Common anthelmintics like, benzimidazole, ivermectin and closantel, are potential substrates for Pgp. The findings of overexpression of P-glycoprotein gene in anthelmintic resistant parasites has led to the basis of our research which focuses on studying the regulation of expression of this gene. A homologue of Pgp gene, Pgp-A, has been isolated and shown to be overexpressed in anthelmintic resistant *H. contortus*. Since overexpression of genes could be a result of gene amplification or changes at the promoter region of the gene, we are attempting to map out the Pgp-A promoter region in *H. contortus*. A 1 kb fragment from the 5' end of the Pgp-A gene have been amplified from the *H. contortus* genomic DNA using polymerase chain reaction (PCR) and sequenced. These fragments have been analyzed for the presence of promoter sequences and sequence homologues a eukaryotic promoter were identified. This included a putative transcriptional start site and a GC-rich region. A *Haemonchus contortus* genomic DNA library is being screened for a corresponding clone to confirm the location of the promoter region and the transcriptional start site. After a putative promoter have been identified, this fragment will be amplified and cloned into an expression vector, to determine if the region identified is a functional promoter region. Later, variations in the promoter region between anthelmintic resistant and susceptible strains will be analyzed and, mutations and their effect on anthelmintic resistance identified.

MITOCHONDRIAL DNA POLYMORPHISMS IN *COOPERIA ONCOPHORA* POPULATIONS

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Background: Populations of *Cooperia oncophora*, a gastrointestinal parasite of cattle, are subject to immunological pressure from the host. To study this effect on population genetics, genetic markers have to be characterised. Because of the high within population sequence variation of mitochondrial (mtDNA) sequences in trichostrongylids, and the strictly maternal inheritance we used mtDNA regions for the identification of genetic markers.

Method: Adult *C. oncophora* individuals were collected from the intestine of the host, larvae were cultured from the faeces of infected animals. PCR amplifications of small mtDNA regions up to 500 bp were directly sequenced. A long PCR system was used to amplify the complete mt genome. Large fragments were sheared and randomly cloned and sequenced. The scanning for nucleotide variation was initiated with restriction enzymes. From the regions found containing polymorphisms, different individuals were sequenced to identify the mutation.

Results: Variation was found within conserved regions of the mtDNA genome. We identified mtDNA polymorphisms in the cytochrome c oxidase subunit I (COI) gene between individuals, which were used in PCR-RFLP assays.

Conclusion: So far we have an indication that the mtDNA genome of *C. oncophora* has numerous polymorphisms. More will be identified and used to characterize different *C. oncophora* populations.

ANALYSIS OF A FAMILY OF ACTIVATION ASSOCIATED SECRETED PROTEIN (ASP) HOMOLOGUES OF *COOPERIA PUNCTATA*

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Background: cDNAs encoding two homologous activation-associated secreted proteins (ASP) of 24-28kDa (ASP-2) and 40-42kDa (ASP-1, apparently arisen by a duplication event in ASP-2) have been identified previously as possible vaccine candidates in the nematodes *Ancylostoma caninum* and *Haemonchus contortus*.

Methods and Results: We employed oligonucleotides derived from conserved regions to clone ASP-1 and ASP-2 homologues from *Cooperia punctata* by RT-PCR. Here we report the cloning and analysis of cDNA and genomic sequences of *C. punctata* and established the presence of two 75 % identical ASP-1 genes in *C. punctata*. Additional ASP paralogues were shown to be present. Analysis of PCR products amplified from genomic DNA from a pool of worms revealed extensive sequence diversity within this family of protein, reflecting the presence of different ASP paralogues in a single worm as well as extensive polymorphisms in between different worms. ASP proteins contain a conserved region called sperm coating protein (SCP) domain of completely unknown function that is present as a single copy in proteins from yeast and a wide range of multi-cellular organisms. Only in three nematodes a protein composed of duplicated SCP-domains has been identified. *C. punctata* is the first organism in which two of such genes are found. Database searches identified similarity of the C-terminal cysteine-rich domain of ASP proteins to a nematode methallothionein motif. Cp-asp-1b was expressed in *E. coli* and both the N-terminal and C-terminal domain were shown to be recognised by sera of *C. punctata* infected bovines.

Conclusions: 1-) Two 75% identical ASP-1 gene homologues are present in *C. punctata* and there are strong indications for several more ASP gene family homologues. 2-) There is and extensive sequence variation between individual worms. 3-) Recombinant *C. punctata* ASP-1 is recognised by immune sera.

CHARACTERIZATION OF *T. SPIRALIS* NBL CDNA CLONES RECOGNIZED BY SWINE INFECTION SERUM.

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Background: Presently, little is known about the mechanism by which *Trichinella* penetrates and modulates re-programing of muscle cells in the formation of the nurse cell. In light of evidence demonstrating strong protective characteristics of antigens derived from this stage, understanding this process may shed light on potential targets for effective abatement of infection.

Methods: A PCR-derived cDNA library consisting of > 100,000 clones was constructed in lambda gt11 using 1 ug of total RNA from *T. spiralis* new born larvae (NBL). Approximately 30,000 clones were screened immunologically using serum from a pig infected experimentally with 5000 *Trichinella* L1 and positive clones were characterized.

Results: One unique clone was identified that encodes an open reading frame of 930 bp and consists of 30.0 % glutamic acid (93/310) and 15.2% serine (47/310) residues based upon the predicted amino acid sequence. The percentage of glutamic acid is greatest at the C terminus (>40%) and is translated from a series of incomplete nucleic acid repeats. Northern blots indicated that the gene is specifically transcribed in the NBL stage where immunohistochemical staining localized the protein to the stichosome of the NBL. Genomic clones from both *T. spiralis* and *T. pseudospiralis* showed substantial sequence conservation between the disparate species. Preliminary data suggest that the gene is multiply spliced within the NBL stage.

Conclusions: Results demonstrated here, and upon screening the library with sera from rabbits immunized with soluble extracts from NBL, suggest that the cDNA library contains sequences unique to NBL and is of sufficient quality to initiate molecular studies on this all important stage of *Trichinella*.

GENETIC VARIATION IN POPULATIONS OF *PROTEOCEPHALUS LONGICOLLIS*

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Background: Genetic variability in the economically important salmonoid fish parasite *Proteocephalus longicollis* (Zeder, 1800) (syn. *P. exiguus* La Rue, 1911) has been surveyed in populations from Palearctic.

Method: Starch gel electrophoresis was used to examine 12 enzyme systems in populations collected from Slovak water reservoirs Dobšiná Východná (host - *Oncorhynchus mykiss*) and lakes Bienne and Geneva in Switzerland (host - *Coregonus lavaretus*).

Results: Striking differences between those of Slovak and Swiss populations were recorded at *Got* and *Pgm-2* loci, in possessing unique alleles in both groups. In addition, Slovak populations substantially differed in the allelic frequencies at *Got* locus. Genetic drift accelerated by the higher degree of inbreeding might be responsible for this geographical variation. On the other hand, a low genetic variation was observed between parasites from Swiss water bodies. Hardy-Weinberg condition valid for randomly mating population was met in Dobšiná population at *Pgm-2* and Swiss populations at *Pgm-2* and *Got* ($P > 0.05$). This pointed out for the extensive exchange of genetic material in these hermaphrodites.

Conclusion: Substantial polymorphism found in *P. longicollis* suggests the marked ecological tolerance of this cestode, being adapted on more fish hosts. A higher degree of the genetic differentiation in Slovak populations was probably due to the artificial barriers of the movement of rainbow trouts in Dobšiná, thus causing disturbances in the continuous gene flow across all locality.

117

IDENTIFICATION OF A NOVEL FLEA CONTROL TARGET ENZYME, ALLANTOINASE

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Background: In an attempt to identify novel targets for immunological or pharmaceutical control of the cat flea, *Ctenocephalides felis*, cDNA libraries representing differentially expressed tissue-specific mRNA were constructed for expressed sequence tag (EST) sequencing.

Methods: mRNA isolated from 20,000 dissected hindguts and Malpighian tubules (HMT) from unfed and blood-fed adult male and female fleas was used to generate a library enriched in genes encoding proteins involved in osmoregulation, excretion, and nutrient reabsorption. mRNA isolated from the remaining carcasses was used in the suppressive subtractive hybridization PCR to effect tissue-specific enrichment.

Results: Approximately 14% of the EST sequences obtained from the subtracted HMT library encoded an enzyme involved in purine degradation, allantoinase. There is no mammalian homologue of allantoinase, as this enzyme has been lost in higher organisms over evolution. A cDNA encoding a full-length flea allantoinase was obtained from an unsubtracted HMT cDNA library. Northern blots showed that the allantoinase transcript was HMT tissue specific and was differentially expressed in only feeding and excreting stages. Native allantoinase was localized immunohistochemically in the hindgut and Malpighian tubule cells using affinity-purified antibodies generated against expressed recombinant allantoinase. An activity assay using crude flea extracts was optimized and used to evaluate the inhibitory activity of a small number of substrate analogs.

Conclusions: EST sequencing of a flea HMT subtracted library identified a potential new and novel target for flea control.

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118

EXPRESSED SEQUENCE TAG ANALYSIS OF AN *SARCOPTES SCABIEI* cDNA LIBRARY

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Background: The parasite *Sarcoptes scabiei* cause scabies in humans and mange in animals, a disease, which inflicts suffering in the hosts as well as financial losses in e.g. swine stocks. Despite the importance of *S. scabiei* as a pathogen very little is known about the organism's genetic background and the factors that contribute to its virulence. In order to gain better understanding about the mite and the mechanisms underlying the infection process, an expressed sequence tag (EST) project was launched.

Method: The ESTs were generated from an *S. scabiei* cDNA library constructed in our laboratory. We have been using a PCR based approach to generate the ESTs. Only PCR products with a size of 500bp or larger were used as templates for the sequencing reactions. Sequence data from each EST clone was edited and compared to public DNA databases.

Results: After sequencing and editing about 75% of the total number of ESTs contained data suitable for DNA database comparison. Significant or informative database matches has been identified for about 50% of the total number of ESTs. Most of the ESTs are homologues to *Drosophila melanogaster* proteins. Examples of identified ESTs of interest from the cDNA library are those matching a cuticle protein expressed on the surface of a spider (*Araneus diadematus*) and proteases from both dust mites and ticks.

Conclusion: We have shown that the sequence data from the ESTs can be used to identify proteins that might be of importance for the *S. scabiei* virulence and therefore potentially useful for immunological control.

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119

EXPRESSION AND PURIFICATION OF RECOMBINANT HORN FLY THROMBOSTASIN

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Background: In an effort to investigate the utility of a novel horn fly salivary protein possessing anti-thrombin activity (thrombostasin, TS) as a vaccine antigen, recombinant protein was expressed, purified, and characterized. This protein was used to immunize rabbits to assess the level of immunogenicity of TS.

Methods: A cDNA encoding a TS isomer obtained from a horn fly salivary gland cDNA library was used to express recombinant protein in both *E. coli* and insect cell expression systems. Various constructs designed to encode tagged and untagged full-length, proenzyme, and mature only versions were expressed and purified by nickel affinity and anion exchange chromatography. Purified recombinant proteins were characterized by SDS-PAGE, Western blot analysis, N-terminal sequencing, mass spectrometry, and anti-thrombin enzymatic activity.

Results: Recombinant TS was expressed as a pool of various C-terminal truncated proteins. At least 5 different expressed versions of TS proteins contained the intact N-terminal sequence, including an N-terminal His tag. Interestingly, not all bands were recognized by a monoclonal antibody directed against a portion of the N-terminal tag. The pool of TS expressed proteins was purified to near homogeneity and neutralizing anti-TS specific antibodies were generated in rabbits.

Conclusions: Purified recombinant TS protein and affinity-purified anti-TS specific antibodies were generated and characterized. These reagents were used to evaluate TS as a vaccine antigen against horn flies.

Acknowledgments: Sponsored by Novartis Animal Health.

120

GENOTYPING CLINICAL AND ENVIRONMENTAL SAMPLES OF *GIARDIA* BY SEQUENCE ANALYSIS OF THE BETA GIARDIN GENE

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Background: The enteric protozoan *Giardia* is one of the most common nonviral causes of diarrhea in humans. In developed nations, water is increasingly recognised as an important vehicle for transmission. Molecular methods are needed to identify the species/genotypes and to evaluate the viability of cysts present in the environment.

Methods: DNA was extracted from 8 human fecal samples and from 21 waste water samples that were collected in different Italian regions. DNAs from 19 reference isolates were also included in the study. The β giardin gene was PCR amplified and the products were sequenced on both strands.

Results: Single amplification products of about 750 bp were obtained from all samples. The detection limit of the assay is about 10 cysts in fecal or in environmental samples. The analysis of the beta giardin sequences showed the presence of extensive polymorphisms that fully discriminate between isolates belonging to the A or B assemblage. All cysts from the 21 waste water samples were typed as *G. duodenalis* (15 assemblage A, 3 assemblage B, and 3 with both assemblages), as were the human fecal samples (6 assemblage A, 2 assemblage B). On the basis of these results, a simple PCR-RFLP assay was developed for the molecular identification of cysts.

Conclusions: Amplification of the β giardin gene has been found to be highly sensitive and specific for *Giardia* parasites present in fecal and environmental samples. Genetic polymorphisms allow for the discrimination of *G. duodenalis* genotypes. *Giardia* cysts present in the water samples have been characterised as potential human pathogens, indicating that the utilization of waste waters in dual networks may increase the risk of waterborne and foodborne transmission of the infection.

CLASSICAL LIGANDS BIND TUBULIN AND INHIBIT TRYPANOSOME GROWTH

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Background: Tubulin is the major building block protein of microtubules which constitute the cytoskeleton and have many other physiological functions in eukaryotic cells. The properties of tubulin of lower eukaryotes such as protozoa differ from those of higher eukaryotes such as mammals, making it possible to selectively target parasite tubulin, for chemotherapy.

Method: We examined whether the classical tubulin ligands would bind trypanosome tubulin and inhibit the proliferation of *Trypanosoma brucei* in culture. The binding of [³H]mebendazole to trypanosome and mammalian tubulin was compared. The classical tubulin ligands examined include various benzimidazole anthelmintics, colchicine and vinblastine. Tubulin purified from *T. b. brucei* or rat brain was used in binding studies.

Results: All compounds tested inhibited trypanosome growth. There were no significant differences ($p > 0.05$) among the benzimidazoles but vinblastine or colchicine caused significantly greater inhibitions than the benzimidazoles ($p < 0.005$ and $p < 0.02$ respectively). In the binding assays there was higher total binding of [³H]mebendazole to trypanosome than rat tubulin.

Conclusion: These results suggest that the ligands tested inhibited trypanosome growth via specific interaction with trypanosome tubulin. Therefore, tubulin may be a reasonable target against which novel drugs can be developed to control trypanosomiasis.

Acknowledgement: This investigation received financial assistance from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR).

PCR AMPLIFICATION AND SEQUENCING OF ITS-2 rDNA OF LEUCOCYTOZOON CAULLERYI

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Background: Except for mature gametocytes, merozoites and other developmental stages of blood-borne protozoa of domestic fowls cannot be differentiated. The second internal transcribed spacer (ITS-2) of ribosomal DNA has been shown to be valuable tool for species differentiation. The aim of the study was to characterize the ITS-2 of *Leucocytozoon caulleryi* and to develop species-specific primer for diagnosis of leucocytozoonosis in a polymerase chain reaction (PCR) assay.

Method: Schizonts of *L. caulleryi* were collected from the muscle of naturally infected domestic fowls sent for post-mortem inspection to South China Agricultural University. Genomic DNA was extracted from frozen parasite materials by a standard small-scale sodium dodecyl-sulphate (SDS)-proteinase K extraction method. The ITS-3 and ITS-4 primers of White et al (1990) were used to amplify a fragment containing ITS-2. PCR products were cloned in pGBM-T Easy Vector (Promega). Automated sequencing was performed using ABI337 system by TaKaRa Biotechnology (Dalian) Co., Ltd. The resulting sequences were aligned for comparison using WDNASIS analysis software.

Results: The ITS-2 fragments of *L. caulleryi* isolates after PCR amplification are shown as an approximate size of 300 base pairs (bp) compared to the DNA marker bands. Sequences of the amplified region were 270 nucleotides long and contained 47.0% GC. The sequence similarities ranged from 8.9% for *L. caulleryi* and *Eimeria tenella* and 9.6% for *L. caulleryi* and *Cryptosporidium parvum*.

Conclusion: The ITS-2 sequences of *L. caulleryi* reported here for the first time are the basis for further studies on genetic marker for diagnosis of leucocytozoonosis.

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NITRIC OXIDE - A NEW NEUROTRANSMITTER IN PARASITIC WORMS

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Background: Parasitic worms have well differentiated nervous system, which play important roles, integrating and co-ordinating their feeding, movements, reproduction and attachment to the host. In order to create new antiparasitic drugs, information about the neuronal signal substances in parasites is needed. Cholinergic, aminergic and peptidergic neuronal signal substances have been described from parasitic worms. During the last decade evidence for the presence of nitric oxide (NO) as neurotransmitter in vertebrates and invertebrates has appeared.

Objectives. The aim of this investigation is to study the role of NO as neurotransmitter in parasitic flatworms.

Methods. 1. NADPH-diaphorase histochemistry for detection of cells containing NO synthase (NOS). 2. TRITC-phalloidin for detection of muscle fibres. 3. Radiometric analysis for assessment of the activity of NOS. 4. Application of the NOS inhibitor N^G-nitro-L-arginine (L-NNA) for detection of the effects of NO.

Results. 1. A large number of NADPH-d positive nerve cells occur in the CNS and PNS of tapeworms and flukes. 2. A very close association between NADPH-d positive nerves and muscle fibres is evident in every worm studied so far. 3. The rate of production of L-[³H]citrulline in *Hymenolepis diminuta* is 0.49 ± 0.026 pmol/min/mg protein, indicating active NOS. 4. L-NNA disturbs the movement patterns of *Mesocostoides corti* and *Gorgoderia synnoides* and inhibits the proper function of the suckers.

Conclusion. Histochemical, radiometrical as well as pharmacological data suggest that NO is a neurotransmitter in parasitic flatworms. This information can be of use in the development of new antiparasitic drugs.

GENETIC MDR1 DEFICIENCY IN COLLIE DOG CAUSES HYPERSENSITIVITY TO IVERMECTIN

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The macrocyclic lactone drug "ivermectin" inhibits GABA-mediated neurotransmission in target parasites and insects. It was previously documented that certain dogs of the collie breed show increased sensitivity to this molecule at doses as low as 100 µg/kg. Clinical signs resulting from treatment at higher levels include: depression, ataxia, salivation, coma and even death. The observation in 1994 that knockout mice deficient in MDR1 P-glycoprotein displayed similar sensitivity led us to speculate that sensitivity in dogs might originate from a putative mutation in the MDR1 gene. Since no molecular data were available for the dog, using consensus primers and liver messenger RNA, we amplified (RT-PCR), cloned and sequenced a set of overlapping cDNA clones used to document the complete cDNA sequence of the beagle dog MDR1 gene.

Using the beagle MDR1 sequence and liver samples from two American collie dogs that were phenotypically characterized as sensitive to ivermectin, we cloned and sequenced the corresponding full length cDNA. The overall nucleotide sequence divergence between the two breeds is 0.11%. The two "sensitive" collies showed an identical homozygous mutation consisting of a four base pair exonic deletion that leads to a premature stop codon in the messenger RNA resulting in the absence of the protein evaluated by western blot in cellular membrane preparations. The lack of active protein in the epithelial cells of the CNS blood vessels results in the impairment of the blood/brain barrier, leading to the adverse effect of ivermectin on sensitive neurones.

This work, which represents the first reported model of naturally occurring gene invalidation in dog, might offer valuable perspectives in improving the accuracy of MDR-related drug transport studies.

13 p

A COOPERIA PUNCTATA GENE FAMILY ENCODING 14KDA EXCRETORY SECRETORY ANTIGENS CONSERVED AMONG TRICHOSTRONGYLOID NEMATODES
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Background: Excretory/secretory products (E/S) released by parasitic nematodes are a complex mixture of proteins with an anticipated critical function in infection. E/S contains highly immunogenic proteins that are hoped to evoke a protective immune response when incorporated into a vaccine. Here we have obtained further insight into the complexity of this approach.

Methods & Results: A polymorphic set of 14 kDa E/S antigen encoding cDNAs, homologous to a previously characterized 15 kDa E/S antigen of *Haemonchus contortus*, was cloned from *Cooperia punctata*. Polymorphisms may reflect differences in between individual worms in a population. However, genomic analyses on individual worms proved the existence of three 14 kDa E/S antigen encoding genes and strongly suggests the presence of additional members of this gene family. Thus, despite the fact that heterologously expressed *C.punctata* 14 kDa E/S products are shown to be recognized by immune sera, potential pitfalls in the development of a recombinant vaccine are presented by this genetic diversity. Vaccine design could be further rationalized by knowledge on the function, and possible redundancy in function, of the E/S products that is presently lacking. We discuss the obstacles encountered in assigning a function to the 14/15 kDa family of E/S proteins that is so far unique to the trichostrongyloid nematodes. **Conclusions:** 1-) Four cDNAs 70 to 80% identical 14kDa homologues cloned from *C.punctata*. 2-) At least three different 14kDa genes are present in *C. punctata* as demonstrated by the presence of introns. 3-) Two *C. punctata* 14kDa recombinant proteins are recognised by immune sera.

14 p

CHINESE FASCIOLA DETEMINATION BY ITS-2 SEQUENCE AND RFLP

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Background: The purpose of this study is to elucidate the taxonomic statues of the Chinese *Fasciola* species by amplifying the rDNA the second internal transcribed spacer(ITS-2) of *Fasciola* isolates from different places of China and RFLP and sequencing analysis of the PCR products.

Method: The total DNA of *Fasciola* spp isolated from Guangxi, Sichuan, Helongjiang, Gansu of China and France was separated by CTAB method. A pair of primers was designed according to the published sequence of ITS2 of ribosomal RNA gene. Restriction enzyme *Hsp92II* and *RcaI* were used to digest the PCR products. The PCR products were cloned in the vector of pGEM-Teasy by standard method. The recombinant clones were sequenced double-strandedly on ABI 377 DNA sequencer.

Results: All *Fasciola* isolates gave a PCR product of about 550bp. The PCR products were digested by *Hsp92II* and *RcaI*, respectively. As a result, all *Fasciola* spp studied in this work could be classified into three groups. Sequencing analysis of all the PCR products virified the classification. The ITS2 of all *Fasciola* spp was 362 bp in length. Sequence comparison also revealed that there were no variations in ITS2 sequence within one species of *Fasciola* and there were five to six bases difference in ITS2 sequence between different species of *Fasciola*.

Conclusions: It was concluded that the *Fasciola* isolated from Sichuan and France were *F. hepatica*, the *Fasciola* isolated from Guangxi were *F. gigantica* and the *Fasciola* isolated from Helongjiang were intermediate *Fasciola*. This is the first time to confirm that there are intermediate *Fasciola* in China in addition to *F. hepatica* and *F. gigantica*.

15 p

PCR FOR THE CYATHOSTOME BETA-TUBULIN CODON 200 PHE/TYR MUTATION

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Resistance against the benzimidazole (BZ) broadspectrum anthelmintics is a widespread phenomenon in sheep and horses. It has been shown that BZ-resistance in sheep gastrointestinal nematodes is linked with an increase in Tyr expressing alleles in the resistant parasite populations. Current techniques for the evaluation of BZ-resistance in horses as for example the focal egg count reduction (FECR) assay or the larval development inhibition assay are laborious and insensitive. In contrast, the molecular detection of the BZ-resistance related beta-tubulin allele provides a very sensitive method for the evaluation of the resistance status in parasite populations.

We have developed an allele specific PCR for the discrimination of the TAC/TTC mutation in the beta-tubulin 200 codon. One forward primer is used in two separate amplifications with one of two reverse primers which only differ in their final 3' nucleotide. The primers flank a facultative intron/exon. Therefore the amplified fragments are either 251 bp or 308 bp in size, depending on the presence or absence of the intron in individual worms. Titration of full length beta-tubulin cDNA revealed that the PCR is capable of detecting as little as 10 copies. Titration of DNA from a pool of 100 small strongyle larvae, resulted in a sensitivity of 1 larva equivalent. PCR with DNA isolated from single larva also reproducibly generated specific fragments.

This method will be applied for the assessment of allele frequencies in susceptible and resistant populations in order to further investigate the mechanism of BZ-resistance in small strongyles.

16 p

SEROTONINERGIC SYSTEM OF RATS INFECTED WITH MESOCESTOIDES CORTI TETRATHYRIDIA
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Background: Serotonin (5-Hydroxytryptamine, 5-HT) has a very wide distribution in the animal kingdom. In vertebrates the function of this compound is studied mainly in mammalia, where it serves as neurotransmitter, neuromodulator and also as true hormone, which is released into the circulatory system. 5-HT is involved in a range of physiological activities. In mammalian brain 5-HT containing neurons affect feeding, attention, sleep, behaviour, mood. It may play a role in temperature regulation, as well as in regulation of immune system, stress response and a lot of other functions. 5-HT has important role in different pathological processes.

Objectives: The aim of this investigation is to study the influence of helminth infection on serotonergic system of host.

Methods: Rats infected with *Mesocestoides corti* tetrathyridia were used in experiments. 5-HT was detected in different tissues, namely, in spleen, lung, liver, intestine by spectrofluorimetrically (Maickel et al, 1968)

Results: The results showed that in rats infected with *M.corti* tetrathyridia 5-HT level in all investigated tissues was more high than that in control (uninfected) animals. The most significant difference was observed in liver of control and infected rats (0.799±0.093 and 15.927±3.600 µg/g of tissue), respectively.

Conclusion: The data received show that infection with parasitic worms influences on serotonergic system of host. It means that 5-HT is one of the factors which plays the role in helminth pathology.

17p

CHARACTERISATION OF THE ITS-1 SEQUENCE OF *THELAZIA LACRYMALIS* (NEMATODA, THELAZIIDAE)

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Background: *Thelazia lacrymalis* (Nematoda, Thelaziidae) is transmitted by some species of *Musca* (Diptera, Muscidae) and is responsible for an ocular infestation in horses. The aim of this work was to assess the efficacy of conserved primers to amplify the first Internal Transcribed Spacer (ITS-1) of the ribosomal DNA of *T. lacrymalis* and to characterise that sequence.

Methods: Genomic DNA was extracted by proteinase-K treatment from four single adult worms of *T. lacrymalis* from native horses bred in the Abruzzo region (Central Italy). PCR was performed with two conserved primers to amplify the ITS-1 plus primer flanking regions using the procedures previously described. PCR products were purified in Ultrafree-DA columns and directly sequenced; the sequences were then aligned with each other and compared to those of *T. rhodesi*, *T. gulosa* and *T. skrjabini* registered in GenBank.

Results: The primers amplified a fragment of 452 bp including 357 bp of ITS-1 plus 25bp and 70bp for the 18S and 5.8S flanking regions respectively. The molecular analysis of ITS-1 showed a GC of 31.1%, with an intraspecific variation (A-G) at position 333. Comparison of the ITS-1 of *T. lacrymalis* with those of the three bovine thelaziæ revealed a homology rate of 57, 63 and 70% for *T. rhodesi*, *T. gulosa* and *T. skrjabini* respectively while the 18S and 5.8S flanking region showed homology rates of 100 and 99%, respectively.

Conclusion: The results obtained permitted the acquisition of information of the ITS-1 sequences of *T. lacrymalis*; these data are a preliminary step in the development of a diagnostic tool (PCR-RFLP, DNA probes) for the detection of *T. lacrymalis* larvae in flies and to identify the main *Musca* species which are responsible for the diffusion of thelaziosis in our country.

18p

DIFFERENTIATION OF *EIMERIA* INTRA-SPECIES BY THE RANDOM AMPLIFICATION OF POLYMORPHIC DNA

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Background: One strain selected for precocious was derived from the Beijing strain of *E. tenella*. We hope to distinguish this strain from other strains of the same species by choosing appropriating method.

Methods: It seemed likely that RAPD assays, using appropriate primers, could distinguish intra-species of most *Eimeria* species from one another. Species-specific DNA fragment mobility patterns were observed in most cases. The genomic DNA of the precocious strain, parental strain embryo-adapted strain, the drug-resistant strain of *E. tenella* and the precocious strain, the virulent strain and the drug-resistant strain of *E. acervulina* were analysed by RAPD.

Results: The results showed that the similarity (SI= 0.8950 — 0.9836) between *E. tenella* strains was higher than that (SI=0.8000 — 0.8592) between *E. acervulina* strains. It was suggested that the intra-specific variance between the strains of different virulence, geography, drug-resistant was not consistent.

Conclusion: Genomic fingerprints generated by RAPD revealed a high degree of similarity (SI) between the strains of *E. tenella* and the strains of *E. acervulina*. The strain-specific fragment of *Eimeria* strains can be amplified by the RAPD technique, and the RAPD method may be an extremely useful tool for studies of the genetic organisation of coccidial parasites. Polymorphisms detected by RAPD-PCR might facilitate the selection of molecular markers and study of the relationship between different strains.

19p

EXPRESSION AND IMMUNIZATION OF SO7 GENE OF *EIMERIA TENELLA* BJ STRAIN IN *E. COLI*

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Background: China has the largest amount of chickens in the world and the avian coccidiosis is a big problem. Presently we are looking at the value of a genetically engineered vaccine for coccidiosis.

Method: SO7 Gene was amplified and cloned with total RNA from 7h sporulating oocysts of *Eimeria tenella* BJ strain. Three expression vectors, designated pGKGS07, pETS07 and pThioHisSO7 were constructed with inserting SO7 gene into pGEX-KG, pET-28b (+) and pThioHisB plasmid, and expressed in *E. coli* Dh5a by inducing of IPTG. Transformant containing pThioHisSO7 could synthesize a fusion protein at high levels (17.1% of protein visible on coomassie blue stained gels) and was approximately 40 kDa in size, but transformant containing pGKGS07 and pETS07 could not produce recombinant protein visible. Young chickens were vaccinated at 4, 11, 17 days of age respectively with SO7 recombinant antigen at 100 µg or 10 µg. Experimental birds were challenged with 3×10⁴ sporulated oocysts of *E. tenella* at 25 days of age.

Result: SO7 recombinant antigen with adjuvant (1% alum) at 100 µg of large dose could induce a partial protection for chickens against coccidiosis by decreasing of 30% in cecal lesion score, but no protection was observed at 10 µg of low dose.

110p

CLONING OF *B. EQUI* GENE ENCODING 82-KDA PROTEIN AND APPLICATION FOR ELISA

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Background: At the present, Japan is free from Babesiosis. It is needed to develop a high-quality diagnosis system for the demonstration of *B. equi* infection.

Method and Results: To isolate *Babesia (B.) equi* genes encoding immunodominant proteins, a cDNA expression library prepared from *B. equi* mRNA was immunoscreened with the *B. equi*-infected horse serum. As a result, 18 positive cDNA clones were obtained and the nucleotide sequence of one of the clones, showing the strongest immunoreactivity, was determined. The cDNA clone consisted of 1,953 base pair nucleotide sequence and contained a putative open reading frame losing the 5'-terminal sequence. The gene was designated as Be44. The antibody rise against Be44 gene product fused with a gene 10 protein in mice could recognize only a 82-kDa merozoite protein of *B. equi*, but not *B. caballi*, in Western blotting analyses. The Be44 gene product fused with a glutathione S-transferase was expressed in *Escherichia coli*, purified, and used as the antigen of enzyme-linked immunosorbent assay (ELISA).

Conclusion: The ELISA was able to differentiate very clearly between *B. equi*- and *B. caballi*-infected horse sera. The results suggest that the Be44 gene product is a useful diagnostic antigen for the detection of *B. equi*-infected horses in both quarantine and epidemiological studies.

111 p DETECTION OF A P-GLYCOPROTEIN IN PSOROPTES SP.

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Background: P-glycoproteins (P-gp) are ubiquitous transmembrane proteins belonging to the ABC transport proteins superfamily, often implicated in multidrug resistance (MDR). Within the framework of a study on therapeutic failures in *Psoroptes ovis*, a bovine scab mite, we investigated the presence of P-gp homologues and their potential role in an observed resistance to acaricides. P-gp have been investigated at both molecular and biochemical levels on *P. cuniculi*.

Methods: Crude protein extracts of *P. cuniculi* were separated by SDS-PAGE electrophoresis and transferred to PVDF membrane. P-gp were detected using the C219 monoclonal antibody from Signet. ECL protocol (AmershamPharmacia) was used as revelation system.

Northern blot was carried out on total RNA from *P. cuniculi* and mouse liver used as positive control. Radiolabelled probes used to screen blots were issued by PCR from murine cDNA (MDR probe).

Results: A mRNA of 4,5 kb was detected with the murine MDR probe which corresponds to the P-gp mRNA molecular weight. Another 2,5 kb spot was observed which is suspected to corresponds to a TAP protein mRNA (Transporter Associated with Antigen Processing). A protein of approximately 170 kDa corresponding to the P-gp molecular weight was observed by western immunoblotting.

Conclusion: Both Northern- and Western-blot results seem to indicate the existence of a P-gp in *Psoroptes* sp. It should be confirmed by sequencing either the mRNA and/or the protein. On the other hand the spot at 2,5 kb observed with the Northern will be identified.

112 p THE MOLECULAR CHARACTERISATION OF THE SHEEP SCAB MITT, PSOROPTES OVIS

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Background: Sheep scab is a highly contagious ectoparasitic disease, which occurs as a form of allergic dermatitis as a result of infestation by the mite, *Psoroptes ovis*. This disease is considered an important welfare issue. The main aims of this study were to identify antigens that may be involved in the protective immune response observed in sheep and to define the main allergens associated with the sheep scab mite.

Method: A cDNA library was generated from *P. ovis* mites using a lambda TriplEx2 vector for the dual purpose of immunoscreening and expressed sequence tag (EST) analysis. The cDNA library was screened with sera from vaccinated and protected animals, then differentially screened with sera from highly protected animals versus sera from weakly protected animals. The EST project consisted of the sequencing and analysis of 500 clones.

Results: Immunoscreening of the library identified 50 positives and the differential screen identified 6 positives which were strongly recognised by the highly protected sera but only weakly recognised by the weakly protected sera. Analysis of the EST's is currently underway, but to-date approximately 5 allergens, glutathione-S transferase (GST) and a cathepsin B-like protease have been identified.

Conclusions: The positives identified by the differential screen may be involved in the protective immune response as they are recognised only by highly protected animals. The EST project has identified many allergenic proteins which may be involved with the pathogenesis of the disease.

113 p FUNCTIONAL ANALYSIS OF 3-HYDROXYKYNURENINE TRANSAMINASE FROM AEDES EGYPTI

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This study concerns the isolation and functional characterization of a mosquito transaminase responsible for catalyzing 3-hydroxykynurenine (HK) to xanthurenic acid (XA). HK is a natural metabolite in the tryptophan oxidation pathway but is oxidized easily under physiological conditions, and when allowed to accumulate, stimulates the production of reactive oxygen species. In this study, we demonstrate the presence of a specific HK transaminase capable of converting HK to XA in the presence of pyruvate in *Aedes aegypti* mosquitoes using various chromatographic techniques plus non denaturing electrophoresis. Analyses of the purified transaminase by gradient native PAGE and SDS-PAGE show that the native enzyme has a relative molecular mass of 80,000 and 40,000, respectively, which indicates that the native enzyme is present as a homodimer. Electric focusing of the enzyme determined its PI at 5.3. The purified enzyme is active to HK and kynurenine, shows a maximum activity at temperature around 55 °C, and also has glutamine aminotransaminase K activity, which is similar to those reported for mammalian kynurenine aminotransferase (KAT). The enzyme is more active to 3-hydroxykynurenine than to kynurenine, prefers pyruvate as amino acceptor, is more active at slightly acid pH around 6.0, and has no aminoadipate aminotransferase activity. Our data suggest that the specific mosquito HK transaminase plays an important physiological role in preventing over-accumulation of the chemically reactive and potentially toxic HK during tryptophan oxidation in mosquitoes during development.

114 p MOLECULAR CHARACTERATION OF A TROPONIN I-LIKE PROTEIN FROM THE HARD TICK HAEMAPHYSALIS LONGICORNIS

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Background: We are interested in the muscle-associated molecules responsible for moving the coxae of the appendages, retracting the chelicerae, controlling pharyngeal action, and other necessary functions for blood feeding of ticks.

Method and Results: A cDNA library of tick eggs was constructed in λ ZAP II vector and immunoscreened using polyclonal rabbit anti-tick immune serum. Twenty of 26 clones had the same open reading frame. The size of mRNA corresponding to the cloned cDNA was confirmed in Northern blotting analysis of total RNA extracted from adult of *H. longicornis*. The cloned cDNA hybridized to a band about 1.3 kb in size. The predicted amino acid sequence of the P27/30 gene shows a rather high homology 69% with *Drosophila melanogaster* troponin I clone E2. P27/30 gene possesses amino acid sequence of actin binding domains of troponin I at the amino acid residues 128 to 148. By immunoblot analysis, mouse anti-recombinant P27/30 serum reacted with major constituent protein bands in extracts of adult ticks, and also immunoreacted with muscle, cuticle, gut, and salivary gland in *H. longicornis*. Moreover, immunohistochemistry using the anti-P27/30 serum showed a strong reactivity in muscle.

Conclusion: P27/30 is a troponin I-like protein and has demonstrated the possibility of candidate antigen for vaccines against *H. longicornis*.

M

Diagnosis of parasitic diseases

M1

DETECTION OF INTESTINAL NEMATODE STAGES BY A MIGRATION AND A MUCOSAL DIGESTION METHOD

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Artificial infestation of dogs (n=16) with *Uncinaria stenocephala* and of cats (n=16) with *Ancylostoma tubaeforme* were established using ~300 infective stages of each parasite, in order to determine if different methods of larval retrieval would lead to the recovery of different developmental stages of larvae. Dogs and cats were necropsied at both 12 (n=8 each) or 16 (n=8 each) days post infection. Parasite stages from the entire intestine contents (stomach to rectum) were recovered and counted. Following washing of the gastrointestinal tracts, small intestines, suspended by wire, were soaked in 0.9% saline at 37°C for approximately three hours to allow migration and subsequent sedimentation of mucosal parasite stages. The mucosal membrane from the small intestine was then stripped off and subjected to pepsin digestion in order to recover any remaining non-migrated parasites. In the case of both *Uncinaria stenocephala* and *Ancylostoma tubaeforme* infections, the larval migration method recovered fourth stage larvae exclusively, while pepsin digestion yielded only third stage larvae. This separation of different larval stages mirrors the in-vivo migration of fourth stage larvae from the intestinal mucosa, while the third stage larvae remain within the tissue for further developmental maturation. Such differential detection of third and fourth stage larvae can be used as a model to study the efficacy of novel anthelmintics directed against specific stages of nematode infections.

M3

TREATMENT OF HAEMONCHOSIS IN GOATS ACCORDING TO DEGREE OF CLINICAL ANAEMIA

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Background: A longitudinal study of the faecal nematode egg counts and cultures for third-stage larvae and the haematocrits of indigenous crossbred goats of resource-poor farmers was carried out. During the summer months of November to April 1998/1999 and 1999/2000, *Haemonchus* was found to be an important species at three sites within the summer rainfall area of South Africa. The FAMACHA[®] system has been developed to identify those sheep suffering from clinical anaemia based on the comparison of the colour of the ocular mucous membranes with a chart of 5 colour categories. Animals considered to be in danger of dying from anaemia caused by haemonchosis are selectively treated.

Method: Goats were scored according to the FAMACHA[®] assay and the sensitivity, specificity, and positive and negative predictive values were calculated for the two summer seasons of 1998/1999 and 1999/2000.

Results: Analyses of the FAMACHA[®] system in goats showed a test sensitivity of 76% and 85% for 1998/1999 and 1999/2000, respectively, meaning that the system may be used to identify correctly 76% to 85% of those animals in need of treatment. However, the test specificity remains low at 52% to 55%.

Conclusion: The FAMACHA[®] system appears to be suitable for use in goats provided the lower three anaemic categories are treated.

M2

PEPSINOGEN PRODUCTION IN SHEEP INFECTED WITH *OSTERTAGIA CIRCUMCINCTA*

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Background: Not all sheep develop elevated serum pepsinogen concentrations following infection with abomasal parasites. One possibility is that animals differ in the amounts of pepsinogen that are stored and secreted by the abomasum.

Method: Sheep were infected with *Ostertagia circumcincta* and killed 5, 10, 20 and 30 days after infection. Other, uninfected sheep were killed as controls. Blood was collected from all animals and serum pepsinogen concentrations were determined. Tissues were collected to measure the amount of stored pepsinogen and the rate of basal and stimulated secretion in vitro.

Results: For the control animals at least, tissue content was a good predictor of the amounts secreted. The correlation of content and unstimulated secretion was good ($R^2 = 0.8$, $P < 0.05$). Tissue pepsinogen concentrations varied markedly between animals, ranging from 840 to 12,800 iU/g. Infection did not appear to affect the amounts of pepsinogen stored by tissues since there were no significant differences between the animals killed on the different days of infection. Several animals with low tissue pepsinogen concentrations were also notable in that they had not developed elevated serum pepsinogen concentrations following infection.

Conclusion: Variation in the production and storage of pepsinogen affects the amounts secreted by tissues and animals that produce little zymogen will as a result have little available for uptake into the circulation during infection.

M4

LOW *ASCARIS SUUM* EGG COUNTS IN PIGS INFLUENCE ESTIMATED PREVALENCES RATES

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Background: *Ascaris suum* is one of the most common helminths in domestic pigs and may be diagnosed by identification of the characteristic eggs in faeces. Low egg counts may, however, be false-positive due to coprophagia. **Method:** *Ascaris suum* faecal egg counts from longitudinal studies in organic and intensive Danish sow herds and a cross-sectional survey in Nordic swine herds were examined to describe the egg excretion patterns in relation to pig age with focus on low egg counts.

Results: Despite *A. suum* being a prolific egg layer, low egg counts made up large percentages of the positive samples, being highest in the youngest age groups (e.g. 73% and 55% of the positive egg counts of the weaners being <200 egg per gram (epg) and <50 epg, respectively) and lowest in the gilts (31% and 20% being <200 epg and <50 epg, respectively). This may be explained by false-positive egg counts due to coprophagia which is common in groupwise penned pigs. Due to the high numbers of low egg counts, the prevalence of egg-positive animals is very sensitive to both the efficiency and the lower detection limit of the used coprological technique. The use of a cut-off value may eliminate some of the methodological problems.

Conclusion: *Ascaris suum* prevalence rates, obtained by coprological examinations, depend to a large extent on the efficiency and the lower detection limit of the faecal egg count technique. Therefore, it may be very misleading to compare prevalence rates obtained in different laboratories.

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M5 EVALUATION OF TRICHURIASIS DIAGNOSIS BY ANTIGEN CAPTURE SANDWICH ELISA

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Background: *Trichuris suis* (*T. suis*) does not excrete eggs during larval stage as well as in particular adult stage. It is impossible to diagnose by use of fecal examination method in those period, therefore, serological diagnostic method is very useful for those stages.

Method: To produce monoclonal antibody, somatic and secretory-excretory (SE) antigens of *T. suis* were identified and analyzed by SDS-PAGE and western blot. Hybridoma producing monoclonal antibody was cloned, which was made of popliteal lymph node of BALB/c mice immunized with 45 kDa somatic antigen of *T. suis*. Five clones (1B9, 2C4, 2C5, 2D7 and 2D8) showing strong responses among yielded monoclonal antibodies were selected and were identified the isotype.

Results: All were IgG1 isotype and the light chains were κ chain. Established monoclonal antibodies were specifically reacted with somatic and SE antigens of *T. suis*. There, however, were no cross-reaction with *Ascaris suum* (*A. suum*), *Trichuris vulpis* (*T. vulpis*), *Trichinella spiralis*. The sensitivity of somatic and SE antigens against monoclonal antibody were significant ($p < 0.01$) associated with those of positive and negative sera. The infection rate for 112 field samples by antigen-capture sandwich ELISA using monoclonal antibody was higher than the rate by fecal examination method. The results by ELISA for *A. suum* infected pigs' coproantigens against this monoclonal antibody were almost same to those of uninfected pigs.

Conclusion: Antigen-capture sandwich ELISA was approved to be more useful method for the diagnosis of swine trichuriasis by all stages of *T. suis* than microscopic fecal examination.

M7 MOLECULAR DETECTION OF TICK BORNE PATHOGENS IN SMALL RODENTS BLOOD SAMPLES

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Background: A fast a reliable diagnosis of vector borne pathogens is a necessary prerequisite for the development of accurate control strategies. Polymerase Chain Reaction (PCR) methodology have been of extensive use in the diagnosis of these infections but its use needs particular attention in the set up of the methodology with particular regard of DNA extraction methods and amplifications conditions.

Method: We have applied the PCR to diagnosis of Ehrlichiosis and Borreliosis in blood samples collected from small mammals (*Apodemus* sp., *Clethrionomys glareolus*) live trapped from May to November 1999 within an endemic Lyme disease site of Trentino region (Northern Italy). Same of these samples have been used to compare different DNA extraction methods. Particularly we have compared an "home made" extraction protocol with a modified standard protocol (Qiagen kit). We have then perform PCR reactions on over 300 samples.

Results: Primarily we have developed a new protocol for tick and blood DNA extraction for the subsequent PCR detection of tick borne disease. This protocol is quite cheap but more time consuming than standard protocol using commercial kit. Also when using these kind of protocol we still get the chance to improve it, particularly by carefully testing the initial volume of blood to be further analysed. Smaller volume of blood than suggested by the manufacturer gave better yield of DNA. Once DNAs were extracted, by the application of specific PCR we were able to perform a diagnosis of Ehrlichiosis and Borreliosis to 384 blood samples of small rodents living in risk area. No samples were positive for Ehrlichia while 21 samples (18.28%) were diagnosed infected by *Borrelia* spp.

Conclusions: Our research offers a contribution to the study of tick borne diseases in North-Italy by the development of new tools for an accurate diagnosis pathogens involved in the infection. The elaboration of new protocols and the refinement of standard ones can improve the epidemiological analysis of these sanitary and veterinary diseases. Particular care as to be dedicated to the choice of the appropriate protocol since this can strongly enhance the efficiency of the experimental work.

M6 DETECTION OF TRICHINELLA SPIRALIS ANTIGENS IN URINE

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Background: The practical inability to diagnose *T. spiralis* antibodies in man before day 20 post infection (dpi) has stimulated interest in the development of immunodiagnostic test to detect circulating antigens. Our previous experience showed that soon after infection immune complexes as well as uncomplexed parasite antigens in sera of infected rats could be detected.

Method: To diagnose the presence of antigen in urine, double sandwich-capture ELISA was applied using a peroxidase-conjugated rabbit immunoglobulin to *T. spiralis* larval antigens. The plates were coated with metabolic (ES) or somatic (S) larval antigens. Mice were infected with 500 *T. spiralis* larvae. The urine samples from experimentally infected mice taken from 1 to 41 dpi. and the urine samples from patients of clinical hospital in Białystok taken from 3 to 120 dpi were examined. Before testing, the urine samples were heated for 6 min. at 100° C and centrifuged for 6 min. at 5000g, supernatants were used in ELISA.

Results: The presence of *T. spiralis* antigens in mice urine samples were detected between 6-26 dpi. using double sandwich-capture ELISA. All samples taken later were negative as samples taken from uninfected mice. 3 from 9 human urine samples taken 3-10 dpi were positive, the remaining samples taken 3-10 and 10-30 dpi showed values near to "cut-off". In both mice and human urine samples the higher level of somatic antigen than metabolic antigen was detected.

Conclusion: The *T. spiralis* antigens were present in urine of infected man and mice in the first phase of infection.

M8 TRITRICHOMONAS FOETUS: VALIDATION OF PCR TO DIAGNOSE INFECTION IN BULLS.

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Tritrichomonas foetus is the causative agent of bovine tritrichomonosis. So far, diagnosis of bovine tritrichomonosis was based mainly upon light microscopical examination or *in vitro* cultivation of diagnostic samples. Morphological diagnosis is hampered by contamination of samples with intestinal or coprophilic (tri-) trichomonadid protozoa or other conditions. PCR can offer a valuable alternative for the sensitive detection of *Tritrichomonas* spp. (Felleisen *et al.*, J. Clin. Microbiol. 36: 513-519, 1998). Now we report on the validation of this PCR with the following strategy: (1) Individual preputial wash fluid pools (originating from 10 different bulls) were each spiked with 5, 10, 100 or 1000 *T. foetus* trophozoites, respectively, and the experiment was repeated four times with four different *T. foetus* isolates. Sensitivity of PCR was then comparatively assessed with the InPouch™TF-Test. Performance was identical for both tests with a mean detectability efficacy of 50 organisms per 5 ml wash fluid. (2) One out of 10 wash fluid samples was spiked with different numbers of *T. foetus* trophozoites, all samples were subsequently pooled and analyzed by PCR, and separately by the InPouch™TF-Test. The detection level now was 8 to 10 trophozoites for the PCR and 10 trophozoites for the InPouch™TF-Test per individual test sample. The conclusion is that pooling samples for PCR does not result in a lack of sensitivity when compared to the time-consuming individual testing with the InPouch™TF-Test. The *T. foetus* PCR underwent a practical test phase by including this test into a large-scale epidemiological case-control-study on bovine neosporosis in Switzerland (Neo'99 study). In this context the *T. foetus* PCR proved high reliability, reproducibility and maintenance of operating characteristics under field conditions.

M9

SENSITIVITY OF A PCR TEST FOR DIAGNOSING TRITRICHOMONAS FOETUS INFECTION IN BULLS.

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Introduction: Trichomoniasis is a venereally transmitted reproductive disease of cattle caused by *Tritrichomonas foetus*. Disease control is based on test and cull practices and relies on the ability of a test to accurately identify infected animals. The current diagnostic test for *T. foetus* is culture but estimates of the sensitivity of the test are variable. A PCR assay was modified to enhance sensitivity.

Method: Using repeated preputial samples from 11 *T. foetus* infected bulls the PCR assay was compared with a culture kit. Negative culture samples were subsequently submitted for PCR testing.

Results: The PCR assay identified 93.2% (82/88) of samples as positive, while the culture kit yielded 80.7% (71/88) positives. Further PCR of cultured samples yielded an additional 5 positives.

Conclusions: The PCR assay performed better than the commercial culture kit in this trial, with an apparent significant difference after adjusting for repeated samples from the same bulls. The PCR assay, which does not rely on the presence of live organisms, could be used to replace the culture test or as a supplementary test.

Acknowledgements: We wish to thank PFRA, Agriculture & Agri-food Canada and MII, CFIA for funding this project.

M10

A Latex agglutination test (LAT) for detection of antibodies to *Neospora caninum* in sera of multiple host species.

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Background: *Neospora caninum* infection can occur in several different host species. The extent to which non-domesticated animal species may play a role in the epidemiology of infection is as yet uncertain. For these reasons, we set out to develop a cost-effective serological assay for *N. caninum* antibodies which did not require the use of species-specific secondary antibodies, an optical density reader, or fluorescent microscope. **Methods:** Antigen was prepared from lysates of NC-1 tachyzoites grown in VERO cells. This antigen was chemically coupled to latex microparticles of defined size range, and the coupled "beads" stored at 4°C until use. Coated beads were mixed in 96-well U-bottomed plates with serial dilutions of sera from cattle, dogs, badgers and foxes of known serological status with respect to *N. caninum*, and incubated at room temperature overnight. The presence of specific antibodies in sera was detected by agglutination in the coated microparticles when specific antibodies were present, and the formation of a discreet button of particles at the bottom wells, when they were not. **Results:** The LAT was found to be capable of specifically detecting antibodies to *N. caninum* antigen in sera from all of the species tested. The shelf-life of coated microparticles was found to be at least 6 months. **Conclusions:** This test has the potential to be a useful tool in serological investigations of *N. caninum* status. It is simple to perform, cheap, and does not require the use of specialised equipment. It is adaptable for use not only by diagnostic laboratories, but also by veterinary practices and where species-specific secondary antibodies are not available.

M11

SERODIAGNOSIS OF TOXOPLASMA GONII IN RABBITS (*Oryctolagus cuniculus*).

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This study was initiated to evaluate the modified agglutination test (MAT) in experimental and natural infection with *T. gondii* in rabbits.

The experimental rabbits were inoculated either orally with oocysts of sheep strain (8 rabbits) or parentally with tachyzoites of RH strain (6 rabbits). Five and 3 rabbits were used as uninfected control for each group respectively. All rabbits were examined serologically at weekly intervals for a period of 8 weeks. Formalized *T. gondii* whole tachyzoites incorporating mercaptoethanol were used as antigen for MAT. Sera obtained from 120 rabbits collected from different localities of Cairo city were also examined by using MAT. The rabbits inoculated with oocysts had their maximum titer (1:100-1:500) either on the 2nd week post-inoculation (PI) or on the 3rd week PI (1:100-1:1000). The tachyzoites inoculated rabbits had their maximum antitoxoplasma antibodies (1:500-1:1000) on the second week PI. In both groups of rabbits the antibody titers started to decrease on the 4th week PI and could be detected until the 8th week PI. Serological examination of 120 rabbits using MAT revealed 31 positive rabbits (25.38%) at a titer of 1:25. Further titration of positive samples indicated that 6 (19.35%), 15 (48.39%) and 10 (32.26%) rabbits had titers of 1:50, 1:100 and 1:500 respectively.

It was concluded that MAT is a reliable test for screening rabbits for antitoxoplasma antibodies.

M12

IgG RESPONSE AGAINST THE WOLBACHIA SURFACE PROTEIN: DIAGNOSTIC APPLICATIONS

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Background: *Wolbachia* intracellular bacteria have been observed in most species of filariae, including *Dirofilaria immitis*. It has been suggested that *Wolbachia* could play an important role in the biology of filarial nematodes and could be implicated in the immunology of filarial diseases. The presence of IgG antibodies against the *Wolbachia* surface protein (WSP) was demonstrated in sera of cats experimentally or naturally infected with *D. immitis*. Here we investigate the possible use of the IgG response against WSP for the diagnosis of dirofilariosis in humans and animals.

Method: We produced in recombinant form the WSP of the wolbachiae of *D. immitis* and *Brugia pahangi*/*B. malayi*. Sera were collected from cats and humans sero-positive and sero-negative for dirofilariosis. Some of the human sera were from patients with a diagnosed pulmonary dirofilariosis. The collected sera were tested by Western blot and ELISA for the detection of anti-WSP IgG.

Results: In cats and humans sero-positive for *D. immitis*, anti-WSP IgG were detected by both ELISA and Western blot. IgG titres in humans patients with pulmonary nodules were higher compared to the titres of sero-positive individuals without nodules. Sero-negative cats and humans had significantly lower IgG titres. Control experiments using the *B. pahangi*/*B. malayi* WSP protein revealed a low level of cross-reactivity.

Conclusions: The results obtained demonstrate that WSP could be useful for the diagnosis of dirofilariosis in both cats and humans. In addition, our preliminary results indicate that ELISA using WSP could allow to differentiate between humans which are simply sero-positive and patients where the development of pulmonary nodules has occurred.

M1 p IDENTIFICATION AND EXPRESSION OF A 50 KDA SURFACE ANTIGEN OF *BABESIA GIBSONI* AND EVALUATION OF ITS DIAGNOSTIC POTENTIAL IN AN ENZYME-LINKED IMMUNOSORBENT ASSAY

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A cDNA expression library prepared from *Babesia gibsoni* (*B. gibsoni*) merozoite mRNA was screened with a *B. gibsoni*-infected dog serum. A cDNA encoding 50 kDa protein was cloned and designated P50 gene. The complete nucleotide sequence of the P50 gene had 1,922 bp. The sequence of the P50 gene contained an open reading frame of 1,401 bp with a coding capacity of approximately 50 kDa. The complete genomic nucleotide sequence of the P50 gene has been analysed and shown to contain a single intron with a size of 37 bp. Southern blotting analysis indicated that the P50 gene contained a single copy in *B. gibsoni* genome. The native P50 protein of *B. gibsoni* with molecular mass of 50 kDa was identified by Western blotting using anti-recombinant P50 mouse serum. The recombinant P50 protein expressed by baculovirus in insect cells was used for antigen in an enzyme-linked immunosorbent assay (ELISA). The ELISA was able to differentiate clearly between *B. gibsoni*-infected dog serum and *B. canis*-infected dog serum or non-infected dog serum. Furthermore, the antibody response against the recombinant P50 was maintained until the chronic stage of infection in dog experimentally infected with *B. gibsoni* was developed. These results demonstrated that the recombinant P50 might be a useful diagnostic reagent for detection of antibodies to *B. gibsoni* in dogs.

M3 p SONOGRAPHIC EVALUATION OF HEARTWORM INFECTION IN FERRETS TREATED WITH MELARSOMINE DIHYDROCHLORIDE

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Introduction: The role of echocardiography for diagnosis of heartworm infection in cats has recently been defined. The role of ultrasonography for diagnosis of heartworm disease in ferrets is poorly defined.

Method: Twenty-eight male and female ferrets were each given 3 pairs of 114-day-old male and female heartworms by subcutaneous transplantation and 75 days later allocated to 4 groups of 7 ferrets each: Group I, IM injection of saline; Group II, single IM injection of 3.25 mg/kg melarsomine dihydrochloride; Group III, 2 IM injections of 3.25 mg/kg melarsomine dihydrochloride, 24 hours apart; Group IV, single IM injection of 3.25 mg/kg melarsomine dihydrochloride, followed 1 month later by 2 IM injections of 3.25 mg/kg, 24 hours apart. Echocardiography was performed 1 day prior to and at 2 and 3 months after the first or only treatment.

Results: Heartworms were identified by echocardiography in all but one ferret prior to treatment. Heartworms could be identified within the right atrium and ventricle, caudal and cranial vena cava and jugular veins. Seven ferrets died by 3 months after treatment. However, live heartworms were identified in 6 of 6 ferrets in Group I, 5 of 5 in Group II, 4 of 6 in Group III, and 3 of 4 in Group IV. Fragments of worms could be identified.

Conclusion: Sonographic evaluation of the right heart, caudal vena cava, cranial vena cava and jugular vein of ferrets suspected of heartworm disease is highly effective in establishing a diagnosis of heartworm disease and in determining the effect of treatment.

M2 p DIAGNOSIS OF *DIROFILARIA IMMITIS* INFECTION IN CATS BY IMMUNOBLOT ANALYSIS

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Purpose: Diagnostic utilization of immunoblotting analysis for canine heartworm, *D. immitis*, infection in cats were assessed because the increasing of infection cases in cats lately have been reported.

Methods: Sera from four experimentally infected cats with L3 of *D. immitis* and 129 (59 males and 70 females) pet cats in Tokyo area were analyzed with PBS extract of *D. immitis* adult by immunoblot analysis. Antigenic cross-reactivity was also assessed to *Toxocara canis* antigen.

Results: Immunoblot analysis in experimentally infected cats and dogs revealed that the expression of antigen bands of 14, 19, 22, 32 and 36 kDa may suggest the infection of living adult worm(s). Only 5 (3.9%) in 129 pet cats were positive for 1 to 3 bands in 19, 22 and 32 kDa, which were indicating of adult worm infection. The 5 pet cats did not cross-reactive for *T. canis* antigen in immunoblot analysis before and after the antibody absorption treatment.

Conclusion: Immunoblot analysis is useful for diagnosing *D. immitis* infection in cats.

M4 p TOOLS FOR THE IMPROVED DIAGNOSIS OF *ISOSPORA SUI* INFECTIONS IN PIGLETS.

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Isospora suis, the causative agent of piglet coccidiosis, receives growing attention as a common primary cause of diarrhoea and production losses in the pig industry worldwide. However, the disease is frequently overlooked because (a) clinical signs occur prior to oocyst excretion and (b) the diagnostic panel often only includes viral and bacterial pathogens. From experiences in experimental and field studies a scheme has been designed to improve the detection of *Isospora* in faecal samples. The proper sampling date and correct handling of the samples can greatly improve the sensitivity of coproscopical examination. A panel of methods for the improved diagnosis *intra vitam* and *post mortem*, including autofluorescence microscopy, histology and molecular techniques, is now available. These methods have been evaluated and should provide more accurate data on the prevalence of piglet isosporosis in the field.

M5 p**KINETIC OF ANTI-HYPODERMA SPP. ANTIBODIES IN NATURALLY INFESTED CATTLE**Frangipane di Regalbano¹, A., Otranto², D., Stella³, V. & Pietrobelli¹, M.¹Istituto di Patologia e Igiene Veterinaria, Università di Padova(Italy); ²Dipartimento di Sanità e Benessere degli Animali, Università di Bari (Italy); ³Medico Veterinario

Background: ELISA tests are currently used on serum and milk samples in many European Countries for the immunoparasitological survey of bovine hypodermosis. Its application is strongly related to the knowledge of the most suitable period for cattle sampling (highest antibody levels) in different climatic conditions. The aim of this investigation was to describe, for the first time, the annual kinetics of anti-*Hypoderma* spp. antibodies in dairy cows bred in the province of Vicenza (North-eastern Italy).

Method: 37 dairy cows from 2 to 12 years old, selected from 2 farms with a history of warble fly infestation, were sampled for blood and milk every 30-50 days from November 1999 to October 2000. All serum and milk samples were individually tested using ELISA kit (Vetoquinol® Diagnostics, France). Animals were also examined once a month from March 2000 for the detection of warbles.

Results: only 4 animals showed negative immunological results during the experimental period and no warbles were detected in their back. Individual changes in the concentration of anti-*Hypoderma* spp. antibodies were registered in the remaining dairy cows. The highest antibody titres were observed in March-May, both in serum and milk samples, while lower values were recorded in September-November. The antibody levels in the serum and milk collected from the same animal (at the same time) were closely related ($r=0.77$). Warbles appeared in May (in 28.6% of the animals) and the larvae reached the third stage in July-August.

Conclusions: March-May may be considered the most favourable period to sample serum or milk in order to perform an immunoparasitological survey in our climatic conditions. Previous experiences in Southern Italy determined that the best period was November-December. These results emphasize the fact that a correct use of immunodiagnostic methods requires knowledge of the kinetic development of anti-*Hypoderma* spp. antibodies at different latitudes.

M6 p**HAND-HELD HAEMOGLOBINOMETERS POTENTIAL TOOLS FOR FIELD DIAGNOSIS OF BOVINE PARASITIC DISEASES IN AFRICA**J.W. Magona^{1,2}, N.N. Jonsson¹, R. Barron¹, N. Machila³ and M.C. Eisler¹¹Department of Veterinary Clinical Studies, University of Glasgow Veterinary School, Bearsden, Glasgow G61 1QH, UK.²Livestock Health Research Institute, P.O. Box 96, Tororo, Uganda.³CTVM, University of Edinburgh, Easter Bush EH25, 9RG.**Background**

Detection of anaemia is an important diagnostic criterion for bovine parasitic diseases such as trypanosomiasis, tick-borne diseases and helminthoses in Africa. In this study, simple hand-held haemoglobinometers (DHT-haemoglobinometer (DHT), WHO-haemoglobin colour scales (HCS) and the HemoCue) were comparatively evaluated with the Cyanmethaemoglobin method under laboratory conditions for measurement of anaemia.

Method

The precision, sources of imprecision, correlation with PCV, sensitivity and specificity of the haemoglobinometers were assessed using 65 cattle samples obtained from routine sampling of cases at Glasgow University Veterinary School.

Results

The CV of the DHT, HCS, HemoCue and the Cyanmethaemoglobin in the Hb range of 4-12 ranged between 7.7-23.1%, 2.9-8.8%, 3.5-10.6% and 3.4-10.1%, respectively. Over 96% of the error in the Hb measurement using the hand-held haemoglobinometer was attributed to diluting. Correlation between PCV and Hb measurements was: $r = 0.93, 0.96, 0.97$ and 0.94 for the DHT, HCS, HemoCue and the Cyanmethaemoglobin, respectively. The DHT, HCS, HemoCue and the Cyanmethaemoglobin had diagnostic sensitivities of 50%, 100%, 81.6% and 89.5% and specificities of 100%, 96.3%, 100% and 89.5%, respectively.

Conclusion

The HemoCue and the HCS, which had a maximum CV of 10%, had high correlation with PCV, high sensitivity and specificity and do not involve a dilution step, are suitable for field conditions and simple to use, could provide quantitative pen-side results for immediate field diagnosis and management of bovine parasitic diseases in Africa.

Acknowledgement

We thank the WHO for supplying the haemoglobin colour scales and DFID for financial support for this study.

M7 p**IMPROVEMENT OF SOIL SAMPLE PCR ANALYSIS BY THE USE OF MAXIMATOR® (CONNEX)**

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Ancylostomosis, toxocarosis and toxascariasis are of importance as helminthological diseases in carnivores. Infectious stages survive in the environment and can be detected in soil samples using flotation methods. Furthermore, they can cause larva migrans in humans.

For an epidemiological study a PCR should be established for the detection of *Ancylostoma caninum*, *Toxocara canis* and *Toxascaris leonina* in soil samples. A number of soil samples was examined for developmental stages of *A. caninum*, *T. canis* and *T. leonina* with species specific PCR primers after proteinase K digest and DNA extraction via silica membranes. Sensitivity of the PCR was then evaluated with and without the addition of Maximator® (Connex, Martinsried). This substance has been developed for the neutralisation of PCR inhibitors. Increased sensitivity could be observed using the substance and thus could allow the establishment of an improved PCR protocol for the examination of soil samples detecting contamination with developmental stages of above mentioned parasites by PCR as an alternative to flotation techniques.

M8 p**Serological diagnosis of bovine cysticercosis by detecting live *Cysticercus bovis* using a monoclonal antibody-based antigen-ELISA**W Wanzala^a, J A Onyango-Abuje^b, E K Kang'ethe^c, H Ochanda^a, L J S Harrison^d

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An ante-mortem Antigen-ELISA-based diagnosis of *Taenia saginata* cysticercosis was studied in artificially (n=24) and naturally (n=25) infected cattle with the objective of further validating the assay as a field diagnostic test. Based on total dissection as a gold standard method of validity, the assay minimally detected 14 live cysticerci in artificially infected calves and 2 in naturally infected steers. In natural infections, the minimum number of live cysticerci consistently detected by Ag-ELISA test was 5 while in artificial infections it was above 14. However, other animals with 12 and 17 live cysticerci in artificially infected calves and 1 and 2 live cysticerci in naturally infected steers, escaped detection for unknown reason. Animals harbouring dead cysticerci gave negative reactions with the assay as was in non-infected experimental control calves. There was a statistically significant positive linear correlation between Ag-ELISA OD readings and burdens of live cysticerci as obtained by total dissection procedures in both artificially infected calves ($r=0.798, n=24; p>0.05$) and naturally infected steers ($r=0.631, n=25; p>0.05$). These results clearly show the potential effectiveness of ante-mortem monoclonal antibody-based antigen detection ELISA in the diagnosis of bovine cysticercosis in cattle.

L

Parasite control: surveillance and prevention

L1

GUIDELINES ON CLINICAL FIELD STUDIES TESTING PARASITICIDES

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Background: The VICH group has finalised a number of new guidelines which are either for implementation or for final discussion. The Guidelines for fleas and ticks in dogs and cats have been adopted. For implementation in July 2001 the VICH GCP will be a more detailed and compulsory ethical and scientific guideline for the conduct of veterinary clinical field studies. A Statistical Guideline has been for consultation resulting in a huge number of questions and comments. At the same time there are reports of investigators who have significantly biased studies.

Experiences: As expected these guidelines give detailed guidance on the conduct of clinical field studies. However all of them change current approaches and in most of the cases challenge the efforts and the expensis to be taken. Some contradictions seem to arise between the different guidelines. While the flea and tick guideline includes the animal numbers to be tested this should be evaluated on an individual basis according to the Statistical Guideline. The GCP guideline obviously asks for much more critical control points than mentioned in the protocols for fleas and ticks.

Conclusion: The development of protocols, conducting and monitoring of studies as well as the analysis will have to be much more detailed and retrospectively assessible than previously done. The objective to assure the public integer, accurate and correct data must be fulfilled. However criminal bias can be limited, but not prevented.

L3

SAINFOIN REDUCES THE ESTABLISHMENT OF NEMATODE INFECTIONS IN GRAZING LAMBS

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Background: Recent studies with sheep have indicated an anti-nematodal effect of several leguminous plants, possibly associated with a high content of condensed tannins. In search for suitable local plants, an experiment was performed under Danish conditions.

Method: Four 0.3 ha plots were established with pure stands of sulla (*Hedysarum coronarium*), white clover, sainfoin (*Oxybrychus vicifolia*) and chicory (*Cichorium intybus*). Five or six parasite naive lambs were grazed for 7 weeks on each plot with repeated moves to avoid reinfection. Lambs were infected 6 times during the first 3 weeks with *Ostertagia circumcincta* and *Trichostrongylus vitrinus*, receiving a total of 42,000 L3 of each species.

Results: The lambs grew at similar rates on white clover, sulla and sainfoin whereas the growth rate was considerably less on the chicory. Faecal egg counts (FECs) on sainfoin and sulla were consistently lower than on white clover and chicory. After 7 weeks of grazing, mean FECs were 1970, 1390, 980 and 900 epg in the white clover, chicory, sainfoin and sulla fed groups, respectively ($p < 0.01$). The worm burdens were 24,800, 20,100, 15,900 and 18,700 ($p = 0.15$) in the abomasum and 28,600, 21,100, 21,700 and 21,400 ($p < 0.02$) in the small intestine in the white clover, chicory, sainfoin and sulla fed groups.

Conclusions: The establishment of nematode infections in sheep was influenced by the species of legume in the diet. Using white clover as a reference, sainfoin had the capacity to reduce infections almost similar to that reported for sulla. The reduction in faecal egg counts was larger (50%) than the reduction in worm burdens (24-36%).

L2

EFFECT OF DIETARY POLYUNSATURATED FATTY ACIDS ON GASTROINTESTINAL NEMATODE INFECTION IN CALVES

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Background: Diet induced changes in the polyunsaturated fatty acids (PUFA) content of immune cells, particularly the ratio of n-6 to n-3 PUFA, can influence the functions of the effector arms of the immune response. 24 pre-ruminant calves were used to investigate the possible effect of dietary PUFA on resistance to gastrointestinal nematode infection.

Method: 12 calves received 25g/day of an n-3 rich PUFA (fish oil) and the remainder 25g/day of an n-6 rich PUFA (palm oil/rapeseed oil) fed as a supplement with milk replacer. Within each treatment group 8 calves were infected with 2000 L3 *Ostertagia ostertagi* and 2000 L3 *Cooperia oncophora* 3 times a week for 8 weeks and 4 calves remained uninfected as controls. Faeces were collected twice weekly for determination of nematode eggs and at the end of the experiment abomasal and intestinal samples were taken for immunohistochemistry and enumeration of the worm burdens.

Results: The mean faecal egg counts and the mean worm burdens tended to be lower in the group of calves offered the n-3 rich PUFA dietary supplement. Infection increased the number of mast cells in the intestinal mucosa relative to controls but there were no consistent dietary effects.

Conclusion: This pilot trial indicates that the composition of dietary PUFA can influence gastrointestinal nematode infection in young calves but the mechanisms involved are unclear.

L4

DIRECT ANTHELMINTIC EFFECT OF CONDENSED TANNINS TOWARDS OVINE NEMATODES

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Background: Consumption of condensed tannin-rich legumes reduces the detrimental effects of parasitism in grazing ruminants. A direct anthelmintic effect of a condensed tannin extract (Quebracho extract, QT) was recently demonstrated towards adult *T.colubriformis*. The aim of the present study was to investigate a possible direct anthelmintic effect of QT towards adult populations of four gastrointestinal ovine nematodes.

Method: 42 sheep (n=6) were infected with a single dose of either 4,000 L3 *H. contortus* (groups 1-2), or 10,000 L3 *T. colubriformis* and *N. battus* (5,000 from each species, groups 3-5) or 10,000 L3 of *T. circumcincta* (groups 6-7). From day 28 until 31 post infection, sheep were drenched with QT at 8% of food intake, or remained as undrenched controls. In the remaining group of sheep infected with the intestinal species, sheep were drenched with 4% of food intake QT. Faecal egg counts (FEC) were determined daily during the drenching period. Sheep were slaughtered on day 36 post infection for worm recovery.

Results: Sheep parasitised with the intestinal species and drenched with 8% w/w QT had lower FEC compared to sheep drenched with 4% QT or the undrenched controls ($P < 0.05$). The lowest intestinal worm burden was recovered from sheep drenched with 8% w/w QT ($P < 0.05$). The administration of QT at 8% w/w for three days did not affect FEC or worm burdens of sheep infected with the abomasal species.

Conclusion: QT clearly showed a direct anthelmintic effect towards intestinal nematodes of sheep. The absence of a direct effect towards abomasal parasites could be due to either species differences or different parasite-tannin interactions.

L5

EFFECTIVENESS OF GRAZING MANAGEMENT IN CONTROLLING GI NEMATODES IN WEANER LAMBS ON PASTURE IN DENMARK

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Background: The problem of anthelmintic resistance calls for alternative control methods, including grazing management. Dose- and -move and move- only strategies of control methods were compared in this study.

Method: In early May, 1999, 16 ewes with twin lambs (2-3 weeks old) were turned out on infected pasture. On 1st July, the lambs were allocated to 4 groups of 8 lambs and weaned to clean pasture. Two groups (dose- and -move) were treated with anthelmintics while the other two (move- only) were not treated. Each group was allocated to a separate paddock sampled every two weeks and set stocked until slaughter.

Results: Moving the weaned lambs with no treatment reduced the faecal egg counts by 70% within 2-4 weeks. The faecal egg counts of the treated groups remained significantly lower for 6 weeks only ($P < 0.01$). The weight gains were comparable in all four groups and no cases of parasitic gastroenteritis were observed. The worm counts were significantly lower in the dose- and -move groups particularly in the small intestines (*Trichostrongylus vitrinus*) ($P < 0.01$).

Conclusion: Weaning lambs in early July and moving them to clean pasture before the expected mid-summer rise in herbage infection may prevent parasitic gastroenteritis and secure good production whether the move is accompanied by anthelmintic treatment or not.

Acknowledgement: The Danish International Development Agency's Enhancement of Research Capacity (ENRECA) Programme for funding.

L6

PRODUCTIVITY IMPROVEMENT IN CATTLE AFTER TREATMENT WITH IVOMEC® GOLD

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In a year-long study, the weight gain of weaned beef cattle on a parasite control program using a long acting ivermectin formulation was compared with that of cattle treated with doramectin in Brazil.

Method: The trial was carried out on four farms, from September 1999 to September 2000, and included 606 cattle. On each farm, about 50 replicates of three animals were formed based on the weight gain observed during the previous 28 days. Within replicate, cattle were randomly allocated to receive two treatments of ivermectin (IVOMEC Gold) at 630 mcg/kg bw SC or doramectin (DECTOMAX®) at 200 mcg/kg bw SC on Days 0, and 224 or 252 (one farm), or were kept as untreated controls. During the evaluation period of 308 to 322 days, cattle were kept in separate pastures according to the treatment groups. Monthly evaluations included weighing, ectoparasite counts and fecal egg counts.

Results: Nematode challenge was present throughout the study on all farms. Ticks (*Boophilus microplus*) were present on only two farms. Treated cattle gained more ($p < 0.05$) weight than untreated controls. Average daily gain was higher ($p < 0.05$) in cattle treated with ivermectin than in cattle treated with doramectin (373 g/day vs. 316).

Conclusion: These results demonstrated that a parasite control program with ivermectin long acting increased the productivity of the herd.

*IVOMEC is a registered trademark of Merial Limited.

*DECTOMAX is a registered trademark of Pfizer

L7

BENEFIT OF TREATMENT WITH IVOMEC® GOLD ON WEIGHT GAIN OF CATTLE

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In a large study involving approximately 73,000 weaned cattle, the effect on weight gain of a single treatment with a 3.15% long acting ivermectin (LAI) formulation, doramectin, moxidectin, abamectin, levamisole and other ivermectin formulations was evaluated.

Method: On each farm, replicates of three animals were formed based on initial body weight. Within replicate, cattle were randomly allocated to receive ivermectin (LAI) (IVOMEC Gold at 630 mcg/kg bw, SC) or the following formulations of macrocyclic lactones at 200 mcg/kg bw, SC or 500 mcg/kg bw, topical: doramectin (856 evaluations), ivermectin (121), moxidectin (67), and abamectin (35); or levamisole at 3.74 mg/kg, SC (3); or were kept as untreated controls. On each farm, cattle were kept on pasture as a single group for the duration of the evaluation period (average 62 days, range 29 to 139 days).

Results: Ticks (*Boophilus microplus*) and grubs (*Dermatobia hominis*) were absent on 57% of the farms, and ticks were present on 27% of the farms at trial start. Average daily gain (ADG) was higher ($p < 0.01$) in treated cattle than in untreated controls. Also, cattle treated with LAI had higher ADG ($p < 0.01$) than animals treated with levamisole (552 vs. 451 g/day), abamectin (502 vs. 464 g/day), moxidectin (474 vs. 436 g/day), other ivermectin formulations (429 vs. 350 g/day) and doramectin (516 vs. 444 g/day).

Conclusion: These results demonstrate that treatment with IVOMEC Gold results in higher weight gains than other formulations of avermectins, milbemycins and levamisole tested in this study.

*IVOMEC is a registered trademark of Merial Limited.

L8

THE EFFECT OF EPRINOMECTIN OR TRICHLORFON ON MILK YIELD OF DAIRY COWS

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We studied the effect of a strategic antiparasitic treatment against *Hypoderma* spp. and other parasites on milk yield and somatic cell counts (SCC) of 742 multiparous dairy cows from 79 farms in Western Switzerland. Cows were randomly assigned to one of the following treatment groups: eprinomectin at 0.5 mg/kg bw, applied topically (EPRINEX®) (n=357); untreated controls (n=133), and trichlorfon at 5 mg/kg bw, applied topically (NEGUVON® 10% Spot-On) (n=252). The treatments were done between October 15 and December 15, 1998. Milk yield and SCC were determined once per month until the end of lactation. The effects of treatment were estimated by hierarchical linear models. Eprinomectin-treated cows produced 652 kg milk/cow more ($p < 0.001$) than the controls during the lactation period (305 days), whereas trichlorfon-treated cows produced 573 kg milk/cow more ($p < 0.001$) than the controls. The effect of eprinomectin on milk production of the cows was persistent throughout the lactation at about 2.14 kg milk/day greater than in the controls. In contrast, milk production in the cows treated with trichlorfon dropped steadily until the end of the lactation by 0.12 kg milk/day per month ($p < 0.05$). Furthermore, SCC in eprinomectin-treated cows increased at a lower rate ($p < 0.05$) than they did in controls.

Acknowledgements: We thank the Swiss Association for Simmental and Red and White Cattle for allowing us to use the lactation data.

*EPRINEX is a registered trademark of Merial Limited.

*NEGUVON is a registered trademark of Bayer.

L9

EFFICACY OF DORAMECTIN AND ALBENDAZOLE IN YOUNG CATTLE OF ZIMBABWE

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Background: Recent reports suggested that gastrointestinal nematode parasites can be controlled by preventing the establishment of these infections during the rainy season. The aim of the present study was to compare efficacy and economic impact of prophylactic treatments with doramectin (sc. 0.2mg/kg) and albendazole (orally 7.5 mg/kg) against naturally acquired nematode infections in permanently grazing young cattle during the wet season.

Method: Five groups of 8 steers and 8 heifers each were treated as follows: nonmedicated controls (CONT), doramectin twice on day 0 and 84 (DOR-2), doramectin once on day 0 (DOR-1), albendazole twice on day 0 and 84 (ABZ-2) and albendazole once on day 0 (ABZ-1). The 243-day study was initiated on January 15 (day 0). The animals were weighed and faecal samples were collected at regular intervals.

Results: Egg counts (epg) of the DOR-treated groups were lower ($P < .05$) than those of CONT and ABZ-treated groups from day 30 to 84. Counts of the DOR-2 group were also significantly lower after second medication to day 210. Epg of ABZ-treated groups differed ($P < .05$) from CONT between day 30 and 120. Cooperia was the predominant genus, followed by Haemonchus and Oesophagostomum. Mean weight gains for the CONT, DOR-2, DOR-1, ABZ-2 and ABZ-1 were 62.2, 86.3, 72.9, 77.9 and 75.4 kg respectively. Weight gains of medicated groups were significantly ($P < .05$) greater than CONT, but differences among last three groups were not significant.

L11

COMPARISON OF LIQUID AND BLOCK SUPPLEMENT FREE-CHOICE INTAKE IN CATTLE. K.C. Garossino¹, T.A. McAllister², D.N. Milligan³, B.J. Ralston¹ and M.E.

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BACKGROUND: Medicating cattle by way of free-choice supplementation reduces the stress and risk of injury to the animals. In order to employ this method a supplement must be consumed on a regular basis and at levels which do not vary significantly on a day to day basis.

METHODS: Two separate groups ($n=17$ for both groups) of pregnant cows were evaluated for two-one month trials, in a cross-over design, with respect to supplement intake, using both a liquid molasses and a urea-molasses block supplement. In a second crossover study the effects of adding monensin to the liquid form was explored using two separate groups ($n=15$ for both groups).

RESULTS: The individual intake was recorded by way of an electronic feeding system. More animals visited the feeder each day and reached or exceeded the manufacturer's recommended intake with less variation between individuals with respect to mean daily intake and at a more rapid rate of consumption when supplied with the liquid supplement. There was little difference between the control and the monensin-containing molasses supplement.

CONCLUSIONS: The liquid form of the molasses supplement appears to be promising as a safe method of administering monensin as an anti-coccidial medication via free-choice supplementation.

L10

CONTROL OF FASCIOLA AND GASTROINTESTINAL NEMATODES OF SHEEP IN THE HIGHLANDS OF KENYA.

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The effectiveness of strategic anthelmintic control of liver flukes (*Fasciola*) and gastrointestinal nematodes (GIT) on the performance of ewes and lambs on pasture in the highlands of Kenya was examined. In July 1999, 45 five Corriedale ewes aged between 2 and 3 years were ear-tagged, weighed and allocated to 3 equal treatment groups. Faecal samples taken at this time revealed low levels of nematode EPG but no liver fluke eggs. All animals were then set stocked for 12 months on a paddock endemic for both *Fasciola* and GIT nematodes. Ewes in group 1 were given a combined anthelmintic treatment against *Fasciola* and GIT nematodes during the periods recommended for the control of *Fasciola* in the area (October, February and June). Ewes in group 2 were given the combined treatment 3 weeks after onset of both the short and long rains seasons (November and April, respectively). Those in group 3 were given separate treatments for *Fasciola* (October, February and June) and nematodes (3 weeks after onset of the rainy seasons).

Nematode EPG for ewes in group 1 were higher than in groups 2 and 3 during the rainy seasons. Nematode EPG did not differ significantly between animals in groups 2 and 3. The prevalence of *Fasciola* eggs (number of positive animals) in ewes in groups 1 and 3 remained very low throughout the study period compared to those in group 2. The highest lambing rate for ewes, birth weights and weight gain for lambs were recorded for the group given separate anthelmintic treatments for *Fasciola* and nematodes (Group 3). The results of this study are discussed in relation to the practice of combined anthelmintic treatments to control liver flukes and nematodes in the area.

L12

STRATEGIC USE OF ANTHELMINTICS IN THE CONTROL OF GASTROINTESTINAL NEMATODES IN BREEDING EWES IN A SEMI-ARID AREA IN KENYA

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A study was carried out to evaluate the effectiveness of strategic use of anthelmintics in the control of gastrointestinal nematodes in breeding ewes in a semi-arid area of Kajiado District of Kenya. In May, 1999, forty Dorper ewes were randomly selected from a breeding stock and assigned to two groups. One group was dosed with albendazole at 5mg/kg body weight 2 weeks before mating, 1 week before lambing and during lactation. The other group remained untreated. Faecal nematode eggs per gram (EPG) of faeces and weight gains for all ewes were monitored every 3 weeks for 1 year. Births and birth weights for lambs were also recorded. The egg counts remained significantly lower ($p < 0.001$) in the treated group throughout the study period. The weight gains and twinning rates for ewe and birth weights for lambs were higher for the treated group. The treatments also significantly reduced the peri-parturient rise in egg production in the ewes.

L13

PREVALENCE OF BENZIMIDAZOLE-RESISTANT GASTROINTESTINAL NEMATODES OF SHEEP AND GOATS IN SWITZERLAND

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The prevalence of benzimidazole (BZ) resistant trichostrongylid nematodes was investigated in 109 sheep and goat farms in Switzerland. The larval hatch assay (LHA) and the faecal egg count reduction test (FECRT) were used according to W.A.A.V.P. guidelines. BZ-resistant nematodes were detected in 83% of the farms. Goat farms tended to be affected more often (91%) compared with sheep farms (81%). *Haemonchus contortus* was the predominating resistant species, but resistance was also detected in *Cooperia curticei* and *Trichostrongylus colubriformis*. In 81% of the farms sheep or goats received 2 to 5 anthelmintic treatments per year, whereas single farmers used 12 annual treatments. BZ were the only used anthelmintic class in 70% of the farms. Moving of animals between farms and to common alpine pastures is widely practiced in Switzerland and is considered to be of major importance for the spread of resistant populations. Since 2000 a parasitological survey programme was initiated by the sheep and goat health service which includes regular screening of pooled faecal samples from adults and lambs and also allows the farmer to submit samples for the FECRT.

L15

A STRATEGIC WORM CONTROL PROGRAM CAN ALSO CAUSE ANTHELMINTIC RESISTANCE

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Background: Control of nematode parasites of sheep in Mediterranean climatic zones of Australia depends on a strategic control program, in which sheep are treated in summer when no larvae survive on pasture to re-infect sheep. Although this provides highly effective worm control, a concern is that resistant worms surviving treatment are the major source of subsequent populations. **Methods:** In field trials in Western Australia, the summer drenching strategy was compared to treatments where some sheep in each flock remained untreated, to allow the survival of some non-resistant worms. Worm burdens, sheep weights and changes in resistance to the anthelmintic (ivermectin) were monitored for 10 months after treatment. **Results:** The summer drenching strategy led to a significant increase in resistance to ivermectin in *Ostertagia* compared to the pre-treatment level, but where some sheep were not treated, resistance did not increase. However, worm burdens were significantly lower in summer-drenched flocks, with sheep weight gains up to 10% greater than where some sheep were not drenched. **Conclusions:** The interaction between environmental effects on worm populations and the time of treatments has been confirmed as a potent cause of anthelmintic resistance, which is likely to be of greater importance than an excessive frequency of treatment in some situations. However, to abandon a highly-effective strategic program will often increase losses due to parasites. New strategies which are both sustainable and effective are required in high-risk environments.

L14

AGE-SPECIFIC EXPRESSION OF IVERMECTIN RESISTANCE IN A FIELD ISOLATE OF *HAEMONCHUS CONTORTUS*.

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Background: The larval-development-assay (LDA) is widely used to detect resistance to macrocyclic lactones (MLs) in field isolates of a number of intestinal nematode species, including *Haemonchus contortus*. The assay relies on the fact that resistance of adult parasites to the effects of the drug *in vivo* is associated with a detectable resistance in larvae to the effects of the drug on development *in vitro*. **Method:** A number of *H. contortus* isolates were examined using LDAs and faecal egg count reduction tests (FECRT). The ML-resistant isolates tested included a new isolate (IVPro) which had survived a recent ivermectin capsule treatment of sheep.

Results: The IVPro isolate showed a high level of resistance as assessed by FECRT. Resistance levels were equivalent to the previously described ML-resistant CAVR and Warren isolates. The CAVR and Warren isolates showed 6-12-fold resistance to 2 ML analogues in the LDA relative to a number of susceptible field isolates, however, the IVPro isolate only showed 1.5-2-fold resistance.

Conclusions: The low level of resistance shown by the IVPro isolate at the larval stage makes it barely distinguishable from susceptible isolates, questioning the likelihood of its detection by the LDA method in diagnostic laboratories. This will have a significant impact on current ML-resistance-monitoring efforts if isolates of this type become widespread.

L16

LOW FREQUENCY OF ANTHELMINTIC TREATMENTS DOES NOT PREVENT FROM NEMATODE RESISTANCE IN SHEEP AND GOATS: FOUR REGIONAL EXAMPLES IN FIELD CONDITIONS.

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Nematode resistance in a farm may be introduced from other farms. The introduction of untreated animals and use of pasture in common to several flocks are ideal for transportation of resistance from one site to another. Nematode resistance may also appear on the farm. Frequently repeated treatments, use of the same class of anthelmintics and under-dosing, are among the main cited reasons for the development of resistance. The role of selective factors (treatments) when resistance is present on the farm has not been fully investigated. All the cases we report have in common a low frequency of treatments and we will evaluate the role of alternative risk factors on the establishment of resistance. Two studies were performed on dairy goats in France (12 Touraine-intensive and 16 Quercy-extensive farms). They did not introduce infected animals since the establishment of the farms (closed farms), treated less than 3 times a year on average and used nearly exclusively benzimidazoles. The meat sheep flocks (Auvergne) were "closed, treated alternatively with benzimidazoles and avermectins (ewes once a year and lambs on 2 to 3 occasions). The 10 farms in the Middle-Atlas of Morocco were "open" (use of pastures also grazed by other flocks, and they treated once to twice a year using tetramisole and additionally benzimidazoles. The putative explanations for resistance are : 1) Touraine: use mostly of benzimidazoles, animal dosed before turn-out (which seeds the pasture with resistant worms) 2) Quercy: exclusive use of benzimidazoles for more than 10 years, animals dosed before turn-out, 3) Auvergne: late turn-out (e.g. infective larvae on the pastures are nearly absent at turn out), animals dosed before turn-out (they seed pasture with resistant eggs only), 4) Middle-Atlas: pasture grazed in common by many flocks (if resistance appears in one farm it will spread in the others), use of non-contaminated pastures possibly after treatment (fallow-fields). The building up of resistance is probably linked to the timing of treatments.

L17 A SURVEY ON THE OCCURRENCE OF ANTHELMINTIC RESISTANCE IN GASTROINTESTINAL NEMATODE OF GOATS IN MOZAMBIQUE

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A survey in order to study the extent of anthelmintic resistance was conducted in Maputo and Gaza, two of the ten provinces of Mozambique, during February and march, 1999.

A total of 12 flocks, 6 in Maputo and 6 in Gaza Provinces, were surveyed. The faecal egg count reduction test (FECRT) was used to access the efficacy of three anthelmintics most used in Mozambique, namely albendazole (Valbazen®, Pfizer), fenbendazole (Ecomintic®, Eco) and levamisole (Levicon®, Milbrow®).

The FECRT was calculated according to two different methods, and varied according to the method used. Resistance to the benzimidazoles was detected in two flocks, one in Maputo and one in Gaza, and to levamisole in four flocks, three in Maputo and one in Gaza using the formula recommended by the World Association for the Advancement of Veterinary Parasitology (WAAVP), (Coles, Bauer, Borgsteede, Geerts, Klei, Taylor & Waller, 1992). Using the calculations of Dash, Hall & Barger (1988), resistance to the benzimidazoles was only detected in one flock in Maputo, and no resistance to levamisole was detected.

The twelve flocks surveyed were too few and no conclusions can be made on the prevalence of anthelmintic resistance in goat farms in Mozambique as a whole. Therefore, an extensive survey involving more farms and other provinces at national level is needed. However, this study gave evidence that anthelmintic resistance in nematode parasites of goats is an emerging problem, to which special attention should be paid.

L19 THE IMPORTANCE OF PRODUCING MANGE-FREE PIGS AT WEANING

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Background: While the relationship between hypersensitive mange and reduced productivity is well established (Cargill and Davies, 1999), the relationships between the presence of mites at weaning and subsequent productivity and clinical disease have not been investigated.

Method: Two groups of 60 infected pigs, housed 5 to a pen, were studied from weaning (21 days) to slaughter (147 to 161 days). Pigs were examined at 21, 37, 76, and 142 days of age and the size of ear and body lesions, the presence of mites and eggs in ear lesions, and rubbing and scratching episodes recorded. Pigs were weighed at 142 days and assessed for dermatitis at slaughter. One group was medicated with IVOMEC® Premix for 7 days post-weaning. The other group remained unmedicated.

Results: Growth rate and days to slaughter in unmedicated pigs were correlated ($P < 0.001$) with the presence of mites and eggs in individual pigs at 21 days but not at 142 days. However, the presence of mites on at least one pig in a pen at day 142 was correlated ($P < 0.001$) with mean pen growth rate and days to market. Growth rate and days to market were also correlated ($P < 0.001$) in individual unmedicated pigs with body lesion and rubbing scores at 142 days, and dermatitis at slaughter, but not in medicated pigs. Medicated pigs were negative for mites at 142 days and did not develop hypersensitive mange. They also grew significantly faster ($P < 0.0001$) than unmedicated pigs.

Conclusion: The results confirm the importance of producing pigs that are free of mites, or eradicating mites at weaning, to achieve maximum production.

Acknowledgment: The support of Merial is gratefully acknowledged.

Reference: Cargill, C & Davies, P (1999) *External Parasites IN Diseases of Swine*, Eds Straw B, Mengeling W, D'Alaire S and Taylor D. (p 669-684) Iowa University Press.

L18 INDICATIONS FOR THE INCREASE IN PREVALENCE OF CONDEMNED PIG LIVERS.

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Background: In a continuous attempt to minimize condemnations of animal carcasses and/or organs, the prevalence of condemned livers of slaughter pigs has nevertheless been doubled during the nineties to well over 10 %. *Ascaris suum* (Ascaris) is mainly responsible for condemnation of livers. Infestation is visible at the surface of the liver and/or after incision by light-coloured connective tissue spots, the so-called *white spots*. Ascaris affects mainly piglets and growing-finishing pigs. In this cooperative study at 4 slaughterhouses, research was conducted to find explanations for the increased prevalence of condemned livers in dutch slaughter pigs.

Method: Per slaughterhouse 12 fattening pig farms (fpf's) with a high (H) and 8 fpf's with a low (L) prevalence of condemned livers were randomly selected out of resp. 50 fpf's with the highest and 30 fpf's with the lowest prevalence. The 48 H farms and 32 L farms were asked (via inquiry) in detail about housing, farm management, hygiene, anthelmintic program and pig introductions from outside the herd.

Results: Concrete fences and fully (concrete) slatted floors were used more frequently in H-farms than in L-farms. H-farms introduced more often piglets from outside the herd and fed more often via a single space feeder. Most H-farms applied anthelmintics on the feed or via the water supply, whereas the L-farms predominantly applied anthelmintics by injection.

Conclusion: The prevalence of condemned livers appears to be affected by the route of anthelmintic treatment and specific on-farm conditions.

Pig farmers and vets have to evaluate the very situation in every fpf.

Acknowledgement: Authors thank CBS, slaughterhouses and farmers for enabling this study.

L20 UPDATE ON DUTCH 'MANGE FREE' CERTIFICATION PROGRAM FOR PIG FARMS.

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Background: AHS runs a nationwide mange (*Sarcoptes scabiei* var. *suis*) certification program in 520 pig farms. Eradication is achieved by 2 injections of *all* pigs with ivermectin, but other methods (in feed, one shot) are accepted under strict preconditions. AHS certification protocol is based on a sum of single parameters, controlled 3 times a year. AHS ran in 1999 a *Sarcoptes* transmission experiment with mange free pigs (17 kg) to study results of AHS Elisa versus earscrappings (scrapes), effect on production and reduction of costs for certification.

Method: 80 pigs were divided in a control and infection group, which was infested by 3 seeder pigs with mange. Controls were conducted: scrapes, dermatitis, scratch-index, AHS Elisa and data of production.

Results: scrapes were positive for max. 25% and only 19 of 40 pigs were ever positive: low sensitivity. A positive AHS Elisa ranged from 5% (D42) to 74% (D112). Growth in controls was significantly ($P < 0.005$) better (41 gr/day) and FCR was 2% lower.

Conclusions: AHS Elisa on *Sarcoptes* antibodies of *preselected* pigs can replace the old AHS protocol with substantial reduction of costs. Mange causes a loss of 1.5 euro/pig i.e. pay back is only ½ year!

References: 1. IPVS98 (Birmingham, p. 121-123). 2. IPVS2000 (Melbourne, p.267). 3. J.Vet.Parasit. 89+93. 4. Vet.Quart., July 2000.

Acknowledgement: The support of Merial is gratefully acknowledged.

L21

CHARACTERISATION OF A TROPICAL ISOLATE OF THE NEMATOPHAGOUS FUNGUS *D. FLAGRANS*

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Background: Anthelmintic resistance has emerged as a major problem in the control of nematode parasites of small ruminants in Malaysia. In response to this emergency, alternative non-chemical approaches are being investigated and amongst these was the search for a local isolate of the nematophagous fungus *D. flagrans*, which could be potentially exploited in this regard.

Method: A screening procedure of more than 1,000 dung samples from a range of domestic, pet and zoo animals, for the presence of nematode destroying fungi was undertaken. Many nematophagous fungal species was identified, but only one isolate of *D. flagrans*, derived from faeces from a sheep farm in Gopeng Malaysia, was made. Testing for growth on a range of cheap and commonly available plant-derived substrates was carried out. Both *in vitro* and *in vivo* testing for nematophagous capacity was conducted.

Results: The most suitable media were moistened, autoclaved unprocessed whole grain padi rice, processed millet grain, or processed wheat grain. The isolate was shown to exhibit voracious trapping of infective larvae of *Haemonchus contortus* when tested as a culture growing on corn meal agar and on autoclaved sheep faeces. Furthermore, *in vivo* trials in which sheep harbouring infections of *H. contortus* were fed spores of this isolate of *D. flagrans*, showed profound reductions (1.5×10^6 spores) to complete elimination ($2.5 - 6.25 \times 10^6$ spores) of infective larvae from faecal cultures.

Conclusion: This tropical isolate of *D. flagrans* has the potential to be exploited as a bio-control agent of anthelmintic resistant nematode parasites of sheep and cattle in Malaysia.

L23

ENVIRONMENTAL IMPACT OF *DUDDINGTONIA FLAGRANS* DEPLOYMENT IN SHEEP FAECES.

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Background: The nematophagous fungus *Duddingtonia flagrans* has great potential for the biological control of nematode parasites of livestock but the environmental effects of introduction of high concentrations of this fungus on the soil-pasture ecosystem are unknown. The impact of *D. flagrans* deployment in sheep faeces was investigated in an improved pasture system.

Method: Sheep faeces containing *D. flagrans* and fungus-free faeces were placed on separate pasture plots on four different occasions (Autumn, Winter, Spring and Summer). At 0, 1, 2, 4, 8, 16 and 24 weeks following faecal deposition, estimates were made of the persistence and dispersal of the fungus and its impact on soil nematode and microarthropod numbers.

Results: For all four series, *D. flagrans* was not found outside the point of faecal deposition indicating that *D. flagrans* did not spread in the horizontal plane. *D. flagrans* was detected at the point of faecal deposition while intact faecal material was present and persisted for 8 to 24 weeks in the soil under the point of deposition and persistence appeared to be dependent on season. Numbers of soil nematodes and microarthropods at the point of faecal deposition varied considerably between samples but there was no negative effect of *D. flagrans* presence on these organisms or on faecal decomposition or the presence of other trapping fungi.

Conclusion: The results of this study indicate that there are no detectable negative environmental impacts of *D. flagrans* use in a typical improved pasture system. Dispersal of *D. flagrans* is limited to the area of application, persistence is short-term and there appears to be no negative impacts of use on organisms associated with the soil/pasture interface.

Acknowledgement: This study was partly funded by International Animal Health Products Ltd, Australia.

L22

ASSESSMENT OF GENETIC VARIATION AMONG *DUDDINGTONIA FLAGRANS* ISOLATES

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Background: Much interest has arisen in recent years in the biological control of animal parasitic nematodes using the nematophagous fungus *D. flagrans*. When embarking on such activities most researchers attempt to isolate a local isolate of *D. flagrans*. It is believed that a local isolate is better suited to the climate of the local area. It also avoids legal complications associated with using an imported isolate. For these reasons, genetic comparisons were undertaken to assess diversity.

Method: Forty-two *D. flagrans* cultures from various countries were used. Key genes were DNA sequenced. Random clones were generated by digesting the DNA from one of the *D. flagrans* isolates with BamHI and PstI. Fragments of sizes between 500-800 bp were isolated, cloned into the plasmid vector pUC19 and primer pairs were designed to amplify a region of ca 500-600 bp in length. AFLP analysis was conducted using the restriction enzyme pair Eco RI/Mse I (Tru I) to cut the DNA, annealing of adaptors, pre-amplification with designed primers and selective amplification with 5 different primer pairs.

Results: Of the 2205 base pairs of DNA sequenced, including 354 base pairs of intron sequence, only 7 base pair differences could be detected. The total number of amplified fragments generated from AFLP analysis was 153, which yielded 121 informative sites.

Conclusions: Little genetic variation could be detected among the *D. flagrans* isolates included in this study. There was no clear clustering of isolates according to geographic origin.

L24

DEVELOPMENT OF AN *IN VITRO* ASSAY TO TEST THE EFFECT OF TANNINS AGAINST GI NEMATODES OF RUMINANTS

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A direct anthelmintic effect of condensed tannins on GI nematodes may be one of the reasons behind the reduction of the detrimental effects of parasitism observed in ruminants, fed on tanniniferous forages. The aim of this study was to apply an *in vitro* methodology to investigate the anthelmintic effect of tannins on the migration ability of various GI nematodes of ruminants.

Larval Migration Inhibition assays were applied to test condensed tannin extracts from *Acacia* spp, *Casuarina* spp and *Leucaena* spp against ensheathed and exsheathed L3 of *Ostertagia*, *Teladorsagia*, *Haemonchus* and *Trichostrongylus*. L3 were incubated in tannin solutions (ranging from 0 to 40,000 ppm) for 2, 8 and 24 hours at 37°C. LD₅₀ values and confidence intervals (95%) were calculated for each tannin.

L3 reduced their migration ability following incubation in tannin solutions. This effect was stronger in higher concentrations of the tannin solutions. The effect depended significantly on the origin of tannin, as incubation in *Acacia* spp extracts caused the most severe inhibition of the migration ability. Long incubation time and the presence of the sheath decreased the LD₅₀ value. *Ostertagia* was the most susceptible worm to tannin effect and *Teladorsagia* the most resistant.

Acknowledgement: This work was carried out via a fellowship under the OECD Cooperative Research Programme: "Biological Resource Management for Sustainable Agricultural Systems".

L25

BAITED TRAPS, A NOVEL APPROACH TO CONTROL *DERMANYSSUS GALLINAE*

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Background: In Sweden, the poultry red mite, *Dermanyssus gallinae*, is the only haematophagous mite of poultry. The parasite affect wild as well as domestic birds and after blood meals it spends most of its life off the host in cracks and crevices where mating and reproduction occur. Therefore, large populations of mites can rapidly be established in poultry production premises. Thus, development of effective measures to control *D. gallinae* are needed.

Method: Cardboard traps (1000x40x3; 160x140x3 and 100x70x3 mm), baited with metrifonate (2%) were placed out of reach for hens in two different floor systems for layers. The parasite populations were monitored by collecting mites in untreated traps (100x70x3 mm). Both monitoring- and baited traps were replaced once a week.

Results: A 97% and 95% reduction of mites, respectively, were recorded.

Conclusion: As *D. gallinae* spends most of its time off the host, alternative strategies where control measures are directed toward the resting sites of the mite should be taken into consideration. The restricted exposure of the active ingredient in this control concept was considered to be environmental friendly and to minimise the risk to obtain pesticide residues in eggs produced. Potential biological control agents may also be incorporated into such traps.

Acknowledgement: Funding by the Swedish Board of Agriculture.

L26

ALTERNATIVE HELMINTH CONTROL METHODS AND INTERNAL PARASITE BURDENS IN DONKEYS

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Method: The helminth burdens and biodiversity of 9 randomly selected donkeys (*Equus asinus*) were examined after necropsy. Prior to necropsy, 8 of the animals were each subjected for a 16-month period to 1 of 3 management interventions (monthly fecal removal, pre-winter moxidectin treatment and a combination of monthly fecal removal and a pre-winter moxidectin treatment) or a control group. Quantitative samples were collected from their gastrointestinal tracts for helminth recovery. The intestinal walls were examined with transmural illumination and thereafter digested with a HCl-peptic method for the enumeration of the mucosal larval stages of the cyathostomes.

Results: Estimated worm burdens ranged from 3,831 to 29,501 and 37 helminth species were recorded. The latter include a previously unknown cyathostome species, *Cylicocycylus asinus* sp. n. *Cyathostomum montgomeryi* was the most abundant cyathostome and *Triodontophorus hartmannae* the most abundant large strongyle. The large strongyles were less abundant compared to the cyathostomes, and both groups preferred the ventral colon. Although both the monthly removal of faeces from the camps and pre-winter treatment resulted in a reduction in the total number of luminal and encysted cyathostome worms, the combination of these two interventions was the most effective. In addition, the latter management system had the largest impact on the total helminth parasite load in the donkeys

L27

A STRATEGIC APPROACH TO ENDOPARASITE CONTROL ON A THOROUGHBRED STUD

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Background: For many years parasite control on a leading UK thoroughbred stud consisted of the monthly administration of fenbendazole, pyrantel and ivermectin in fast rotation. In 1996 the stud changed to monthly administration of ivermectin only. However, the 1996 crop of weanlings was not considered to be in optimal condition and so a new parasite control program was implemented in mid 1997 and monitored.

Method: Non-benzimidazole anthelmintics were used during the summer grazing season at 6-8 week intervals and strategic treatments for encysted cyathostome larvae, tapeworms and bots given at the appropriate time of the year. All incoming horses were treated with larvicidal doses of fenbendazole followed by a double dose of pyrantel. Faecal egg counts (+culture) were monitored at the time of treatment and again 10-12 days later if positive. Pasture larval counts were done monthly and meteorological data collected. Clinical chemistry analyses (weanlings) was done in the autumn.

Results: A small number of the resident mares shed low numbers of eggs mainly during the summer months in the first 2 years of the study but visiting mares were an important source of infection on both years with individual counts of >1800 epg being recorded. Low level intermittent egg shedding was observed in a few of the resident youngstock during the first autumn and again in the spring but following slight modification of the control program the numbers of youngsters with positive egg counts decreased further over the subsequent year.

L28

PREVENTION OF HEARTWORM INFECTION AND FLEA CONTROL IN DOGS USING IVERMECTIN AND IMIDACLOPRID TOPICAL SOLUTION (Advantage® DUO)

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Purpose: The trials were performed with a combination of ivermectin for canine heartworm prevention and imidacloprid for flea treatment and control. The results for a single low volume topical formulation are reported.

Method: Dose confirmation studies with concentrations between 40 and 320 mcg/ kg body weight (b.w.) ivermectin and 10 mg/ kg b.w. imidacloprid applied topically were performed. Dogs were artificially infected 34 days prior to treatment with 50 L3 larvae of *Dirofilaria immitis* each. The dogs were euthanased and examined for adult heartworm infection at 154 dpi. In a second dose confirmation study dogs were artificially infected with L3 larvae and treated with the test formulation 30 or 45 dpi. Furthermore these dogs were infested weekly with 100 unfed adults of *Ctenocephalides felis*. Flea comb counts were performed 1 dpt. as well as 7, 14, 21, 28 and 35 dpt., one day after weekly reinfestations.

Results: The combination of ivermectin and imidacloprid was 100% effective in all dosages between 40 to 320 mcg/ kg b.w. to prevent heartworm disease in dogs. Against 30 or 45 day old *D. immitis* larvae, the test product was 100% effective. The flea reduction was 99% through to day 28. At day 35 the flea reduction was 98%. The combination of 10% imidacloprid (10mg/ kg b.w.) and 0.08% ivermectin (80 mcg/ kg b.w. (Advantage® DUO) applied monthly in a single spot, was highly effective to prevent heartworm disease and treat and control flea infestations on dogs.

L1 p

A GLOBAL GIS NETWORK FOR CONTROL OF SNAIL-BORNE DISEASE

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Background: At an April, 2000 team residency sponsored by the Rockefeller Foundation in Bellagio, Italy, a plan was conceived to create a global network of collaborating medical and earth scientists dedicated to the development of computer-based models for control of schistosomiasis and veterinary snail-borne diseases.

Method: Models will be assembled using GIS methods and data on earth observing satellites, global climate, disease prevalence, snail intermediate hosts, and key environmental features.

Results: The plan calls for research collaboration, data sharing, new members, and model access to other medical workers. Compatible formats, software, methods and data resources will enable seamless incorporation of regional results into a global model. A central resource group is linked to regional sites in East, South and West Africa, Latin America and Asia. An Internet site (www.gnosisGIS.org) allows interaction as a 'virtual research group'.

Conclusion: The network will provide assistance on use of GIS in national control programs and predictions on the health impacts of water development projects and climate variation.

L2 p

VICH HARMONIZATION OF VETERINARY ANTHELMINTIC EFFICACY GUIDELINES.

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The VICH (International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products) Anthelmintic Working Group (WG) includes veterinary pharmaceutical and government regulators representing the European Union, Japan, US and Australia and New Zealand. VICH activities are carried out under the auspices of the Office International des Epizooties (OIE). The WG developed a main document entitled "Effectiveness of Anthelmintics: General Recommendations" (EAGR) that describes the basic aspects that were identified as common in all the studies used to demonstrate efficacy of veterinary anthelmintic drugs in target species. The EAGR document was complemented by species-specific guidelines for bovine, ovine, caprine, equine, porcine, canine, feline, and poultry. An important definition in the EAGR is the standard of effectiveness for an anthelmintic: A compound should be declared effective in principle when product effectiveness against each parasite declared on the labeling stands at 90% or above, based on geometric mean worm counts, and there is a statistically significant difference in parasite burden between control and treated animals. These guidelines will be reviewed regularly based on changing science and updated as required to ensure that they remain contemporary. In the future, the WG believes that other efficacy parasitic guidelines should be harmonised e.g. ectoparasitocides.

251 Words

1770 Characters with spaces.

L3 p

WHY A NATIONAL FORUM FOR VETERINARY HELMINTHOLOGY IN SOUTH AFRICA?

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Background: In response to a dynamic environment, workers and stakeholders in the field of veterinary helminthology in South Africa chose to co-ordinate efforts within research, training, technology transfer and marketing.

Method: This occurred in a series of workshops during 1999-2000. Priority areas were identified and specific objectives set to be achieved in addressing these constraints and a framework of action plans developed. Two themes running through these activities are the resource-poor and commercial agricultural systems.

Results: A report summarizing the proposed framework/process was circulated during 1999 to more than 200 stakeholder organizations and identified persons. During April 2000 a two-day major workshop, a National Forum, was held which was attended by 46 individuals from 30 organizations (academic, government at all levels, industry, international donors, non-governmental organizations).

Conclusion: This workshop developed a structure to coordinate efforts leading up to the present stage of the process. A continuation committee drafted a Working Document, which is available at www.worms.org.za

L4 p

PARASITES OF IMPORTED CATS IN ICELAND

Eydal M., Skirnisson K., Richter S.H.

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Background: Import of cats to Iceland has been prohibited or restricted since 1926. In 1989 the ban was lifted. From 1989-2000 altogether 236 cats from at least 18 countries have been imported and subjected to 6-12 weeks of quarantine period. During quarantine each cat is treated three times against helminths, at least once against ectoparasites and against protozoans if they are found.

Method: Faecal samples, collected from the cats at the beginning and at the end of the quarantine period, were examined for intestinal parasites using formalin-ethyl acetate sedimentation technique. Ectoparasites detected during isolation have also been examined.

Results: At least seven parasitic species were found. Endoparasites: *Giardia* sp. (in 6 cats), *Isospora felis* (2), *Ancylostoma* sp./*Uncinaria* sp. (1), *Toxocara cati* (6), *Toxascaris leonina* (1) and *Opisthorchis felinus* (1). Ectoparasite: *Ctenocephalides felis*. In addition, cats outside the quarantine station were found parasitised with the non-endemic mite *Cheyletiella parasitovorax* after contact with imported cats.

Conclusion: The species *Ancylostoma* sp./*Uncinaria* sp., *T. leonina* and *C. parasitovorax* had not been confirmed in or on the native cat population. Measures against parasites during quarantine appear to have been successful in most cases, and only one species (*C. parasitovorax*) is suspected to have been transmitted to the native cat population.

L5 p

PARASITES OF IMPORTED DOGS IN ICELAND Eydal M., Richter S.H., Skirnisson K.

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Background: Import of dogs to Iceland has been prohibited or restricted since 1909. In 1989 the ban was lifted. From 1989-2000 altogether 608 dogs from at least 27 countries have been imported and subjected to 6-12 weeks of quarantine period. During quarantine each dog is treated three times against helminths, at least once against ectoparasites and against protozoans if they are found.

Method: Faecal samples, collected from the dogs at the beginning and at the end of the quarantine period, were examined for intestinal parasites using formalin-ethyl acetate sedimentation technique. Ectoparasites detected during isolation have also been examined.

Results: At least 16 species were found. Endoparasites: *Giardia* sp. (in 36 dogs), *Isospora canis* (2), *I. bahiensis* (2), *I. rivolta* (4), *Sarcocystis* sp. (1), *Ancylostoma* sp. (3), *Capillaria aerophila* (1), *Strongyloides stercoralis* (2), *Toxocara canis* (26), *Toxascaris leonina* (7), *Trichuris vulpis* (4), *Uncinaria stenocephala* (1) and an unidentified digenean (2). Ectoparasites: *Ctenocephalides felis*, *Ixodes ricinus* and *Rhipicephalus sanguineus*. In addition, the non-endemic species *Cheyletiella yasguri*, *Demodex canis* and *Trichodectes canis* were found on dogs soon after they finished their quarantine period or on dogs which had been in close contact with imported dogs.

Conclusion: Most of the parasite species found had not been confirmed in or on the native dog population. Measures against parasites during quarantine appear to have been successful in most cases. However, as a result of this import, it seems likely that at least two parasite species (*C. yasguri*, *D. canis*) have been transmitted to the native dog population.

L6 p

DIFFERENCE ON THE WEIGHT GAIN IN CALVES TREATED WITH FOUR ANTHELMINTICS

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Background: Gastroenteric nematodes have a strong economic impact in cattle all over the world, due to the effect in the weight gain. The objective was to compare the effect of anthelmintic treatments on the weight gain and the egg output of gastrointestinal nematodes.

Methods: Four groups (G) each of 12 calves were weighed and dewormed every 56 day during six month. G-1 with fenbendazole, G-2 with albendazole sulfoxide, G-3 with ivermectin, G-4 (control) with albendazole. Faeces were analyzed by the McMaster technique. Coprocultures were made to identify genera on third stage larvae.

Results: The difference on weight gain against the control group (G-4) was: G-1, 10.9 kg, no statistical difference was observed; G-2, 28.0 kg ($P \leq 0.05$); G-3 23.7 kg ($P \leq 0.05$). The percentage of rate reduction on the mean of eggs per gram (epg) versus the control group was: G-1, 32 %; G-2, 12 %; G-3, 55 % and G-4 0 % (control). The genera identified were: *Haemonchus*, *Chabertia*, *Strongyloides*, *Cooperia*, *Trichostrongylus* and *Toxocara* eggs.

Conclusion: The effect of treatment with ivermectin and albendazole sulphoxides showed significant differences on the weight gain compared with fenbendazole and fenbendazole.

Acknowledgement: To the staff of the Experimental Centre "Las Margaritas of Hueytamalco, Puebla, and Intervet México, S.A. de C.V.

L7 p

CONDUCTING ON-FARM TRIALS IN HELMINTH CONTROL AMONGST SMALLHOLDER FARMS-PROBLEMS AND POSSIBLE SOLUTIONS FROM KENYA.

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On-farm trials in Kenya to evaluate the merits of a strategic drenching regime against the existing control measures showed that it was difficult to get statistically significant sample sizes within individual farms because of small flock sizes. Increasing the number of farms resulted in some farms falling under different ecological zones. Differences in farm management existed especially where animals were communally grazed. The many variables rendered production data difficult to analyse and interpret. It was equally difficult to achieve a synchronised reduction in pasture larval contamination as anticipated in the trial farms. Unlike in on-station trials, it was not possible to have untreated control groups since farmers in different treatment groups could share information on the treatment regimes owing to the close proximity of the trial farms. The large number of smallholder farms (up to 160) that was required for realistic statistical comparisons clearly conflicted with the limits of logistics and funding.

Possible solutions identified in these studies included use of clusters of smallholder farms that are far apart in different treatment groups. Communally grazed flocks can either be excluded completely in the trials or if that method of grazing is predominant, entire villages or clearly designated locations could be used in different treatment groups. Even with the above, the use of production data (e.g. growth rate) need to be handled with great caution. It is suggested that extension messages, especially those to do with helminth control, should address the unique circumstances of smallholder farmers practising different husbandry

L8 p

A QUESTIONNAIRE SURVEY OF THE MANAGEMENT AND USE OF ANTHELMINTICS IN CATTLE AND GAME ON MIXED FARMING SYSTEMS IN ZIMBABWE

Madzingira, O., Mukaratirwa, S., Pandey, V.S., and Dorny, O.

Background: Cattle and game rearing in a mixed farming system, officially commenced in 1985, when government permitted farmers to introduce and rear game species on private farms (Knottenbelt, 1990).

Methods: A survey of the management of mixed farming of cattle antelope and use of anthelmintics on eleven randomly selected mixed beef cattle and game farms around Harare was conducted between August and December 1999 through a self administered questionnaire.

Results: Seventeen antelope species ranging from the smaller duiker (*Sylvicapra grimmia*) to the enormous eland (*Taurotragus oryx*) occurred on the farms. The impala (*Aepyceros melampus*) was the common antelope species on the farms. Results from the survey on the deworming of game indicate that farmers perceive helminth infections in antelope to be important.

Acknowledgements: We wish to thank the DGIC, Belgium for financial support.

L9 p

Fitness studies with an ivermectin-resistant field isolate of *Trichostrongylus colubriformis*
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Background: This study was conducted to investigate the biological fitness of an ivermectin-resistant strain of *Trichostrongylus colubriformis* by comparing it with two ivermectin-susceptible field isolates.

Methods: Forty-five Romney lambs were randomly allocated into three groups of 15 animals each. The lambs in each group were infected with 15,000 larvae of either an ivermectin-resistant strain or one of two ivermectin-susceptible field strains of *T. colubriformis*. The efficiency of development of eggs to larvae on pasture was estimated from faecal samples collected from five animals at random from each of the three groups of sheep at 22 days post infection (DPI) and 24 DPI. The survival of infective larvae from the same five animals in each group, was monitored at 10°C, 20°C, 25°C and 30°C at weekly interval for 17 weeks and then at 4 weekly interval for a further 20 weeks. Five animals from each group were slaughtered at 28, 42 and 84 DPI. At each sampling, faecal cultures from individual animals were conducted in triplicate and worm counts were performed on 10% aliquots of the intestinal contents. In addition, the lengths of 20 male and 20 female worms from each animal were measured. The number of eggs in each female uterus was also counted. Faecal egg counts were also monitored at weekly intervals from 21 to 84 DPI.

Results: There was no significant difference ($p > 0.05$) in the infectivity, survivability of adult worms, fecundity or development of eggs to larvae under laboratory and natural conditions between the ivermectin-resistant strain and the two ivermectin-susceptible field isolates. The survivability of the ivermectin-resistant larvae was intermediate between the two ivermectin-susceptible field isolates at 10°C, 20°C, 25°C and 30°C under laboratory conditions.

Conclusions: The ivermectin-resistant strain of *T. colubriformis* is equally fit as the two ivermectin-susceptible field isolates.

high producers have most worms

Hoste-Charlier 1993 Am Vet Res 54:1886-1893
Hoste et al 1999 INRA Prod An 12:377-381

L11 p

EFFICACY OF TARGETED APPLICATION OF ANTHELMINTICS TO CONTROL TRICHOSTRONGYLOSIS IN DAIRY GOATS.
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Resistances to anthelmintics (AHs) in nematodes of the digestive tract is a major concern in domestic ruminants and particularly in goats. The targeted administration of AHs has been proposed as one possible solution to combine control of infections and reduced selection for AH resistances. The efficiency of selective treatments to control parasitism in dairy goats was previously examined in experimental station. The drenches were restricted to goats in first lactation and to adults with the highest level of milk production. In this study, we assessed the method in farm conditions. Overall, 16 farms were surveyed for 2 years, in 3 main French areas of goat production. Comparison was made between year 1 (all animals within a flock were treated) and year 2 (treatments restricted to high producers and goats in 1st lactation). These 2 treated groups represented 66% of each flock. On each farm, the evolution of trichostrongyle infections were compared between the 2 years by individual faecal egg counts, 4 times per year. The number of annual treatments and the mean annual milk yield per flock were recorded. The passage from systematic to selective treatment 1) do not lead to any increase in the mean level of infection 2) was not associated with any increased frequency of treatment 3) do not induce any decrease in mean milk production (yr1:805 l; yr2: 835 l). These results suggest that selective AH application during grazing seasons might provide sufficient control of trichostrongylosis while contributing to delay the onset of AH resistance.

L10 p

COST-BENEFIT OF STRATEGIC ANTHELMINTIC TREATMENTS OF CALVES IN BRAZIL

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Background: Milk cattle breeders in the state of Minas Gerais have serious money losses with nematode infection of calves. The main parasites are *Cooperia*, *Haemonchus*, *Oesophagostomum*, *Trichostrongylus* and *Bunostomum*, in this sequence, and there is a seasonal variation in the infections, with two peaks: one in the beginning and the other in the end of the rainy season. In order to control the parasitoses, two different strategic anthelmintic treatments were tested:

Method: Treatment a: Ivermectin 200µg/kg was administered 3 times/year: November (beginning of rainy season) April (end of rainy season) and August (middle of dry season).

Treatment b: Ivermectin 200µg/kg was administered 2 times/year: November and April.

For the evaluation of the cost-benefit of the treatments, it was calculated the weight gains, the EPG and the deaths that occurred in each Group. A mathematical formula was used to calculate the final data of the cost/benefit.

Results: The group with 2 anthelmintic treatments got the better results. This treatment should be one of the main choices for the cattle in this region

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L12 p

IMPACT OF NUTRITION ON THE PATHOPHYSIOLOGY OF ANIMAL TRYPAOSOMIASIS.

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Trypanosomiasis is a major veterinary problem over much of sub-Saharan Africa and is frequently associated with under nutrition. There is growing evidence that nutrition can have a profound effect on the pathophysiology features of animal trypanosomiasis. These features include anaemia, pyrexia, body weight changes, reduced feed intake and diminished productivity including reduced draught work output, milk yield and reproductive capacity. Anaemia is a principal characteristic of trypanosomiasis and the rate at which it develops is influenced both by protein and energy intakes. Pyrexia is associated with increased energy demands for maintenance which is ultimately manifested by reductions in voluntary activity levels and productivity. Weight changes in trypanosomiasis are markedly influenced by the levels of protein intake. High intakes allow infected animals to grow at the same rate as uninfected controls providing energy intake is adequate whilst low energy levels can exacerbate the adverse effects of trypanosomiasis on body weight. This paper presents a series of pathophysiological observations in sheep and goats infected with trypanosomes and given various levels of protein and energy intakes.

EFFICACY OF TARGETED APPLICATION OF ANTHELMINTICS TO CONTROL TRICHOSTRONGYLOSIS IN DAIRY GOATS.



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INTRODUCTION / OBJECTIVES of the STUDY

The control of gastrointestinal trichostrongylosis in small ruminants is now severely impaired by the increasing development of anthelmintic resistances. The prevalence of anthelmintic resistance is particularly high in dairy goats. This is due to several factors identified as favouring the development of anthelmintic resistance, i.e. the high frequency of treatments, the restricted choice of anthelmintic family during lactation in order to avoid milk residues and repeated underdosing due to the lack of knowledge on the peculiarities of drug metabolism in goats. Therefore, there is an urgent need in this host species for alternative or complementary solutions to anthelmintics and also for methods to improve the use of the drugs currently available in order to preserve their future efficiency.

Recommendations to reduce the selection pressure for anthelmintic resistance include a reduced frequency of treatments, the respect of appropriate dose and alternation of drugs. Another recommended option is to prefer selective treatments instead of systematic ones. The principle of selective treatment is to target exclusively the most infected animals within a flock. By diluting the genes of resistance in the nematode populations, the method contribute to slow down the spread of resistance. However, one of the main difficulty is to identify the most susceptible animals within the flock. Assays of targeted applications of anthelmintics have been conducted in horses, based on the results of coproscopical examinations (Krecek et al, 1994). It has also been developed in South Africa, based on clinical signs (the FAMACHA system) to reduce resistance to anthelmintics in the haematophagous species, *Haemonchus contortus* (Vatta et al, 1999; 2001).

In dairy goats, we proposed previously to apply selective treatments within a flock to goats in first lactation plus to high producers, as these animals were identified repeatedly as highly susceptible to infection (Hoste et Chartier, 1993; Hoste et al, 1999). A previous study conducted on an experimental flock showed that selective treatments were as effective as a systematic one to control gastrointestinal infection and allow a similar level of milk production (Hoste et al, 1999). The aims of the current study were to examine whether similar conclusions could be drawn in farm conditions.

MATERIALS and METHODS

The survey was conducted for 2 years in 15 dairy goat farms, from 3 main areas of goat production in France i.e. South-East (5 farms) South-West (4 farms) and Centre (6 farms) (see Map). The goats were using pastures under different epidemiological conditions for nematode parasitism.

During year 1, systematic treatments were applied to the whole flock.

In year 2, targeted treatments during the grazing season were restricted to 2 subgroups: the goats in first lactation and the goats with the highest level of milk production. All together, both subgroups represented 2 thirds of the flock. The treatments were benzimidazoles with no withdrawal period for milk (fenbendazole, febantel or oxfendazole).

During lactation, which corresponded approximately to the grazing season, 50 adult goats per farm were sampled individually on 4 dates during each year, i.e. at the beginning and the end of spring, at the beginning and the end of autumn. Depending on the farm, the sampled goats represented 50 to 100 % of the flock (see Table 1). In each farm, the proportion of goats in first lactation which were sampled corresponded to the proportion in the whole flock. In year 2, the proportion of treated and non treated goats was respected in the sample for egg counts analysis.

Individual faecal egg counts (FECs) were performed using a modified Mc Master method. Mean annual milk productions were recorded from 10 out of the 15 farms as they belonged to a local registration system.



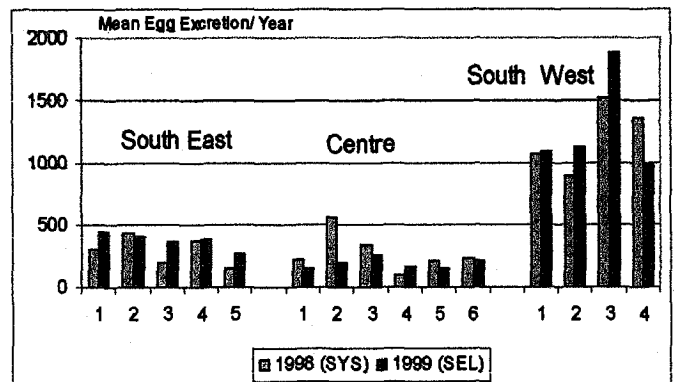
RESULTS

In year 1, clear differences were recorded in egg excretion from the different farms depending on the production area. In contrast, no difference was found in the mean annual egg output from year 1 to year 2 when the farms moved from systematic to selective treatments. The reduced number of treated goats per flock was not compensated by an increased frequency of treatments. Instead, the mean annual number of administrations of anthelmintics was reduced by 40 % (Table 1). Lastly, the switch from systematic to selective treatments was not related to any decrease in milk production (Table 1).

Table 1: Frequency of anthelmintic treatments and milk production during the 2 successive years of study in the 15 surveyed farms.

Farms	Total Number of goats	Number of treated goats on year 2	Number of treatments Year 1	Year 2	Mean milk production/day Year 1	Year 2
South West						
SW1	61	37	3	3	3.16	2.74
SW2	96	52	3	2	2.75	2.77
SW3	80	54	3	2	1.92	2.80
SW4	100	67	3	3	2.80	2.60
South East						
SE1	92	62	4	3	Data not available	
SE2	46	33	4	4	Data not available	
SE3	45	33	3	2	3.06	2.73
SE4	70	47	4	2	2.44	2.59
SE5	90	60	3	2	3.27	3.70
Centre						
CTR1	78	52	5	2	2.74	2.84
CTR2	82	54	5	1	3.26	3.36
CTR3	36	25	4	1	Data not available	
CTR4	70	47	2	1	2.61	2.71
CTR5	90	60	2	1	Data not available	
CTR6	79	52	4	3	Data not available	
MEAN	73.8	49	3.5	2.1	2.80	2.88

Figure 1: Comparison of mean annual egg excretions during year 1 (Systematic treatment) and year 2 (Selective treatment) in the 15 flocks of the survey.



CONCLUSIONS

The results indicate that the control of gastro intestinal nematodes in dairy goat flocks can be achieved through a targeted application of anthelmintic treatments, without any negative consequences on milk production.

From these results as well as from those of previous studies, the following recommendations can actually be proposed to manage concurrently both nematode parasitism and development of anthelmintic resistance in dairy goats.

These measures have obviously to be applied using the adapted doses for goats. They also have to be associated with alternative or complementary methods to control trichostrongylosis such as the improvement of nutrition and integrated measures for grazing management.

ACKNOWLEDGEMENTS

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1. Assessment of the status of the flock for resistance to benzimidazoles.
2. At housing, when goats are out of lactation, systematic treatment of the whole flock with levamisole or endectocides, in alternation every year.
3. During the grazing season (lactation), selective treatment of goats in first lactation and high producers with authorised drugs: benzimidazoles, when resistance is absent; pyrantel or eprinomectin, if not.

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L13 p

NUTRITIONAL CONTROL OF NEMATODE PARASITISM IN YOUNG MERINO EWES

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Background: Young sheep are highly susceptible to nematode parasite infection during the period immediately after weaning. Previous studies have shown long-term benefits of a short period of supplementation with protein. This study investigates whether long-term benefits can be derived from short term feeding of young ewes after weaning while grazing pasture. **Method:** 180 Merino ewes (4-5 months) were divided into 12 equal groups and allocated to 0.8ha pasture plots. Four groups were fed cottonseed meal (CSM) pellets at 170 g/h/day (H) and 4 groups 85 g/h/day (L) for 10 weeks while the remaining 4 groups were not fed (Nil). Faecal egg counts (FEC) and liveweights were recorded each 2 weeks up to week 18 when all sheep were treated with anthelmintic and combined into one flock. Liveweight and FEC were then recorded at 29, 42 and 52 weeks and wool production at week 31.

Results: Over the 10 weeks of feeding, supplementation lowered FEC ^{0.33} (H<L<Nil) and this effect continued up to 8 weeks after supplementation ceased (p<0.05). At 29, 42 and 52 weeks there was no effect of supplementation on FEC ^{0.33}. Supplementation increased liveweight gain (H>L>Nil) during the feeding period (p<0.05) and this difference was still apparent at week 29 but not at weeks 42 and 52. Fleece weight at shearing at week 31 was greater in supplemented groups (H>L>Nil; p<0.05).

Conclusion: Supplementation with CSM pellets reduced faecal egg output and increased liveweight gain and wool production in young ewes. Effects of supplementation persisted beyond the initial 10 week feeding period but were not evident beyond 19 weeks after supplementation ceased.

L14 p

PROTEIN INTAKE ON CARCASS COMPOSITION OF CALVES INFECTED WITH *H. placei*

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Background: Nutrient fluctuations (quantity and quality) throughout the year, especially that of protein, form major constraints for cattle production in tropical areas. This situation becomes worse when the animals present parasite infections. However, the association of these factors on the development of growing calves is poorly studied.

Methods: Thirty worm free, male Holstein (2 month old) calves were allocated in 3 groups of 10 calves each: high (HP), medium (MP) and low (LP) protein in the diet. After 4 weeks, 6 animals of each group were infected with 5000 *Haemonchus placei* L3, twice a week for 9 weeks (infected - I) and the other 4 animals were used as control (uninfected - C). Four weeks after the last infection all calves were slaughtered and the 7th and 10th rib joint was dissected to evaluate carcass composition and worm burdens.

Results: The degree of parasite infection observed was significantly higher in MPI group when compared with HPI and LPI groups. The gain in liveweight was higher in MP (C and I) when compared with LP (C and I) (p<0.05), but not with HP (C and I). The mean killing out percentage in LP (I) was lower than LP(C) (p<0.05). Chemical analysis of the carcass showed lower protein and higher fat deposition in the LP (C and I) than HP (C and I) (p<0.05), but no influence on mineral composition was observed between all groups.

Conclusion: Despite high infection rates with *H. placei*, groups fed medium to high protein levels showed increased live weight gain and protein composition as well as lower fat levels than low protein groups.

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L15 p

EFFICACY OF CONDENSED TANNINS AGAINST TRICHOSTRONGYLES IN GOATS

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In sheep, several studies suggest that condensed tannins (CT) have an effect on infections of the gastrointestinal tract with parasitic nematodes. In contrast, no comparative data are available in goats despite main differences in physiology in the 2 hosts species. Therefore, we investigated the effect of CT given to experimentally infected goats. Fourteen naive kids were initially infected with a mixture of 6000 L3 of *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*. Eight weeks after infection, half of the goats received daily a solution of CT, for 7 days, representing 5 % of the diet. The remaining animals composed an infected control group. Faecal and blood samples were taken regularly to measure parasitological and pathophysiological parameters. Three weeks after distribution of CT, the animals were killed. The abomasum and small intestines were taken to estimate the worm burdens and the fertility of female worms. The distribution of CT decreased significantly, by approximately 50 %, the faecal egg counts and this reduction in excretion persisted even after CT distribution was stopped. No difference was found in the numbers of *Teladorsagia* and *Trichostrongylus* and in pathophysiological parameters. The reduction in FEC was associated with decreases in female worm fecundity, particularly for *T. colubriformis*. These results indicate that CT are as effective to reduce nematode egg excretion in goats as in sheep. This result seems mainly due to an effect on worm fecundity.

L16 p

THE EFFECT OF SOME ANTHELMINTHICS ON *DUDDINGTONIA FLAGRANS* GROWTH

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Background: In the integrated method of animal parasitic nematode control, chemotherapy could be used in combination with biological control methods. Our work was aimed at the investigation of some anthelmintic preparations' (chemical components) effect on the predaceous fungus *D. flagrans* (biological agent).

Methods: Studied were four anthelmintics commonly used in Ukraine: "Brovalzen" (7.5% albendazol), "Brovadazol" (5% fenbendazol), "Univerm" (0.2% aversectin) and "Equisect" (1% aversectin C). The preparations were diluted in fluid sterile Chapek medium in 1:200 ratio. Observations were conducted on the object-plates with paraffin rings (13 mm in diameter). Three drops of diluted preparation were poured in each ring and a piece of fungal colony 1 mm² in size was placed inside. Plates were kept in a "moisture-chamber" under 23 °C. Calculation of fungus trapping structures was carried out on the 14th day of experiment under the light microscope. Fluid sterile Chapek medium was used as the control.

Results: In control samples, 9.6 (100%) trapping rings were observed on average in a field of vision. In the samples with "Brovalzen", 2.4 trapping rings (25.2% to the control number) were observed on average. The index equalled 0.7 (7.1%) for "Brovadazol", 4.2 (43.7%) for "Univerm". In the samples with "Equisect" preparation, 11 trapping rings (110%) were observed on average.

Conclusions: Strong inhibitory effect of benzimidazole preparations on the trapping structure formation of *D. flagrans* was observed. "Equisect" preparation, in contrast, was found to stimulate trapping structure growth and formation. The possibility of such an influence should be taken into account in the schemes of parasitic nematodes' integrated control.

L17 p

SURVEY OF NEMATOPHAGOUS FUNGI FROM AGRICULTURAL ENVIRONMENTS

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Background: The nematophagous fungus, *Duddingtonia flagrans*, has been shown to be effective in reducing pasture contamination with the larvae of various nematodes, including *Haemonchus contortus* from sheep. The resistance of *H. contortus* to anthelmintics in South Africa necessitates the formulation of alternative strategies for control. Among these is the possibility of isolating a local strain of *D. flagrans* and testing it for efficacy in reducing pasture contamination with the infective larvae of *H. contortus*.

Method: The modified sprinkling technique was used for the isolation of nematophagous fungi from soil, faeces and compost samples. The plates were incubated at 26°C, baited with *H. contortus* larvae at least twice a week for three weeks and examined for signs of nematophagous activity every two to three days. Conidia and chlamydospores associated with trapped larvae were sketched, measured and photographed for identification purposes.

Results: 26 isolates of nematophagous fungi were obtained from 244 samples.

Conclusion: 10 percent of the samples cultured were positive for nematophagous fungi but none of these isolates are known to survive passage through the gastrointestinal tract of sheep. The focus of this study is the search for a local strain of *D. flagrans*, adapted to local environmental conditions to be used in an integrated worm control programme.

L19 p

USING NEMATOPHAGOUS FUNGI - *DUDDINGTONIA FLAGRANS* - TO ECOLOGICAL CONTROL OF NEMATODES IN HORSES IN THE CZECH REPUBLIC.

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Background: Since anthelmintic resistant nematodes have been reported from horse herds in the Czech Republic, their possible presence was investigated on 5 horse farms in Moravia and 2 in Bohemia, as well as in horse clinic in Morocco. Chlamydospores of *Duddingtonia flagrans* can survive gut passage of horses, donkeys, mules, climatic conditions on pasture and can reduce the number of infective larvae of strongylides in faeces and on pasture for monitoring and biological control of parasitic nematodes.

Method: We used high active predator microfungi of species *Duddingtonia flagrans*. Chlamydospores were fed to a group 1 million spores daily for 1 month. Individual samples of faeces were collected during the following 48 hours. Faecal egg counts, pasture larval burden of the plot grazed were compared with those of infected fungi unfed control. In vitro experiments were carried out using chlamydospores produced on PDA (24 hours at 26°C).

Results: „Trapping fungi“ could be isolated from faeces of horses, donkeys and mules following 36 hours after peroral application. There was significant reduction in the larval harvest of spore-fed horses compared to controls (from 45% to 8%).

Conclusion: *Duddingtonia flagrans* is able to survive passage through horse, donkey and mule gut and reduce the number of infection larvae on pasture. It is effective on reducing the number of infective larvae of horse strongyle transmitted to herbage in European and African ecosystem (saving biodiversity on pastures and water sources against ecotoxicity of avermectins). The study was supported by Research Project VZ290001.

L18 p

IN VITRO EVALUATION OF NEMATOPHAGOUS FUNGI AGAINST PARASITE NEMATODE

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Biological control is a promising alternative of pre-parasitic stages through of nematophagous fungi's action. The present study evaluated the patogenicity of both *Arthrobotrys musiformis* and *A. conoides* fungi on “infectante larvae (or L3 state larvae)” of the *Haemonchus contortus*, L3 state larvae of *Ancylostoma* spp. and larvated eggs of the nematode *Ascaridia galli*. Nine groups were formed: six treated groups (G1A and G2A: L3 of *H. contortus*; G1B and G2B: L3 of *Ancylostoma* spp.; G1C and G2C: larvated eggs of *A. galli*, being that G1 treated with *A. musiformis* and G2 treated with *A. conoides*) and three untreated groups (G3A, G3B and G3C: larvae of *H. contortus*, *Ancylostoma* spp. and eggs of *A. galli*, respectively, without fungi). Each treatment had ten repetitions (ten Petri dishes with an average of 120 larvae of *H. contortus* and 200 larvae of *Ancylostoma* spp. and, approximately, 100 larvated *A. galli* eggs). The results showed *A. musiformis* plundered 66% and 94% of *Ancylostoma* spp. L3 larvae and *H. contortus* L3 larvae, respectively. While that *A. conoides* plundered 51,7% and 89,4% at the same larvae. The nematophagous fungi evaluated haven't presented any predation type on *A. galli* larvated eggs. Afterwards it has concluded that both are promising fungi as to there use for biological control of parasitic helminthics of animal hosts, except for control of *A. galli*.

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L20 p

ABSENCE OF ENVIRONMENTAL IMPACT OF THE NEMATOPHAGOUS FUNGI *D. FLAGRANS*

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Supplementation of domestic animals with *D. flagrans* has been shown to be effective in the control of gastrointestinal parasites. However, the relationship of this fungi with biotic and abiotic factors in the feces is poorly understood. The aim of this work was to evaluate the environmental effects of the massive use of a Brazilian strain of *D. flagrans* in the control of nematode parasites in sheep. Sixty sheep supplemented with *D. flagrans* (S) and sixty unsupplemented (C) sheep were allocated in separate paddocks from February to April, 1999. Over his period, feces were collected 7 and 14 days post-deposition. A fraction of the feces was cultivated in agar-water (2%) with *Panagrellus* spp. as bait and the presence of *D. flagrans* and other fungi verified. Another fraction was baermanized, the free-living nematodes recovered and counted in graduate cameras. Samples of the soil surface in contact with the feces were collected 1, 2, 3 and 4 months after the collection of the feces of the S group and placed in agar-water (2%) with *Panagrellus* spp. to verify the presence of *D. flagrans*. No significant differences (P < 0.05) were observed between S and C in the population of free-living nematodes and in the population of fungi that colonized feces after deposition. *D. flagrans* was isolated in soil samples only on the first and second month after feces collection. *D. flagrans* did not affect the colonization of free-living nematodes and nematophagous fungi in sheep feces nor survive for long periods in the environment.

L21 p**CHARACTERIZATION OF AN ARGENTINE STRAIN OF DUDDINGTONIA FLAGRANS**

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The discovery of fungi with nematophagic activity on nematode parasites of domestic animals, constitutes an alternative in the integrated control of these parasites. *D. flagrans* seems to be the most promissory fungi among those studied because it produces chlamidospores and its nematophagic activity is not lost during gastrointestinal transit. The objective of this work was to determine some biological characteristics of a *D. flagrans* strain isolated from bovine feces in Tandil, Argentina. Bovine feces were cultivated in agar-water (2%) at 20-24°C, with *Panagrellus* spp. added as bait. An isolate of *D. flagrans* was cultivated in agar-corn meal (2%) at 22°C and its radial growth measured. The isolate was also cultivated in Roux bottles with half solid (birdseed) for 4 weeks at 22°C and the chlamidospores production quantified in camera of Neubauer. The micelial growth (mean $\pm$ sd) in millimeters at 3, 5 and 7 days of culture was 19.5($\pm$ 4.2) x 19.1($\pm$ 3.3), 49.8($\pm$ 5.5) x 48.9($\pm$ 4.4) and 89.8($\pm$ 7.4) x 86.9($\pm$ 7.7), respectively. Chlamidospores production was 1,760,000 mature chlamidospores per gram of humid material. The *D. flagrans* strain isolated from bovine feces has potential for being used in formulations for the control of nematodes parasites of domestic animals. Experiments with liquid media are in progress.

L23 p**EFFECTS OF NEMATODE CONTROL AND ANABOLIC IMPLANTS ON LIVELINEWEIGHT GAIN OF YEARLING CATTLE**

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The effects of nematode control and anabolic implants on liveweight gain of yearling cattle were measured during 342 days on cattle grazing alfalfa naturally infected. The parasitic control was made through the Test of Parasitic Alarm, where the moment of anthelmintic treatment is determined by reductions in performance of the control group (with strategic treatments) compared with animals given systematic anthelmintic treatment. 45 Aberdeen Angus yearling calves averaging 8 months of age with initial weights of 142, 2 kg (CV 13.1) were allotted at random in 3 treatment groups: G1) control strategic group with 2 doses of 200 μ g/kg of ivermectin 1%, at weaning, each dose separated by 30 days; G2) systematic anthelmintic treatment (200 μ g/kg of ivermectin 1%) every 30 days, and G3) strategic anthelmintic treatment + 4 anabolic implants every 3 months (3 x 36 mg of zeranol and the last one with 36 mg of zeranol and 140 mg of trembolone). The 3 groups grazed together and were weighted monthly. Initial faecal egg counts of groups were, 210, 140 and 180 respectively. G2 stayed with epg count below 2 during the whole study. The test of parasitic alarm detected a decreased over 10 % of the weight gain in G1 toward the end of the winter and in the second autumn that indicated the need for 2 extra treatment to G1 and G3. After 342 days, the average bodyweight and daily weight gain of the different groups were: G1: 202,2 kg (591 g/day), G2: 213,4 kg (624 g/day) and G3: 234 kg (684 g/day). The liveweight gains of G2 and G3 were 11,2 and 31,8 kg, respectively, greater than group 1. There were significant differences in liveweight gain ($P < 0.01$) between G3 and the others. The largest weight gain obtained with the application of anthelmintic treatment and anabolic implants allowed to reduce on 53 days the feeding period of animals.

Key words: anthelmintic control, anabolic implants, liveweight gain, yearling cattle.

L22 p**ANTAGONISTIC EFFECT OF NEMATODE-TRAPPING FUNGI ARTHROBOTRYS ON INFECTIVE LARVAE OF OESOPHAGOSTOMUM RADIATUM; COOPERIA PUNCTATA AND HAEMONCHUS PLACEI:**

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In vitro experiments and for passage through the gastrointestinal tract of calves were performed to test two isolates of *Arthrobotrys robusta* and one isolate of *A. conoides* without losing the ability to entrap larvae of *Oesophagostomum radiatum*, *Cooperia punctata* and *Haemonchus placei*. Two Groups with six repetitions were formed: treated group, 1,000 conidia of *Arthrobotrys* sp and 10,000 L3 and control group, 10,000 L3. Daily, for 20 days, on the dishes from the groups were examined and the number of freely migrating larvae counted. Eight housed male Holstein x Zebu calves, six to eight months old, were randomly separated into two animals in the test of each isolate. The animals received 20 x 10⁶ conidia; orally and in the control each calf received 100 ml water. In vitro assay the isolates of fungi were efficient against the nematodes ($P < 0.05$). All the fungi passed through the gastrointestinal tract of the calves and they got to prey more infective larvae than control group without fungi treatment, however there were no statistically significant differences ($P > 0.05$). These results demonstrate that infective *O. radiatum* larvae are preying equal to infective larvae of *C. punctata* and *H. placei* and after the passage through the gastrointestinal tract the fungi can lose the predatory capacity.

L24 p**HORN FLY (HEMATOBIA IRRITANS) CONTROL WITH DIATOMACEOUS EARTH CONTAIN 2% PYRETHRINS**

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Diatomaceous earth (DE) is a geological deposit consisting of fossilized skeletons of numerous species of siliceous marine and fresh water unicellular organisms (diatoms and other algae), with certain abrasive properties and the ability to absorb lipids. Any DE with high oil absorbing capacity is a potential insecticide, that causes insects to lose moisture and die due to dehydration. Two fields studies were conducted to evaluate the efficacy of DE contain 2% pyrethrins against natural populations of *Haematobia irritans* in cattle. The evaluation was made comparing the effectiveness of DE with that of commercial insecticide-repellent products, by means of counting flies on the animals. Trial 1: 70 A. Angus heifers, 18 months old, were divided in 3 groups according to the load of flies. On day 0 (January, 20th), Group 1 was treated with one ear tag containing 20% diazinon, and Group 2 was treated dewing the back of the animal with 40 cc of 3% DE, 2 % oil adherent and water. Group 3 (control) received no insecticide treatment. Trial 2: has the same design that trial 1, except that 76 mature A. Angus cows were used and the ear tag treatment was replaced by cypermethrin 5%, piperonyl 5% and trichlorfon 10% applied as a pour-on treatment at the dosage rate of 5 ml/100 kg.b.w on day 0 (February 9th). During the trials animals grazed similar pasture plots, at least one km apart of each other. Counts of the flies were carried out between 9:00 and 10:00 a.m. in the day of application of the different treatments (day 0) and in days 7, 14, 22, 29, 35 and 49 post treatment. During the first 7 weeks, the weekly percentage of efficacy of Group 1 ranged between 75 to 95,1 % in trial 1 (ear tag) and 78 to 97,3 % in trial 2 (pour-on), compared with 30 to 65,4% and 28,6 to 55,7% in Groups 2 respectively ($P < 0.01$). According to the results obtained in these trials DE did not control *Haematobia irritans* during de first 7 weeks after the treatments.

Key words: *Haematobia irritans*, diatomite, insecticides, efficacy, control

L25 p SHEEP SCAB CONTROL PROGRAM IN NORTHERN PATAGONIA, ARGENTINA

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¹INTA, CC. 277, (8400) Bariloche, Río Negro. ²South Region Development Agency, Maquinchao, Río Negro. ³Ministry of Economy, Río Negro, Argentina. **Background:** Patagonia is the largest fine Merino wool producing area in America, being sheep scab the most important health problem. Despite existing base knowledge, control programmes have suffered considerable setbacks in the last 10-15 years. The decline of funding at national and farmers levels and the need to control sheep scab, have led to a voluntary charged program based in multisectorial participation. Farmers involved were small-scale producers.

Method: Rural leaders and local authorities at national, provincial and communal levels, were invited, in 1999 by the South Region Development Agency (SRDA), to start a 7 year control program, based in a cost recovery system. A Committee of Representatives was created. Rural leaders (acting as technology transfer workers) were trained and developed a common approach based on a shared experience. The farmers were organised by groups of neighbours to apply for assistance, and the time and amount to return the costs from each group was determined by common agreement. Each group decided date and type of acaricide application, but under close monitoring by trained personnel. SRDA administered the resources and bought the acaricides used.

Results: Up to day, 13 groups comprising 802 farmers that owned 303.000 sheep grazing on 2 million hectares., are involved in the program. 280.000 doses of injectable acaricide were distributed. Dipping treatment were carried out by less than 20 % of the farmers. Sheep scab is still detected in a few identified careless managed farms. This considered successful program, builded stronger links between farmers and institutions and address the need of resources-poor farmers, that are motivated to pay the costs incurred in drugs and assessment.

Acknowledgement: We wish to thank our colleges from SENASA and FECORSUR, for their advice and cooperation.

L26 p EFFICACY OF EAR TAGS IMPREGNATED WITH 20% W/W PIRIMIPHOS-METHYL TO CONTROL HAEMATOBIA IRRITANS POPULATIONS ON BEEF CATTLE IN CORRIENTES, ARGENTINA.

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Background: The efficacy of ear tags impregnated with 20% w/w of pirimiphos-methyl to control *H. irritans*, natural field populations, was studied during a period of 5 months in a farm situated in the province of Corrientes, (Argentina).

Method: In a herd of 250 cross bred heifers, 30 animals were selected at random, identified by numbered ear tags and then tagged, using a 20% w.w. pirimiphos-methyl ear tags.

In order to determine horn fly infestation level the heifers were checked prior to treatment and weekly thereafter the 5 months study.

In addition another group of 30 heifers, same age and breed, identified by numbered ear tags, served as control and were hosted in a paddock about 4 km. far from the paddock where treated heifers were grazing. Also these cattle were checked weekly as treated ones.

The percentage of efficacy was established according to Abbot formula, modified by Henderson & Tilton.

Results : During 20 weeks the 20% w.w. pirimiphos-methyl ear tags provided more than a 90% control of horn flies stationed on treated heifers; in the following two weeks *H. irritans* reduction ranged 84%.

Conclusions: Ear tags impregnated by 20% w.w. pirimiphos-methyl (2 ear tags/head) has a 5 months efficacy to control *H. irritans*.

L27 p DERMATOBIA HOMINIS (TROPICAL WARBLE FLY) CONTROL BY CHLORPYRIPHOS 10% w/v POUR-ON

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Background: The A.A. report in this paper a field trial carried out in the province of Corrientes (Argentina) during the *Dermatobia hominis* infestation season 1999 - 2000.

Method : In this study 47 cattle of different breeds, sex and age, were treated by Chlorpyrifos 10% w/v pour-on at the dosage rate of 10 ml/100 kg.b.w. and, in order to evaluate the efficacy against the larval stages of *Dermatobia hominis* (tropical warble fly), all the treated cattle were checked every 7 days during a 42 days period.

Results : The tested product was highly effective for the control of *Dermatobia hominis* (tropical warble fly) because it killed all the larvae into the nodules up to 22 days after treatment and prevented new reinfestation for another 18 days.

Conclusions : In this area of the province of Corrientes, where this trial was carried out, the A.A. estimated by repeating the pour-on treatment with Chlorpyrifos 10% w/v every 40 days, it is possible to control this parasite during the *Dermatobia hominis* infestation season as well as to control *Haematobia irritans* (horn fly) another harmful fly infesting cattle in this region

L28 p DERMANYSSUS GALLINAE: TESTING ACARICIDE RESISTANCE AND NEW PRODUCTS FOR CONTROL IN GERMANY

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Decreasing efficacy of acaricides for control of *D. gallinae* is common, however figures about spread, kind and degree of resistance were missing.

Methods: A mite-packed-test was based on the tick-larval-packed test (CSIRO /FAO). Folders of filter papers were impregnated with the active substances (OPs, pyrethroids and carbamats) and mites of the field strains and of a laboratory reference strain were placed between the folders. A baseline was established with the non resistant HO-*D. gallinae* reference strain, comparable to the „Yeerongpilly“ *Boophilus* tick strain. After 24 h incubation the count of dead and living (locomotion) mites gave the degree of resistance. The same test was used to evaluate in vitro the efficacy of new acaricidal formulations. In semi-field trials cages with hens became infested with the reference strain of mites and were sprayed with the new products. Hollow trap perches with a groove forming a channel between a lower and an upper part were placed into the cages in order to collect and to count the mites. Cages were treated with the effective dose of the acaricide, as determined in the mite-packaged-test.

Results: *D. gallinae* strains from 10 big poultry from different parts of Germany were tested. In all farms resistance against pyrethroids occurred to a certain degree (11-96%), against OP-compounds in 9 of 10 farms (degree 0 -96%), and against carbamats in 3 of 10 farms ((degree 1-69%). Multiple resistance against all 3 groups of acaricides was found in 3 farms, against OPs and pyrethroids in 9 of 10 farms. From in vitro tests and in vivo trials good (more than 90%) efficacy against *D. gallinae* in cages for egg laying hens resulted with 3 new formulations containing the active compounds deltamethrin, phoxim and ortho-phenylphenol.

L29 p

A METHOD FOR *IN VITRO* DETERMINATION OF IVERMECTIN EFFECTS ON PSOROPTES SP.

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Background: *Psoroptes* sp. are ectoparasites responsables of scabies in domestic animals. *Psoroptes ovis* induces a range of economic importance on the Belgian White and Blue Breed. Since few years, therapeutic problems occur with the avermectin molecules resulting in treatment failures. We have found interesting to develop an *in vitro* test allowing detection of resistant forms of parasites.

Methods: *In vitro* culture conditions were determined using *Psoroptes cuniculi* model. Mites were collected from rabbits ears and put into antibiotic / antifungic solution for 24 hours. They were then transferred into 24 wells plates within Sf9 medium supplemented with baytril and amphotericin B and incubated at 37°C. NaCl 0.9% was used as control medium. Ivermectins was prepared at the concentrations of 8, 12, 16, 32 and 40 ng/ml in glycol propylen.

Results: Survival curves are similar in control and Sf9 media. Nevertheless yield of alive mites is always two times more important in Sf9 than in NaCl. Results observed with ivermectin show a dramatic effect in all concentrations after a one day incubation in Sf9.

Conclusion: Several attempts were previously carried out with bacteriological media without success. Use of Sf9 medium allows us to keep alive both adults and juveniles for period which exceeds 6 days. Ivermectin experiment clearly show that this *in vitro* model would be usefull for testing acaricide molecules as well as for identifying potential resistance to these molecules.

Acknowledgment : This work has been supported by a grant from the Région Wallonne and the FRIA.

L30 p

EFFICACY OF IMIDACLOPRID ON CAT FLEA INFESTATION OF RABBITS AND FERRETS

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Background: Rabbits and ferrets are popular as pets and are vulnerable to infestation with the cat flea (*Ctenocephalides felis*). Imidacloprid is widely used for control of fleas on cats and dogs. These studies were designed to assess efficacy on artificially infested rabbits and ferrets as care is needed when extrapolating drug usage recommendations between species.

Method: Animals were allocated by sex and flea count to either untreated control or treatment groups. A dose of 0.4ml 10 % imidacloprid spot-on formulation was given in 1 rabbit and 1 ferret study, while 10mg/kg bodyweight was used in a second ferret trial. Rabbits were infested with 100, and ferrets with 50 or 60 unfed adult *Ct. felis* on the day prior to treatment and at weekly intervals thereafter. Flea counts were performed 8 h, 24 h or 7 days post-treatment, and at 24 h after each re-infestation until day 28 or 29.

Results: Flea burdens were reduced by 96 and 95 %, respectively, on rabbits and ferrets, at 8 hours post-treatment and 100 % by 24 hours. In rabbits, protection against re-infestation at 1, 2, 3 and 4 weeks post-treatment, was 95, 81, 79 and 68 %, respectively. In ferrets 10mg/kg gave 93 % efficacy at day 8 but little useful activity thereafter. The fixed dose of 0.4ml gave 100 % protection at day 7, 94 - 95 % at days 14 and 21 and 73% at day 28.

Conclusion: Imidacloprid was safe when used on rabbits and ferrets and eliminated resident flea populations. The 0.4ml dose was highly effective for 1 and 3 weeks post-treatment in rabbits and ferrets, respectively, and continued to give substantial protection against re-infestation for 1 month.

L31 p

EFFECT OF RAIN ON THE EFFICACY OF TOPICAL IVERMECTIN AGAINST PARASITES OF CATTLE.

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Background: Grazing cattle treated with a topical formulation may be exposed to unpredictable weather conditions. The study was designed to evaluate the influence of heavy rainfall on the efficacy of Virbamec® Pour-On on cattle exposed to natural nematode infection.

Method: 48 healthy weaners were ranked by D-7 faecal egg counts, allocated into 6 groups of similar body weight and hair length (n = 8). Group A remained untreated (Placebo), Group B received the topical ivermectin formulation (D0) and both were not exposed to the artificial rain. The Groups C, D, E and F were treated (D0) at 1 ml/ 10 Kg and exposed to rain (20 mm over 30 minutes) 1 hour prior to treatment (C), 2 hours after treatment (D), 6 hours after treatment (E) and 24 hours after treatment (F). Individual faecal samples were taken on D-7, D0, D3, D5, D7, D14 and D21.

Results: The larval culture showed a mixed nematode infection (*Haemonchus* spp., *Trichostrongylus* spp., *Ostertagia* spp., *Oesophagostomum* spp. and *Cooperia* spp.). Before treatment (D-1), the geometric mean count ranged between 42 to 349 with no significant difference between groups (P > 0.05). The GM count in Group A (placebo) remained high (209 on D21) From D5 onward, the efficacy (Abbotts formula) in Groups C, D, E and F was 100%. No significant difference was shown (P > 0.05) between Group B (no rain) and other treated groups.

Conclusion: Exposure of cattle to precipitation had no effect on anthelmintic efficacy of the topical ivermectin formulation*.

* Virbamec® Pour-On: Ivermectin 0.5% w/v pour-on solution from Virbac Laboratories

L32 p

METAPHYLACTIC EFFECT OF TOLTRAZURIL (BAYCOX® 5% ORAL SUSPENSION) AGAINST COCCIDIOSIS IN SUCKLING PIGLETS UNDER PHILIPPINE CONDITIONS

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Background: Although a previous study was not able to establish *Isospora suis* coccidiosis locally, we initially examined 3 pig farms using flotation and sporulation techniques and found that 2 of the farms had *I. suis* infection in their suckling piglets. We then conducted a trial to determine the metaphylactic effect of toltrazuril against coccidiosis in one of the positive farms that had already started a preventive medication program against diarrhea and coccidiosis in their suckling piglets.

Method: 24 litters were divided equally into two groups: Group 1 was given enrofloxacin at 1-3 days of age then sulfaclozine at 4-7 days of age, which formed the current medications of the farm, whereas Group 2 was given toltrazuril alone at 4 days of age. Weight gain, fecal consistency score, number of oocysts per gram, and preweaning mortality were gathered.

Results and Conclusion: Based on the results, the toltrazuril-treated group had a higher but not significant mean weigh gain at 21 days of age, significantly improved fecal consistency, and lower preweaning mortality rate compared to Group 1. With the OPG, oocyst shedding ceased earlier in Group 2 than Group 1.

L33 p

LICE AND LEATHER DAMAGE IN CATTLE

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Background: The European leather industry loses hundreds of millions of \$s annually due to loss of leather quality caused by the defects light spot and fleck. A trial was undertaken to confirm that infection with chewing lice (*Bovicola bovis*) is the major cause of light spot during the winter housing period by comparing hide damage in first and second year animals.

Methods: 60 calves were reared either lice free or naturally infested with lice (*B. bovis* and *Solenopotes capillatus*). Some of the infested calves were treated with fenvalerate spray and eprinomectin pour-on in February to determine the rate and extent of reversion of damage. Hides were manually removed at slaughter, professionally tanned and assessed for light spot and fleck in terms of extent and severity of damage.

Results: First and second years calves kept lice free had the lowest damage from light spot. They also had lower levels of scratch damage indicating infestation with lice increases irritation of the skin. In infested calves that were treated with insecticides at the time of peak infestation damage persisted for at least 12 weeks although there was a significant reduction in area of damage by 12 weeks.

Discussion: Chewing lice are the major cause of winter light spot. In additional they result in damage to hides caused by cattle rubbing to relieve irritation. To produce top quality hides animals require treatment when housed in the autumn rather than when lice are seen in January or February.

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L34 p

CURRENT CHANGES IN THE CONTROL OF *Boophilus microplus* IN ARGENTINA

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Official campaigns for the control of *B. microplus* commenced in Argentina in 1906, and by 1998 had confined the parasite to 12 M cattle in 11 NE and NW provinces, covering 80 M Ha. However in 1999, resistance to pyrethroids, a drastic reduction in campaign personnel and funds, and current farm economics, caused a virtual paralyzation of the programme with a sharp regression in the control of treatments and cattle movements, with a reinfestation of farms in tick-free areas covering more than 5 provinces. Between 1997/99, in Entre Ríos alone, 184 new reinfested farms were quarantined and cattle submitted to repeated supervised treatments. By late 1999, approximately 2000 reinfested farms in Entre Ríos, Santa Fe, Córdoba, S. del Estero and in the rich humid pampas of Buenos Aires, had added to the general confusion. In early 2001, control methods were modified, more responsibility given to cattle owners, and a new order of priorities given to programme officers. Because of resistance to pyrethroids, the Tick Control Government Agency after strict stable and field evaluation trials approved the use of new products, including 2 injectable ivermectins and a flazurion 2.5% pour-on. Of these, a 1% w/v injectable ivermectin with a modified excipient^(*) reached 19 days residual effect and 31 days effective control post-treatment, with a large acceptance and efficacy under high challenge in the field, applied either alone or in a combined 1% ivermectin injectable/amitraz dip programme. The paper outlines the product profile, details of approval trials and results of official field performance and efficacy evaluations.

L35 p

THE EFFECT OF FLY ABUNDANCE ON THE MILK PROPERTIES IN 16 ITALIAN DAIRY FARMS.

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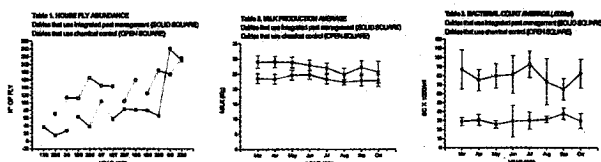
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House flies and stable flies are a nuisance to dairy cattle, and they can increase bacterial contamination of milk and milk losses.

The purpose of this research was to quantify the effects of fly abundance on milk production.

Sixteen dairies located in the province of Ferrara (Northern Italy) were sampled, with sticky traps, for house flies and stable flies from May to September in 1999. In order to control flies nuisance, eight of the above-mentioned dairies carried out an integrated pest management with releases of pupal parasitoids, while the other eight used insecticides in case of necessity. Dairy farmers provided quantitative and qualitative data about the milk production from March to October.

Fly and milk data from the 16 dairies are summarised in tables 1, 2 and 3. The low presence of house flies (37% less), in the eight dairies that use an integrated pest management, compared to those that use a chemical control (table 1) seems to have two effects: the first is a 15 per cent increase of milk average production (table 2), the second is a remarkable (66%) reduction in bacterial count average (table 3).



L36 p

BIOASSAYS OF *BACILLUS THURINGIENSIS* AGAINST HOUSE AND STABLE FLIES

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The house and stable flies are two of the most widespread Diptera of medical and veterinary importance. Control of these pests is based on the use of chemical insecticides, with the risks of development of resistance in target pest and contamination of the environment. Due to these problems, scientist have placed a great effort in alternative environmentally compatible products, particularly microbial insecticides. In our experiences, 18 isolates of *Bacillus thuringiensis* (*B.t.*) obtained from samples of soil collected in Angola were tested in laboratory bioassays against adults of house-fly (*Musca domestica*) and stable-fly (*Stomoxys calcitrans*) reared in laboratory. Isolation of spore-forming aerobic bacteria was performed as described by Ohba and Aizawa (1986). One gram of the soil was diluted in sterilised water, heated at 80°C for 10 min and the suspensions plated on nutrient agar. Crystal containing colonies were tested against the adult flies, using 300 µl of *B.t.* suspensions (at the concentration of ca. 10¹⁰/ml) per day added to 300 µl of 60% sugar solution or 300 µl of blood for house-fly and stable-fly, respectively. Groups of 10 adults, 1-day old (?) were kept in plastic containers and fed with the liquid diet. Every day for 7 consecutive days the dead adults were counted. The tests were replicated 4 times. The 18 strains of *B.t.* showed typical parasporal inclusions of different sizes and shapes. The higher mortality was caused by the strains AF14 and AF17 either against *Musca domestica* and *Stomoxys calcitrans*. These preliminary results need further experimentation, but are promising for the development of new strains of *B.t.* to replace synthetic chemical insecticides.

L37 p

CONTROL OF *Melophagus ovinus* IN THE ARGENTINE PATAGONIA WITH A OP/SP EMULSION USING A PRESSURE SPRAY

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The sheep ked *M. ovinus* is a common parasite in the Argentine Patagonia, causing fleece and hide damage in approximately 7.5 M sheep. The prolonged pupal stage which can reach >40 days make single treatments ineffective. Repeat treatments are not always feasible under range management, the size of the flocks and the cost, an important factor in the light of current low wool prices. In order to evaluate the efficacy in *M. ovinus* control by using a low-cost combination of an organic-phosphate (OP) and synthetic pyrethroid (SP)⁽⁴⁾ in a water emulsion, applied under high pressure with a spray equipment adapted for sheep installed at the exit of a shute, 35,600 Merino sheep on two farms in the Province of Santa Cruz, were sprayed twice with an interval of 40 days. The post-shearing pre-trial one side rib/shoulder average *M. ovinus* counts in 15 ear-tagged rams, 15 ewes and 15 wethers were 1.85, 2.85 & 1.85, respectively, and prior to the second treatment, 0 in all groups. The jetting was applied with a portable PPP Super Jetter equipped with 24 nozzles, at a pressure of 70-75 lbs/sq.inch, which assured a thorough wetting of each animal. Excess emulsion and run-off was recovered and recycled; the average quantity used per adult animal was approx. 1 lt, at a current average price of US\$ 0.06 for the 2 treatments. The life-cycle of *M. ovinus*, of special importance for treatment, is discussed. Conclusion: the combined OP/SP emulsion proved highly efficient for ked control.

L38 p

EFFICACY OF FIPRONIL AGAINST *WERNECKIELLA EQUI* IN HORSES

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Background: Treatment of 15 horses naturally infected with *Werneckiella equi*.

Method: On the horses such a clinical signs were diagnosed: agitation, itching and pathological changes (skin irritation and loss of hair) on the neck and at the base of the tail. In parasitological examination all developmental stages of *Werneckiella equi* were found. Fipronil in spray was used on skin with pathological changes.

Results: Change for the better was observed in third day after drug application (lack of agitation and itching). After 2 to 4 weeks changes on skin disappeared.

Conclusions: Fipronil in spray was the drug of very high effectiveness against biting louse of horses.

L39 p

THE DYNAMIC OF PARASITIC COMMUNITIES OF HORSES AND SEASONAL VARIATIONS IN DIETS

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Background: The aim of our project was to determine the species with anthelmintic effects in the structure of the plant communities on pasture. The seasonal variation of the horses feeding and influence on dynamics parasitic communities for wild horses on the pasture of the Chernobyl exclusion zone were studied at the present research.

Method: Nematode faecal eggs counts using sedimentation methods. The feeding of the horses was studied by the method of observing of the vegetation and horses migration tracks during the all season of the years (1999-2000)

Results: Helminthoscopy of adult animals shows the 100% contamination by strongylids, and foals are contaminated by strongylids and parascaris. The relationship between the dynamic of parasitic communities wild horses and seasonal variations in diets was determined for a breeding group for all seasonal during 1999-2000 years. The fact of the feeding *Artemisia* and *Tanacetum* were noted in the end of the summer. High level of the strongylids contamination which is fixed during the spring, gradually became lower for summer, with following peak for autumn.

Conclusion: The horses usually change their feeding for plant species with anthelmintic effects during the year (*Artemisia*, *Tanacetum*). This is the one of the natural mechanism of biological control of the parasitic burden.

Acknowledgment: I would like to thank the scientific direct Dr. Dvojnjos G.M. for his assistance and interest in my work. I would especially like to thank Dr. Balashov L.S. for his advise on botanical part of this work.

L40 p

NEW RECORD ON HORSE CYATHOSTOMES' RESISTANCE TO BENZIMIDAZOLES IN UKRAINE

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Background: The aim of our work was to test the efficacy of a range of anthelmintics in horses on Dubrovskiy horse farm, where the resistance of cyathostomes to cambendazole has been found in 1995 (Borgsteede et al., Vet. Parasitol., 1997, 68: 113-117). Our investigations were carried out in summer, 2000.

Material and methods: Five anthelmintic of wide action spectrum were used: "Brovalzen" (7.5% albendazol), "Brovadazol" (5% fenbendazol), "Avertin" (0.2% abamectin complex), "Univerm" (0.2% aversectin), "Equisect" (1% aversectin C). Sixty horses 1-1.5 years-old (200-250 kg weight) divided into 5 experimental and 1 control groups were involved in experiment. Faecal samples were collected on a day before and 15 days after treatment. Efficacy of each preparation was determined by faecal egg count reduction (FECR) test. Faecal samples were cultivated during 15 days at 20-25 °C for infective larvae identification.

Results: In the horses treated by "Brovalzen" and "Brovadazol", the FECR test revealed 45.8 % and 71.9 % reduction of nematode eggs' number correspondingly. Treatment by avermectin preparations resulted in comparatively higher FECR test values: 98.8 % for "Univerm", 98.9 % for "Avertin" and 99.7 % for "Equisect". Before treatment, 74.8% of nematode larvae in samples were of the cyathostome type. After treatment only cyathostome larvae were found in the cultures.

Conclusions: The continuing resistance of cyathostomes to benzimidazole preparations was revealed in horses from Dubrovskiy horse farm. Avermectin preparations were effective against both cyathostomes and other intestine helminths.

L41 p NEMATODE CONTROL PRACTICES ON THOROUGHbred HORSES IN SOUTH AFRICA Mathee¹, S., Dreyer², F.H. & Van Niekerk¹, F.E.

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Method: A questionnaire was developed to obtain information on helminth control practices and management. It was sent to 110 horse stud farms during 2000. The farms consisted of a minimum of 15 animals and were distributed all over South Africa.

Results: A total of 55 (50%) questionnaires were returned and most of these (76%) came from farms located in two provinces (Western Cape and KwaZulu-Natal). The average number of horses per farm was 107 (± 81) with a minimum of 15 and a maximum of 410. The study revealed that foals, yearlings and adults were, on average, annually treated 7.28 (± 3.01), 6.52 (± 2.69) and 5.26 (± 2.35) times, respectively. An average of 3.29 different drugs were used annually with ivermectin being the most commonly used drug for the period 1997-2000. The eye measure was used on 44% of the herds and the body tape measure on 41% to obtain the weight estimate. Individual weight and the average group weight were equally preferred at dosing. Most (81%) of the herds were subjected to faecal egg counts, which were performed at an average of 2.55 times per year. Ninety percent of herd owners practiced pasture rotation, 44% performed alternated grazing with cattle, 37% spread the horse dung on the pastures while 60% removed the dung from the pastures. Thirty-four percent performed anthelmintic treatment at rotation, 94% treated new horses at introduction and 79% treated visiting horses. Colic seems to be of little importance within the first three weeks following deworming and 81% of the herd owners performed disease registration.

L42 p EFFICACY TESTING OF A COMBINATION OF IVERMECTINE AND PRAZIQUANTEL Bruni, R., Vizio, E., Gimenez, R.A. & Basso, N.*

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Introduction: Endoparasitism leads to the development of inespecific signs in horses, particularly lower sporting profiles. This is important for our country, owing to the fact that is the third sports horse breeder in the world. This work was conducted in order to test the efficacy of an endoparasiticide combination, based on ivermectine and praziquantel, for which there is no available commercial presentation.

Materials and methods : A combination of 2% ivermectine and 10% praziquantel, prepared as a paste, was administered in a single dose of 1 g/100 kg p.o. to thirteen polo ponies (250- 500kg). Three animals were used as blanks. Feces were collected before the paste was administered. Second and third stage larvae (L2-L3) of *Gasterophilus* were scanned with a videogastroscope at days 0, 7 and 21. Coproparasitologic analyses were performed on these same days. Egg count was carried out using a parasitometer and larvae culture was done. The Teuscher method was carried out to count Anoplocephalidae eggs in feces. Data was processed following a non-parametric procedure in order to test the media between groups.

Results: A significant decrease in the egg count was observed for large and small Strongylidae, *Oxyurus equi* and *Parascaris equorum* from day 7 onwards. Results were negative for L2 and L3 of *Gasterophilus* as observed through the videoscopes. The results of the Teuscher method for cestodes, showed negative results for Anoplocephalidae, from day 7 onwards.

L43 p EFFECT OF DIETARY PROTEIN SUPPLEMENTATION ON RESPONSES OF YOUNG GRAZING SHEEP TO INFECTION WITH GASTROINTESTINAL NEMATODES.

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Background: Poor nutrition and helminth parasitism constitute two major constraints in small ruminant productivity in the tropics. The two factors severely restrict the growth of both sheep and goats.

Methods: The trial was conducted over a 9 month period using eighty 3-4 month-old Corriedale sheep of mixed sexes allocated to one of four groups (n=20): protein supplemented-strategic treatment (PST), supplemented-untreated (PNT), unsupplemented-treated (NPST) and unsupplemented-untreated (NPNT). Supplemented sheep were fed ad libitum in the evenings with a well mixed feed (cotton seed cake; 35% CP + wheat bran) at a level equivalent to 200mg day⁻¹. Each group was housed separately but grazed together during the day. Liveweight and rectal faecal egg counts (FEC) were monitored fortnightly and worm burdens assessed at the end of the trial. Nematode control regimes were done with ivermectin (0.2 mg kg⁻¹) according to a strategic drenching programme or not drenched unless survival of individual animals was threatened.

Results: There was significant reduction in FEC of trichostrongylids and number of GI nematodes in the (PST) animals as well as daily liveweight gain advantage compared with the other groups of animals. *Haemonchus contortus* and *Trichostrongylus colubriformis* were the predominant nematodes with small numbers of *Nematodirus filicollis* and *Oesophagostomum columbianum*.

Conclusion: The trial suggested that protein supplementation had an effect on the establishment and survival of GI nematodes in grazing weaner sheep.

Acknowledgements: The financial support from the Danish International Development Agency (DANIDA) is appreciated.

L44 p EFFICACY AND SAFETY OF PERMETHRIN, TETRAMETHRIN AND PIPERONYLBUTOXIDE IN COMBINATION IN CAGE AND AVIARY BIRDS. Roussel¹ E., Fossati² A., Genchi³ C

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Background: The combination of the three active ingredients allows efficacy against a greater number of insect species and also action against acarids. The aim of the present study was to assess the efficacy and therapeutic safety of the combination in a sample of cage and aviary bird populations.

Methods: An open, randomised study, controlled against untreated infested birds. The combination was tested in three formulations: powder, spray and liquid. Forty-two birds divided into equal groups for each formulation were admitted to the study. Treatment was carried out twice at a distance of 5 days. After 10 days' follow-up the birds were examined and the following efficacy parameters were assessed: itching, weight loss, excitement, alteration of plumage, cutaneous alteration, and presence of parasites. For the whole study period any adverse reactions were monitored.

Results: The birds affected by parasitosis responded positively to therapy, which resolved the pathology in course. Symptoms regressed in a statistically significant fashion following treatment, and the birds were completely cured by the end of the study. During the study no adverse reactions due to treatment were observed.

Conclusion: The present study has confirmed the therapeutic efficacy of the formulations examined and demonstrated their good safety profile.

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Background: Laboratory trials showing a high anti-feeding effect of deltamethrin-impregnated collar (Scalibor® ProtectorBands) against *Phlebotomus* have been reported by Killick-Kendrick (1997) and Lucientes (1997). A field validation has been recently provided by a field trial carried out in Italy (Maroli, 2001). In the present study, the efficacy of deltamethrin-impregnated collars has been assessed in dogs living in a heavy endemic area for *Leishmania infantum* in northern Italy (province of Imperia and Savona, north-western coast of Ligurian sea).

Method: Out of 176 asymptomatic dogs sampled at the beginning of the sand fly season in Spring-early Summer 2000, 168 were found negative to anti-*Leishmania* antibodies (IFAT, cut off 1:40) and were included in the study. Hematological parameters, total protein and electrophoretic pattern were also assessed. One hundred twenty nine dogs were referred by the owners at the end of the sand fly season during Winter-early Spring 2001. Reference data on seasonal canine seroprevalence in the same area for the years 2000-2001 (20%-30%) were from the routine yearly surveillance programme by the Istituto Zooprofilattico Sperimentale.

Result and Conclusion: Among 129 collared-dogs, two seroconverted at 1/80, two at 1/160 and two at ≥ 1280 (seroprevalence 4.65%). The seroconversion rate (0.062) was markedly lower than expected in the study area and significantly lower than reported in previous, recent, surveys (Zaffaroni, 1999: 0.112) ($p < .001$). This provides an indirect evaluation of the suitability of deltamethrin-impregnated collar as a cost-effective and widespread-applicable method of dog protection against sand fly bites for a complete season.

O

Helminth infection in horses

01

PASTURE EXPOSURE AND ACQUIRED RESISTANCE TO CYATHOSTOMES IN EQUIDS

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The effect of pasture exposure on acquired resistance to reinfection of cyathostomes was investigated using 17 parasite naive ponies, 2 to 3 years of age. These were divided into 3 groups: one to graze a contaminated pasture for 4 months, one to graze a "clean" pasture, not previously grazed by parasitized animals, and one to group to remain in the barn under helminth free conditions. Following pasture exposure, all ponies were housed in stalls in the barn and de-wormed with IVM and 10xOBZ, a treatment that eliminated mucosal encysted cyathostomes as well as luminal parasites based on necropsies of 3 animals, 17 days after treatment. Three weeks after treatment ponies were challenged with 100,000 cyathostome L3 per os. Necropsies were performed 7 weeks after the challenge. Recoveries of luminal cyathostomes (adults and L4) ranged from a mean of 11,000 (contaminated pasture group) to 18,000 (PF group) but did not differ significantly from one group to the other (adults $P=0.220$; L4, $P=0.343$). A total of 13 cyathostome species were recovered, with a mean of 8.4 per pony. Digestion of the mucosa to liberate encysted stages showed a significant increase in encysted larvae in the ponies that had pasture exposure vrs. those that remained in the barn; however differences in recoveries of encysted larvae were not significantly different in ponies grazing the contaminated pasture vrs the clean pasture. Total cyathostome burdens (luminal plus encysted stages) were not significantly different among any of the groups ($P=0.536$). It would appear that four months exposure on a contaminated pasture is insufficient to evoke a strong acquired resistance to re-infection.

02

LONGITUDINAL STUDY OF CYATHOSTOME SPECIES IN NATURALLY INFECTED HORSES.

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Background: Research on cyathostome species is mostly done in post-mortem studies that give a still picture of the adult parasites in the gut lumen of a horse. In order to follow the evolution of species patterns in individual horses or farms, we studied the parasite population that could be recovered after anthelmintic treatment.

Methods: To check the validity of our method, we classified all specimens recovered after Ivermectin treatment, from 0 to 50 hs post treatment (p/t) in one highly and one moderately infested horse. The greatest amount of total worms and of worms in a single defecation was at 24/28 hs p/t. All species that appeared before and after the 24-28 hs period also appeared then, and the pattern was homogeneous during the whole study. Then, worms recovered at any defecation 24-28 hs p/t are a valid representation of all sensitive species found in a horse. Using one defecation 24/28 p/t, we carried out a 2 year bimonthly longitudinal study of 5 horses in a farm that had had a 50% prevalence of *C. insignis*. Two adult and one weanling mares imported from France and immediately lodged in that farm and 2 adult mares born and raised in the same farm were studied.

Results: Four species: *C. catinatum*, *C. longibursatus*, *C. pateratum*, and *C. nassatus*, were prevalent in the 5 horses, but their relative prevalence varied, not following a regular pattern. During this study, *C. insignis* practically disappeared from the entire farm's horses.

Conclusion: Our method is useful for the study of cyathostome species naturally infecting valuable horses and shows the existence of variation of the species infecting a given population of horses.

03

STRONGYLES IN WILD VENEZUELAN HORSES: PREVALENCE AND INTENSITY

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Key words: Strongyles-horses-prevalence-Venezuela

Purpose: To estimate prevalence, intensity of strongylid infection and present species of these nematodes on 6 populations of wild Venezuelan horses never or rarely treated with anthelmintics. **Methodology:** 1. Study area and horses: 6 farms (F1 to F6) and 271 adult horses (h) distributed and located as follow: F1(61h)-F2(26h)-F6(24h): Cojedes State. F3(94h)-F5(45h): Apure State. F4(21h): Yaracuy State. F1-F3-F6 treat horses with anthelmintics once a year. F2-F5-F4 never treat. In all farms horses live free in nature only grazing all year. 2. Faecal samples: Taken individually from each horse and faecal egg counts performed using Mc Master modified Method (minimum detection 50 eggs per gram "epg"). Prevalence (P)= % of sampled horses with epg over or equal to 50epg. Intensity (I) consider 3 level I1: epg=0-500, I2: epg=500-1000, I3: epg>1000. Bulk fecal samples from positive horses at each farm were prepared for the cultivation of third stage larvae and identification of species present. 3. Intestinal content: a 5 liters aliquotes from each caecum and ascending colon were taken from randomly selected necropsied horses and a total of helminths recovered to identify Strongylidae species. **Results:** 181(66,78%) of all horses were positive. P had significant differences ($p<0,05$) when comparing horses treated once (58,76%) with horses never treated (91,90%). 45,38% of horses showed level I3 of infection. On cultures, 84-100% of larvae were Cyathostominae and *Strongylus vulgaris* was present in 4 of the 6 farms (1-10%). Parasitic burden was between 700-31200 parasites/horse. *Cyathostomum coronatum* and *C. catinatum* were the most prevalent and abundant nematodes. *Strongylus equinus* and *Gyaloccephalus capitatus* were found only in one horse. **Conclusion:** Intensity level and species present rather than prevalence makes the difference between strongylid infection in these horses and those regularly dewormed. Large strongyles can be an important part of the parasitic burden and is more likely to observe typical signs of parasitic disease.

04

PRAZIQUANTEL PLUS IVERMECTIN: A 14 DAY CRITICAL TEST IN HORSES

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Purpose: A Praziquantel / Ivermectin formulation (oral paste) was used in a 14 day critical test against field strains of tapeworms (*Anoplocephala perfoliata*) in horses.

Method: 20 horses with proven tapeworm infestation (eggs detected in faeces with an improved centrifugation-flotation method) were selected from pastures in 1998. On D0, 15 horses received a combined paste* (20mg Ivermectin + 150mg Praziquantel per 100 kg) and 5 horses received a placebo. During the 14 days post-treatment, all faeces from each animal were collected and screened daily for expelled tapeworms. Only tapeworms found in faeces were considered as "removed". To avoid overestimation of efficacy, all tapeworms found at necropsy (D14) were considered as "remained": normal or abnormal (if disintegrating or with a brown discoloration), attached or detached, usual or unusual location.

Results: At necropsy, the 15 treated horses had 0.43 remained tapeworms on average (9 had none), while they had expelled 12.63 tapeworms on average (4 to 24). These figures yield a calculated efficacy of 88% (n=1) 95% (n=5) and 100% (n=9) for the treatment by the critical test (97% on average). In the meantime, the placebo group had expelled no tapeworm in their faeces, and 10.13 (3 to 21) tapeworms on average remained at necropsy. These figures yield 95.8% efficacy by the controlled test.

Conclusion: This study demonstrated >95% cestodicidal efficacy of Virbac's new equine wormer against *Anoplocephala perfoliata*.

* Equimax®: Ivermectin + Praziquantel Virbac Laboratories

05

A survey of anthelmintic susceptibility in cyathostomine nematodes of horses in New Zealand using a larval development assay

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Aim: To use a Larval Development Assay (LDA) to conduct a limited survey of anthelmintic susceptibility in horse cyathostomine nematodes in New Zealand.

Method: The LDA involved culturing eggs to ensheathed 3rd stage larvae in doubling concentrations of ivermectin, ivermectin aglycone or thiabendazole. The mean + 2X standard deviation (SD) for the LC₅₀ values for feral horses (n=22) were estimated and used as the cutoff for the upper limit of normal. For the survey, faecal samples (n=47) were collected from horses from around New Zealand. Resistance factors were calculated by dividing the LC₅₀ by the mean of that from the feral horses.

Results: For ivermectin, 6 of 47 horses had nematodes which gave an LC₅₀ value above the nominal cutoff with resistance factors ranging from 2.3 to 5.2 suggestive of increased tolerance by these nematodes. For ivermectin aglycone, nematodes from 7 of 47 horses gave an LC₅₀ value above the nominal cutoff value with resistance factors ranging from 2.6 to 7.1. These included the same 6 horses that had high LC₅₀ values with ivermectin. For thiabendazole, nematodes from 22 of 47 horses gave LC₅₀ values above the nominal cutoff value with resistance factors ranging up to 6.0. Nematodes from the 6 horses with LC₅₀ values for ivermectin above the cutoff also had LC₅₀ values for thiabendazole above the cutoff.

Discussion: These results suggest an increased tolerance by some cyathostomine nematodes to ivermectin and that approximately 50% of the horses contained cyathostomine nematodes which have increased tolerance to BZs. Of concern is the observation that horses with nematodes that had high LC₅₀ values for ivermectin and ivermectin aglycone also had high LC₅₀ values for thiabendazole.

07

A NATIONAL SURVEY FOR ANTHELMINTIC RESISTANT NEMATODES IN THOROUGHBREDS

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Background: The development of anthelmintic resistant nematodes poses a threat to the health of horses but the extent of the problem is not known.

Methods: 57 Thoroughbred yards sent faecal samples at the time of dosing, 1 week after pyrantel treatment, 2 weeks after benzimidazoles and 3 weeks after ivermectin/moxidectin (ML). Eggs were counted by the McMaster or flotation techniques and *in vitro* sensitivity determined with the microagar larval development test (MALDT).

Results: 86.6% of 732 horses had <50 epg at the time of treatment. 13 horses from 8 yards tested positive for eggs after treatment. The numbers treated, where eggs were present at the time of dosing, and % cured (<50 epg) for benzimidazoles was 16, 56%, for pyrantel was 28, 82% and for ML was 59, 97%. On 4 yards mixed cyathostome/large strongyle eggs were present in 7 horses 1-3 weeks after treatment. Using the MALDT LD₁₀₀ values for ivermectin were above the delineating doses.

Discussion: The small numbers of eggs seen in most yards suggests that pyrantel and/or MLs are effective in controlling most nematodes. However the failure of MLs to eliminate egg counts in two horses combined with high MALDT values suggests that ML resistance may be starting.

Acknowledgements: Work funded by Intervet (UK).

06

COMPARING THE EFFICACY OF MOXIDECTIN WITH IVERMECTIN IN THE DONKEY.

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Background: Previous studies on the use of Ivermectin for the control of strongyles in donkeys had been carried out at The Donkey Sanctuary to investigate the possibility of slowing down the development of resistance. Rotation of anthelmintics was also considered and as a result Moxidectin was adopted as the anthelmintic for the control of strongyles for two years. Collection of data from the use of both drugs allowed comparison of certain parameters.

Method: Conducted at two Sanctuary farms in Devon, where the herds varied in age, population densities, pasture management and parasitological burdens. The trial took place over a four-year period; prior to spring turn out the donkeys were treated with Ivermectin in the first two years and moxidectin in the second two years. The faecal egg count was monitored using the Modified McMaster technique. 15% of the resident herd was sampled and sampling commenced eight weeks post worming and then fortnightly until the ERP was reached. The ERP being 25% of the sample group reaching > 200epg. In addition to descriptive analyses, a general linear model (GLM) was used to analyse the data with time, year and treatment as fixed effects and farm as random. Because of the change in policy during the period of study, year was considered nested within year. Individual samples were considered as replicates. In all analyses, significance was set at the 5% level.

Results: In the GLM, there was a significant time*treatment interaction (P=0.02), suggesting that the treatments differed over time within years.

Conclusion: There was a significant difference in the efficacy of Ivermectin with Moxidectin over time.

Acknowledgements:

The Donkey Sanctuary, especially the Veterinary Department and farm staff.

01 p

STRONGYLES INFECTION AND EOSINOPHILES LEVEL IN WILD VENEZUELAN HORSES.

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Purpose: In order to stimate the impact of strongyles parasitism on some physiological parameters of horses the strongylid infection and eosinophiles level were assesed in three populations of venezuelan horses coming from three different farms. **Methodology:** 1. Area Study and animals: 3 groups (G) of adult horses, males and females, numbered 1-3. G1 (n= 62), and G2 (n=30), from 2 farms located in Tinaco, Cojedes State. G3 (n=51): from 1 farm located in Mantecal, Apure State (Venezuela). 2. Prevalence (P) and Intensity of strongyles infection (SI) were measured by coprological examination using the Mc Master modified Method, by calculating the number of eggs per gram (epg). SI had 3 levels: SI1= epg>0<500epg; SI2=500-1000epg; SI3=>1000epg. 3. Eosinophiles level (E) was measured from EDTA preserved blood samples using an automatic coulter and examining the blood films. None of the horses had been received anthelmintic therapy the last 6 months (G2 had never been received anthelmintics). 4. Statistical analisis was performed using the non parametric Kruskal-wallis method. **Results:** G1: P=63,99%(82,05% of samples in SI1); E=526cell/cm3 (3,38%). G2: P=92,3%, (50% of samples in SI3), E=1236cell/cm3 (8,56%). G3: P=41,48%, (27,65% of samples in level SI3) E=767,43cell/cm3 (5,03%). There was no significant difference on the eosinophiles level between negative and positive animals in any of the levels of SI when comparison was made sharing all groups. **Conclusion:** Eosinophiles level in practice is not always a good indicator of strongyles infection.

02 p

CORRELATION OF EOSINOPHILS IN PERIPHERAL BLOOD TO ENDOPARASITES OF HORSES

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Background: In the course of cellular immunity, the general function of eosinophils against parasites is well known. In the peripheral blood eosinophils exist only for few hours, but with a wide range (physiological values: 40-350/ μ l) depending on parasitic infections or allergic reactions.

Method: In 1998, during a pasture period a herd of 15 horses was examined in order to investigate the correlation of eosinophils to the quantity of strongyle-eggoutput of horses. In autumn, all animals were treated with ivermectin. In 2000, out of these animals 11 horses were kept free of parasites with a periodical treatment of ivermectin and moxidectin during the grazing season. Blood and faecal samples were taken monthly.

Results: During the pasture period of 1998, a high significant correlation of strongyle-eggoutput and increase of the number of eosinophils from ϕ 200/ μ l in April to ϕ 750/ μ l in November could be verified. After treatment with ivermectin a significant reduction of eosinophils was detected. In contrast to this two years later the numbers of eosinophils increased only for a time of six weeks (ϕ 500/ μ l) followed by a continuous decrease to ϕ <100/ μ l.

Conclusion: The range of the physiological limits of eosinophils in the peripheral blood of horses should be critically revised. Further investigations about the influence of pasture-contamination with strongyle-larvae and blood-sucking flies will be conducted.

04 p

PARASITE CONTROL ON THOROUGHBRED TRAINING YARDS

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Background: Published evidence suggests that anthelmintic resistance is likely to be most common in Thoroughbreds. However there is little information on helminth control practices on racing yards in the UK and on those responsible for designing control strategies.

Methods: Approximately 1/6 of the training yards were randomly selected and a telephone questionnaire completed. Yards with the majority of horses below 4 years old were classified as flat, above 4 years as national hunt.

Results: 23% were flat yards, 42% national hunt and the remained mixed. 89% of animals had access to grass during some part of the year. 44% of trainers said they had seen clinical signs of infection the most common being weight loss. There was no logical programme for treatment of new arrivals. Trainers designed 65% of the control strategies and vets only 29%. 40% of yards treated horses every 4-6 weeks, 25% every 7-8 weeks. Only 38% removed faeces from grass twice weekly.

Discussion: There is a very high emphasis on chemical use to control nematodes in Thoroughbred yards and in terms of control this appears to be successful. Despite almost daily visits to yards veterinary surgeons have little input into parasite control. As anthelmintic resistance becomes a greater problem considerable changes in worm control will have to occur.

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03 p

THE SIGNIFICANCE OF FAECAL NEMATODE EGG COUNTS IN HORSES.

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Background: Faecal egg counts are often used to decide whether horses should be treated with anthelmintics. Based on studies in other species it would be expected that there would be a poor correlation between egg counts and nematode numbers in the lumen of the large intestine except possibly in very young animals.

Materials and methods: Rectal samples and large intestines from abattoir horses were taken to the laboratory for 5 replicate McMaster egg counts per sample and 10% worm counts (large strongyles - 11 horses) or 1% worm counts (cyathostomes - 13 horses).

Results: Below 300 epg there was a large variability in replicate egg counts. There was no correlation between cyathostome numbers in the lumen of the large intestine and cyathostome egg counts and no correlation between large strongyle numbers and egg counts. However, with total numbers large strongyles up to 100 there was a correlation with large strongyle egg counts.

Discussion: Faecal egg counts do not indicate the numbers of worms in the large intestinal lumen, although they will obviously indicate the approximate levels of contamination reaching the pasture. When over use of anthelmintics selects for resistance, what should the decision to treat be based on? A better way of estimating worm burdens is required so that worm burdens and pathology can be correlated and there is a scientific basis for administration of anthelmintics

Acknowledgements: Supported by Intervet (UK).

05 p

PARASITES IN THE STOMACH AND SMALL INTESTINE OF HORSES IN THE SOUTH WEST OF ENGLAND

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Background: Changes in parasite burdens in abattoir horses will provide information on anthelmintic use by owners and may indicate changes in climate (global warming).

Methods: Stomachs and small intestines were collected from abattoir horses and the numbers and weights (bots and tapeworms) determined. Blood from horses was assayed with an ELISA for antibodies to tapeworms.

Results: There was no significant change in bot (*Gasterophilus intestinalis*) and tapeworm burdens from two years before, but no *G. nasalis* were found. *Anoplocephala perfoliata* was the only tapeworm found. Only one *Habronema* was seen and *Trichostrongylus axei* was relatively uncommon. Up to 400 worms there was a linear relationship between numbers of tapeworms and total mass. There was a low correlation between ELISA values and tapeworm mass.

Discussion: The control of tapeworms and bots in privately owned horses requires improvement. The linear relationship between tapeworm numbers and mass indicates that crowding is not limiting growth. The correlation between ELISA values and tapeworm mass was not as good as previously published. The apparent disappearance of *G. nasalis* suggests it is very sensitive indicator of local climate change.

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06 p

CYTOKINES PRESENT IN INTESTINAL MUCOSA OF CYATHOSTOMIN-INFECTED HORSES

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Cyathostomins are an important cause of equine colitis. These nematodes spend a substantial part of their lifecycle as larvae in the large intestinal mucosa. Large numbers of larvae (~millions) can accumulate and reactivate synchronously to cause severe, often fatal, colitis. This project is investigating the underlying mechanisms of colitis by measuring levels of pro- and anti-inflammatory cytokines in equine colonic mucosa. So far, we have used RT-PCR to detect IL-2, TNF α , INF γ , IL-4 and TGF β at 6 different sites. Levels of cytokines have been established in clinical colitis cases and in a large number of parasite-infected and uninfected samples taken post mortem at an equine abattoir. In each case, local mucosal cyathostomin burdens are being enumerated and inflammation scores performed histologically. The presence of intestinal inflammatory disease and its severity has been correlated with the local expression of each cytokine. IL-2 and IL-4 were detected at all sites, whilst TGF β and INF γ expression varied. Expression of the latter two cytokines was not correlated with anatomical site or disease status. TNF α was detected most frequently in those horses with colitis, particularly those with infiltrative colitis of unknown origin. This suggests that TNF α may have an important role in pathogenesis of diarrhoea in the horse.

07 p

FAECAL SURVEY ON ENDOPARASITES OF CAB HORSES IN BALI, INDONESIA, AND FECR TESTS FOR MOXIDECTIN AND DORAMECTIN

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Background: According to the official veterinary census, the horse population in Bali consists of approximately 1000 animals, most of them are cab ('Dokar') horses. To date, knowledge about equine parasite infections in Bali is missing. The aims of the present study were (i) to obtain first data on endoparasites of horses in this tropical island and (ii) to evaluate the efficacy of two new anthelmintics against equine strongyles.

Methods: Faecal samples were collected from 148 adult cab horses of different areas in August 2000 and examined using the McMaster technique and the formalin-ether sedimentation method. Horses with ≥ 150 strongyle egg counts were orally treated with moxidectin (Equest® 1.89% gel, Fort Dodge; n=12) or doramectin (Dectomax® 1% solution, Pfizer; n=12) both at a dose rate of approximately 0.2 mg/kg bwt. and re-examined 10 days later, and the mean faecal egg count reductions (FECR) were calculated.

Results: Strongyle (mainly cyathostomes) and *Parascaris* eggs were found in 81% and 2% of the horses, respectively. The strongyle egg counts were relatively low (median: 200 EPG; arithmetic mean: 360 EPG; maximum: 2650 EPG). Faecal stages of other helminth parasites or of protozoa were not detected. Both moxidectin and doramectin reduced the geometric mean strongyle egg counts by >99%.

08 p

THE INTERNAL PARASITES OF DONKEYS IN KIAMBU DISTRICT, KENYA

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Background

The main objective of the above study was to identify the internal parasites in donkeys in Kiambu District of Kenya.

Methods

Donkeys with no history of deworming were considered in this study. They were put down and the various portions of the gut examined for parasites. L3 were cultured from pooled faeces and identified. In total, 353 donkeys were considered.

Results

Both large and small Strongyles were isolated. In addition, *Dictyocaulus arnfieldi*, *Paranoplocephala mamillana*, *Anoplocephala perfoliata*, *Gasterophilus intestinalis*, *Habronema muscae* and *Parascaris equorum* were also isolated.

Conclusion

The donkeys, like any other domestic animal are infected by a number of internal parasites.

Acknowledgement

I am grateful to the Danida - funded, Livestock Helminth Research Project at the University of Nairobi, Kenya.

09 p

THE DEVELOPMENT OF INFECTIVE CYATHOSTOME LARVAE DURING THE WINTER

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Background: In the early 1970s it was reported that eggs did not develop to L₃ larvae during the winter in Bristol. Global warming with mild winters suggests that this may no longer be true.

Materials and Methods: 100 gr faecal samples from a naturally infected pony were placed outside in large petri dishes in screened boxes every two weeks from late October until early February. Triplicate samples were brought inside every two weeks and larvae recovered on a mini Baermann apparatus. In addition faecal samples held at room temperature at different stages of development (0-7 days) were cooled to -16°C for 24 hours to simulate hard frosts.

Results: Freezing did not affect the numbers of larvae recovered on days 1, 2, 5, 6 and 7 but on days 3 and 4 recovery was reduced by up to 93%. With daytime temperatures averaging 10°C up to mid December up to 41% of eggs were recovered as L₃ in mid December. When temperatures fell with frosts at night the numbers recovered were reduced to 9% but when mild weather resumed the numbers of larvae collected from the Baermann apparatus from the same initial batch increased. Some development occurred whenever dishes were placed outside.

Discussion: Mild spells in winter can be important in the development of infective larvae on pasture although heavy frosts can kill developing larvae. This stresses the need for collection of faecal samples during the winter. The temperature outside affects the numbers of larvae collected by Baermannisation, suggesting larvae are slow to 'wake up' from cooling.

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010 p

DELINEATING DOSES FOR DETECTION OF ANTHELMINTIC RESISTANT EQUINE NEMATODES.

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Background: Previous studies have shown that use of LD₅₀ values for the diagnosis of thiabendazole resistance is insufficiently sensitive. Therefore delineating doses were established for use in the egg hatch (EHT) and microagar larval development tests (MALDT). The optimum conditions for equine nematodes in the MALDT were also determined.

Material and methods: Faecal samples were collected from Dartmoor (wild and untreated) ponies and taken anaerobically to the laboratory for extraction and testing. Standard EHT and MALDT were performed on the samples. MALDT: 10µl anthelmintic, 150µl 2% agar, 10µl eggs plus amphotericin B and 10 µl yeast extract were used in MALDT tests in 96 well plates with water in outside wells.

Results: The optimum conditions for cyathostomes in the MALDT gave 48% recovery of eggs as L₃ larvae and contained 725 µg/ml Amphotericin B and 2.5x dilution of yeast extract. The delineating doses for this test were thiabendazole 0.125 ppm, levamisole 0.4 ppm and ivermectin 1.5 ppm. The delineating dose for thiabendazole in the EHT was 0.185ppm.

Discussion: Delineating doses provide a more sensitive method of detecting anthelmintic resistant nematodes than LD₅₀ values and will detect 2% of resistant nematodes in the EHT (unpublished). It would be of value to relate the test to both PCR probes for benzimidazole resistance and to faecal egg count reduction (FECRT) in treated horses.

Acknowledgement: Supported by Intervet (UK).

012 p

NEMATODE CONTROL ON THOROUGHBRED STUDS

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Background: Foals are particularly at risk from nematode infections but the practical actions taken by horse owners in general and Thoroughbred breeders in particular to control infections in foals and mares in the UK is not known.

Methods: A random sample of Thoroughbred breeding studs were made from 'The Directory of the Turf 1999'. Managers at 84 yards were contacted by letter and a questionnaire completed during a telephone interview. Data was analysed using EPINFO.

Results: Mares were grazed all the year round with a mean of 10.4 hours on grass during the winter and 23.2 hours in summer. Weanlings and yearlings were grazed separately on 74% of yards. Visiting mares were grazed separately on 56% of yards. Only 24% removed faeces twice weekly with a further 20% removing faeces weekly. 73% grazed foals on the same pasture each year. Almost half treated animals with anthelmintic every 4 weeks. However, only 46% used a double dose pyrantel to remove tapeworms and only 18% planned to treat for encysted cyathostomes. 46% used faecal egg counts if a worm problem was suspected but otherwise egg counts were hardly used.

Discussion: On most Thoroughbred studs control of nematodes is highly reliant on frequent use of anthelmintics. Obvious worm avoidance strategies such as not using the same pasture each year for foals are not widely used. Using faecal egg counts to plan control is ignored and inadequate use is made of management of pasture such as removal of faeces.

Acknowledgement: Supported by Intervet (UK).

011 p

COMPARISON OF FIVE METHODS FOR THE RECOVERY OF MUCOSAL CYATHOSTOME LARVAE IN EQUINES

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Background: This study was performed to compare five methods for the recovery of mucosal larvae of cyathostomes from the large intestine of naturally infected pony foals.

Methods: The foals were housed in December and necropsied 6-8 weeks later, following fenbendazole or moxidectin treatment or remaining untreated. Mucosa samples of caecum, ventral and dorsal colon were examined for inhibited early L₃ (EL) and developing L₃ and L₄ (DL) by the following methods: transmural illumination (TMI; Reinemeyer & Herd 1992; Klei et al. 1993), pepsin-HCl digestion (DIG; using large or small amount of mucosal tissue) and a compressorium method (COM; former used for the detection of *Trichinella* larvae).

Results: Higher numbers of DL were found in tendency using the TMI techniques when compared to the DIG methods independent of the intensity of infection. There was no significant difference in DL counts between both the TMI techniques. The COM method showed a tendency in higher infections to overestimate DL counts in comparison to the other methods. EL were only detected using the DIG or COM methods, and there was no significant difference of EL counts in higher infected animals between these methods. In low infections, however, higher numbers of EL were found when a large amount of mucosal tissue was examined by the DIG method as compared to a small amount of mucosa examined by the DIG or COM methods.

Conclusion: The COM method seems to be an inexpensive and fast alternative for the recovery of EL, at least in higher cyathostome infections, if the DIG equipment is not available.

013 p

EFFICACY OF AN IVERMECTIN-PRAZIQUANTEL COMBINATION IN EQUIDS.

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Background: Praziquantel has high activity against tapeworms and its combination with ivermectin would make a valuable product for treatment of nematodes, cestodes and bots in horses. Currently praziquantel is not registered in the UK for the control of tapeworms in horses.

Materials and methods: Wild Dartmoor ponies were used in this trial. Animals were randomised based on faecal egg counts (epg ≥ 200). Worm burdens were counted by standard procedures in 8 untreated and 8 treated animals 14 days after the oral administration of a paste formulation of the combination product.

Results: 100% of the control ponies were infected with *Gasterophilus intestinalis* and nematodes and 75% were also infected with *Anoplocephala perfoliata*. A single treated animal was withdrawn due to incomplete dosing. A 99.0% reduction in *G. intestinalis*, 100% efficacy against nematodes of the stomach, small intestine and humeral stages of the large intestine and a 100% reduction in *A. perfoliata* was found when treated animals were compared to controls.

Discussion: The trial allowed the assessment of the efficacy of ivermectin (200 µg/kg) in combination with praziquantel (1.5 mg/kg) in concomitant infection with bots, tapeworms and nematodes in horses. Both nematodes and *A. perfoliata* are recognised as important causes of colic in horses. In controlling both nematodes and cestodes the combination of ivermectin and praziquantel as a paste will make a valuable contribution to equine health.

014 p FIELD EFFICACY OF AN IVERMECTIN+PRAZIQUANTEL ORAL PASTE IN HORSES

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Purpose: The aim of this controlled and randomised field study was to assess the efficacy of the combination Ivermectin+Praziquantel in the treatment of natural nematode and tapeworm infections in horses.

Method: 357 horses from various areas in 6 European countries were treated on D0 with 1 graduation per 100 kg of an anthelmintic oral paste. 269 horses received the combined paste* (20mg Ivermectin + 150mg Praziquantel per 100 kg) and 88 horses received a reference Ivermectin paste (20mg Ivermectin per 100 kg). Faecal samples were collected on D0, D14 and D21, processed for nematode egg count (McMaster flotation) and improved tapeworm egg detection (specific centrifugation-flotation method). Percentages of nematode FECR were calculated. On D14 and D21, horses with no cestode egg were considered as cestode-free. **Results:** The percentage of nematode FECR was higher than 99% in both groups on D14 and D21. 98.7% of the cestode infected horses treated with the combination of Ivermectin-Praziquantel paste (n=166) were cestode egg free on D21.

Conclusion: As previously shown by necropsy means, this large-scale field trial clearly confirmed by coprological means the high nematocidal and cestodicidal efficacy of a new equine wormer.

* Equimax®: Ivermectin + Praziquantel Virbac Laboratories

015 p EFFICACY OF IVERMECTIN AGAINST BOT AND NEMATODE INFECTIONS IN HORSES.

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Purpose: The aim of this blind and controlled trial was to confirm the high efficacy of Ivermectin (given at 200 µg/kg b.w. in a combined oral paste with Praziquantel*) against natural bot and nematode infection in horses

Methods: 61 horses from different countries (Poland, Argentina, Australia, Hungary) were randomised on the basis of D-7 nematode faecal egg counts (counts >200 epg) and orally treated (on D0) either with the combined paste (group IP) or with a Praziquantel paste only (group P). Worm counts at necropsy were performed by standard procedures on D14.

Results: D14 faecal egg counts were reduced by 100% in group IP compared to group P. At necropsy, horses from group P were infected with adult small strongyles (65%), larval small strongyles (65%) and adult large strongyles (69%). In the following parasites species, a statistically significant difference was shown on D14 between groups (% reduction based on geometric means between brackets): adult and larval *Parascaris equorum* (99.6%, 97.6%), adult *Trichostrongylus axei* (97.3%), larval *Oxyuris equi* (98.3%), *Habronema muscae* (100%), adult *Strongylus edentatus* and *vulgaris* (100%, 99%), adult *Tridontophorus* spp. (88.6%), adult and larval *Cyathostominae* (99.5%, 94.5%) and third instar *Gasterophilus nasalis* and *intestinalis* (100%, 98.4%).

Conclusion: This study conducted in various geographic areas confirmed the high efficacy of Ivermectin (given at 200 µg/kg b.w. in a combined oral paste with Praziquantel) against natural bot and nematode infection in horses.

* Equimax®: Ivermectin + Praziquantel Virbac Laboratories

016 p CESTODICIDAL EFFICACY OF AN IVERMECTIN+PRAZIQUANTEL ORAL PASTE IN HORSES

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Purpose: The aim of this controlled and randomised study was to assess the efficacy of the combination Ivermectin+Praziquantel in the treatment of naturally *Anoplocephala perfoliata* (A.p), *Anoplocephala magna* (A.m.) and/or *Paranoplocephala mamillana* (P.m.) infected horses.

Method: 48 positive horses (on the basis of tapeworm eggs in faeces) were selected from pasture where A.p., A.m. and P.m. have been previously detected in congeners at necropsy. 24 horses received the combined paste* (20mg Ivermectin + 150mg Praziquantel per 100 kg) and 24 were left untreated (Control). At necropsy, 10 days after treatment, tapeworms were identified and counted. For each species, geometric means were calculated and compared between groups.

Results: At necropsy, 83% of Control horses harboured tapeworms. A.p. was recovered in 75% (max.: 128 worms), A.m. in 17% (max.: 2 worms) and P.m. in 38% of horses (max.: 67 worms). A. m. or P. m. were present in 50% of Control horses. All the horses treated with the Ivermectin-Praziquantel combined oral paste were cestode-free. For each tapeworm species, geometric means were statistically different between Treated and Control groups.

Conclusion: This study demonstrated a high cestodicidal efficacy of a new equine Virbac's dewormer against the three tapeworm species described in horses: *Anoplocephala perfoliata*, *Anoplocephala magna* and *Paranoplocephala (Anoplocephaloides) mamillana*.

* Equimax®: Ivermectin + Praziquantel Virbac Laboratories

017 p TREATMENT INTERVALS FOR ANTHELMINTIC PASTES IN NATURALLY INFECTED HORSES

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Background: The frequency of anthelmintic treatment must strike a balance between the need to minimise the number of eggs in faeces and the need to discourage the development of resistant parasites. Selecting the most appropriate interval between treatments is just as important as selecting the most reliable anthelmintic.

Method: A total of 80 horses naturally infected by gastrointestinal nematodes were selected in 2 sites (Australia & Brazil), ranked by D-7 egg counts and randomly allocated into 5 groups (n=16). On D0, horses were treated by oral route with 4 endectocide-based pastes and remained on the pasture (natural challenge). Group A received a combination of ivermectin/praziquantel, Group B received a generic ivermectin, Group C received a reference ivermectin, Group D received a moxidectin and Group E remained untreated (Placebo). Individual faecal samples were taken at weekly interval from D14 to D70 post-treatment. Treatment interval was based on ERP*, time after treatment to reach 200 epg or more (Arithmetic Mean).

Results: From D14 to D56, egg counts of all groups were statistically different from Placebo (P<0.05). Group C reached the fixed threshold on D49 while Group B exceeded this point on D56 and Group A and D on D70. The mean number of days to recurrence of eggs in faeces was 60 days Group A, 56 days Groups B and C and 64 days Group D.

Conclusion: The interval between 2 treatments (ERP) can strongly vary according to the fixed threshold egg. In this study, the treatment interval recommendation was 2 months whatever the product used. Faecal sampling remains the simplest method for equine parasite control. If treatments are combined with other methods, the number of treatments required will be reduced even further.

* egg reappearance period

018 p

AN ABATTOIR SURVEY FOR ANTHELMINTIC RESISTANCE IN EQUINE NEMATODES

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Background: To date all surveys for anthelmintic resistant equine nematodes have been based on samples collected from horse yards or farms. As an alternative, faecal samples were collected from horses at an abattoir.

Materials and methods: Faecal samples were collected at slaughter, transported on ice to the laboratory and eggs extracted from samples containing over 100 epg. Eggs were tested with the egg hatch (EHT) and microagar larval development tests (MALDT) using thiabendazole, levamisole and ivermectin.

Results: In 33 horses no LD₅₀ values with thiabendazole for the EHT were above 0.1 ppm. However, 72% had some eggs hatching at or above 0.2 ppm with some hatching above 0.5 ppm. All levamisole tests were within the 95% confidence limits, but one ivermectin test was outside the 95% confidence limits.

Discussion: Using the WAAVP definition of anthelmintic resistance there was no benzimidazole resistance, but using a concept of a delineating dose with eggs hatching at or above 0.2 ppm thiabendazole being resistant, the majority of horses contained some benzimidazole resistant worms. One horse had nematodes with a changed response to ivermectin. The data suggests the need to revise the interpretation of the EHT.

Acknowledgement: supported by Merial Animal Health.

019 p

PREVALENCE OF BENZIMIDAZOLE-RESISTANCE OF HORSE STRONGYLES IN SLOVAKIA

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A survey was conducted to determine the prevalence of benzimidazole-resistant small strongyles in Slovak horse herds.

The present study included 26 horse stud farms with total number of 355 horses. The number of horses on the farms varied from 9 to more than 100, and horses of all ages were included. The criteria used were that minimum ten horses were available for coprological examination in each farm and animals had to be left untreated at least 10 weeks. Minimum of 7 horses were used for faecal egg count reduction (FECR) tests. Horses were distributed among 1, 2 or 3 equally sized groups. Group 1 was a benzimidazole treatment group, Group 2 ivermectin treatment group and Group 3 served as pyrantel treatment group. Egg hatch test and larval development test were also used to measure the sensitivity of eggs *in vitro* to thiabendazole.

Resistance to benzimidazoles was detected on 16 (61.5 %) farms, with FECR values ranging from 31.1 % to 86.3 %. Larval cultures after fenbendazole treatment revealed exclusively cyathostome larvae. Ivermectin was tested on 11 farms and proved to be effective on all. Pyrantel was tested on 4 farms and FECR test indicated high efficacy (92-100 %). Results from *in vitro* egg hatch assays indicated that mean concentrations of thiabendazole that inhibited hatching in 50 % of the eggs (ED₅₀) in resistant populations were over 0.1 µg.ml⁻¹.

The results of our study suggest widespread resistance to fenbendazole in equine cyathostomes in Slovakia and need for innovative approaches such as alteration between drugs and using modern anthelmintics in an attempt to minimize the rate of selection of anthelmintic resistance.

N

Chemotherapy and pharmacokinetics

EFFICIENCY OF ANTHELMINTICS IN CATTLE

N1

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A survey to verify the efficacy of three groups of broad-spectrum anthelmintics was carried out in 49 farms, chosen at random from 26 different counties in Southern Brazil. At each farm, calves ageing 6-9 months old were used in a faecal egg count reduction test along with larval culture for larval differentiation. Animals were as homogeneous as possible regarding age, weight, body condition and then, at random, allocated to four different groups of 10 animals each and treated as follows: untreated control, ivermectin (0.2mg/kg), levamisole phosphate (4.7mg/kg) and albendazole (5mg/kg). Results obtained 10 days post-treatment revealed that from the farms evaluated, 20%, 6%, and 4% of the animals examined showed egg reductions below 90% after treatment with ivermectin, levamisole phosphate and albendazole, respectively. Percentage reductions were in the range of 80 to 100% for albendazole, 83 to 100% for levamisole phosphate and 57 to 100% for the group treated with ivermectin. Results from the larval cultures indicated that in the group treated with albendazole the prevailing genus surviving treatment were *Cooperia* spp. and *Haemonchus* while in animals treated with levamisole phosphate were *Ostertagia* spp and *Haemonchus* whereas in the group treated with ivermectin there was a high percentage of *Cooperia* surviving treatment with this drug. These results indicate a less than expected efficacy under field conditions, mainly for the group of ivermectins and this should be further monitored due to the very common use of this chemical group by Brazilian farmers.

N2

ANTHELMINTIC ACTIVITY OF ALBENDAZOLE ON *PARAMPHISTOMUM DAUBNEYI* IN CATTLE

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Background: Due to European Agricultural Policy there has been husbandry extensification in France and concomitantly, exportation of Limousin and Charolais breeds from their original areas. The use of flukicides only efficient on *Fasciola hepatica* is also frequent. This has resulted in the expansion of *Paramphistomum daubneyi* over the last few years. Treatment drugs are scarce and data regarding MRL of Bithionol sulfoxide are incomplete and its future is uncertain. Thus testing of other drugs is required for assessment of efficacy. Recently, several drugs (albendazole, closantel, netobimin, nitroxylin, thiophanate) have been tested but failed to cure paramphistomosis.

Method: The purpose of this study was to confirm and complete data concerning albendazole efficacy, used at 15 mg/kg BW against *Paramphistomum daubneyi*, using Bithionol sulfoxide as a control drug. Faecal egg counts performed over 6 weeks allowed a calculation of curative efficacy.

Results: From Day 8 to Day 43, the parasitic infestation level is significantly superior albendazole group than in bithionol sulfoxide group ($p=0.0005$).

Conclusion: the use of albendazole at 15 mg/kg BW is not satisfactory in curative treatment of rumen fluke infection in cattle. Other investigations should be carried out with other galenic forms or other dosage regimes.

N3

THE BIOAVAILABILITY OF CLOSANTEL IN CATTLE DEPENDS ON PRODUCT QUALITY

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Background: It is well known that the plasma concentration profile of closantel is an excellent predictor of its efficacy against haematophagous parasites. The quality of the closantel-containing product administered accordingly is of crucial importance.

Method: Two groups of 6 calves received a 2.5 mg/kg BW subcutaneous dose of closantel, either with the original product Flukiver® (50 mg closantel/ml) or with the generic product Ditavet® (50 mg closantel/ml). Plasma kinetics and safety parameters were monitored for up to 42 days after administration.

Results: The absorption rate and maximum plasma concentrations with Ditavet® were lower than with an equal dose of Flukiver®, but the elimination rate was slower. Ditavet® provoked severe swellings in 5 calves. It was found that Ditavet® contained less active ingredient than stated on the product information and more closantel-related impurities than those accepted for Flukiver®.

Conclusions: The study makes evident that Ditavet® is an inferior product compared to the original product Flukiver. with regard to efficacy, tolerance and consumer safety. This could be due to an inferior quality of the active ingredient included and/or of differences of the product vehicle. When used according to its label instructions, Ditavet® will achieve only about 70 % efficacy against adult stages of fluke.

N4

IS INHIBITION OF FEEDING BY MACROCYCLIC LACTONES A PART OF THEIR ACTION *IN VIVO* ?

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Background: Macrocyclic lactones (MLs) are known to cause paralysis of somatic musculature and also inhibition of nematode feeding by paralysis of the pharynx *in vitro*. The relative importance that these 2 factors play in the *in vivo* action of the drugs is unclear. This study aimed to determine whether significant inhibition of feeding occurred in *Haemonchus contortus* in sheep dosed with ivermectin.

Methods: Uptake of [³H]inulin by nematodes following intravenous (IV) administration to sheep was used to assess nematode feeding. Levels of feeding were compared in ML-susceptible nematodes recovered from sheep treated with ivermectin or left untreated. Feeding levels were also compared in ML-susceptible and ML-resistant nematodes contained within the same sheep dosed with ivermectin. **Results:** ML-susceptible nematodes recovered from sheep treated with ivermectin showed equivalent feeding levels to those from untreated sheep. In addition, in 3 separate animals, ML-susceptible nematodes showed equivalent feeding to ML-resistant nematodes contained within the same sheep which had been treated with ivermectin.

Conclusions: Ivermectin had no effect on nematode feeding under our experimental conditions. As expulsion of *H. contortus* nematodes from sheep following ivermectin treatment is known to occur at a time point soon after the sampling time used in this study, inhibition of feeding appears not to be a significant part of the toxic action of MLs towards this species *in vivo*.

N5

DISTRIBUTION OF TOPICALLY ADMINISTERED MOXIDECTIN TO TISSUES OF ENDO- AND ECTO-PARASITES LOCATION

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Background: Topical formulations of endectocide compounds are currently available for administration to cattle. The plasma profiles of ivermectin, doramectin, eprinomectin and moxidectin (MXD) administered pour-on have been recently determined. However, there is a lack of data on the tissue distribution of topically applied endectocides. The tissue concentrations of pour-on administered MXD in lungs, different sections of the gastrointestinal (GI) mucosa (abomasum, small and large intestine) and in skin from different anatomical sites (face, chest and thigh), are reported.

Methods: Parasite-free Holstein calves (n=15) were treated with MXD pour-on (Cydectin®, Fort Dodge, USA) at 500 µg/kg. Three (3) animals were sacrificed at days 1, 7, 14, 24 and 35 post-treatment to obtain samples of lung tissue, GI mucosa and skin from different body sites. Samples were processed and analysed by HPLC to determine MXD concentrations.

Results: MXD was detected in the different sampled tissues up to 35 days after its pour-on administration. The highest MXD tissue concentrations were measured in the skin of the chest and thigh (peak concentrations between 88 and 200 ng/g). MXD tissue availabilities (expressed as the ratio AUC_{tissue}/AUC_{plasma}) were 2.7- (lung), 6.4- (abomasal mucosa), 8.9- (duodenal mucosa), 18.5- (ileal mucosa), 5.7- (caecal mucosa), 4.7- (colon mucosa), 29.8- (chest skin), 20.7- (thigh skin), 8.4-fold (face skin) higher than that obtained in plasma.

Conclusion: Sustained low plasma profiles, extensive tissue distribution and an important skin depot characterise the kinetic behaviour of topically administered MXD to cattle. The differences observed on the distribution of pour-on administered MXD to different skin sites and GI sections, may explain the variation in its efficacy/persistence of activity against endo- and ectoparasites of different location.

N6

QUANTITATIVE DISPOSITION OF MOXIDECTIN IN THE GASTROINTESTINAL TRACT OF SHEEP.

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Background: The pharmacokinetic behaviour of Moxidectin (MOX) has been reported in the peripheral plasma of sheep but there is no information on its quantitative kinetic disposition in gastrointestinal fluid and particulate digesta nor on its quantitative disposition in bile.

Method: Sheep were fitted with rumen and abomasal sampling cannulae and digesta fluid flow was monitored using intraruminal (IR) infusion of a Cr-EDTA marker. A re-entrant bile cannula with *in situ* monitoring pump quantified biliary secretion. MOX was administered (200µg/kg) IR and subcutaneously (SC) and its kinetic disposition determined in plasma and the cannulated compartments.

Results: IR administered MOX associated with rumen particulate digesta with less than 3% of dose exiting the rumen in fluid digesta; the flow rate from the rumen extending drug presence in the abomasum. MOX remained particulate bound with only 0.5% of the IR dose traversing the abomasum in fluid digesta. No SC administered MOX was detected in the rumen and less than 1% of the equivalent IR dose was secreted into the abomasum. After absorption, MOX was cleared at a half life of 340-380h following both routes of administration. Almost half of the IR dose and 130% of the SC dose was secreted in bile demonstrating that biliary metabolites underwent extensive enterohepatic recycling.

Conclusion: The passage of particulate digesta-associated MOX from the rumen significantly influenced the duration of MOX in the upper gastrointestinal tract with minimal presence in digesta fluid. Thereafter, the extended presence of MOX in peripheral plasma with extensive recycling of biliary secreted drug is believed to contribute to greater availability of MOX to intestinal compared to abomasal parasites.

N7

EFFECT OF PARASITISM ON THE PHARMACOKINETICS OF MOXIDECTIN IN LAMBS

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Most pharmacokinetic studies on anthelmintics have been carried out in non-parasited animals. However, it seems likely that parasitic burdens could influence the disposition of anthelmintics drugs. The pharmacokinetic of moxidectin administered orally and by subcutaneous injection were compared in lambs exposed to parasitic infection and in parasite naive lambs. Plasma samples were analysed for moxidectin over a 40 days period following administration. Pharmacokinetic parameters demonstrated significantly lower peak levels, area under the curve (AUC) and mean residence time (MRT) in the parasitized group as compared with naive group for both administration of moxidectin. The mean decrease in AUC was 52% and 46% for moxidectin respectively administered by subcutaneous and oral route. There was also a significant reduction in the remanence of the drug in the parasited lambs as compared with non-parasited animals. This important decrease of the exposition level to moxidectin could be due to an increase of the clearance of the drug in infected animals. These results provide evidence of the parasitism infection influence on the disposition of moxidectin and deserve to be correlated with efficacy studies.

N8

EVALUATION OF ANTHELMINTIC ACTIVITY OF HERBAL PREPARATIONS USED BY PASTORALISTS IN KENYA

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Background: *Haemonchus contortus* is the most important nematode parasite of small ruminants in the tropics and subtropics and control is compromised by development of resistance to conventional anthelmintic drugs. The use of ethnoveterinary preparations that have a strong tradition and widespread use amongst pastoralist and smallholder communities in Kenya, provide an alternative method of control. Four plants *Myrsine africana*, *Rapanea melanophloeos*, *Hagenia abyssinica* and *Albizia anthelmintica* were tested in the following model:

Method: Young sheep infected with *H. contortus* were used in all trials. 15 animals were used for each treatment and control group. Dose-titration was conducted for all plant preparations. Five sheep were administered with the 'traditional' dose rate while 5 others were given half and double the dose rate respectively. Efficacy was indirectly assessed by faecal egg count (FEC) depression. Effects of parasite infection were also monitored by packed cell volume (PCV) determination. Animals were monitored post-treatment for behavioural responses to determine the safety of the preparations.

Results: No adverse effects on behaviour were observed after treatment. No significant differences in FEC reduction occurred between the control and treatment groups. No significant dose effect was observed on PCV, FEC and weight in the treatment groups.

Conclusion: None of the above plant preparations tested have shown an efficacy to warrant animal slaughtering and worm recovery. Further studies are planned using mouse nematode *Heligmosomoides polygyrus*, as a model for estimating anthelmintic effects of plant preparations against nematodes in monogastric animals.

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N9

ANTHELMINTIC RESISTANCE IN SMALL-RUMINANT FLOCKS IN SPAIN: RISK FACTOR INVESTIGATIONS.

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Background: First description on the extension of small-ruminant AR in Spain has shown a general flock-prevalence of around 20%. Despite of the absence of epidemiological studies on AR, several variables have been pointed out as potential risk factors.

Method: 77 small-ruminant flocks were surveyed for AR and a questionnaire on farm characteristics and management was administered to the farmers. Questionnaire and AR results were cross-tabulated and chi-squared (χ^2) univariable analyses were performed to assess significant relationships.

Results: The main variable associated with AR or AR-suspect was breed. Flocks with foreign breeds or their crosses had a higher odds of presenting AR compared to local breeds (37.1% vs 14.2%; $P=0.07$). Higher odds were also observed in flocks that bought replacement animals (females) (50% vs 27.9%), but this relationship was not statistically significant ($P=0.26$). On the other hand, the use of common pasture seemed to reduce the odds of AR compared to that for flocks grazing private pasture areas (26.3% vs 46.6%; $P=0.2$). Farmers with flocks with AR complained significantly more about poor anthelmintic efficacy than farmers with flocks with susceptible parasite strains (49% vs 22%, $P=0.024$).

Conclusion: The potential importance of breed on the development of AR is stressed. Most of the variables previously described as potential risk factors for AR have not been found as such in this study. Only the introduction of new animals and the use of private pastures presented a certain degree of association with AR, but the small sample size of the survey precluded obtaining significant results. The impact of AR on the flocks seemed to be perceived by many of the affected farmers.

N11

MACROCYCLIC LACTONES: DOMINANCE OF RESISTANCE IN *OSTERTAGIA CIRCUMCINCTA*.

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Confusion exists on the relative effects of moxidectin (MOX) and ivermectin (IVM) on selection for drug-resistance. While oral MOX has higher therapeutic efficacy than oral IVM, it also has a period of declining activity during which resistant parasites may establish. There may, therefore, be conflicting effects between the therapeutic 'head' and the prophylactic 'tail' on selecting resistant parasites. As the dominance of the resistance gene R is a key factor in the development of treatment failure, the present study was designed to measure head and tail efficacy against populations of *O. circumcincta* known to be either homozygous-susceptible (SS), -resistant (RR) or heterozygous resistant (RS). Therapeutic efficacy, or 'head' selection, was determined by treating established infections of each parasite population with oral IVM, capsules containing IVM (IVM-CRCs), oral- or injectable-MOX or no treatment. Prophylactic efficacy, or 'tail' selection, was determined by treating animals with IVM, IVM-CRCs or oral-MOX 9-10 days prior to challenge with 10,000 larvae of each parasite population. Dominance of resistance was then determined by comparing efficacy of each treatment against RS and RR. Results indicated that resistance to MOX was recessive during 'head' selection but dominant during the residual 'tail'. Resistance to oral IVM 'head' selection was dominant while resistance to IVM-CRCs was also dominant in 'head' selection but recessive during the period of the prophylactic 'tail'.

N10

HOST RESPONSE IN DIMINAZENE DIACETURATE WITH ANTIPYRINE AND PROCAINE (TRYPAN) TREATMENT AGAINST *TRYPANOSOMA EVANSI* INFECTED BUFFALO CALVES

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Background: Trypanosomosis or "surra" in Indian subcontinent is caused by *Trypanosoma evansi* in domestic livestock which is highly underestimated in view of its subclinical nature in cattle and buffaloes. The drug, Diminazene Diaceturate with antipyrine and Procaine (Trypan) has painless, antipyretic and long lasting effect. The present study deals with host response and immunochemical changes in *T. evansi* infected, treated and immuno-modulated buffalo calves.

Materials and Methods: Fourteen indigenous buffalo-calves were grouped into five groups. Group 1 (Infected and treated), Group 2 (Infected and immuno-modulated), Group 3 (Infected immuno-modulated and treated), Group 4 (Infected control) and Group 5 (Uninfected control). The dose of Trypan used was @ 3.5 mg / kg b.w., procaine @ 0.2 mg / kg b.w. and antipyrine @ 4.16 mg / kg b.w. The clinical response and immunomodulatory changes were studied.

Results and Discussion: The experimental infection was established by s/c infection of 1×10^7 tryps / calf. The infected animals were treated with Trypan, at the peak of parasitaemia on 7 days post infection. The results showed decreased clinical response, albumin level, A/G ratio, higher CIC and quick absorption of drug following I / m route in the plasma. The parasites were cleared within one and half to two hours of administration. The minimum effective concentration of Trypan was achieved within two minutes and was maintained for six days. The value of pyruvate were decreased. However, the values were increased after the relapse which occurred on 42 days post infection in two buffalo-calves after the treatment. The detail observations have been discussed keeping in mind the role of chemotherapy in "surra" in cattle and buffaloes.

N12

FENBENDZOLE TREATMENT IMPROVES INTESTINAL STRUCTURE AND FUNCTION IN *GIARDIA* INFECTED CALVES

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Background: *Giardia duodenalis* is an enteric protozoan parasite that has been identified in up to 100% of beef and dairy calves. Infected calves and lambs may have diarrhea and decreased weight gains. We have previously shown that fenbendazole is effective in treating giardiasis in calves. The aim of this study was to determine if morphological and physiological improvements occur following fenbendazole treatment in naturally infected calves.

Method: *Giardia* infected calves were treated with fenbendazole ($n=6$, 5 mg/kg for 3 days) or saline ($n=6$). Calves were euthanized 7 days following treatment. Antiparasitic efficacy, morphological measurements and disaccharidase activity were evaluated.

Results: Fenbendazole eliminated the *Giardia* infection in all treated calves. Treatment was associated with a decrease in intraepithelial lymphocytes, an increase in microvillus surface area and an enhanced disaccharidase activity.

Conclusion: These results demonstrate that fenbendazole is an effective treatment for giardiasis in calves. Fenbendazole eliminated *Giardia* trophozoites from the small intestine of calves resulting in increased microvillus surface area and greater intestinal enzyme activity. Controlling *Giardia* in calves may result in improved intestinal function, thereby providing economic benefits through increased weight gains and feed efficiency.

N13

THE EFFICACY OF IVOMEC® PRE-MIX IN ERADICATING MANGE FROM GROWING PIGS

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Background: Many acaricides used to treat mange in pigs are injectable products, requiring one or two injections over a short interval of time. Because of welfare considerations, the risk of muscle damage, and the labour associated with handling pigs for treatment, many swine producers prefer to use in-feed preparations, especially for growing pigs.

Method: 24 groups of heavily infested pigs were allocated alternatively to 12 groups medicated with IVOMEC® Pre-mix and 12 control groups, based on weaning weight and the presence of mites and ear lesion scores. Pigs were medicated for 7 consecutive days at a dose rate of 100 mcg ivermectin/kg body weight. All pigs were examined pre-weaning (day -2), and 9, 48 and 114 days after medication ceased, and the size of ear and body lesions, presence of mites and eggs, rubbing and scratching episodes, body weight, dermatitis (at slaughter) and antibodies to *Sarcoptes scabiei* var *suis* (day-2 and day 114) recorded.

Results: No live mites or eggs were detected on medicated pigs 9 days following removal of medication. At 48 and 114 days, mean rubbing and body lesion scores were both <0.03 in medicated pigs, compared with 0.52 and 1.1 ($P < 0.0001$) respectively in non-medicated pigs. Dermatitis scores and ELISA values were also significantly lower ($P < 0.001$) at slaughter and medicated pigs grew significantly faster (4.9%; $P < 0.001$) than non-medicated pigs.

Conclusion: Medicating pigs post-weaning for seven days with IVOMEC® Pre-mix resulted in the rapid eradication of mites and elimination of clinical lesions associated with hypersensitive mange.

Acknowledgment: The support of Merial is gratefully acknowledged.

N15

ANTICOCCIDIAL EFFICACY OF SALINOMICIN SEMDURAMICIN COMBINATION IN CHICKEN BROILERS

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The anticoccidial efficacy of salinomycin semduramicin (Pfizer) combination (full or half doses) were compared with each of these drugs alone in floor pen experiments with broilers from one day of age until 42 days.

Three hundred and sixty, 1 day old chicks were assigned into ten groups (36 chicks in each group, 3 replicate pens of 12 chicks each). The groups include infected medicated chicks with either salinomycin semduramicin combination (full dose or half dose), salinomycin (alone) or semduramicin (alone) in addition to infected unmedicated control group. Uninfected medicated control groups (4 groups) were also included in a similar design together with uninfected unmedicated group.

Each chick of the infected groups received an inoculum of a mixed field isolates of *Eimeria* spp. oocysts (10^5) collected from 10 commercial broiler houses. Treatment was assessed according to the following parameters, weight gain, feed to gain ratio, mortality, lesion scores, oocyst counts in faecal and litter samples.

Satisfactory results were achieved with either mixed treatments or single treatments with the studied anticoccidials. The highest definite anticoccidial activity was recorded with full doses of salinomycin semduramicin combination.

Accordingly it seems that this mixture is the optimal drug for controlling both intestinal and caecal coccidiosis in chickens.

N14

ACQUIRED SULPHONAMIDE RESISTANCE IN CHICKEN COCCIDIA IN BANGLADESH

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Over the years there have been increasing reports of failure of sulphonamides in the treatment of chicken coccidiosis, a major disease in Bangladesh. This report describes a high degree of resistance in 12 field isolates of chicken *Eimeria* spp. The chicks received treatment @ 1 gm, 2 gm or 2.5 gm Esb₃^R (sulphaclozine sodium)/litre of drinking water from 3-5 days after experimental infection with 10^4 sporulated oocysts. The results were assessed on the basis of clinical signs, faecal oocysts count, weight gain and/or pathological changes. The chicks received treatment @ 1 gm showed very similar clinical signs and pathological changes, but passed relatively fewer oocysts compared with the untreated control chicks. Except in chicks infected with 5 of the isolates and receiving treatment @ 2.5 gm, the weight gains were not significantly different ($P > 0.05$) amongst the treated and untreated chicks. The faecal oocysts count in chicks treated @ 2 gm or 2.5 gm were very minimal or even nil in few cases. But on histopathological examination stunted parasitic stages were seen during the period of treatment which showed flourish development with sub-acute to chronic ulcerative caecitis on withdrawal of treatment. These suggest a varying but high degree of resistance in chicken coccidia in Bangladesh. Increasing the dosage of the drug as suggested by the manufacturer can not be recommended since this may cause toxic effects to the birds. Therefore an alternative to sulphonamides needs to be found out for treatment of chicken coccidia

N16

EFFICACY OF FRONTLINE® SPOT-ON™ AGAINST Ixodes ricinus TICKS IN DOGS

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INTRODUCTION: The aims of this study were to determine the efficacy of Frontline® Spot-on™ (fipronil) against *Ixodes ricinus* tick infestations in comparison with a reference product on dogs.

METHODS: Twelve female and 12 male Beagle dogs were used. They were 8 months to 8 years old and weighed 5.7 to 13.7 kg. The dogs were housed in individual pens. Based on a pre-treatment *I. ricinus* tick count at 48 hours after infestation, dogs were ranked within sex. One dog in each replicate was randomly allocated to each of the following three treatment groups: 1) untreated control; 2) Frontline Spot-on for Dogs; and 3) EXspot® (permethrin) for Dogs. Treatments were applied once topically on Day 0. On Days -1, 7, 14, 21, 28 and 35 each dog was infested with 50 unfed *I. ricinus* (males and females in approximately equal numbers). Attached and unattached live ticks were counted and then removed on Days 2, 9, 16, 23, 30 and 37.

RESULTS: Dogs treated with Frontline Spot-on had significantly ($p < 0.01$) fewer *I. ricinus* ticks than did the controls at 48 hours after treatment and at 48 hours after each post-treatment infestation. Efficacy against existing tick infestation on Day 2 was 98.8% and 94.7% in Frontline Spot-on and EXspot treated groups, respectively. Numbers of ticks on dogs treated with Frontline Spot-on were reduced by >95% at all times of challenge. By 5 weeks after application, efficacy of Frontline Spot-on was still 97.9%, while efficacy of EXspot was 91.8%.

DISCUSSION/CONCLUSION: Frontline Spot-on was shown to be highly effective (>95%, $p < 0.01$) for the treatment and control of tick infestations (*Ixodes ricinus*) for up to 5 weeks following a single topical application.

Nom eggs post R_r

N17

EFFICACY OF FIPRONIL/(S)-METHOPRENE AGAINST IXODES SCAPULARIS TICKS

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Background: The objective of this study was to evaluate the efficacy of a fipronil/(S)-methoprene spot-on formulation against the black-legged tick, *Ixodes scapularis* (Say) on dogs.

Methods: Twenty-four dogs were allocated to 8 replicates of 3 dogs each based on Day -5 tick counts. Dogs in Group 1 were not treated, dogs in Group 2 received fipronil/(S)-methoprene (pipette size based on weight), dogs in Group 3 received selamectin (tube size based on weight). Treatments were applied on Day 0. Each dog was infested with approximately 50 *I. scapularis* adult ticks on Days -7, -1, 7, 14, 21, 28, and 35. Counts were conducted 24 and 48 hours after treatment or after challenge infestation.

Results: Based on 48 hour counts, the efficacy of fipronil/(S)-methoprene ranged from 97.7% to 100% ($P < 0.01$) against established infestation and against challenge infestation through Day 37. The efficacy of selamectin ranged from 47.3% against established infestation to 0% against challenge on Days 14, 21, and 35.

Conclusion: Fipronil/(S)-methoprene provided >95% control of *I. scapularis* for up to 5 weeks following a single application.

N18

BOVINE NEMATODES RESISTANCE TO AVERMECTINS IN ARGENTINA

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Over the last years I have found residual nematode eggs in the routine effectiveness control of treatments made with avermectins in bovines. This have evolved to what I call "treatment failures" with EPG levels unacceptable for treated animals. These occurred in autumn 2000 and then I made some FERCT to determine if the failure was caused by one of three possible factors: application, product or resistance. Using different drugs, and making the application by myself, I found that the cause of the "treatment failures" was resistance.

In 2000 there were also two publications of resistance in my country (Fiel et al; Fulano et al). One is in the same zone were I work, and the other is at a province (Corrientes) where cow-calf operations are common. This year I have found that the animals arriving from that province were difficult -if not impossible- to be effectively treated with avermectins in many cases.

I will discuss these points:

- Procedures that led to the appearance of resistance, with special reference to management of the farms and application of drugs.
- History of some farms where resistance is present. EPG data previous to the resistance, how I founded it, and my "practitioner view" of the FERCT.
- Measures applied on the farms with resistance and the poor results we have had on the reversion of it.
- Reality of farms, farmers and how parasites is only "something more" to look for.
- The poor communication between research and researchers with the practitioners, and how far is the everyday control from science.

N1 p

CHEMOTHERAPEUTIC APPROACHES TO NEMATODES: CURRENT KNOWLEDGE AND OUTLOOK

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Classification of drugs against cestodes on the basis of their mechanism of action: 1. Drugs which interfere with carbohydrate - and/or energy metabolism: *cyanine dyes* (*pyrvinium*, *dithiazanine iodide*) inhibit glucose uptake; their antifilarial action is probably due to irreversible inhibition of oxygen uptake. 2. Drugs which interfere with polymerization of microtubules: *phenothiazine*, *thiabendazole*, *cambendazole*, *parabendazole*, *oxibendazole*, *mebendazole*, *flubendazole*, *fenbendazole*, *oxfendazole*, *albendazole*, *cyclobendazole*, and prebenzimidazoles (*febantel*, *netobimin*, *thiophanate*) bind to β -tubulin subunit. 3. Drugs which impair membrane functioning: *diethylcarbamazine* leads to the destruction of the microfilarial sheath by lysosomal enzymes; thereby host immune factors and also non-antibody mediated mechanisms are involved. 4. Drugs which interact with neurotransmitter function: a) drugs affecting acetylcholine neurotransmission: *coumaphos*, *haloxon* and *naphthalophos* inhibit acetylcholinesterase; *bephenium*, *thiethyl*, *pyrantel*, *morantel*, *oxantel*, *levamisole*, and *butamisol* interference with nicotinic acetylcholine receptor; b) Drugs affecting GABA-gated chloride channels: *piperazine* is a selective GABA agonist; macrocyclic lactones (*ivermectin*, *abamectin*, *doramectin*, *epinomectin*, *selamectin*, *milbemycin oxime*, *moxidectin*) induce opening of the ivermectin-sensitive glutamate-gated chloride channel. The situation of the chemotherapy of nematode infections in human as well as veterinary medicine is currently satisfactory. There are different drugs available: the *benzimidazole* (*carbameles*), *levamisole*, *pyrantel*, and the different macrocyclic lactones. In human medicine, *mebendazole* and *albendazole* are mainly used against the intestine nematodes, *ivermectin* and *diethylcarbamazine* are microfilaricidal drugs used in prophylaxis against onchocercosis and filarial infections. The situation in veterinary medicine is quite different because it is governed by the great resistance problem against all important anthelmintics in some animal hosts. There are a variety of reports on resistance against *benzimidazoles*, *levamisole*, *pyrantel* and macrocyclic lactones in trichostrongylides in small ruminants (sheep, goats) and also strongyles in horses. There are minor resistance problems in cattle, dogs and cats. Thus there is always an urgent need for new anthelmintics with a new mode of action in veterinary medicine which can easily break resistance against the existing anthelmintics. The situation of the chemotherapy of filarial infections in human medicine is not satisfactory. There is an urgent need for a new macrofilaricidal drug with only slight side effects. Today the only way is the prophylactic control of *Onchocerca volvulus* infections and those with lymphatic filariasis by *ivermectin* or *diethylcarbamazine* or by *ivermectin/DEC* or *ivermectin/albendazole* combinations.

N2 p

THE EFFICACY OF ABAMECTIN POUR-ON AGAINST NEMATODES OF IMPALA

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Introduction: The study was designed to evaluate the efficacy of an abamectin pour-on against nematode parasites and oestrid larvae in game animals in South Africa.

Experimental design: The trial animals consisted of a family group of free ranging Impala (*Aepyceros melampus*) captured together. They ranged from approximately 1 year to approximately 3 years of age. A negative control group consisted of 6 untreated impala and the treatment group consisted of 6 animals treated with the abamectin pour-on. The 2 groups were selected so that the composition of the groups was comparable for age and sex. The groups were numbered and a number drawn for each treatment. Natural infections confirmed by coprological examinations were used in the study. The animals were slaughtered on days 10-11 after treatment and parasites were recovered and counted.

Housing and Management: The 12 free ranging impala were captured and transferred to game holding pens at a commercial game enclosure. Lucerne hay was available *ad libitum*. Portable drinking troughs were cleaned and filled with water daily. Each of the two groups was housed in separate pens.

Results and Conclusion: When compared to the untreated animals, the abamectin pour-on was highly effective (>90%) against immature *Haemonchus* species, adult *Trichostrongylus* spp and immature *Oesophogostomum* spp., and 86.2% and 85.9% effective against immature *Cooperia* spp and immature *Impatiata tuberculata*, respectively.

N3 p PERSISTENT ANTHELMINTIC EFFICACY OF IVERMECTIN IN CATTLE

J.S. van der Merwe¹, J.P. Louw², J. Minárik³ and A.M. Durand⁴

Introduction: The study was designed to generate data to confirm the persistent anthelmintic efficacy of a proprietary injectable long-acting ivermectin formulation against nematode parasite strains in South Africa. To obtain a "persistent" efficacy claim under the South African evaluation system, an anthelmintic has to achieve an efficacy of at least 90%.

Experimental design: 24 recently weaned and healthy calves were divided into 4 groups of 6 each. 3 groups were treated with the formulation: the first group on 42 days, the second on 35 days and the third on 28 days prior to a larval challenge. The remaining group was not treated (negative control) and received a larval challenge simultaneously with the other group. A random group was selected from the herd for each successive treatment (the trial animals could not be randomized all at once due to the time scale of the trial). The larval challenge consisted of 2475 *Bunostomum phlebotomum* given to all calves per-cutaneously, and 4230 *Cooperia* spp given per os to the control group and the group treated 28 days earlier. 38 to 41 days after the last infection the animals were slaughtered, the worms recovered and counted, the geometric means determined and the anthelmintic efficacy evaluations done.

Housing and Management: The trial animals were housed in a north-facing stable with concrete floors, steel post and rail fence. A roof covered one half of each pen. The stable was open on one side leading to a loafing area. Every group was housed in its own pen from immediately after selection and treatment.

Results and Conclusion: The efficacy of the ivermectin formulation against *Bunostomum phlebotomum* 28, 35 and 42 days after treatment varied from 99.5 to 100 % and the efficacy against *Cooperia* spp after 28 days was 92.4%. The ivermectin formulation qualifies for a persistency claim against *Bunostomum phlebotomum* and *Cooperia* spp at the time points indicated above.

N4 p ENDECTOCIDE EFFECTIVENESS OF A IVERMECTIN LA* FORMULATION IN CATTLE

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This study evaluated the efficacy of a 1% subcutaneous long acting (LA) formula of ivermectin* (200 mcg/kg of body weight) against nematodes, warble-fly larvae (*Dermatobia hominis*) and ticks (*Boophilus microplus*) in naturally (*D. hominis* and *B. microplus*), and experimentally (helminths) parasitized cattle hosts. For nematodes, 36 bovine hosts were experimentally infected 56, 49, 42, 35 and 28 days post-treatment, and then necropsied for assessment of parasite burden. The effect on warble-fly larvae was evaluated by counts of *D. hominis* larvae nodules present on the host's bodies on days zero to 140. Female *B. microplus* ticks (4,5 to 8,0 mm of length) present on one side of the body of each animal were counted on days zero to 63. The formula showed a 93,5% efficacy in preventing infection by *Haemonchus placei* on the 28th DPT. Against *Oesophagostomum radiatum* the effective protection was 100,00% on the 35th DPT, with high levels for adult forms up to 56 DPT. The LA ivermectin totally eliminated (100% efficacy) warble-fly larvae from days 14 to 105, with high level of efficacy up to the 140th DPT (98,5%). The treated animals showed significant reduction in number of ticks, with maximum efficacy observed on day 28 (92,4%). It can be concluded that the evaluated formula has persistent activity against endo and ectoparasites of bovine hosts.

* Produtos Veterinários Ouro Fino Ltda.

N5 p CONCENTRATIONS OF IVERMECTIN IN PLASMA, AND SKIN SECRETIONS OF CATTLE AND SHEEP AFTER SUBCUTANEOUS ADMINISTRATION

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Introduction: Ectoparasitic disease is of major concern in south america. The arrival of avermectins to skin in animals infested with ectoparasites is a key factor on its efficacy. Ivermectin (IVM) concentrations in skin secretions of animals with mange has been studied previously (1). The aim of the present paper is to determine the concentrations of IVM in skin normal secretions, to correlate them with the concentrations attained in pathological skin.

Materials and Methods: Six castrated male healthy calves and 6 healthy, female sheep were the experimental animals. Blood and skin secretions samples were obtained at different times after administration of 200 µg/kg IVM formulated classically in 1 % solution through the s.c. route. Concentrations of IVM were determined by HPLC. Kinetic analysis was performed linearly.

Results and Discussion: IVM presented concentrations in skin higher than in plasma either in cattle and sheep. This is a result of real interest related to the previous one that IVM concentrates in skin pathologic secretions in sheep with mange (1).

References: Echeverría et al (2001). Concentrations of ivermectin in plasma, scabs and mites of sheep carrying natural mange infestations. 8TH international congress of the European Association for Veterinary Pharmacology and Toxicology (EAVPT). Jerusalem.

N6 p CONCENTRATIONS OF IVERMECTIN IN PLASMA AFTER SUBCUTANEOUS ADMINISTRATION OF THREE 3,15 % FORMULATIONS IN CATTLE

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Introduction: Formulation of avermectins in controlled release or long release vehicles has been a common approach in the last years. Shortly ago, a tixotropic ivermectin (IVM) suspension was registered by Merial in some markets. The aim of the present paper is to compare the IVM plasma profiles after the administration of three different 3,15 % formulations.

Materials and Methods: Twelve castrated male healthy calves were the experimental animals. The treatments were T1: 3,15 IVM suspension (Ivomec Gold®), T2: experimental 3,15 % IVM suspension in silicon, and T3: experimental 3,15 % IVM solution in sesame oil, administered subcutaneously. Concentrations of IVM were determined by HPLC. Kinetic analysis was performed linearly.

Results and Discussion: The three formulations behaved in very different way. T1 Cmax was 54.34 ng/mL and concentration at 117 days (C117) was 2.33 ng/mL. T2 Cmax was 135.30 ng/mL and C117 days was 0.93 ng/mL. T3 Cmax was 38.78 and C117 was 0.62 ng/mL. T1 profile showed a tendency to plateau between 21 and 117 days. Interestingly, the oily solution not only reached the lowest C117, but the lowest Cmax. Undoubtedly, although with a Cmax of half the T2, the T1 gave the most favorable pharmacokinetic profile, maintaining average concentrations above 2 ng/mL.

N7 p

CRYSTALLINE POLYMORPHY OF CLOSADEL SODIUM AND ITS BIOAVAILABILITY

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Background: The plasma concentration profile of closadel is an excellent predictor of its antiparasitic activity. Bioavailability from oral suspensions can be dependent of the crystalline polymorphy of the particles. Chemical synthesis of closadel sodium results in amorphous product or particles with polymorphy type I or II

Method: Four groups of 10 sheep received a 10 mg/kg BW oral dose of closadel, either with the commercial product Flukiver[®] 5 % oral suspension (containing polymorph type II) or with suspensions with the same formula, but containing type I, type II or amorphous closadel sodium. Plasma kinetics were monitored for up to 42 days after administration.

Results: The suspensions with polymorph I and II attained t_{max} after 3 days, as Flukiver[®]; for the amorphous particles t_{max} was already at 1.7 days. The mean C_{max} was around 50 mg/l for Flukiver[®], the amorphous particles and polymorph II, but only 22.5 mg/l for the polymorph I. Accordingly, the AUC for polymorph I was less than half of that of the other suspensions.

Conclusions: The study makes evident that the polymorph I has a lower bioavailability than the polymorph II of closadel sodium, which is included in the commercial Flukiver[®]. The presence of polymorph I in formulations will result in impaired product efficacy. The amorphous particles display acceptable bioavailability, but must be avoided for reasons of substance and product stability.

N9 p

COMPARATIVE EFFICACY OF IVERMECTIN AND DORAMECTIN AGAINST MICROFILARIAE OF SETARIA LABIATOPAPILLOSA IN CATTLE.

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BACKGROUND: Ivermectin plays a major role as a microfilaricide for both humans and domesticated animals in endemic areas. Resistance to the drug, toxicity and damage to the environment makes a search for alternative drugs imperative.

Doramectin, another avermectin has been found to be less toxic, more efficacious and more environmentally friendly than ivermectin.

METHOD: Thirteen naturally infected White Fulani cattle were randomly assigned into two treatment groups of 5 animals each and a control group with 3 animals. Animals in group A and B were treated with either Ivermectin (IVM) or Doramectin (DOR) at 200 mcg/ body weight given subcutaneously at the neck region. Group C animals were given a placebo. Microfilarial densities were determined at days 0, 3, 7, 14, 21 and 28 post treatment and percentage efficacies computed for each treatment group.

RESULTS: All animals in the IVM and DOR treatment groups were cleared of microfilariae by the third day post treatment and remained free till the end of the study. Control animals did not show any significant reduction in the number of microfilariae detected post treatment.

CONCLUSION: The high efficacy rate for Ivermectin further confirms its usefulness as a microfilaricide. However, a similar rate for Doramectin (100%) indicates its potential as an alternative or adjunct to Ivermectin therapy for both human and animal filarioses.

N8 p

EFFICACY OF NILZAN PLUS[®], WORMICID PLUS[®], VERMITAN[®] AND IVOVEC[®] AGAINST NEMATODES IN UGANDAN GOATS

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Background

This study was undertaken to assess the efficacy of the common brands of anthelmintics sold in rural veterinary drug shops and farm stores in Uganda.

Method

The efficacy of Nilzan Plus[®] (levamisole 1.5% + oxclozanide 3.0%, Cooper Ltd, Nairobi, Kenya), Wormicid Plus[®] (levamisole 1.5% + bithional 8.0%, Cosmos Ltd, Nairobi, Kenya), Vermitan[®] (albendazole 10%, Sanofi Ltd, Budapest, Hungary) and Ivmec[®] (ivermectin, Merck Ltd, U.S.A) was assessed using the faecal egg count reduction test on 43 naturally infected goats divided into 4 treatment groups and 1 control. Faecal worm egg counts were performed on day 0, 10, 14, 21, 28, 35, 49 and 56 on all groups, while larval cultures were conducted on day 0, 10, 14 and 28 on faecal samples from treatment groups.

Results

Ivmec[®], Vermitan[®] and Wormicid Plus[®] displayed high efficacy against nematode infections in goats, but Nilzan Plus[®] was grossly ineffective. Ivmec[®] maintained an efficacy of 100% up to day 35 then dropped to 92% by day 56. Vermitan[®] maintained an efficacy of 100% up to day 28 then dropped to 98% by day 56. Wormicid Plus[®] maintained an efficacy of 100% up to day 35 then dropped to 70% by day 56, but Nilzan Plus[®] had an efficacy of 85% on day 10 and thereafter it declined to 19% by day 56. *Trichostrongylus* spp., *Haemonchus* spp. and *Oesophagostomum* spp. were the most predominant nematodes in Nilzan Plus[®] treated goats post-treatment.

Conclusion

There is a need for strict quality control of anthelmintics sold in many rural veterinary drug shops and farm stores in Uganda, since development of resistance against Nilzan Plus[®] is highly suspected. This may also guard against the sale of fake or adulterated anthelmintics in rural veterinary drug shops and farm stores.

Acknowledgement

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N10 p

EFFICACY OF AN IVERMECTIN CONTROLLED-RELEASE CAPSULE AGAINST SMALL LUNGWORMS IN SHEEP

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INTRODUCTION: To evaluate the efficacy of the ivermectin controlled-release capsule (CRC), which delivers 1.6 mg ivermectin/day intraruminally for 100 days to sheep weighing 40-80 kg (IVOMEC[®] Maximizer[™] CR Capsule, Merial), against small lungworms two trials with 48 naturally infected adult female Merino Landrace sheep were conducted.

METHODS: The sheep were allocated by restricted randomization based on bodyweight to untreated controls or received an ivermectin CRC. Eight sheep per group were necropsied 35, 70 or 105 days post treatment (p.t.). Lungworms were recovered by dissection or peptic digestion of the lungs. Faecal lungworm larval counts were conducted at weekly intervals.

RESULTS: The efficacy of treatment was 100% against *Dictyocaulus filaria* and *Protostrongylus* (*P.*) *rufescens* ($p < 0.05$) at each necropsy day. The efficacy against *P. brevispiculum*, *Cystocaulus ocreatus* and *Neostrongylus linearis* was found to be 100% ($p < 0.01$), 96.6% ($p < 0.01$) or 99% ($p < 0.01$), respectively, at 105 days p.t. The reduction of *Muellerius* (*M.*) *capillaris* counts varied from 96.2% ($p < 0.05$) at 70 days p.t. to 44.6% ($p > 0.1$) at 105 days p.t. Faecal lungworm larvae were eliminated from 3 weeks after CRC administration for all protostrongylid species including *M. capillaris* indicative that pasture contamination would be reduced following treatment.

CONCLUSION: The results indicate that the ivermectin CRC is a useful instrument to control the multispecies nature of ovine lungworm infection.

N11 p CONCENTRATIONS OF ABAMECTIN IN MILK OF LACTATING COWS AFTER POUR-ON ADMINISTRATION

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Introduction: Abamectin (ABA) has been largely used in large animal medicine. Pour-on formulations avermectin based, have been in use since some time. Milk penetration and persistence has been a problem that lead to the contraindication of avermectin use in milk producing animals. ABA acceptable daily intake has been fixed in 0.1 µg/kg b.w., maximum residue levels (MRL) for fat and liver are 100 µg/kg, and for kidney 50 µg/kg. There is not a (MRL) for milk. It is essential to strictly control this kind of drugs that, because of liposolubility, can easily be transferred to milk.

Materials and Methods: Eight lactating cows were treated with an ABA based pour-on formulation (Necaverm® Rosenbusch) at a dose rate of 500 µg/kg. Milk samples were obtained at different post-administration times. Concentrations of ABA were determined by HPLC. Kinetic analysis was performed linearly.

Results and Discussion: ABA was detected in milk with some concentration oscillations during the first samples, probably consequence of irregular absorption from skin. Average maximum concentration was 9,07 observed 1 day after administration. Twenty five days after administration no concentrations were detected in milk. Considering ADI, MRL, and the concentrations found in the present experiment, the conclusion is that the studied ABA formulation can be used without withdrawal period in milk.

N13 p DETERMINATION OF IVERMECTIN IN BOVINE PLASMA FOR BIOEQUIVALENCY STUDIES

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The present work evaluated the bioequivalence of two commercial 1% Ivermectin formulae (Ivermectina OF and Ivomec), one used as reference product, in cattle. The plasmatic concentrations of Ivermectin were determined in 504 blood samples taken from 18 steers, distributed in two groups, each submitted to administration of the two formulae (200µg/kg, sc) in two phases. The values were determined by high performance liquid chromatography (HPLC), with a fluorescence detector, after a derivation process. Clinical and hematological (hematocrit, white cell count and blood urea nitrogen levels) examination showed no alterations in the medicated animals. The pharmacokinetic parameters was determined according to Brazilian law that regulates the production of generic medicaments for humans and the FDA Bioequivalence Guidance. The values of the C_{max} (maximum plasma concentration), T_{max} (time to reach maximum plasma concentration), AUC_{0-4} and $ASC_{0-\infty}$ (area under the curve of concentration versus time), K_{el} (elimination rate constant), $t_{1/2}$ (mean half-life), MRT (mean residence time) of both formulae did not show significant statistical differences ($p \leq 0.05$) when compared. The results suggest that, in the studied conditions, the reference and test formulations are bioequivalent.

[®]Ivomec is a registered trademark of Merial Limited.

[®]Ivermectina 1% Ouro Fino is a registered trademark of Produtos Veterinários Ouro Fino Ltda.

N12 p CONCENTRATIONS OF IVERMECTIN IN PLASMA AND TISSUES AFTER SUBCUTANEOUS ADMINISTRATION TO SWINE

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Introduction: Avermectins were largely used in different animal species. Pharmacokinetic studies in swine, however, are rather scarce. The objective of the present paper was to study the kinetic behaviour and the tissue residue profile of ivermectin (IVM) after subcutaneous (s.c.) and intravenous (i.v.) administration of a 1% w/w solution.

Materials and Methods: Twenty young healthy male pigs were divided into three groups: G1 (4 pigs) received 1 mL/30 kg of a 1% p/p formulation intravenously, G2 (6 pigs) and G3 (10 pigs) received 1 mL/30 kg of the same formulation subcutaneously. Blood samples at different post-administration times were drawn from animals of G1 and G2. Animals belonging to G3 were sacrificed in groups of 2 at 2, 3, 4, 5 and 6 weeks post-administration. Concentrations of IVM were determined by HPLC. Kinetic analysis was performed linearly.

Results and Discussion: Ivermectin concentrations were detected after i.v. administration until 20 days. After s.c. administration the C_{max} was 18.80 ng/mL at T_{max} : 4,17 days, and the half-life of elimination was 4.30 days. Muscle residues were detected until 4 weeks, liver residues until week 5, kidney residues until week 5, adipose tissue residues until week 5 and injection site residues until week 6.

N14 p CATTLE BREED DIFFERENCES ON THE PLASMA KINETICS OF MOXIDECTIN

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Background: Endectocide compounds, including ivermectin, doramectin, eprinomectin and moxidectin (MXD) are now available for topical administration to cattle. Animal's breed is among the numerous factors affecting the kinetics and metabolism of different drugs used in Veterinary Therapeutics. It has been suggested that differences in body composition may influence the kinetic behaviour and the resultant persistent efficacy of endectocides in cattle. The comparative plasma kinetics of MXD after its pour-on administration to two cattle breeds is reported.

Methods: Parasite-free Aberdeen Angus (AA) and Holstein (HL) calves (n=16) were treated with MXD pour-on (Cydectin®, Fort Dodge, USA) at 500 µg/kg. Plasma samples were collected between 2 hours and 35 days post-treatment, processed and analysed by HPLC.

Results: MXD was detected in plasma from 2 hours up to 35 days after its topical administration in both bovine breeds. The observed MXD peak plasma concentration in AA calves (2.33 ng/ml) was significantly lower ($P < 0.05$) than that obtained in HL calves (5.08 ng/ml). The peak concentration was attained significantly earlier ($T_{max} = 2.06$ days) in HL calves compared to AA animals ($T_{max} = 5.0$ days post-treatment). The total plasma availability, expressed as AUC values, was significantly greater ($P < 0.01$) in HL calves (47.2 ± 15.5 ng.d/ml) compared to AA calves (21.7 ± 8.62 ng.d/ml). The inter-animal variations, measured as the ratio maximum-to-minimum MXD AUCs, were 3.03 (AA) and 3.32 (HL).

Conclusion: Marked differences in the plasma availability of MXD were observed between the cattle breeds under evaluation. Breed-related differences on the absorption of MXD from the skin and/or in its fat tissue reservoir, may account for the observed kinetic results. These kinetic differences between animal breeds along with the large individual variation observed within breeds, should be considered to establish parasite control strategies based on pour-on treatments.

N15 p

ASSESSMENT OF THE PHARMACOLOGICAL INTERACTION BETWEEN LOPERAMIDE AND ENDECTOCIDE COMPOUNDS

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Background: Large amounts of active unchanged endectocides are excreted in bile and faeces of treated animals. Different pharmacological approaches are currently being tested to delay the bile-faecal elimination and to obtain increased systemic availability for endectocide molecules. Loperamide (LPM) is an opioid derivative that reduces gastrointestinal (GI) secretions and motility. This work reports on the effects of LPM on a) the tissue distribution of ivermectin (IVM) in laboratory animals and, b) the plasma and faecal disposition of moxidectin (MXD) in cattle.

Method: Male Wistar rats received IVM (300 µg/kg) either alone or co-administered with LPM (1.5 mg/kg) by subcutaneous injection. Three rats of each group were sacrificed between 6 and 72 h post-treatment to obtain plasma and different tissue samples. Aberdeen Angus calves were treated intravenously and subcutaneously with MXD alone (200 µg/kg) or co-administered with LPM. Blood and faecal samples were collected over 40 days post-treatment. Plasma, faecal and tissue samples were analysed by HPLC with fluorescence detection to quantify IVM and MXD concentrations.

Results: The presence of LPM caused marked changes to the kinetics of both molecules. The plasma availability of IVM in rats was significantly higher in the presence of LPM. IVM tissue concentrations were markedly higher after the co-administration IVM+LPM. For instance, significantly greater IVM concentrations in plasma (3.2-fold) and liver (2.8-fold), compared to the IVM alone treatment, were measured 24 h after the co-administration. LPM induced a significantly delayed appearance of MXD in faeces. Significantly higher plasma profiles and delayed elimination of MXD were obtained after its co-administration with LPM in cattle.

Conclusion: The LPM-induced delay in GI transit may extend the plasma-intestine recycling time, accounting for the observed increased systemic availability of IVM and MXD. Additionally, a potential competition between LPM and endectocides for the P-glycoprotein-mediated bile/intestinal secretion processes, may contribute to their enhanced systemic concentration profiles.

N16 p

INCREASING PRAZIQUANTEL PERFORMANCE USING PHARMACOKINETICALLY-BASED FORMULATION MODIFICATIONS

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Background: Resistance to praziquantel (PZQ) by *Schistosoma spp.* is increasing and with no real replacement therapeutic there is urgent need to develop new formulations / delivery concepts to improve PZQ performance.

Method: The pharmacokinetic disposition of PZQ and its hydroxy metabolite (OH-PZQ) were determined in pigs after oral and intravenous (IV) administration. Based on these results, modified formulations to reduce PZQ availability in the stomach, and thereby absorption from the proximal intestine, but to maximise quantitative passage to the distal intestine were tested in pigs infected with *Schistosoma japonicum*.

Results: IV administered PZQ was rapidly cleared from the bloodstream with minimal presence of OH-PZQ. Conversely, large quantities of OH-PZQ were present, for a prolonged period, in blood after oral administration. This suggests that PZQ might be hydroxylated in the stomach prior to absorption which can reduce PZQ availability in the distal gut lumen.

Compared with conventional liquid PZQ dosing, formulations which limited PZQ exposure in the stomach, increased PZQ efficacy against *S. japonicum*. **Conclusion:** Administering PZQ in formulations that are believed to limit contact with the pig's stomach environment reduced circulating OH-PZQ and increased the efficacy of PZQ against *S. japonicum*. This indicates that there is potential for such formulation modifications to increase PZQ treatment of human Schistosomiasis.

N17 p

ANTHELMINTIC RESISTANCE IN SMALL-RUMINANT FLOCKS IN SPAIN: EXTENSION IN THE LEON PROVINCE (NW).

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Background: During the last decades tricostrongylid anthelmintic resistance (AR) has been detected throughout Europe. In some countries, reports show high flock prevalences, ranging between 40-80%. In Spain, so far, only two reports have shown AR against pro and benzimidazole (BZ).

Method: 77 randomly selected small-ruminant flocks were investigated for AR. Faecal egg count reduction test (FECRT) was used for BZ (52 flocks), macrocyclic lactones -ML- (50) and imidazothiazole -IM- (12). Egg hatch assay (EHA) with thiabendazole was also carried out on 50 flocks to detect BZ-resistance. A flock was considered resistant, AR-suspect or susceptible in accordance with the WAAVP criteria. Faecal cultures were used to identify major resistant-nematode genera.

Results: When FECRT was used, 3 flocks (5.8%) presented AR to BZ and 1 (2%) was AR-suspect; for ML, 5 (10%) were resistant and 5 (10%) AR-suspect; and for IM, 4 (33.3%) were resistant and 2 (16.7%) AR-suspect. EHA detected AR in 7 of the flocks (14%) analysed (2 of them FECRT-resistant). Resistance was observed in *Teladorsagia circumcincta* and *Trichostrongylus spp.*

Conclusion: Although AR was observed with some frequency in NW of Spain (21%), the total flock-prevalence was lower than that reported for other European countries. Despite of BZ has been the most used anthelmintic during the last decade, BZ-resistance is low. Higher AR-prevalence was observed for other less-used anthelmintics. EHA seemed more sensitive for detecting BZ-resistance flocks than FECRT.

N18 p

SOME PROBLEMS RELATED TO THE USE OF ALBENDAZOL IN SHEEP FLOCKS FOR THE CONTROL OF MIXED NATURAL INFECTION BY GASTROINTESTINAL AND LIVER FLUKE PARASITES

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Background: Natural infections with both gastrointestinal (GI) nematodes and *Fasciola hepatica* (Fh) are very common in sheep and goats throughout the world. Their control is mainly based on anthelmintic therapy, albendazole (ABZ) being the most commonly used. The use of ABZ against GI nematodes at the manufacturer's dose rates (5 mg/kg) could then favour side and crossed Fh anthelmintic resistance (AR).

Method: 2 groups (A1 and A2) of 10 animals each were randomly selected from a flock with a mixed infection by GI nematodes (mean epg=331) and Fh (mean epg=81). A1 was drenched with ABZ and A2 with triclabendazole (TBZ) at the recommended dose rate for Fh (7.6 mg/kg and 10 mg/kg respectively). Faecal egg counts were carried out for each group at day 0 (treatment day) and 12 days post treatment. Some blood parameters and hepatic enzymes were also recorded at the same time.

Results: Efficacy, measured as % of Fh egg reduction, was 100% at day 12 for TBZ and 0% for ABZ. Significantly higher levels of RBC, Hb, PCV, albumin and P were observed in A2 at day 12, while higher hepatic enzymes (GGT and AST) values were recorded in A1.

Conclusions: The continuous use of ABZ against GI nematodes could be a risk factor for AR development in Fh, as recommended ABZ dose rates for Fh did not effectively reduce the adult worm population in this flock. In addition, the use of ABZ at these dose rates besides allowing the maintenance of adult parasites could favour the development of immature stages thus increasing the Fh pathogenic effect.

N19 p FAUNA COLONIZATION AND DEGRADATION OF DUNG OF AVERMECTIN TREATED CATTLE

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Background: The effects of faecal residues of avermectins on dung colonizing invertebrate and dung degradation in the East of La Pampa during autumn were reported.

Methods: Faeces from 3 groups of 10 steers were used: GT, control group no treated, and GI and GD treated groups with 0.2 mg/Kg of injectable ivermectin LA and doramectin respectively. Fifty dung pats per group of 550 g were prepared and deposited on the field on days 3, 8, 16 and 28 post-treatment (pt). Eight pats per group were recovered after 7, 14, 21, 42, 100 and 180 days on the field. Weight, dry matter, number of arthropods and nematodes on faeces and soil N concentration were determined.

Results: After 21 days on the field, total number of arthropods of GT were higher ($P < 0.001$) than those of GI and GD from 3° to 28° pt days. A decrease ($P < 0.001$) of Coleoptera larvae, Diptera larvae and Collembola was noted in pats from GI and GD in all depositions exposed since 21 day. Numbers of Staphylinidae and Acari of treated groups were fewer ($P < 0.05$) than those of GT during 3, 8 and 16 days pt. There were no differences in adult Scarabaeidae recovered. Dung specific nematodes were reduced ($P < 0.05$) in GI and GD pats at 21 days in the field. Weight of GT pats from the total depositions were lower ($P < 0.001$) than treated groups. No differences were detected in soil nitrogen levels.

Conclusions: Comparisons evidenced that the effects of ivermectin and doramectin were similar. These results showed a depression in invertebrate colonization and degradation of dung recovered from endectocide treated cattle during early autumn.

N20 p Fenbendazole reverses pathology associated with *Trichuris suis* infection

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Background: Antibiotic treatment ameliorates colonic pathology due to secondary bacterial infection in *Trichuris suis* infected pigs. This experiment was designed to determine if colonic pathology and cytokine responses associated with *T. suis* infection are reversed by anthelmintic treatment.

Methods: Weaned pigs were infected either experimentally or naturally with *T. suis*. Once infection was confirmed, half of the pigs were treated with fenbendazole. Seven days later, pigs were killed, and examined for pathology. Gastrointestinal lymphoid tissues were sampled for IL-10 and IL-12 measurement by competitive RT-PCR.

Results: Fenbendazole treatment eliminated adult *T. suis* and reversed colonic pathology. Method of infection impacted cytokine expression, however, anthelmintic treatment had little effect. Expression levels varied significantly between lymphoid tissues.

Conclusions: We conclude that disease, pathology, and cytokine expression in some gastrointestinal tract lymphoid tissues, associated with *T. suis* infections in pigs can be reversed within seven days after fenbendazole treatment.

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N21 p CHEMOTHERAPEUTIC APPROACHES TO CESTODES CURRENT KNOWLEDGE AND OUTLOOK

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Classification of drugs against cestodes on the basis of their mechanism of action: 1. Drugs which influence carbohydrate - and/or energy metabolism: *dichlorophene*, *hexachlorophene*, *bithionol (sulfoxide)*, *bunamidine*, *niclosamide*, *nitroscanate* suppress ATP synthesis by uncoupling of oxidative phosphorylation. 2. Drugs which interfere with DNA metabolism: *acridine derivatives* (e.g. *mepacrine*) intercalate into DNA, and may impair disulfide reductases. 3. Drugs which inhibit protein synthesis: *paromomycin* inhibits translation which leads to the formation of nonsense proteins; The mechanism of action of *axenomycin* and *streptothricin* is unknown. 4. Drugs which interfere with polymerization of microtubules: *albendazole*, *fenbendazole*, *flubendazole*, *mebendazole* and *oxfendazole* bind to β -tubulin subunit. 5. Drugs which cause desintegration of membrane integrity: *praziquantel* and *epsiprantel* disrupt tegument membranes accompanied by alterations of their physico-chemical properties (e.g. ion gradients, compartmentation etc.). 6. Drugs which interact with neurotransmitter function: a) *metrifonate*, *dichlorovos*, *diuredosane* and *fospirate* inhibit acetylcholinesterase; b) *pyrantel* interferes with nicotinic acetylcholine receptor. The situation of the chemotherapy of cestode infections in human as well as veterinary medicine is currently satisfactory. *Praziquantel* is the drug of choice and is effective against all important adult and larval cestode spp. There are two exceptions where the situation of chemotherapy is not satisfactory: the alveolar and cystic echinococcosis can only be treated with high dosages of *albendazole*, *mebendazole* or with a combination of *albendazole* (*mebendazole*) with *praziquantel*. However, surgical removal cannot be circumvented. The second exception is coenurus, caused by *Multiceps multiceps*, which can only be cured by surgery.

N22 p CESTODICIDAL EFFICACY OF PRAZICQUANTEL COMBINED WITH IVERMECTIN

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Purpose: To achieve 1.5 mg/kg bodyweight of Praziquantel and 0.2 mg/kg of Ivermectin, an oral paste was used in a 14 day controlled test against field strains of tapeworms (*Anoplocephala perfoliata*) in horses.

Method: 12 horses were split in two equally sized groups constituted on day of treatment (D0), balanced for D-3 nematode faecal egg counts. All horses had proven to be tapeworm infected via tapeworm eggs detected in faeces. On D0, six horses received a combined paste* (20mg Ivermectin + 150mg Praziquantel per 100 kg) and six horses received a placebo. On D14, the 12 horses were slaughtered and the stomach, small and large intestines were removed and examined individually for remained tapeworms. All tapeworms found at necropsy were noted as normal or abnormal (if disintegrating or with a brown discoloration), attached or detached, and their location in gastrointestinal tract was recorded.

Results: At necropsy, remained tapeworms were found in all untreated horses, with a group arithmetic mean of 20.03, ranging from 1 to 207 specimens. All tapeworms were identified as *Anoplocephala perfoliata*. The Praziquantel / Ivermectin treated horses had no remained tapeworm two weeks after treatment. The combined paste provides 100% reduction in tapeworm burden by the controlled test ($p = 0.0023$, Mann-Whitney U).

Conclusion: This study demonstrated 100% cestodicidal efficacy of Virbac's new equine wormer against *Anoplocephala perfoliata*.

* Equimax®: Ivermectin + Praziquantel Virbac Laboratories

N23 p THE EFFECT OF COPPER SULPHATE ON RABBIT RESISTANCE TO CYSTICERCOSIS

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Background: The rabbit cysticercosis is a widely distributed disease that causes considerable damage to rabbit-breeding in Armenia.

Methods: Three groups of rabbits of 1.5 months of age (6 in each group) were tested. The 1st group was made to be intact, the 2nd - infected with oncosphere of *Taenia pisiformis*, the 3rd infected with *T. pisiformis* was provided with three-course treatment with 25mg copper sulphate in each course for three days: 14 days before the infection, during the infection and 10 days after the infection. The animals were infected with 1000 *T. pisiformis* eggs for three days in row. They were dissected on the 25th day after the infection.

Results: The 25mg copper sulphate has positive effect on the infection process: there is increase in the number of erythrocytes (15%), vitamin C in blood serum (7%) and in liver (9%) and decrease in the number of leucocytes (18%), titer complement (60%), lysozyme (33%). Also, there is rise in copper content in liver and muscles to the norm (0.27mg% - in the liver, 0.16mg% - in muscles) and sharp limitation in the number of cysticercus compared to infected rabbits.

Conclusion: The use of copper sulphate in mentioned doses has favourable effect on the process of experimental cysticercosis of rabbits.

N25 p SELECTION OF THE OPTIMUM DOSE LEVEL OF AN EXPERIMENTAL FASCIOLISCIDE IN CATTLE

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Background: Efforts on the development of a new fascioliscide for cattle have been undertaken. The aim of the present study was to determine among three different dose levels the most suitable for treatment of cattle with the 6-cloro-2-metiltio-5-(1-naftiloxi) bencimidazole named "alfa".

Methods: Twenty five crossbred steers, naturally infected with *Fasciola hepatica* were used. After positive faecal diagnosis of *F. hepatica* eggs, animals were divided into five groups of 5 animals each and were treated as follows: Groups (G) G-1, G-2 and G-3 received compound alfa at a dose of 10, 12 and 14 mg/kg/po, respectively. G-4 was treated with triclabendazole at 12 mg/kg/per os. G-5 served as non-treated control. Faecal samples of the steers were taken on week 12 and 14 postinfection. One month after treatment, animals were sacrificed to count in liver the number of flukes present. Efficacy was assessed as percentage of fluke reduction in treated animals relative to the control one.

Results: The efficacy obtained for groups G-1 to G-4 was of 84.2%, 100%, 100% and 100%, respectively.

Conclusion: The best dose level selected of compound alfa was 12 mg/kg/per os.

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N24 p EFFICACY OF AN EXPERIMENTAL FASCIOLISCIDE IN NATURALLY INFECTED CATTLE

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Background: The aim of the present study was to determine the efficacy of 6-cloro-2-metiltio-5-(1-naftiloxi)bencimidazol, named compound "alfa", compared with three commercial fascioliscides through the percentage of *F. hepatica* egg-reduction in naturally infected cattle.

Methods: Fifty adult Holstein Freisian cows previously determined as positive to fluke eggs by the sedimentation test were used. They were divided into 5 groups of 10 animals each. Group 1 was treated with compound alfa at 12 mg/kg/per os. Group 2 with triclabendazole at 12 mg/kg/per os. Group 3 with closantel at 3.5 mg/kg/sc. Group 4 with clorsulon at 2.0 mg/kg/sc and Group 5 remained as non-treated control. All animals were sampled for feces to carry out coprological examinations on days -15, -8, 0, 7, 14, 21, 30, 60 and 90. Efficacy was assessed on the reduction of egg output on days 14 and 21 after treatment with respect to the control group.

Results: The efficacy obtained was of 87% and 94% for compound alfa, 92.3% and 100% for triclabendazole, 89% and 87% for closantel, 94.8% and 92.3% for clorsulon. No statistical differences were demonstrated between treatments.

Conclusion: Compound alfa shows similar efficacy to commercial fascioliscides regarding fluke-egg reduction.

Acknowledgements: Study supported by the project No. IN227998 PAPIIT-UNAM.

N26 p CHEMOTHERAPEUTIC APPROACHES TO SCHISTOSOMES: CURRENT KNOWLEDGE AND OUTLOOK

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Classification of drugs against schistosomes on the basis of their mode of action: 1. Drugs which influence carbohydrate - and/or energy metabolism: a) *antimony compounds* (potassium antimony tartrate, stibophen) inhibit enzymes with essential thiol groups (phosphofructokinase and other glycolytic enzymes); b) *nitrofurane derivatives* act by specific inhibition of glycogen phosphorylase phosphatase. 2. Drugs which interfere with DNA metabolism: a) *tubercidine* as a purine analogue becomes incorporated into nucleic acids of schistosomes, thereby toxicity to parasites is enhanced; b) *lucanthone*, *lucanthone*, *oxamniquine* inhibit nucleic acid synthesis since they become activated by a schistosomal sulfotransferase resulting in an irreversible alkylation of schistosomal DNA and delayed onset of death; c) *acridanone hydrazone derivatives* reduce the rRNA and mRNA content which is followed by a decrease of total expression of schistosome genes, d) *niridazole* covalently binds to reactive intermediate products following nitroreduction and leads to formation of adducts with macromolecules (DNA alkylation); e) *altipraz* induces a decrease of glutathione concentration in schistosomes, a reduction of activities of glutathione S transferase and glutathione reductase and thus to a reduced supply of desoxyribonucleotides. 3. Drugs which inhibit protein synthesis or - function: a) *emetine* inhibits protein synthesis on the level of peptide chain elongation; b) *amoscantate* covalently binds with primary amino groups in proteins (very reactive isothiocyanate group); c) *cyclosporin A* inhibits cyclophilin properties which results in a loss of rotamase and chaperone activity with lethal effects on schistosomes by impaired folding of nascent and denaturated polypeptide chains. 4. Drugs which cause desintegration of membrane integrity and - function: a) *Ro-11-3128* causes strong spastic paralysis of schistosomes by quick Ca^{++} - influx into the muscles and tegument vacuolisation; b) *praziquantel* leads to death of schistosomes within 16 hours, muscle contracture, spastic paralysis, tegument rupture and vacuolisation by impaired Ca^{++} - homeostasis. 5. Drugs which interact with neurotransmitter function: *metrifonate* inhibits acetylcholinesterase located on the tegument of schistosomes. The chemotherapy of schistosomiasis is currently satisfactory. There are three drugs available: *metrifonate* (activity only against *Schistosoma haematobium*), *oxamniquine* (activity only against *Schistosoma mansoni*) as narrow spectrum drugs and *praziquantel* as drug of choice. It is effective against all important adult *Schistosoma* spp. and their immature stages. There are some tolerance effects against praziquantel observable during mass treatments in Africa (Senegal, Egypt).

N27 p CHEMOTHERAPEUTIC APPROACHES TO TREMATODES (EXCEPT SCHISTOSOMES): CURRENT LEVEL OF KNOWLEDGE AND OUTLOOK

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Classification of drugs against trematodes (except schistosomes) on the basis of their mechanism of action: 1. Drugs which influence carbohydrate - and/or energy metabolism a) *antimony compounds* (*Fasciola hepatica*, *Opisthorchis sinensis*) which inhibit enzymes with essential thiol - groups (phosphofructokinase and other glycolytic enzymes); b) *monophenoles*, *bisphenoles* and *salicylanilides* (mainly *F. hepatica*) which uncouple oxidative phosphorylation; c) *clorsulon* (*F. hepatica*) which act by inhibiting phosphoglyceromutase / phosphoglyceroisomerase. 2. Drugs which inhibit protein - synthesis: a) *emetine* (*F. hepatica*) inhibits peptide chain elongation; b) *diamfenethide* (*F. hepatica*, *Dicrocoelium dendriticum*): the mechanism of action is fairly unknown. 3. Drugs which interfere with polymerization of microtubules: *triclabendazole* (*F. hepatica*), *cambendazole*, *thiabendazole*, *albendazole*, *mebendazole*, *fenbendazole*, *febantel* (*F. hepatica*, *D. dendriticum*, cestodes, mainly nematodes) bind to β -tubulin - subunit. 4. Drugs which cause desintegration of membrane integrity: a) *carbontetrachloride*, *hexachloroethane*, *tetrachloroethylene*, *hetol*, *hetolin* (*F. hepatica*) induce the formation of free radicals and peroxidation of macromolecules - lipids, proteins, nucleic acids; b) *praziquantel* (*D. dendriticum*) leads to desintegration of membrane integrity and thus of membrane function (e.g. ion gradients, compartmentation etc.). 5. Drugs which interact with neurotransmitter - function: *bramphenophos* inhibits acetylcholinesterase.

The situation of the chemotherapy of most of the liver fluke -, lung fluke - and intestinal fluke - infections of man is currently satisfactory since the introduction of *praziquantel*. In the treatment of *Fasciola hepatica* infections *triclabendazole* becomes more and more important. In veterinary medicine infections with *Fasciola hepatica* in ruminants are most important. Here, against all the different classes of compounds there are worldwide great resistance problems. This may be surmounted by use of drug combinations such as *salicylanilides* with *clorsulon*, *triclabendazole* or *luxabendazole*, respectively. Moreover, since *diamfenethide* and *triclabendazole* are effective against juvenile and adult *Fasciola hepatica* only these drugs can be used for chemoprophylaxis and treatment of an outbreak of liver fluke infections. Thus, there is an urgent need for new drugs against liver flukes with new mode of actions.

N29 p ONCHOCERCA GUTTUROSA: SENSITIVITY TO A RANGE OF ANTIBIOTIC AND ANTIPARASITIC DRUGS

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Background: A number of recent studies have demonstrated that certain antibiotics have antifilarial properties, thought to be mediated by the destruction of the intracellular endosymbiotic bacterium *Wolbachia*. To further examine this, we have evaluated the effects of a range of antibiotics, some used in combination with antiparasitics, against adult male *Onchocerca gutturosa* in long-term *in vitro* culture assays. **Methods:** Adult male *O. gutturosa* were obtained from the nuchal ligament connective tissues of naturally infected cattle. Each drug concentration was tested against 6-10 worms, with each worm maintained individually in a well of a 24 well plate containing 1.8 ml of Minimum Essential Medium + 10% calf serum + a monkey kidney cell feeder layer. Medium (+ or - drug) was replaced every 5 days. Worm viability was measured using motility levels and MTT colorimetry after 40 days incubation, with the exception of ciprofloxacin (21 days). **Results:** Drugs active at a concentration of 5×10^{-5} M or lower included rifampicin, clofazamine, minocycline, doxycycline. Drugs not active at 5×10^{-5} M included erythromycin, oxytetracycline, isoniazid, trimethoprim, sulphamethoxazole, ethambutol, dapson, pyrazinamide, ciprofloxacin. No synergistic effects were seen using drug combinations of rifampicin/isoniazid, rifampicin/chloroquine, rifampicin/mefloquine, rifampicin/ivermectin, rifampicin/moxidectin, trimethoprim/sulphamethoxazole. **Conclusions:** This study has identified 4 commonly used antibiotics with significant anti-*Onchocerca* activity *in vitro*. There is growing evidence that *Wolbachia* provides a target for treatment with antibiotics that may result in filarial effects, which raises the possibility that new therapeutic agents could become rapidly available for the treatment of filarial diseases. **Acknowledgement:** Financial support from UNDP/World Bank/WHO.

N28 p DETECTION OF PRAZIQUANTEL RESISTANCE IN SCHISTOSOMES

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Background: *Schistosoma japonicum* is a zoonosis in which water buffalo are an important reservoir host. Buffaloes are treated with praziquantel but not always cured. A simple test resistance for praziquantel resistance is therefore required.

Methods: Susceptible and praziquantel resistant *S. mansoni*, whose status was confirmed in drug trials in mice, were maintained in CD1 mice and *Biomphalaria glabrata*, *S. japonicum* in *Oncomelania hupensis* and mice. Miracidia were incubated in solutions of praziquantel and then treated with Lugol's iodine.

Results: Incubation of miracidia in praziquantel results in a change in shape which can be counted quantitatively. Incubation for 60 secs in 10^{-6} M praziquantel clearly differentiated between susceptible and resistant isolates of *S. mansoni* with percentage changes from 52-100% in susceptible and 3-15% in resistant isolates. *S. japonicum* was more sensitive to praziquantel than susceptible *S. mansoni* with 50-70% changing in 5×10^{-7} M praziquantel compared with 0% in *S. mansoni*.

Discussion: Incubation of miracidia for 60 sec in praziquantel provides a simple objective test to look for praziquantel resistance in animals and man. Since *S. japonicum* is more sensitive to praziquantel than *S. mansoni*, resistance should be slower to develop.

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N30 p TREATMENT BY IVERMECTIN 1% L.A. TO PREVENT NAVEL MYIASIS CAUSED BY COCHLIOMYIA HOMINIVORAX IN NEWLY BORN CALVES.

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Background: *Cochliomyia hominivorax* is one of the main ectoparasitic problems in the Province of Corrientes, a subtropical area of Argentina.

Method: In order to evaluate the prophylactic effect of Ivermectin 1% L.A. (Long Acting) administered subcutaneously at a dose level of 200 μ g per kg. b.w., some studies were carried out on a farm situated in the central area of Corrientes province during 1999 and 2000 calving season. In these studies, all newly born calves were treated within 24 hours of calving, in order to prevent navel myiasis caused by *Cochliomyia hominivorax*. Untreated calves as well as treated ones remained with their dams in the same paddock where they were naturally exposed to *Cochliomyia hominivorax* infestation.

The navels of the treated and untreated calves were examined on day 3, 5, 7, 10, 14 and 20 after the treatment in order to control the presence of myiasis.

Results: Calves treated with Ivermectin 1% L.A. had a significant lower incidence of navel myiasis ($p < 0.01$) than untreated calves.

Conclusions: According to these results the A.A. suggested to the farmer a prophylactic treatment for navel myiasis injecting calves, immediately after birth, with Ivermectin 1% L.A. (Long Acting) at a dose level of 200 μ g/kg.b.w.

N31 p INHIBITION OF LARVAL DEVELOPMENT OF *COCHLIOMYIA HOMINIVORAX* BY CYROMAZINE AND TRIFLUMURON

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Background: bioassays were carried out to evaluate the effects of the addition of cyromazine and triflumuron in larval rearing medium on larval survival.

Method: The insect grow regulators were added to the larval rearing medium to obtain concentrations of 0.05 % and 0.01 % for cyromazine and 0.024 % and 0.048 % for triflumuron. Twenty first instar *C. hominivorax* larvae were inoculated in twelve replicates of each concentration. Similar control groups of larvae were inoculated in untreated larval rearing medium. After 24 h the mean number of surviving larvae in each treatment and concentration were recorded in half of the replicates and compared with control groups. The same procedure was performed in the another half of replicates at 48 h post larvae inoculation.

Results: The mean number of live larvae was significantly ($p < 0.001$) reduced after remaining 24 or 48 h in the cyromazine and triflumuron treated mediums. However, the higher mortality rate was observed when the exposition period to treatment was longer. In this context, no surviving larvae were observed in both triflumuron concentrations after 48 h while cyromazine showed mortality rates of 90.8 % and 94.2 % at the lower and higher concentration respectively.

Conclusions: cyromazine and triflumuron addition to larval rearing medium produced a significant reduction of larvae number. Time dependent effects were observed with both drugs. Larval development was completely inhibited after 48 h triflumuron exposition. Both drugs could have a potential use in the control of *C. hominivorax* larvae infestation.

N33 p TREATMENT OF GIARDIA INFECTIONS IN DOGS AND MICE WITH FEBANTEL AND PYRANTEL-EMBONATE (DRONTAL® PUPPY)

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50 out of 161 young dogs, which had shown diarrhoea (often bloody and slimy) were found to excrete *Giardia*-cysts. 42 of them were orally treated with febantel and pyrantel-embonate (Drontal® Puppy), 8 remained untreated as control. 34 dogs were treated once, 8 were treated twice (over two consecutive days). In the group treated once 27 dogs did not excrete cysts for several days, but only in 15 dogs the *Giardia* infection remained completely suppressed. Dogs from the group treated twice stopped cyst-excretion completely, as demonstrated by results of three separate feces examinations.

Immunosuppressed Balb/c mice were orally infected with 1.7×10^6 *Giardia*-trophozoites and developed an intensive infection, proven by fecal sample-investigations and in histological analysis. Four days after infection one group of mice obtained 20 µl of a febantel and pyrantel-embonate suspension (Drontal® Puppy) /mouse over three consecutive days, while two other groups remained untreated (one of them uninfected). Four days after last treatment treated mice contained only 8% of the parasitic load of untreated animals, while they were still present in huge numbers in untreated ones as seen in histological sections. However, cyst excretion had stopped in treated animals on the 1st day after treatment, while it was still present in the others. The present study reveals that apparently more than one application of the drug is needed for a complete cure. No signs of toxicosis were seen in any of the treated animals.

N32 p EFFICACY OF PYRANTEL EMBONATE, FEBANTEL, AND PRAZIQUANTEL AGAINST *GIARDIA* IN DOGS

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Giardiasis is recognized as an important disease in humans and in young animals of several species in most areas in the world. The prevalence of *Giardia* spp. in dogs varies greatly, but has been reported to be very high in breeding kennels and in puppies. The objective of this study was to prove the therapeutic efficacy of pyrantel embonate, febantel and praziquantel (Drontal® Plus)^a against *Giardia* spp. in naturally infected dogs under field conditions.

A test for *Giardia*-specific antigen (ProSpecT® *Giardia* Micoplate Assay) performed in dogs from private owners identified 38 dogs to be naturally infected with *Giardia* spp. All 38 dogs were treated orally with combination tablets (144 mg pyrantel embonate, 150 mg febantel and 50 mg praziquantel)^a at the recommended dosage on two consecutive days. The therapeutic response was assessed on day three after the last medication by means of the coproantigen test for *Giardia*-specific antigen. On the third day after the final administration of the tablets 35 of the 38 treated dogs were negative for coproantigen of *Giardia* and 3 were positive. Signs of toxicosis were not observed in any dog.

The results of the study indicate that the combination tablets (144 mg pyrantel embonate, 150 mg febantel and 50 mg praziquantel)^a given at the recommended dosage on two consecutive days are effective for treating naturally acquired giardiasis in dogs.

^a Drontal® Plus, Bayer AG

N34 p EFFICACY OF MOXIDECTIN AGAINST *THELAZIA CALLIPAEDA* INFESTATION IN DOGS

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Background: *Thelazia callipaeda* (Nematoda, Thelazidae), dog eyeworms, cause ocular infestation characterized by epiphora, conjunctivitis and keratitis and requiring drug treatment. This infestation, which is not very common in Italy, has recently been described in a large number of dogs in Basilicata (Southern Italy). There is not much information on therapy for dog thelaziosis: levamisole and ivermectin (s.c. injection) have been used. Given the availability of a milbemycin in aqueous solution we tested the efficacy of this agent for local applications. This paper reports the results of a field treatment of affected animals with moxidectin[®] (Fort Dodge) 1% by ocular instillation.

Method: 78 dogs, (1-12 years old) which belonged to different breeds, had always lived in the towns of Accettura, Oliveto Lucano, and Pietrapertosa (Basilicata region, Southern Italy), and showed heavy *T. callipaeda* infestation (single or double) were randomly selected and allocated to 2 groups. 1) Treatment group – comprising 48 dogs, that received an ocular instillation of 1 -2 drops moxidectin per eye. The dosage was determined according to the degree of infection. 2) Control group – comprising 43 dogs left untreated.

Results and conclusions: In all treated dogs nematodes died a few minutes after drug instillation and within 24 -48 hours the worms and clinical signs disappeared. No adverse local and/or general reactions were observed on the treated animals. All the untreated dogs remained parasitized and exhibited clinical lesions. These results suggest that ocular instillations of moxidectin 1% are effective for the control of *T. callipaeda* infestation in dogs.

N35 p SEX-INFLUENCE ON THE PHARMACOKINETIC OF SELAMECTIN IN DOGS.

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The pharmacokinetic of selamectin (monosaccharide oxime derivative of doramectin) was determined in dogs following spot-on application at a dose rate of 6 mg/kg. The dogs (adult beagles, n=10) were allocated into 2 groups based on their sex. Selamectin was detected in plasma at the first sampling time and thereafter for at least 35 days. The maximum plasma concentration occurred 5 days post-administration. The plasma kinetic was analysed using a one-compartment model. The mean of each parameter was calculated for the 2 groups of dogs. The most striking results were the significant differences registered between the 2 groups for the area Under the Curve (AUC) and clearance. Sex influenced greatly the AUC, which was about 90% higher in females than in males. The clearance values were significantly higher in males compared to females. The higher plasma selamectin concentrations in female dogs appears mainly to be related to difference in the clearance of the drug. This observation is consistent with previous results reported for selamectin in rats and doramectin in cattle. This experiment provides evidence of sex influence and deserves to be correlated with efficacy studies in dogs.

N37 p PYRIPROXYFEN IN THE COAT OF CATS AND DOGS AFTER TREATMENT WITH FLEEGARD®

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Purpose: Distribution of active ingredients and persistence in the hair coat of pets after treatment is essential to maintain high efficacy over extended periods. This study evaluated the kinetics of the insect growth regulator and juvenile hormone mimic, pyriproxyfen, on cats and dogs for 8 weeks after a single treatment using the new flea control product Fleegard®. **Method:** Twelve dogs and 12 cats were treated with Fleegard® topical solution (1% pyriproxyfen) with a dosage of 0.04 ml/kg and 0.1 ml/kg, respectively. Hair samples were taken from cats and dogs on days 0, 1, 4, 7, 14, 21, 28, 42, and 56 after treatment and analysed by GC/MS for pyriproxyfen concentrations. The limit of quantification was 0.02 mg/kg. **Results:** The concentration of pyriproxyfen found in the samples from both dogs and cats demonstrated that Fleegard® dispersed very rapidly in the hair and persisted for the 8 week test period. On day 1, levels from 0.15 mg/kg to 4.74 mg/kg and from 0.2 mg/kg to 4.16 mg/kg were found in the hair from dogs and cats respectively. At 8 weeks post treatment the content of pyriproxyfen found was 0.05 mg/kg to 0.33 mg/kg in dogs and <0.02 mg/kg to 0.21 mg/kg in the samples from cats. The samples close to the application site, contained more pyriproxyfen than those collected from the back of the animals. A high standard deviation was observed within the measured values. This is believed to be due to different dermatological factors as well as differences in intensity of grooming behaviour. **Conclusion:** Efficacy studies have demonstrated a 100% inhibition of flea development from eggs to adults, when adult fleas and their eggs were exposed to dosages of pyriproxyfen as low as 0.0001 mg/kg in hair. A complete sterilisation effect on eggs was achieved exposing adult fleas for six hours to 0.01 mg/kg hair. Thus, in spite of the limit of quantification in this study being 0.02 mg/kg, the results obtained here indicate that one treatment with Fleegard® provides sufficient levels of pyriproxyfen in the hair coat to prevent development of fleas for up to 8 weeks after treatment.

N36 p ENDECTOCIDE EFFICACY OF "POUR-ON" IVERMECTIN AND IMIDACLOPRID IN DOGS

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This study evaluated the efficacy of a 0.25% ivermectin and a 10% imidacloprid (each 500mcg/kg of body weight) "pour-on" formulations (Prod. Vet. Ouro Fino Ltda.), applied concomitantly, against nematodes, ticks, fleas and scabies in naturally (for helminths, *Sarcoptes scabiei* and *Ctenocephalides felis*), and experimentally (for *Rhipicephalus sanguineus*) parasitized dog hosts. For evaluation against nematodes, 18 dogs were treated and then humanely killed on the 7th day post-treatment for assessment of parasite burden. The effect against scabies was evaluated by skin scrapings of 16 dogs, once before treatment and five times afterwards until 28 DPT. Counts made of all dead or repelled fleas until 72 hours after treatment, as well as all fleas still present on the dogs, were used to evaluate the effect of the formulae against these parasites. Experimental infestation of dogs was performed with adult ticks with the aid of feeding chambers glued of the hosts. Biological parameter of engorged ticks from the control group (n=4) were compared to those from the treated animals (n=6). The evaluated formulae, applied concomitantly, showed 100% efficacy against *R. sanguineus*, *S. scabiei* and *Ancylostoma caninum* and showed a prevention rate of 94,18% for *A. braziliensis* infections, and 92,17% against immature *Toxocara canis* forms. The compounds showed no efficacy against immature forms of *T. canis*. In all, the evaluated formulae applied concomitantly showed efficacy against endo and ectoparasites of dog hosts.

N38 p THE USE OF PYRIPROXYFEN (FLEEGARD®) ON DOGS AND CATS IN CONTROLLED CLINICAL AND FIELD STUDIES IN FRANCE AND GERMANY

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Three negative controlled clinical studies in Germany and 2 field studies in France were performed to examine the efficacy of 1% spot-on Pyriproxyfen (PPF, Fleegard®, Bayer, Germany) for control of the cat flea (*Ctenocephalides felis*). Animals of the clinical studies (group size of 10 animals, one group as untreated control, weekly reinfestation, incubation of collected flea eggs and blanket samples after animal contact) were treated with a single dose of PPF spot-on. In dogs, the development inhibition (d.i.) effect on flea offspring lasted 64 days (100%) and >71 days (>95%), in cats the d.i. lasted for 43 days (>95%). The efficacy of impregnation of environment lasted at least 24 days for dogs (100%) and 17 days for cats (>95%). In field studies (flea counts and clinical exam. on days 0, 14, 21, 28, 56 and 84, blanket samples on day 13 after treatment) 101 naturally flea-infested dogs and 99 flea-infested cats were treated with PPF 1% spot-on every 8 weeks. (81 dogs and 63 cats were treated with Lufenuron every 4 weeks.) No additional treatment was conducted. After treatment with PPF, the percentage reduction in infestation level was 78.7% (day 28), 91.2% (day 56) and 94.7% (day 84) for dogs and 81.5% (day 28), 91.6% (day 56) and 93.9% (day 84) for cats. The impregnation effect on the blankets (d.i. 47.1% for dogs and 42.9% for cats) was obvious after animal contact.

N39 p

EFFICACY OF NON-ACARICIDAL-CONTAINING OTIC PREPARATIONS IN THE TREATMENT OF CANINE AND FELINE OTOACARIASIS

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Background Otoacariasis or ear mite infestation is a relatively common disease in dogs and cats, caused by *Otodectes cynotis*. This condition is typically treated with products containing an acaricide or with systemic ivermectin. However, some products without acaricide also appear effective. This paper summarizes the results of an international field trial comparing the efficacy of two such products in the treatment of otoacariasis.

Method 89 cats and 38 dogs, naturally infested with the ear mite *Otodectes cynotis* were randomly allocated to treatment with either a product containing miconazole, polymyxin B and prednisolone (Surotan®, Janssen), or with a product containing diethanolamine fusidate, framycetin sulphate, nystatin and prednisolone (Canaural®/Fucidine®, Boehringer Ingelheim).

Treatment (5 drops in each ear) was applied twice daily for 14 days. Efficacy was assessed on days 7, 14 and 21 on the basis of an otoscopic examination, microscopical examination of scrapings for the presence of ear mites and evaluation of clinical signs of pruritus, pain, erythema and/or exudate.

Results Both products were highly effective in the treatment of otoacariasis in dogs and cats with no significant differences in efficacy nor in clinical improvement. Apart from an allergic reaction in 1 cat treated with Canaural®, no adverse events were observed.

Conclusion Both non-acaricidal containing products are highly and equally effective in the treatment of canine and feline otoacariasis. It is hypothesized that this activity could be attributed to the vehicle (liquid paraffin for Surotan®, sesame oil for Canaural®), which either alters the habitat of the external ear canal in such a way that it becomes unsuitable for the ear mites or simply drowns, suffocates or immobilizes the mites. Otoacariasis can safely be treated with this type of products, which offers an alternative to products with potentially toxic acaricides or the off-label use of ivermectin.

N40 p

REPELLENCY OF CHEMICALS TO THE CAT FLEA, *Ctenocephalides felis*

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Background: An effective chemical repellent to fleas could provide an alternative method to direct insecticidal action for their control. A natural product with such activity could be of interest to certain groups of consumers. The repellency of selected chemicals to fleas was determined in a simple avoidance test.

Material and methods: Unfed, artificially reared adult fleas were placed in a box with alternating strips of chemically impregnated paper. The box was kept in the dark and numbers of fleas present on each individual strip were counted at intervals over a 24 period.

Results: Although a standardised extract of Neem as a single compound showed no repellent activity it enhanced the repellency of IR3535. Methoprene at 0.5% showed repellency but over time proved lethal to fleas. A 10% solution of permethrin also showed initial signs of repellency but within six hours the fleas had died. The strongest repellency was obtained with Deet (N,N-diethyl-m-toluamide) with fleas actively seeking to avoid the impregnated strips by collecting under the control strips.

Discussion: Offering fleas a choice of untreated or treated papers provided a simple test for estimating repellency. Using this test it was confirmed that the insect repellent, Deet, is strongly repellent to fleas. Other chemicals, including permethrin, showed some repellency, but this activity was largely masked by their insecticidal effects.

N41 p

MELARSOMINE DIHYDROCHLORIDE AS A HEARTWORM ADULTICIDE FOR FERRETS

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Background: Melarsomine dihydrochloride has been shown to be highly effective in the treatment of adult and 4-month-old *Dirofilaria immitis* in both naturally and experimentally infected dogs. Unfortunately, no FDA-approved heartworm adulticides are available for treating infected ferrets.

Method: Twenty-eight ferrets were each given 3 pairs (3M, 3F) of 114-day-old *D. immitis* worms by subcutaneous transplantation and 75 days later allocated to 4 groups of 7 ferrets each. Group I served as a saline-treated control. Groups II, III, and IV were given melarsomine dihydrochloride intramuscularly (IM) at a dose rate of 3.25 mg/kg; Group II was given a single IM injection; Group III was given 2 IM injections, 24 hours apart; Group IV was given a single IM injection, which was followed 1 month later by 2 IM injections, 24 hours apart. Seven ferrets died early and all remaining ferrets were necropsied between Days 104 and 112 after the first or only treatment.

Results: All ferrets in Groups I and II had adult worms, with an average of 3.6 worms per ferret (59.9%) and 2.5 worms per ferret (41.7%), respectively. Four of 7 ferrets in Group III had adult worms, with an average of 0.6 worm per ferret (9.5%). Three of 6 ferrets in Group IV had adult worms, with an average of 0.7 worm per ferret (11.1%). Percent efficacies of melarsomine dihydrochloride for Groups II, III, and IV were 30.6, 83.3, and 80.6, respectively.

Conclusion: Melarsomine dihydrochloride at the dosage of 3.25 mg/kg was equally effective (80.6-83.3%) when given twice 24 hours apart or 1 injection followed 1 month later by 2 injections 24 hours apart.

N42 p

ANTIFUNGAL ACTIVITY OF SOME *INULA VISCOSA* EXTRACTS

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Background: In previous investigations some essential oils and sesquiterpenes from several plants (*Artemisia verlotorum*, *Lavandula angustifolia*, *Ocimum gratissimum*) have proved to have acaricidal, antifungal and antibacterial activity. *Inula viscosa* Ait. (Asteraceae), a plant growing spontaneously in the Mediterranean area, is currently used by popular medicine for its therapeutic effects. Flavonoids, azulenes, sesquiterpenes, and essential oils have been isolated and identified from its leaves. This paper reports the results of chemical analyses and antifungal activity *in vitro* of different leaf and flower extracts of *I. viscosa*.

Method: Different extraction procedures (chloroform, hexane and hydro-distillation) were used. The extracts were dried and the residues diluted in ethanol to evaluate their efficacy at 1mg/ml and 0.1mg/ml. Their antifungal activity against *Microsporum canis*, *Trichophyton mentagrophytes*, *Microsporum gypseum*, and *Trichophyton terrestre* was tested as described by Lattanzio (Lattanzio et al., Ital. J. Food Sci., 1994, 1, 23-29). Thin layer (TLC) and gas/mass (GC/MS) chromatography were used for the chemical analysis.

Results and Conclusions: All the extracts revealed a high antifungal activity against dermatophytes (>40%). The hydro-distillate extracts, mainly from the leaves, exhibited the greatest antifungal efficacy. Chemical analyses detected many compounds (flavonoids, carboxylic acids, monoterpenes, sesquiterpenes, diterpenes, oxygenated aromatic compounds, waxes and isopropyl cuminic acid). The high concentration of isopropyl cuminic acid revealed in the hydrodistillate of leaves may explain its greater antifungal activity.

P

Echinococcosis and hydatidosis

P1

ECHINOCOCCUS MULTILOCULARIS INFECTION IN RED FOXES

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Occurrence of *E. multilocularis* in red foxes was observed within the territory of Slovak Republic throughout the years 1999-2000. In 1999, mainly red foxes from border districts were examined, however, in the next year they came from the whole territory.

E. multilocularis was isolated from the small intestines of red foxes using the scraping technique. Samples were examined for the presence of coproantigens by use of sandwich ELISA method. For the identification of juvenile tapeworms and *Taenia*-type eggs, nested PCR method was applied.

In 1999, 10.7 % occurrence of *E. multilocularis* was recorded based on the finding of proglottides and/or eggs in faeces of red foxes coming from three districts. Coproantigens of *Echinococcus* spp. were detected in 24.4 % of faecal samples being obtained from five districts. In 2000, survey of the occurrence of this cestode throughout all Slovak territory was undertaken in foxes captured in spring and in autumn. In foxes examined in spring, *E. multilocularis* occurred in 25.9 % (autopsy and coproantigens). Coproantigen were assessed in 16.7 % of foxes investigated in autumn. A total coproantigen positivity of 21.6 % was detected in year 2000.

Our results indicated that during last two years *E. multilocularis* infection has been transmitted to the most of Slovak territory.

P3

ECHINOCOCCUS MULTILOCULARIS IN RED FOXES IN NORTH-EASTERN ITALY

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Background: In central Europe an extension of the endemic area of *Echinococcus multilocularis* to regions, where this parasite was not described before, has recently been recognised. Therefore, a study was initiated to investigate the occurrence of this parasite in north-eastern Italy.

Method: During the years 1997-2000, 360 red foxes (*Vulpes vulpes*) from the provinces of Trento and Bozen were sampled. In most cases the foxes were shot in the course of the official local population control programme. A faecal sample for each red fox was collected and analysed by ELISA (CHEKIT Echinotest) in order to detect *E. multilocularis* coproantigens. Ambiguous or positive ELISA results were confirmed by the parasitological examination of the intestine with the sedimentation and counting technique and partially by PCR, using the faecal sample for isolation of taeniid eggs.

Results: 23 of 360 of the red fox faecal samples were coproantigen positive and 13 had an ambiguous ELISA result. *E. multilocularis* worm burden of > 100 worms were found in the intestine of two red foxes, and in both these cases egg identification by PCR was positive. Cestode infections by *Taenia* spp., dipylidids or *Mesocestoides* sp. were found in the remaining foxes.

Conclusion: This is the first description of *E. multilocularis* in Italy, and further studies are in progress to better define this endemic area.

P2

IS A HIGH PREVALENCE OF *E. multilocularis* IN WILD AND DOMESTIC ANIMALS ASSOCIATED WITH INCREASED DISEASE INCIDENCE IN HUMANS?

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A highly endemic *E. multilocularis* area was subjected to specific investigations for (a) assessing the multiannual persistence of high endemicity among rodents, (b) exploring the transmission potential of the parasite to domestic carnivores and (c) rating the putative exposure versus infection frequency in inhabitants of this region. For 6 years, the prevalence of *E. multilocularis* in rodents had persistently ranged between 9% and 39% for *Arvicola terrestris*, and between 10% and 21% for *Microtus arvalis*. In the same time period, 7% of feral dogs and 3% of cats living in the study area tested positive for intestinal *E. multilocularis* infection. The test procedure included egg detection, copro-antigen detection (ELISA) and parasite DNA amplification by PCR. Thus, infected domestic carnivores may notably increase the *E. multilocularis*-egg exposure risk of the human population. Consequently, a sero-epidemiological survey - including 2'943 blood donors residing in the study area - was carried out using Em2-ELISA. Four out of 6 Em2-positive persons agreed to further clinical investigations by hepatic imaging procedures. One person exhibited a hepatic lesion compatible with an abortive (died-out) status of the infection, as assessed by CT. This person represents the first Swiss case with an abortive course of alveolar hydatid disease. The other three persons did not exhibit any signs of AE. Comparative statistical analyses of seroprevalences and clinical incidences provided a significant ($p < 0.005$) increase of the Em2-seroprevalence between 1986 and 1996/7, whereas the clinical incidence of alveolar hydatid disease did not increase ($p = 0.378$). Consequently, a high prevalence of *E. multilocularis* in wildlife and domestic animals seemed not to have a direct short-term impact on the incidence of AE in humans (Gottstein et al., *Emerging Infectious Diseases*, in press).

P4

RECOMBINANT EM-14-3-3 AS A VACCINE PROTECTS AGAINST EXPERIMENTAL *E. multilocularis* INFECTION IN MICE.

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The *Echinococcus multilocularis* 14-3-3 protein has demonstrated biological properties related to the tumour-like growth characteristics of the *E. multilocularis* metacestode (Siles-Lucas et al., 1998; 2000). Basic immunogenicity of the Em14-3-3 protein has been shown in mice upon both infection with eggs (primary AE) or inoculation with metacestode vesicles (secondary AE). Immunization (vaccination) with recEm14-3-3 protein confers protectivity at an efficacy range of 97% upon oral challenge infection with 1'500-3'000 *E. multilocularis* eggs. The immune response elicited by vaccination was characterized by a high production of antigen-specific IgG₁ and IgG_{2a} antibodies. The vaccination also evoked an antigen-specific cellular immune response, reflected in the corresponding production of Th1- and Th2-associated cytokine patterns upon *in vitro* stimulation of splenocytes with the specific antigen. Conversely, vaccination of animals subsequently challenged with i.p. inoculation of *E. multilocularis* vesicles did not protect against secondary AE, as demonstrated by equivalent parasitic masses between vaccinated and non-vaccinated mice. Our results demonstrate (1) the possibility to protect mice with the recEm14-3-3 vaccine against primary infection and (2) that protection is unsuccessful against metacestode vesicles (secondary AE). As vaccination seems to protect against the oncospheres but not against the vesicles, the results add to our knowledge about the role of the laminated layer which seems to protect efficiently the parasite vesicle against immune effector mechanisms.

P5

IMMUNOLOGICAL COMPARISON OF THE LAMINATED LAYER OF *ECHINOCOCCUS GRANULOSUS* ISOLATED FROM FIVE DIFFERENT HOSTS SPECIES

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Background: All species of *Echinococcus* are characterized by the existence of the laminated layer (LL). This layer in cystic hydatid cysts, is unique and of variable thickness, and is involved in the survival of the metacestode. An attempt to identify both parasite and host components and to detect an antigenic/or immunogenic nature of different host species of LL extracts.

Method: The primary source of hydatid cyst fluid, protoscoleces (Px) and LL was from natural infections in the liver and lungs of sheep slaughtered. Additional material was obtained from natural infections in horse and from human hydatid cysts removed at surgery. Secondary hydatid cysts were obtained by intraperitoneal inoculation of gerbils and BALB/c Mice. The samples were therefore analyzed using SDS-PAGE and immunoblotting.

Results: The SDS-PAGE results indicated that each LL isolate produced different binding profiles although there was evidence of common bands between different extracts. Results of potentially antigenic components by rabbit anti-sheep-LL, at least confirmed that the crude LLs had an antigenic nature. On the other hand immunoblotting data using monoclonal Ab to Px, hydatid cyst fluid, sheep serum, IgG showed that the LL extracts contained both parasite and host molecules in their structures.

Conclusion: The current study indicated that the most important antigenic molecules of LLs were concentrated to two regions (50 - 66 KDa and 25 - 29 KDa). In addition, the common bands had a much stronger reactions and some bands (at 34, 23, 17, 16 and 15 KDa) were also restricted to LL isolated from sheep in comparison with other host species of LL extracts.

P1 p

THE CARRIAGE OF *ECHINOCOCCUS MULTILOCULARIS* BY THE RED FOX IN SOUTHERN BELGIUM

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Background: *E. multilocularis* is a small tapeworm which is responsible for a serious zoonotic disease in many parts of Europe. In Belgium, the presence of *E. multilocularis* in its final host, the red fox (*Vulpes vulpes*), was reported for the first time in the Ardenne area. The present study was conducted to assess the distribution of the parasite in other parts of Wallonia (Southern Belgium).

Method: Between 1998 and 2000, 475 red foxes killed in Wallonia were available for parasitological examination of the gut. The identification of the tapeworm was based on morphological criteria.

Results: A total of 120 foxes were found positive (25%). The analysis of data revealed marked differences between the different geological areas of Wallonia; the highest prevalence (34%) was found in the Ardenne and the lowest (0%) North of river Meuse.

Conclusion: *E. multilocularis* is a very frequent parasite in the fox population of Wallonia and it has an increasing incidence between the low land and hilly areas of Central and South-East Belgium respectively.

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P2 p

EPIDEMIOLOGICAL SITUATION ON ECHINOCOCCOSIS IN ARMENIA

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Background: From 1986 through 2000 we have done an epidemiological analysis of the dynamic distribution of echinococcosis among the population in Armenia.

Methods: We have used archive data of the Health Ministry of Armenia and personal research materials.

Results: The following natural developments have been identified. In 1986 the index of intensity calculate for the 100,000 population made 1.25, in 1989 - 0.7 and in 1990-1991 - 0.6 and 0.52 respectively. This natural decrease of the infection was the result of a large-scale veterinary and antiepidemiological measures. In 1992 and 1996 same index made 1.8 and 3.1 respectively. The last years were marked by an increased intensity of echinococcosis infection among the population. This is explained by a transition in the animal husbandry from state to the private sector, which makes impossible implementation of a number of control measures.

Conclusion: The problem of echinococcosis in Armenia is popular and needs elaboration of control measures at the new stage of social and socio-economic development which can be implemented in close collaboration of epidemiological and veterinary services.

P3 p

EPIDEMIOLOGY AND CONTROL PROGRAMMES OF ECHINOCOCCOSIS / HYDATIDOSIS IN IRAN

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Echinococcosis or Hydatid disease is one of the most prevalent and important helminthic zoonosis in the world, and it is widely distributed throughout the mediterranean region as well as in Iran. Its results are health damage and economic losses in human and affected livestock, specially in sheep raising areas. Transmission to human may occur whenever infected dogs live in close to them. In domestic and wild cycles of *E. granulosus* hydatid cyst occurs in herbivores by ingestion of eggs passed out by carnivore hosts. The adult worms are acquired by carnivores feeding on infected herbivores that are discarded after slaughtering animals at home or abattoirs. Of genus *Echinococcus* two species *E. Granulosus* and *E. multilocularis* are reported in Iran. Three distinct transmission cycles are reported, domestic cycle between dogs and livestock, desert cycle between dogs and cammels, and aylvatic cycle between wild carnivores and wild ruminants. Human hydatidosis has been reported from all parts of the country. The incidence of hydatidosis is estimated by hospital records, so, it dose not give us the true incidence. Any how a large countrywide study on some 3,370 patients operated on for hydatid cysts between 1980 - 1985 and estimated prevalence rate of 1.12 /100,000 population, 4.5 /100,000 in north east and 0.1/100,000 in the southern province along the Persian Gulf coast. Infection rates among females were 56.4% and males 43.6%. Reported studies during 1967- 1994 within the dog population infection rates was 6- 50% in stray dogs and 3.3-63.3%, in farm dogs, and in wild life final hosts jackals, 22%, wolves, 33- 100% and red fox (*Vulpes vulpes*), 12.5%. Significant rates of hydatid cyst was 1.5- 16.8% in sheep and goat, 6-14.7% in cattle, 11.2- 64% in cammels and 57.7% in buffalo and in wild ruminants such as wild sheep 4.8% and gazelle 62%. Unlike *E. granulosus* which is very common parasite in our country, *E. multilocularis* has been reported only in the north west of Iran, Dasthmogan. Reporting during 1994- 1996 prevalence rate was 22% in red fox and 23% in jackals, and in wild cat 50%. According to hospital records between 1946- 1993, 37 human cases are reported.

The most successful control programs are, a) regular deworming of dogs, destruction of stray dogs, registration and pasportization of dogs, changing dog feeding habits, b) prevention of illegal slaughtering, c) proper washing vegetables before use, d) increasing medical and veterinary education of people, and e) cooperation among all related health departments.

P4 p

ONCOSPHERAL HOOK-MUSCLE SYSTEMS IN *ECHINOCOCCUS GRANULOSUS* AND *E. MULTILOCULARIS*
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Purpose: The aim of this study was to describe functional ultrastructure of the oncospherical hook muscle system and its role in the mechanism of host infection by the oncospheres of *E. granulosus* and *E. multilocularis*.

Method: Tissue samples of gravid proglottids of *E. granulosus* and *E. multilocularis* were fixed in 4% glutaraldehyde in 0.1M sodium cacodylate buffer (pH 7.4), postfixed in 2% OsO₄ and routinely processed for TEM examination.

Results: Ultrastructure of hook-muscle system has been reconstructed from this and semi-thin serial sections of mature oncospheres. Oncospheres consist of one pair of medial hook and two pairs of lateral hooks. Each lateral pair is composed of one dorso-lateral and one ventro-lateral hook. The hook muscle cells of each hook are organized functionally into the 3 following systems: (1) the protraction system, for hook extension; (2) the abduction for drawing the hooks together toward the median plane of bilateral symmetry, and; (3) the retraction system for pulling the hooks back into the body.

Conclusion: The interconnections observed between the different muscle fibers provide a structural basis for coordinated hook action remaining under control of oncospherical nerve cells. Both oncospherical hook-muscle system and penetration glands play an important function in the mechanism of the intermediate host infection.

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P5 p

ONCOSPHERAL PENETRATION GLANDS IN *ECHINOCOCCUS GRANULOSUS* AND *E. MULTILOCULARIS*
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Purpose: Our aim is to describe the functional ultrastructure of the oncospherical penetration glands in relation to the mechanism of host infection by the oncospheres of *E. granulosus* and *E. multilocularis*.

Method: Tissue samples of gravid proglottids were fixed in 4% glutaraldehyde in 0.1M sodium cacodylate buffer (pH 7.4), postfixed in 2% OsO₄, dehydrated and routinely processed for TEM examination.

Results: Two types of oncospherical glands have been identified in the oncospheres of *E. granulosus* and only one type in these of *E. multilocularis*. A common type for both species is a large U-shaped, syncytial gland resembling the oncospherical penetration glands described for other cestode species. It is four-nucleated in *E. granulosus* and bi-nucleated in *E. multilocularis*. The shape and electron-density of secretory granules in the U-shaped syncytium change progressively from flattened, very dense granules in the perinuclear regions to spherical electron lucent granules observed in gland arms near their exits. The second type of gland observed in the oncospheres of *E. granulosus*, with evidently different type of secretory granules is situated between the arms of the U-shaped gland. Two oncospherical nerve cells, produce typical neurosecretory granules in a form of membrane-bound vesicles (97 nm in Ø) with a characteristic electron-dense content surrounded by a narrow peripheral halo of clear space.

Conclusion: Better knowledge of oncospherical penetration glands and nerve cells ultrastructure, directly involved in the mechanism of host infection may provide practical means for preventing infection. In targeting penetration gland secretion or nerve cell action, the hope lies in finding new chemotherapeutic agents that can act specifically on some secretory or neuronal signal substances or receptors of the infective oncosphere, thus preventing its activation, hatching and host tissue penetration, producing in effect the nonviable eggs.

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P6 p

UNUSUAL LOCATION OF *ECHINOCOCCUS MULTILOCULARIS* METACESTODES IN A DOG

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Background: Recent data on the occurrence of *Echinococcus multilocularis* in red foxes have indicated that this tapeworm is present in all federal states in Germany. A larger well investigated focus is situated in the north western part of the Brandenburg.

Case history: A 17 months old female dog (race: Berner Sennenhund) originating from a rural place north of Berlin with a chicken egg size subcutaneous swelling located at the right thorax was presented and a cyst like and chambered tumour was removed. A second fist size swelling located near the right elbow was detected 10 months later. Histologically, the surgically removed tumour was identified as a metacestode of *E. multilocularis*. Serologically, a positive IFAT antibody titre against *E. multilocularis* was found. Due to the poor general condition the dog had to be euthanased three month post operationem. At necropsy, an other fertile metacestode of smaller size infiltrating muscle tissues of the thorax was found. Internal organs were free of hydatids.

Discussion: Canids, especially foxes usually act as final hosts of this tapeworm. Voles (Microtidae) are the main intermediate hosts. However, few cases of metacestode findings in dogs have been described in the literature. In all these cases internal organs were always involved. The route of infection and the restriction of bladder worms to subcutaneous and muscle tissues remain unclear.

P7 p

OUTFOXING ECHINOCOCCUS USING A REMEDY AGAINST THE SOURCE OF HUMAN INFECTION OF *E. MULTILOCULARIS* IN JAPAN

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In 1999, *E. multilocularis* metacestodes were found in pigs in Aomori prefecture on the mainland of Japan. Thus, the endemic area for this cestode, which had been hitherto confined to Hokkaido, has been extended to the mainland of Japan. The most important measure to control human alveolar echinococcosis is against the source of infection of this disease. There is a need to identify and control definitive hosts that disperse the parasite eggs into environment.

Presently, the prevalence of echinococcosis in Hokkaido foxes is more than 50% and the parasite has also been detected in pet dogs and cats. The group at Hokkaido University developed a diagnostic method for fox detecting antigen in the faeces of the infected fox, whereby it is not necessary to autopsy the animal in order to diagnose the infection. In 1998, a deworming program to eradicate echinococcosis in foxes was initiated in a pilot area facing the Sea of Okhotsk in Hokkaido, covering 200 km². The wild foxes were fed fish sausage containing the anthelmintic praziquantel. Using the coproantigen detecting method and faecal examination for taeniid eggs, it was observed that the number of foxes excreting *Echinococcus* eggs had been reduced significantly despite the fact that some foxes were still positive for the coproantigen test, indicating there were still infected intermediate voles in the area during operation. Thus, it is expected that if deworming program will be carried out more extensively to reduce the infection of intermediate hosts, there is a possibility to eradicate the source of infection of the parasite from fox population.

Workshops

Relationship between pharmacokinetics and antelmintic efficacy of endectocides

THE RELATIONSHIP BETWEEN PHARMACOKINETICS AND ANTHELMINTIC EFFICACY OF ENDECTOCIDES

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Influence of routes of administration and species differences:

Anhelmintic activity of endectocides relates to both : action of the drug on the parasite and presence of effective concentration in terms of level and duration at the site of action. The pharmacokinetic profile of an endectocide depend upon the formulation of the dosage form, route of administration, and pattern of metabolism. The influence of these factors varie due to differences with the species of animal. Pharmacokinetic investigations have demonstrated large differences in endectocides bioavailability by regarding both, the different routes of administration and the various animal species. As a result of the highest endectocides bioavailability observed after subcutaneous administration in all investigated species, this route of administration can be considered as the reference route of administration in contrast of the very low bioavailability reported for pour-on administration. The quantitative disposition of equivalent dose rate and route of administration can markedly differ between species. This is of therapeutic concern, and we can assume that endectocides administered to goats at the sheep dose rate contribute to potentially subtherapeutic parasite exposure. The pharmacokinetic results obtained in various animal species by using different routes of administration deserve to be correlated with efficacy studies to decreased chances of development of resistant parasites.

THE RELATIONSHIP BETWEEN PHARMACOKINETICS AND EFFICACY OF MACROCYCLIC LACTONES; INFLUENCE OF HOST PHYSIOLOGY

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Background: Disposition of antiparasitic drugs in the central compartment (blood pool) has largely been assumed to directly reflect drug availability, and therefore efficacy, in other physiological compartments. These pools are interconnected but different pool size and exchange rates dramatically affect quantitative drug / metabolite presence at sites of parasite habitat.

Method: Accurate description of drug in the blood pool and physiological compartments of interest requires studies in animals permanently fitted with appropriate sampling cannulae. The behaviour of the matrix in the compartment through which the drug is distributed (digesta flow rate, biliary secretion / recycling) and physiological parameters (body condition, feed type / quantity, exercise) must be determined to compare quantitative drug availability at the site(s) of parasite predilection.

Results: Transit time of digesta particulate material affects exchange of associated drug into gastrointestinal fluid for absorption. Turnover of bodyfat affects the residence time of absorbed lipophilic compounds. Abomasal secretion of drug is generally low but there is significant secretion of absorbed or parenterally administered drug in bile. Enterohepatic recycling increases availability in the proximal intestine while further absorption extends parasite contact in the distal intestine.

Conclusion: Host physiology profoundly influences antiparasitic drug kinetic disposition and only with full understanding of compartmental interactions can maximum therapeutic effect be realised.

RELATION DRUG-HOST-ECTOPARASITE

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The idea of developing this subject is based in the economical importance of ectoparasitic disease and the difficulties in understanding the drug-ectoparasite-host relationship. The objective was to contribute to the understanding of the drugs arrival to the ectoparasite site and the ectoparasite itself, and the evaluation of possibilities of improving this arrival. We developed studies determining plasma, ticks, eggs and larvae abamectin (ABM) concentrations in cattle with tick disease. We determined the ABM concentrations and the time after administration at which *B. microplus* larvae are unable to develop. In other experiments the differences in ivermectin (IVM) pharmacokinetics between healthy sheep and sheep with mange were determined, finding some interesting differences. The diseased animals concentrates drug in the central compartment and eliminates it faster. In a study directed to understand the distribution of IVM, a group of sheep with mange was used to determine concentrations in plasma, scabs and mites. We concluded that the high efficacy of avermectins (AVM) in mange is related to its distribution to skin, scabs and to the alimentary habits of mites. Screw-worm and horn-fly are two important parasitic diseases. Some AVM-based formulations prevents the establishment of screw worm strikes, but not all of them are able to successfully interrupt the clinical course of the disease. The reasons of these differences are probably pharmacokinetically based. The knowledge of the arrival of the drug to the parasite will contribute to better preventive and therapeutic results.

MACROCYCLIC LACTONES: COMPARATIVE CONCENTRATION PROFILES IN TARGET TISSUES

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Background: The time of parasite exposure to active concentrations determines the persistence of the potent and broad-spectrum antiparasitic activity of macrocyclic lactones (endectocides). The evaluation of the concentration profiles attained in target tissues may contribute to understand the differences on the therapeutic and preventive efficacies observed for endectocide compounds in cattle.

Results: These highly lipophilic molecules are extensively distributed from the bloodstream to different tissues; this extensive tissue distribution pattern agrees with the large availability of ivermectin (IVM), doramectin (DRM) and moxidectin (MXD) measured at different sites of parasite location such as the gastrointestinal (GI) mucosa, lungs and skin over 50 days after their subcutaneous administration to cattle. Drug concentrations were markedly higher in the mucosa compared to its respective digestive fluid, and in the dermis compared to the hypodermic tissue. The more sustained concentration profiles and larger availability of DRM in the GI tract, compared to IVM, may account to an increased time of parasite exposure to therapeutic concentrations and to its prolonged preventive efficacy against GI nematodes. The extended residence of MXD in the abomasal/intestinal mucosa agrees with its reported anthelmintic persistence for at least 35 days post-treatment.

Conclusion: The endectocide concentration required at the site of parasite location to inhibit parasite establishment/development has not been determined. However, the characterisation of drug concentrations in target tissues may provide information to predict the drug level below which the effectiveness against larval and adult parasites begins to decrease. There is a good correlation between active concentrations measured in tissues of parasite location at critical times post-treatment, and drug levels that affect basic nematode functions in *in vitro* pharmacological assays. The progress achieved in the comprehension of the target tissues disposition kinetics contributes to optimise the use of endectocide compounds in livestock.

**MACROCYCLIC LACTONE/ P-GLYCOPROTEIN
INTERACTIONS, EFFECTS OF REVERSING
AGENTS**
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P-glycoproteins (Pgp) are transmembrane proteins that can efflux lipophilic drugs with ring structures from the cell membrane to the extracellular space. Pgps can be expressed at relatively high levels in some normal mammalian tissues, in mammalian tumour cells that display multidrug resistance (MDR), and in the pharynx and anterior intestine of nematodes as well as in other nematode tissues. Macrocyclic lactone (ML) anthelmintics such as ivermectin (IVM) are excellent ligands for Pgp and strong MDR reversing agents. Pgps play a key role in keeping the concentration of certain lipophilic substances, including IVM, relatively low in the central nervous system (CNS) of mammals and defects in the expression of Pgp in the blood brain barrier greatly increased susceptibility to CNS toxicity from certain drugs such as IVM. Anthelmintic selection with IVM, moxidectin (MOX), and probably other MLs, has been clearly demonstrated to select for certain alleles of Pgp genes in *Haemonchus contortus*. Selection on Pgps appears to be involved in the mechanism of resistance to IVM and of other types of drug resistance, although it may not be the principal mechanism of IVM resistance. MDR reversing agents such as verapamil, which block Pgp efflux mechanisms enhance the activity of ML anthelmintics such as IVM and MOX against ML resistant strains of *H. contortus*. This effect of MDR reversing agents on ML efficacy may be due to several factors, (1) a direct effect on the nematode Pgp, inhibiting its ability to efflux the anthelmintics from receptor sites in the worms, (2) an inhibition of hepato-biliary excretion of IVM resulting in an increase in mean residence time of IVM in the mammalian host, and (3) possibly an inhibition of cytochrome P450 liver metabolism of some drugs.

Harmonization of diagnostic methods in veterinary parasitology in the EU

Serum pepsinogen determination for diagnosis and monitoring of ostertagiosis in cattle.

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Background: Serum pepsinogen levels have been recommended as an aid in the diagnosis of parasitic gastro-enteritis in cattle for some years. Because serum pepsinogen levels at housing are also a very good measure of exposure to *Ostertagia* infections experienced by naturally infected first grazing season calves, they have also been used to monitor both the epidemiology of gastro-intestinal nematode infections, and the efficacy of preventive treatment strategies during a first grazing season. The potential of pepsinogen values for herd health monitoring has been demonstrated.

Methods: Major disadvantages of pepsinogen determination were that the methods used are very labour intensive and therefore, too expensive, and that there was little standardisation, making comparison of results from different laboratories difficult. Recently an inexpensive, simple micro-method for the routine determination of pepsinogen concentration in bovine serum samples was described. This technique has the advantage that equipment to carry out the test is available in virtually any diagnostic laboratory.

Conclusions: Since its introduction the micro-method has been adopted by several laboratories. Reference bovine serum samples should be made available to allow a quality control of the technique.

A proficiency test of the modified McMaster technique

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Background: A proficiency test of the McMaster technique, a method to count parasite eggs and oocysts in faecal samples, was organised among some European veterinary parasitological laboratories.

Method: Faecal samples of the most common mammalian husbandry animals may be used. These samples must be freshly collected and delivered to the collaborating laboratories within about 24 hours. Samples are distributed by a door-to-door service. Three to five faecal samples are distributed at each time thrice per year. The managing laboratory carries out 10 separate tests, named pre-tests 1-10, for each faecal sample. These are consecutively performed by the same operator. The modified McMaster technique using 3-4g faeces and yielding 50 epg as the minimum detectable limit was employed.

Result: Most results of the participating laboratories will agree with the results of the pre-tests within the range $\pm$ one SD value. The between laboratory variation was greater than the within laboratory variation.

The aim of the test today is to demonstrate how well the tests are accomplished at different laboratories performing the tests with their actual modifications. The next step will be to upgrade the procedure in accordance with ISO 41

THE USE OF THE AGAR-GEL TECHNIQUE FOR POST MORTEM ISOLATION OF HELMINTHS

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Background: Post mortem isolation of nematodes may be a very labourious task, especially when recovering minute larvae from voluminous samples. Therefore, an easy and time-saving agar-gel method has been refined in our laboratory for routine quantifying of nematodes.

Method: In principle, a fresh subsample of gastro-intestinal contents or of a blended internal organ is mixed with 2% agar (1:1), poured onto a cotton cloth in a thin layer and allowed to solidify. When incubating the gels in 0.9% NaCl at 38°C, the worms will move out of the gels and settle.

Results: The agar gel technique has proved to be excellent for lumen dwelling larval and adult *Oesophagostomum* sp. and *Hyostromylus rubidus* and larval *Ascaris suum* from the small intestine of pigs. For the latter helminth, for example, the method may be used to estimate the daily uptake of infective eggs of tracer pigs. The technique may also be used for isolating migrating *A. suum* larvae from the liver and lungs of pigs although the recovery may be lower and more variable. The method does not work for *Trichuris suis*. The agar-gel technique allow us to handle a considerable number of rather voluminous samples per day, harvesting almost clean suspensions of living worms/larvae within a few hours or by the next morning. Hereby we avoid a long-lasting job recovering for example fixated worms under a microscope or on a light table. The most essential drawback of the method is that the incubation of the gels should start within approx. 4-5 hrs after the death of the host animal.

Conclusion: When the agar-gel technique is carried out properly, it makes isolation and quantifying of even minute nematodes easy and reproducible.

Monitoring gastrointestinal nematode and lungworm infections in cattle using antibody-detecting ELISA.

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The detection of parasite-specific antibodies by ELISA is at present not used routinely as a tool for monitoring gastrointestinal (GI) nematode and lungworm infections in cattle. The main reasons for the limited use of ELISA in practice are a lack of standardisation and the difficult interpretation. A range of different ELISA's has been developed for detection of antibodies against GI nematodes and *Dictyocaulus viviparus*. Some tests use crude worm antigen, others use a purified adult worm protein or a recombinant protein as antigen source, and while some laboratories use titres to express antibody levels, others use optical densities. Threshold levels of antibodies against GI nematodes and *D. viviparus* that are associated with reduced production or with acquired immunity have not yet been determined.

More effort should be put into the harmonisation of the ELISA tests that are currently available and into the determination of threshold antibody levels for (1) the assessment at housing of the efficacy of control systems for GI nematodes in first grazing season calves; (2) the estimation of infection levels with GI nematodes in adult dairy cows so as to decide for anthelmintic treatment; (3) the early detection of lungworm infections in a herd; and (4) the determination of the immune status of first grazing season calves against GI nematodes and *D. viviparus* at housing in order to decide on preventive measures next year.

Dirofilaria infection in humans and animals

What is changing in the epidemiology of *Dirofilaria* infection

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Several species of filarid worms infect dogs and cats worldwide: *Dirofilaria immitis*, *D. (Nochtiella) repens*, *Acanthocheilonema reconditum*, and *Dipetalonema grassi*. *Acanthocheilonema* spp. and *Dipetalonema grassi* are transmitted mainly by fleas, ticks and flies, whereas both *Dirofilaria* species are transmitted by mosquitoes. The most prevalent species are *D. immitis*, *D. (Nochtiella) repens* and *Acanthocheilonema reconditum*, the latter two being the ethiological agents of canine and feline subcutaneous dirofilariosis. *Acanthocheilonema reconditum* is cosmopolitan, whereas *D. repens* is diffuse in Europe, the Middle East, Asia and Africa. From a clinical point of view, *D. immitis* is the most important species. As the agent of canine and feline heartworm infection, it induces severe/extremely severe pathological changes in both dogs and cats. Furthermore, *D. immitis* and *D. repens* are zoonotic and where canine and feline disease is endemic, humans are at risk for developing pulmonary and subcutaneous lesions (human dirofilariosis). Many countries are endemic for *D. immitis* infection and, despite efforts aimed at control and prevention, infection appears to be spreading towards previously disease-free areas. The increasing number of dogs and cats in the established endemic areas are important factors contributing to the spread of the heartworm (HW) infection. The most important factor, however, is the abundance of that mosquito species which are able to transmit infective larvae but are resistant to being damaged by larvae as they migrate through their body. Furthermore, the general climatic trend which is strongly influenced by global warming, tends to extend the risk season for HW infection and to maintain a high incidence level, thereby reducing the effective reservoir of infection. This emphasizes the need to maintain prophylactic treatment in all the areas where the infection is endemic and carefully evaluate the disease in animals which have travelled in risk areas.

Feline heartworm infection: just a medical curiosity or a real danger?

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Dirofilaria immitis infection in cats is reported worldwide and it is consistently diagnosed in canine heartworm (HW) infection endemic areas. Although cats are susceptible hosts, they are more resistant to infection with lower prevalence and lower worm burden (usually less than 5 adult worms) than dogs. The clinical importance of HW infection is however amplified in cats because of their relative small body size and prognosis, in case of infection, has always to be considered guarded. There are significant differences between feline HW disease and its canine counterpart. Clinical signs usually develop during two stages of the parasite life cycle. The first coincides with the arrival of immature adult worms (fifth stage) in the pulmonary arteries 4 to 6 months post infection. The latter with the death of the adult worms, 2 or 3 years after the infection has been established. Clinical features are generally referable to the cardiopulmonary, gastrointestinal or nervous systems and are typically acute and elusive. Despite few cats may exhibit chronic signs, in most cases symptoms arise suddenly and death can occur so rapidly to preclude diagnosis or treatment. Serology to detect antibodies against the parasite and antigens from adult female worms, thoracic radiographs and echocardiography have in most cases to be used combined for achieving a definitive diagnosis. A causal therapy against the heartworm gives unpredictable results. Both adulticide therapy and surgical removal of the heartworm may be attempted in selected cases but they are considered risky procedures and have a high incidence of fatal adverse side effects. A conservative treatment based on the control of symptoms is usually elected giving the cat the chance to overcome the infection. For these reasons, chemoprophylaxis is the best option. Infection can be fully prevented throughout monthly doses of either ivermectin or milbemycin oxime per os, or topical selamectin. It is safe and fully effective and should be advised in cats living in HW endemic areas, even if the prevalence of the disease in dogs is low.

DIROFILARIASIS IN HUMANS: AN UPDATE

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The definition of the areas where *Dirofilaria* spp. are endemic in the canine populations, the degree to which these populations are affected and the species of vectors present are primordial factors for the determination of the risk of infection for the human populations. With regard to pathology, the characteristic lesion of pulmonary dirofilariosis is a solitary pulmonary nodule, which is revealed by radiological exploration, though in some cases multiple nodular lesions have been observed. Subcutaneous dirofilariosis appears as a small subcutaneous nodule which gradually grows during weeks or months. When the location is ocular, the worms are situated in the conjunctiva. The distribution of human dirofilariosis is difficult to define. Retrospective review of previously published clinical cases show that there are far fewer geographical points where human dirofilariosis has been reported than those where dirofilariosis has been found in animals. Approximately 550 subcutaneous/ocular cases and 280 pulmonary cases have been published so far. The frequency of the reports has increased in parallel to the increasing interest in these parasites, and human cases are detected more and more frequently in areas considered as non endemic until now. Seroepidemiological studies conducted in South Europe have shown that a significant percentage of the individuals living in endemic areas are infected and present specific IgM, IgG and IgE antibodies, in spite of the fact that only a few develop pulmonary or subcutaneous nodules. In an endemic area of Western Spain in which *D. immitis* infects 33% of the resident dogs, IgM appear in the young, IgG in the old and IgE in all age groups of the human population. Specific IgG antibodies against a recombinant molecule (WSP) from the *Wolbachia* endosymbiont present in the body of *D. immitis*, have been also identified in humans diagnosed as having pulmonary dirofilariosis. More studies are required to know if the *Wolbachia* antigens participate in the inflammatory reaction associated with the development of pulmonary nodules.

LABORATORY AND IN-CLINIC METHODS FOR THE DIAGNOSIS OF *DIROFILARIA IMMITIS* INFECTIONS

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Serologic (immunodiagnostic) tests are now used routinely in the diagnosis of heartworm infection in dogs and cats. For dogs, only antigen (Ag) detection tests are used. They detect Ag released by adult female worms, and infections are not consistently detected unless they are over 7 months old and 3 or more female worms are present. ELISA-, immunochromatography-, and hemagglutination-based Ag test kits are available to veterinarians for single (stat) and multiple (batch) unit testing and samples may be submitted to diagnostic laboratories. We recently compared sensitivity and specificity of 10 (7 stat; 3 batch) test kits and 3 diagnostic laboratories using split serum samples from necropsy-confirmed heartworm-positive (108) and -negative (100) dogs. Sensitivity and specificity were excellent for all test kits and lab tests, and there was no statistical difference between tests. Thus, the choice of test should depend mainly on clinic preference, ease of use, technician capabilities, and cost.

Both antibody (Ab) and Ag tests are used for cats. Antibody test kits and diagnostic laboratory tests are highly sensitive for prepatent (≤ 2 mo. PI), male-worm only, and recently self-cured infections, as well as infections with adult worms (~90% positive), and they are transiently positive on some cats exposed to infection while on preventative. A positive Ab test should be followed by Ag testing. Antigen tests are only 35 to 40% sensitive for cats with adult heartworms, but sensitivity is much higher in symptomatic cats. A positive Ag test is generally considered diagnostic. False-negative Ag test results are common in cats due to low worm burdens (<3 females), young infections, and single-sex infections (15% have only males), which are common in cats. Symptomatic, Ab-positive cats require additional diagnostic procedures and tests.

IMMUNE RESPONSES TO LARVAL *DIROFILARIA IMMITIS*

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Dogs and ferrets can be protected against infection with *D. immitis* by immunization with either radiation-attenuated L3 or chemically abbreviated infections. However, detailed investigations into the mechanisms of larval killing have not been done. With the advent of new reagents to investigate canine immune responses, and the use of intradermal infection and biopsy techniques, the ability to assess larval killing mechanisms is now possible. Naïve dogs, patently infected dogs, and dogs rendered immune to *D. immitis* infection were intradermally injected with L3 and punch biopsies of injected skin taken at intervals afterward. The biopsy material was split and part was fixed, sectioned and stained. An unfixed portion of the biopsy was placed immediately in culture allowing the larvae to migrate out of the tissue plug. Preliminary data demonstrated striking differences in the cellular responses in the skin of immune dogs as compared to naïve or infected dogs. Tissue sections from immune dogs demonstrated an immediate acute dermatitis (by 24 hours) that evolved over time to a more chronic and granulomatous inflammation (by 7 days). The predominant cells from early biopsies were eosinophils, while macrophages increased by day 7. Larvae were found in most tissue sections, and were surrounded by cellular infiltrates. This is in contrast to biopsies from patently infected dogs and naïve dogs, where the biopsies were normal or showed very mild dermatitis. In addition, larvae recovered from biopsies of immune dogs were dead or dying and were covered with eosinophils. This was in contrast to the normal-appearing larvae recovered from infected and naïve dogs. The data present compelling evidence that the intradermal infection model in dogs is a useful tool to evaluate the protective immune responses in dogs.

ROLE OF HISTAMINE IN HEARTWORM EXTRACT-INDUCED SHOCK IN DOGS

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Purpose: Intravenous administration of *Dirofilaria immitis* extract induces shock in dogs. Clinicopathologic findings of heartworm (HW) extract-induced shock are similar to those of anaphylactic shock and endotoxin-induced shock in dogs. The purpose of this study is to compare the mechanisms of HW extract-induced shock with those of anaphylactic shock and endotoxin-induced shock.

Methods: 1) 6 mixed-breed dogs were injected intravenously with 2 ml of HW extract and plasma histamine and serum tumor necrosis factor (TNF) concentrations were determined; 2) 4 specific pathogen-free (SPF) Beagles were injected intravenously with 2 ml of HW extract and examined for shock; and 3) mast cell degranulation by HW extract was tested, using canine cultured mast cells.

Results: 1) Six dogs developed shock, and plasma histamine concentrations increased significantly from 0.3 ± 0.2 (mean $\pm$ SD) ng/ml before HW extract injection to 44.6 ± 68.9 ng/ml at the onset of shock. However, serum TNF concentration was detected at a low concentration after recovery from shock; 2) All SPF dogs developed shock and had an increase in plasma histamine concentrations; 3) HW extract released histamine directly from canine mast cells.

Conclusions: Results of our study indicate that an unknown mast cell degranulating substances contained in HW extract might degranulate mast cells directly, consequently releasing histamine that might participate in the onset of shock in HW extract-induced shock in dogs.

Revisiting Chemoprophylactics of Heartworm Infections and Their Effect on Gastrointestinal Parasites in Dogs and Cats

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The introduction of the tablet formulation of ivermectin for dogs initiated the era of monthly chemoprophylactics for heartworm prevention. The subsequent introduction of milbemycin oxime expanded the choices available to the veterinarian, and introduced the potential for simultaneous control of gastrointestinal nematode parasites. Later, the development of an ivermectin/pyrantel pamoate chewable formulation and the introduction of a topical formulation of selamectin further expanded the alternatives for development of integrated programs for control of both gastrointestinal nematode parasites and *Dirofilaria immitis* in dogs. One of the macrocyclic lactone compounds, moxidectin, recently entered the monthly heartworm prevention marketplace in a tablet formulation. However, this product does not reach gastrointestinal nematodes of veterinary importance. The correlation of use of the monthly heartworm preventatives with the prevalence of gastrointestinal nematode parasites of dogs under veterinary attention has been studied in at least two surveys, one of which showed statistically significant reductions ($P < 0.05$) in the prevalence of *Ancylostoma caninum* and *Trichuris vulpis*. The reduction in *T. vulpis*, however, was apparent only when the monthly preventatives were administered year round. The situation in cats is different since only two monthly heartworm preventives (ivermectin and selamectin) are commercially available for cats and their use is still limited in spite of the high medical importance of *D. immitis* in cats. Although both formulations are also effective against some species of gastrointestinal nematodes of cats, their effect on the general prevalence of these parasites is probably negligible, in view of the limited use of heartworm preventives in cats.

Cryptosporidiosis

GENOTYPIC CHARACTERISATION OF CRYPTOSPORIDIUM: IMPACT ON MOLECULAR EPIDEMIOLOGY AND TAXONOMY.

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Considerable genetic variability has been demonstrated between isolates of *Cryptosporidium parvum* from various hosts. This has been achieved using numerous loci of which the 18S and ITS regions of rDNA, HSP, COWP, AcetylCo A synthase and B-Tubulin genes, have proved to be the most useful. Several novel genotypes have been identified which appear to be genetically conserved and geographically widespread. Although genotypic variation is often not reflected in morphological differences, molecular epidemiological investigations indicate that most of the newly identified genotypes are probably host-adapted and may represent distinct species, thus supporting the need for a taxonomic revision of the genus *Cryptosporidium*. A formal nomenclature is an essential pre-requisite for effective communication in studies on the epidemiology of cryptosporidiosis.

PCR-based procedures have been developed for the genotypic characterisation of *Cryptosporidium* isolates using oocysts recovered from environmental and clinical sources. This will provide information on the source(s) of infection in outbreak situations and the public health significance of contamination events. Molecular epidemiological tools are also being developed which are capable of detecting genetic diversity at different levels of specificity. Such highly variable genetic markers are needed to discriminate between "clones" of the major genotypes/species so that the transmission dynamics of *Cryptosporidium* can be studied in endemic foci such as day care centres, farms and in outbreak situations, as well as providing markers for phenotypic differences such as virulence.

IDENTIFICATION OF CRYPTOSPORIDIUM PARVUM USING MICROSATELLITE SEQUENCE ANALYSIS

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Background: It has been recently shown that isolates of the parasite *Cryptosporidium parvum* that belong to the human (H, or anthroponotic) and animal (C, or zoonotic) genotypes, can be further distinguished into several subgenotypes using highly polymorphic microsatellite sequences as molecular markers.

Methods: PCR amplification of two loci containing microsatellites was performed on a large number of human isolates of *Cryptosporidium parvum*, which represents both sporadic and outbreak cases, and on isolates collected from livestock animals. PCR products were directly sequenced on both strands and the sequences were aligned for comparison.

Results: The sequence analysis revealed the presence of several alleles at the investigated loci among isolates typed as genotype C (zoonotic), whereas less variability was observed among isolates typed as genotype H (anthroponotic). These alleles differ by expansions/contractions of the microsatellite repeats, which generate length polymorphisms.

Conclusion: The data obtained so far indicate that certain subgenotypes are either host-specific or are geographically restricted. With the use of microsatellites as markers, the source of infection of cryptosporidiosis can be traced back by comparing fingerprints of oocysts shed by infected individuals with those present in the suspected source(s) of infection (water, food, animals or humans). Moreover, the availability of these markers will allow the structure of the parasite's population to be critically evaluated, and it will be possible to confirm or reject the hypothesis of clonality, as discussed for other protozoan parasites.

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THE MOLECULAR EPIDEMIOLOGY OF HUMAN CRYPTOSPORIDIOSIS

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Background: The coccidian parasite *Cryptosporidium* is increasingly recognised as a major cause of diarrhoeal disease world-wide. However, the exact modes of transmission, and the role of infected animals are often unclear. Recent advances in the application of molecular methods for analysis of *Cryptosporidium* have allowed more rational approaches to the identification of different species together with changing the understanding of the epidemiology of this group of organisms.

Methods: Simple methods were devised for the extraction of cryptosporidial DNA from whole faeces. Cryptosporidial gene fragments amplified by PCR were characterised by PCR, PCR-RFLP and direct sequencing from >2000 samples from human and other animals.

Results: *Cryptosporidium parvum* was identified as the major cause of cryptosporidiosis in humans, however, *C. meleagridis*, *C. felis*, and an as yet un-named species (designated the 'dog-type') were recovered from both immunocompetent and immunocompromised patients. Analysis of multiple loci indicated that *C. parvum* comprises two genotypes: genotype 1 is exclusive to humans, and genotype 2 occurs in humans, livestock animals plus other hosts. Both genotypes 1 and 2 were involved with waterborne outbreaks. Amongst sporadic cases: patients reporting contact with animals and/or farms were almost all infected by genotype 2; the spring peak in cases was almost exclusively due to genotype 2; genotype 1 was significantly more common in patients infected during the late-summer/autumn peak and in those with a history of foreign travel; there were distinct geographical variations in the distribution of the genotypes.

Conclusions: Further insights into the epidemiology of cryptosporidiosis will be obtained by the application of genetic analysis to *Cryptosporidium*.

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Third WAAVP Workshop on the Systematics of the Cyathostominae of Horses

CYATHOSTOMES EXPELLED AFTER DEWORMING OF HORSES IN SWEDEN

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Objective: A survey was conducted in Sweden to investigate the species composition of cyathostomes expelled in the faeces of horses after deworming using 3 different anthelmintic preparations.

Methods: 27 horses excreting =200 strongyle EPG were divided into 3 groups and dewormed with either 0.2 mg ivermectin, 19 mg pyrantel pamoate or 7.5 mg fenbendazole, per kg body weight. For each of the 3 days following anthelmintic treatment all faeces produced from each horse was collected, and subsamples were examined for strongyle nematodes. 75 ft 258 worms per horse were identified to the species level.

Results: The majority of the worms recovered were expelled during the first day from horses treated with ivermectin or pyrantel pamoate, and during the second day from horses treated with fenbendazole. 15 strongyle species were identified and the 5 most prevalent were *Cylicostephanus longibursatus*, *Cylicocyclus nassatus*, *Cyathostomum catinatum*, *Cylicocyclus leptostomus*, and *Cylicostephanus minutus*. These species composed 83% of the total burden of cyathostomes. The number of species found per horse ranged from 6 to 13, with an average of 9. No significant differences in species composition or distribution were found between the treatment groups.

Conclusion: This study showed that it was possible to identify small strongyles recovered from the faeces of dewormed horses, and that the most prevalent species corresponded to those found in autopsy surveys performed in other countries.

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ECOLOGICAL ANALYSIS OF STRONGYLIDS NEMATODES OF COLON DORSAL OF EQUINES

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The interspecific associations of strongylids nematodes in dorsal colon of 33 equines from Metropolitan Rio de Janeiro were analyzed. The study was conducted at W.O. Neitz Center Research at the Federal Rural University in Rio de Janeiro, Brasil. Each compartment of large intestine were isolated and ten per cent of nematodes of dorsal colon were collected, recovered, clarified and identified. The Chi-Square (χ^2) test and correlation coefficient of Spearman (r_s) were used to ecological analysis. Twenty three species belonging to *Cylicostephanus*, *Cylicocyclus*, *Cyathostomum*, *Cylicodontophorus*, *Parapoteriostomum*, *Coronocyclus*, *Gyaloecephalus*, *Triodontophorus*, *Strongylus* genera were recovered. The most prevalent and abundant species were *Cylicostephanus longibursatus* (90.9%; 1,078) and *Cylicostephanus goldi* (81.8%; 236) respectively. One hundred and seventy and one species pairs were analyzed, but only 33 pairs were significant to r_s , they presented negative correlation, there was competition, then their abundance were inversely proportional and seven species pairs were present significance to both tests (χ^2 , r_s) showing a dependence in spite of the competition. It can be inferred that strongylids nematodes species of equine show different distribution into compartments of large intestine based on prevalence, physiologic conditions of hosts and associations of species on multiple infections that favour the establishment of ecological niche.

Keywords: Ecological analysis, Strongylidae, equines, Brasil.

SPECIES-SPECIFIC DNA PROBES FOR IDENTIFYING CYATHOSTOMINS

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Cyathostomins are important intestinal nematodes of horses. There are 51 different species in this group and only the parasitic adult stages can be identified. The association of an individual species with pathogenicity or resistance is not known, hence it is important to study these parasites at a single species level. Oligoprobes were designed from intergenic spacer (IGS) sequences for identification of 4 species, *Cylicocyclus ashworthi*, *C. nassatus*, *Cylicostephanus longibursatus*, *C. goldi*, and a fifth probe was designed to identify all members of this tribe. Southern blotting was used to validate the specificity of each probe. Sixteen cyathostomin species were examined and all 4 probes were shown to be species-specific, whilst the fifth probe hybridised to all cyathostomin species tested but not to members of the related genus *Strongylus*. Furthermore, these probes were used to identify individual non-parasitic stages, such as infective L3, L4 and eggs. These probes were adapted to a PCR-ELISA format, to allow high throughput analysis of the different cyathostomin stages and have been used to identify different stages from various equine populations. To investigate the role of these species in pathogenicity and anthelmintic resistance, these probes were used to identify species of eggs from faecal samples and larvae from diarrhoea and pasture samples.

Nutrition and parasites: feasibility of dietary manipulation for parasite control

NEMATODE CONTROL IN PERIPARTURIENT SHEEP THROUGH PROTEIN SUPPLEMENTATION

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Background: Periparturient breakdown of immunity to parasites (BIP) coincides with times of a high protein demand for reproductive functions (pregnancy and lactation). When protein supply does not meet this high protein demand, i.e. protein becomes scarce, reproductive function but also immune functions are expected to be penalised. It has been suggested that periparturient BIP results from allocation of relatively more scarce protein to prioritised reproductive functions, rather than to immune functions.

Current situation: An increasing body of evidence is in agreement with this nutritional basis for periparturient BIP, and supports the view that lowering the degree of protein scarcity reduces the extent of periparturient BIP in sheep. Single-rearing ewes have a relatively low protein demand, which reduces the likelihood of protein scarcity, and consequently, show a lower degree of periparturient BIP than twin-rearing ewes. For the latter, dietary protein supplementation can overcome protein scarcity and has repeatedly resulted in a reduced extent of periparturient BIP (decreased worm burdens and faecal egg count). Such a reduction was accompanied with an increased number of globule leukocytes in the gastrointestinal mucosa. Labile body protein (body protein reserves) can also act as a source of protein to overcome protein scarcity and thus reduce periparturient BIP.

Future research: Elucidating the temporal changes of local humoral and cellular immune responses over the periparturient period may help to identify which underlying immune mechanism(s) during BIP are most sensitive to protein scarcity. Such information is much needed to establish whether reduced periparturient BIP through protein supplementation is due to changes in rate of establishment of incoming larvae, number of arrested larvae, adult nematode expulsion and/or nematode fecundity.

Tanniferous forages: a New Zealand perspective.

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Grazing livestock on forages which contain condensed tannins can result in significant increases in animal performance. Such forages also appear capable of at least partially ameliorating the impacts of gastrointestinal parasites. Lambs grazed on these forages often acquire fewer dags and subsequently suffer less from flystrike. With some forages reduced gastrointestinal worm burdens and faecal egg outputs have been noted. It remains unclear, however, whether this is due to direct anthelmintic effects or whether the amelioration of disease is an indirect product of increased immunity through improved nutrition or to physiological changes in the intestine. Despite their potential advantages use of tanniferous forages is not widespread, primarily due to their higher establishment and maintenance costs. However, tanniferous forages could still play a significant role in livestock production, particularly under reduced chemical inputs. Some farmers currently use such forages as 'hospital' blocks for sick or undersize stock. Other uses have included the production of winter lambs. Weaning lambs early on to forage may not only improve growth and protect against parasites but also facilitate an early reduction in stock density and hence overall pasture contamination, leading to improved herd health.

OPTIONS FOR PARASITE CONTROL USING TANNIFEROUS FORAGES IN LIVESTOCK IN NORTHERN TEMPERATE AREAS

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Leguminous forages with a relatively high level of condensed tannins (CT)(4-8 % of DM) fed to sheep may lead to lower faecal nematode egg counts and worm burdens. Scottish studies using a CT, quebracho in the diet have confirmed this effect in *Trichostrongylus colubriformis* infections, and Danish *in vitro* studies have indicated a direct effect of purified CTs on preparasitic stages of ovine nematodes. The legumes greater trefoil (*Lotus pedunculatus*), sulla (*Hedysarum coronarium*), sainfoin (*Onobrychis viciifolia*) and birdsfoot trefoil (*L. corniculatus*) are presently of major interest. In Denmark, nematode infected lambs grazing pure stands of sulla and sainfoin for 5 weeks have shown up to 50% reductions in egg counts compared to grazing clover. A Swedish study in lambs found no effect on worm counts of 10% birdsfoot trefoil in the diet. Preliminary results have thus been promising but the following problems have to be addressed for a future, successful application of tanniferous forages in nematode control in Northern temperate areas:

1. Species better suited locally should be found. Sainfoin is not competitive in leys, and weeds dominate, particularly in organic farming without herbicidal use. Sulla, a Mediterranean plant, do not overwinter.
2. Efficacy relatively low. Knowledge of the mechanism of action may indicate ways of improving the efficacy. Selection of appropriate cultivars or growing conditions is needed.
3. The activity in cattle and goats needs to be investigated.
4. The forages need to be integrated in other systems of control, most likely repeated moves or biological control. The implementation is governed by the seasonality of the forages and the epidemiology of the infections.

UREA-MOLASSES BLOCKS FOR NEMATODE PARASITE CONTROL

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Background: Supplementation with high quality protein has been shown to enhance the ability of sheep to resist infection with gastrointestinal nematode parasites. Economic restrictions imposed by the high cost of protein feeds for livestock limit the application of this knowledge in most sheep rearing systems. Recent research has indicated that provision of non-protein nitrogen, to stimulate feed intake and increase rumen outflow of microbial protein, can also enhance the ability of infected hosts to overcome the detrimental effects of nematode parasitism. In situations where low quality forages are the predominant ruminant feed resource urea-molasses blocks (UMB) can provide a ready means of delivering non-protein nitrogen, energy and minerals to promote efficient rumen function. **Results:** Field application of UMB for small ruminants have resulted in variable responses in production and nematode parasite control. In Fiji, provision of UMB to young Fiji ewes, halved the requirement for anthelmintic treatment based on faecal egg count and doubled the number of lambs born and weaned. In Indonesia, provision of UMB to young Javanese Thin Tail sheep resulted in improved liveweight gains during supplementation but had no effect on nematode parasites as indicated by faecal egg count.

Conclusion: These results emphasise the need to tailor supplementation strategies to suit local environmental conditions. In particular, it is critical that animals have access to suitable quantities of fermentable carbohydrate to maximise the potential for UMB to influence the productivity and ability to resist infection with nematode parasites.

NUTRITION – PARASITE INTERACTIONS IN GOATS

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Parasitism of the gastrointestinal tract with nematodes is a major pathological threat in goats as in sheep. Moreover, the need for alternative control solutions in goats is urgent due to the high prevalence of anthelmintic resistance. Nevertheless, very few studies are dedicated to interactions between nutrition and parasitism in goats compared to sheep. The need for direct information is also supported by host specificities in physiology, metabolism or immune response.

Recent results in dairy goats or Criollo growing kids have shown that protein supplementation was generally associated with beneficial effects on resilience whereas an improvement of resistance was more inconstant. Both effects were usually more obvious in animals with high nutritional stress, related to high level of production or to insufficient food quality. Studies on interactions between nutrition and genotype selection did not show any effect of supplementation on expression of immunity in selected lines of Cashmere goats whereas divergent results were found with meat producing goats. Last, a few results from natural or experimental infections suggest that plants rich in condensed tannins represent also an alternative for the control of nematodes in goats. However, several questions remain to be addressed. Basic studies have to state precisely the impact of nutrition on the various processes related to expression of immunity. Possible interactions between energy and protein diet contents should also be examined. For field development, it seems essential to determine which source of protein to use and when to use it, particularly in tropical conditions. Last, due to their ability to exploit rangelands, specific studies on use of tannins in goats offers also an attractive field for future researches. Overall, the results underline the value of goat as a model for nutritional studies and the interest to compare data in the 2 small ruminant species.

Myiasis

SEROPREVALENCE OF *OESTRUS OVIS* INFECTIONS IN SHEEP IN SOUTHWEST GERMANY

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Background: Knowledge of ovine oestrosis in Germany is very scanty. The aim of this study was to determine the seroprevalence of *Oestrus ovis* infections in flocks in southwest Germany where approximately 7200 flocks with 285,000 sheep existed in 1997 according to the official veterinary census.

Methods: Serum samples were collected from a total of 1497 sheep (>6 months of age; 0.5% of all) from 110 flocks (1.5%) in 1997 and 1998. Sera were examined for antibodies to *Oestrus ovis* antigen using an ELISA technique described by Dorchies et al. (1999). Data on farm management were collected by a questionnaire.

Results: Overall, the estimated seroprevalence of *Oestrus* infection was 50% (95%-CI: 48–53%) in sheep and 76% (68–85%) in flocks. The seroprevalence was significantly higher in sheep from large (>50 ewes) and medium flocks (11–50 ewes) than in those from small flocks (≤10 ewes). There was no significant difference in the seroprevalence between the grazing systems used (paddocking versus herding or transhumance).

Conclusion: The *Oestrus ovis* infection is widespread in sheep in southwest Germany. Further studies seem to be necessary to estimate the economic importance of ovine oestrosis and the necessity of control measurements in this country.

ONE CASE OF EQUINE MYIASIS CAUSED BY RHINOESTRUS USBEKISTANICUS

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Background. *Rhinestrus usbekistanicus* is an insect of family Oestridae normally distributed in Africa and in the central part of Asia, whose larvae are parasitic of equine nasal cavity. The authors described one case of parasitosis occurring in a horse living in Sicily.

Method. A female, native breed, 14 months old, horse was slaughtered for sudden neurological symptoms.

Macroscopically numerous larvae of first (L1), second (L2) and third (L3) stage were observed. Nasal cavity, ethmoid labyrinth, frontal sinuses and brain were collected for histopathological examinations. The tissues were paraffin-embedded and cut to obtain 5 µm sections stained with haematoxylin and eosin.

Results. Macroscopically 65 larvae, 6 L1, 23 L2, and 36 L3 were observed into the nasal cavity and the ethmoid labyrinth. Purulent exudate was present into these areas. Histopathological examination revealed a diffuse, intense, non suppurative infiltration into mucosal lamina propria of nasal cavity and necrosis of epithelium. Severe suppurative effusion of polymorphonuclear cells and necrotic debris were observed into the ethmoid labyrinth. Cerebral involvement was not present.

Conclusion. The origin of this infection is not identified. Epidemiological studies have to be done to evaluate the prevalence of pathology in Sicily.

Acknowledgement: for technical collaboration Mrs Rubino G. and Mr. Failla G.

DEVELOPMENT OF AN ANTIGEN CAPTURE ELISA FOR DIAGNOSIS OF HYPODERMOSIS

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Antibody detection ELISA for hypodermosis does not give an accurate indication of the status of the infestation because antibodies persist in animals that have no active larvae (i.e. older animals with previous exposure and in animals treated with parasiticides). An ELISA for diagnosis of active infestations would be useful for developing more accurate picture of infestations in all age classes of cattle. Migrating first instar cattle grubs secrete large quantities of a specific serine proteinase, hypodermin C. Secretion appears to cease as the larvae molt the second instar. There is thus an opportunity to develop a diagnostic tool that detects the presence of this abundant enzyme during the time when the larvae are migrating in the host. Briefly, the antigen capture ELISA was carried out by sensitizing microtitre plates with a monoclonal antibody raised against a recombinant hypodermin C. Subsequently the plates were blocked with BSA and test sera were added. Plates were then treated with a second antibody (purified polyclonal bovine anti-Hypoderma IgG). Subsequently, conjugate and substrate were added and colour development measured. Preliminary assessment was carried out using known negative sera spiked with either purified, native hypodermin C or with recombinant hypodermin C. Serial dilution of spiked sera gave an extinction curve showing the lowest level of detectability was approximately 0.03 µg/ml. Evaluation using sera from known negative animals and animals with confirmed infestations indicated a sensitivity of 96.0% and a specificity of 93.4%. Dynamics of detectable circulating HyC was determined in cattle artificially infested with *Hypoderma lineatum*. Antigen was detectable 6 weeks post-infestation and generally increased until weeks 21–30 p.i. Little antigen was detectable by 39–43 weeks post-infestation when grubs were beginning to appear on the back.

MOLECULAR METHODS FOR THE STUDY OF MYIASIS CAUSING DIPTERA: OESTRIDAE

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In the last 30 years several aspects (i.e. taxonomy, immunology, control methods) of myiasis causing larvae belonging to the Family Oestridae have been focused on and their economic impact on animal productions has been recognised. Despite scientific interest in this topic over the past decade, very few molecular studies have been performed on myiasis causing Oestridae compared to the Calliphoridae and Sarcophagidae families and they have mainly regarded the following areas:

Molecular Differentiation. A PCR-RFLP based technique has been developed on the subunit I of the cytochrome oxidase (COI) gene of *Gasterophilus* spp., *Hypoderma bovis*, *H. lineatum*, *Oestrus ovis* and *Przhevalskiana silemsis*. This assay is a first step to overcoming constraints imposed by damaged or badly stored samples, lack of operator skills and variation within species of larvae collected from different animals and countries.

Diagnosis. Hypodermin C, produced by first instar larvae of *Hypoderma* spp., which shares epitopes of many Hypodermatinae larvae, has been characterized, sequenced and expressed. These results will make it possible to produce a recombinant antigen to prepare a sero-diagnosis of the most common Oestridae myiasis causing larvae.

Vaccination. The biochemical characterization of hypodermins (A and B) and the isolation of cDNAs encoding entire translational products, opens a new era for the preparation of recombinant vaccines effective against *Hypoderma* spp. infestation.

Phylogenetic Analysis. The COI gene of Oestridae larvae coming from many European and African countries has been characterized to highlight the inter-specific relationship within this taxon.

MODEL SIMULATIONS OF THE EFFECT OF HUSBANDRY PRACTICES ON THE INCIDENCE OF SHEEP BLOWFLY STRIKE

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Background : Ovine cutaneous myiasis is a significant economic and welfare problem in many areas of the world.

Method: The effects of farm management strategies on the incidence of ovine cutaneous myiasis are considered using comprehensive simulation models, which have been developed to simulate the seasonal incidence of sheep myiasis on farms in Britain.

Results: The simulations show that familiar farm management strategies, such as shearing, lambing date, reduction in scouring caused by endoparasitic worms and the timing and type of insecticide used can have an important role in the reduction of strike incidence in ewes and lambs. However, the effectiveness of these techniques can be highly dependent of their timing in relation to seasonal changes in blowfly abundance and the effects of temperature and rainfall.

Conclusions: The models developed provide a valuable tool in the development of improved animal management strategies for sheep blowfly strike control.

Acknowledgements: we wish to thank NERC and the Bristol Enterprise Centre for financial support.

MOLECULAR METHODS FOR THE STUDY OF MYIASIS CAUSING DIPTERA: CALLIPHORIDAE J.R. Stevens

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Abstract: The evolutionary origins of the myiasis habit in the Calliphoridae, a family which includes the screwworm flies and numerous blowflies, remains unclear. Species associated with an ectoparasitic lifestyle can be divided generally into three groups based on their larval feeding habits: (1) saprophages, normally living in decaying matter, which may secondarily invade existing infestations; (2) facultative ectoparasites, which can live as saprophages or can initiate myiasis and live as ectoparasites; and (3) primary obligate parasites, which feed only on the tissues of living hosts. It has been proposed that this functional division may reflect the progressive evolution of parasitism in the Calliphoridae. In support of this proposed evolutionary progression, species displaying a range of dependencies on ectoparasitism can be identified within several Old World calliphorid genera, e.g. *Calliphora*, *Chrysomya* and *Lucilia*. Sequence data from genes with differing, but complementary levels of resolution (e.g. mitochondrial cytochrome oxidase I + II, nuclear 28S large subunit ribosomal RNA gene and the ribosomal ITS spacer regions) have been analysed, permitting comparison of gene phylogenies. Subsequently, comparative analysis of life history characters, including the ectoparasitic status of species, has allowed new insights into the evolution of myiasis in the Calliphoridae.

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MAGGOTS IDENTIFICATION BY MEANS OF CONVENTIONAL AND MODERN TECHNIQUES Sotiraki, S.T.¹, Hall, M.J.R.², Adams, Z.J.O.², Himonas, C.A.¹

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Background: Myiasis, which has a grate impact as an infestation of animals, is caused by dipterous larvae, which, at least for a certain period, feed on the host's living or dead tissue, liquid body substances, or ingested food. There are usually three larval stages in those flies involved in myiasis and in most of the cases the first two stages are very difficult to identify to species except by association with mature larvae or subsequently reared adults. Two of the most important genera regarding myiasis in the Sarcophagidae family are *Wohlfahrtia* and *Sarcophaga*, which are virtually indistinguishable in the larval stages, and consequently extremely difficult to identify to species.

Method: In this paper a number of structures of taxonomic importance are presented using examples of the three larval stages from various species of *Wohlfahrtia* and *Sarcophaga* by means of standard light microscopy, scanning electron microscopy (using a Hitachi S2500 SEM at 15kV) and auto-montage (using a microscope fitted with a digital video camera connected to a PC loaded with Synoptics Auto-Montage software) techniques. The species that were studied were *Wohlfahrtia magnifica*, *W. pattoni*, *W. vigil*, *Sarcophaga argyrostoma* and *S. ruficornis*.

Results: The new techniques used were found to be more effective in demonstrating surface structures of the larvae than the conventional ones. Differences in the mouth hooks, the anterior and posterior spiracles, the modified spines on the head and on the posterior face of the terminal segment, and the body spinulation between larval species were noticed.

Conclusion: Both of the above techniques used seem to be quite promising and having the ability to serve well taxonomy in the field of entomology.

OESTRUS OVIS (DIPTERA : OESTRIDAE) : EXCRETORY-SECRETORY SERINE PROTEASES AND ASSOCIATED MUCOSAL IMMUNE RESPONSE

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Oestrosis is a common myiasis of sheep and goats nasal and sinus cavities caused by *Oestrus ovis*. The aims of this work were to study the biochemical and immune mechanisms involved in the host-parasite relationships and to identify key molecules of larval biological functions which could represent interesting targets for a vaccinal approach.

Trypsin-like serine proteases of digestive origin were identified in excretory-secretory products of each *O. ovis* larval stage. These proteases were able to degrade mucous and plasmatic components suggesting that they are involved in larval nutrition and immune evasion. The homeostasy of the nasal epithelium is disturbed by the release of such molecules resulting in strong inflammatory and regenerative processes of mucosae. Presence of larvae was associated with an important local cellular recruitment and local IgA and IgG production. Inflammation also induces plasmatic protein leakage.

Thus datas obtained in this study suggested that lesions and symptoms observed in sheep oestrosis are the result of an immunopathological phenomenon modulated by larval excretory-secretory products.

Psoroptic mange

THE BIOLOGY OF *PSOROPTES OVIS* AND THE NATURE OF SUB-CLINICAL OVINE PSOROPTIC MANGE (SHEEP SCAB).

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Background: *Psoroptes ovis* is the cause of a debilitating allergic dermatitis resulting in wool loss, intense irritation and scab formation (psoroptic mange/sheep scab). Early lesions are undetectable and a "sub-clinical" phase (characterised low mite numbers and small lesions) can last for a matter of days, weeks, months or even years before the lesion progresses into the active phase, eventually covering the whole of the sheep. This paper investigates the nature of sub-clinical disease and the factors that can extend its duration.

Method: A number of laboratory or field studies were carried out to investigate the potential factors that could influence the duration of the sub-clinical phase: eg the influence of the host (eg: sheep breed, individual susceptibility to infestation, the site of challenge or acquired resistance to previous scab exposure), the influence of the parasite (eg: the virulence of the mite population or the size of the infestive dose) or the effects of previous chemotherapeutic agents (eg. ineffective acaricidal treatments or the use anthelmintic drenches).

Results: Results demonstrated that sheep presenting sub-clinical disease can look perfectly healthy and can unknowingly be introduced to a flock and thus be responsible for the spread of infestations within flocks and between properties. The duration of the sub-clinical phase was found to be extended through a number of host modulated factors (open fleeced breeds of sheep, low individual susceptibility to infestation, natural challenge on body areas less susceptible to colonisation and on sheep previously exposed to infestation), parasite modulated factors (eg: mite populations of low virulence or small number of challenging mites) or factors modulated by previous chemotherapeutic agents (eg. the use of synthetic pyrethroid pour-ons or endectocides administered orally as anthelmintic drenches).

CHANGES OF BEHAVIOUR IN SHEEP AFFECTED BY PSOROPTIC MANGE

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Purpose: Behavioral changes are an important clinical feature of psoroptic mange of sheep. This study describes and quantifies the effect of psoroptic mange on the behaviour of sheep.

Method: The behaviour of a group of 10 housed sheep was recorded using focal and scan sampling techniques. The animals were studied before infestation and weekly for 8 weeks following experimental infestation with *Psoroptes ovis* mites. The development of the mange lesions was recorded.

Results: The duration and frequency of rubbing and scratching increased throughout the infestation. Mouthing associated with rubbing and scratching behaviours, and mouthing alone, was seen increasingly throughout the infestation. There was a positive correlation between time spent rubbing and the area of the mange lesions. The duration of cud chewing declined throughout the study, possibly due to adaptation to a new diet introduced at housing, one week before the study started.

Conclusions: It is suggested that mouthing is a stereotypy, induced by animals' attempts to cope with the disease. The increases in the self traumatising behaviours, rubbing and scratching, together with the mouthing stereotypies are important diagnostic features of this disease and indicate the poor welfare of sheep with psoroptic mange. Increased rubbing as the lesions progress is important in the spread of the disease.

Acknowledgement: This work was funded by MAFF and Pfizer Ltd..

IMMUNOHISTOPATHOLOGY OF THE SHEEP SCAB LESION

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Background: The mite *Psoroptes ovis* is an obligate, non-burrowing ectoparasite, which causes sheep scab. This cutaneous disease is characterised by pruritis and scab formation, and is of considerable economic and welfare importance.

Method: Immunohistopathological analysis of the scab lesion has been monitored through the course of infection with 25 ovigerous mites. Biopsy tissue, taken at the outer perimeter of the advancing scab lesion, were analysed histo- and immuno-histologically for inflammatory cells, and for cells of the immune system including B and T cell subsets.

Results: The most striking finding was the presence of a pronounced eosinophilia, as early as 24 hours following infection. Many of these cells appeared to migrate transdermally, accumulating in sub-corneal eosinophilic pustules and together with mast cells formed the main cell constituent of the scab. A pronounced increase in CD45R (mature B cells, naïve T cells), CD 4⁺ (T helper) cells and CD 21⁺ (dendritic) cells occurred during infection, with little or no increase in CD 8⁺ (T cytotoxic) cells.

Conclusion: The immunopathology of the scab lesion has the characteristics of a profound hypersensitivity reaction, involving the rapid influx of eosinophils and mastocytosis. The scab lesion appears not to involve a significant increase in CD 8⁺ cytotoxic cells, indicating that immunity is through an antibody mediated mechanism.

SEROEPIDEMIOLOGICAL SURVEY OF PSOROPTIC SHEEP SCAB IN SWITZERLAND

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Sheep scab caused by the astigmatid mite *Psoroptes ovis* is notifiable in Switzerland and compulsory prophylactic treatments were carried out in spring only for sheep going to alpine pastures. Despite these compulsory control measures the number of outbreaks has been increasing in recent years, raising the question of how this epizootic disease is spreading. To improve the traditional microscopical diagnosis we developed an ELISA for the specific determination of antibodies against psoroptic antigen. The diagnostic sensitivity and specificity were 93.7% and 96.5%, respectively, as evaluated with experimentally and naturally infested sheep. With this test we screened a serum bank which is representative for the sheep population in Switzerland (approximately 15% of registered sheep flocks randomly chosen, collected between February and May 1998). The prevalence using 16'439 sera was 10.3%, and in 13.2% of the 2'508 flocks we found seropositive sheep. Spatial analyses of the results confirmed known epizootic foci and revealed regions with high prevalences where no cases have officially been notified for years. Additionally, a significant correlation of scab and flock size was found. The prevalence is increasing from 11.3% to 22.7% and 30.3% in flocks with 4-10, 11-30 or more than 30 adult sheep, respectively. Large flocks seem to be an important high risk factor for sheep scab which probably is caused by more frequent animal movement. Our next goal is to establish a more efficient control strategy based on serological monitoring of the total sheep population.

EVALUATION OF THE PERSISTENT ACTIVITY
OF INJECTABLE ENDECTOCIDES
AGAINST *PSOROPTES OVIS*

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Background: This study compared the persistence of activity of three injectable endectocides based on ivermectin, doramectin or moxidectin against *P. ovis* in sheep.

Method: Forty-one sheep were allocated randomly to four groups: two groups of 15 were treated with a single injection of either moxidectin (0.2mg/kg, subcutaneously) or doramectin (0.3mg/kg, intramuscularly); one group of 5 with a single injection of ivermectin (0.2mg/kg, subcutaneously); and the last group of 6 acted as untreated controls. At various intervals after injection (day 17 for ivermectin (IVER-17) and on days 17, 22 and 29 for moxidectin and doramectin (DOR-17, DOR-22, DOR-29, MOX-17, MOX-22, MOX-29) groups of 5 sheep from each treatment group were challenged with *P. ovis* mites and housed in close confinement for 24 hours with two infested seeder sheep. Two controls were likewise challenged on days 17, 22 and 29. The sheep were monitored for approximately six months.

Results: All the controls developed mange, as did the IVER-17 group, by three weeks after challenge. Live mites were observed in all DOR groups: in DOR-17 on day 106, in DOR-22 on day 63 and in DOR-29 on day 70. No live mites were detected in any of the MOX groups after challenge.

Conclusion: Moxidectin has a persistent activity of at least 29 days against *P. ovis* compared to <17 days for ivermectin and doramectin.

FUNGAL PATHOGENS AS NOVEL CONTROL
AGENTS FOR PSOROPTES MITES

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Background: mange caused by species of the genus *Psoroptes*, results in significant economic and animal welfare problems in many areas of the world.

Method: the effects of fungal pathogens on *Psoroptes* mites were considered in laboratory bioassays, with a view to evaluating their use as biocontrol agents.

Results: the fungus *Metarhizium anisopliae* (Metschnikoff), showed a high level of pathogenicity against mites; in bioassays three days after exposure to fungal conidia all mites were dead and six days after exposure 60% of the dead adult females, 10% of the adult males and 30% of the female nymphs had fungal hyphae protruding from their cuticular surface. There was a significant effect of conidial concentration on the number of infected mite cadavers.

Conclusions: entomopathogenic fungi show good promise as potential novel control agents for parasitic mites. On the host, the fungus could proliferate in the cadavers of dead infected mites forming sources of conidia which could infect uninfected mites. Infected, not-yet dead mites may also disperse over the surface of the host, carrying the source of infection to all parts and again liberating conidia after death. Infected mites may be transferred from sheep to sheep carrying the pathogen.

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TOOLS FOR SURVEILLANCE:
MICROSATELLITE MARKERS FOR SHEEP SCAB
MITE, *Psoroptes* sp.

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Abstract: One of the most economically important members of the astigmatid mites (Acari) is *Psoroptes* sp. This group of mites is most well known for causing a severe skin disease in sheep, known as scab. The taxonomic status of this genus has been called into question continually since its discovery in the 19th century, and it has yet to be resolved. A number of morphological studies and, more recently, sequencing analyses have found little variation between putative species. Hence, a novel approach, using a more sensitive marker system, has been attempted to clarify the species relationships within the Psoroptidae. A set of microsatellite markers has been isolated, characterized and used to assess DNA variation between populations of mites from different hosts and geographical locations. This data, combined with DNA sequencing analyses, will provide a more thorough understanding of mite species relationships. This research will contribute to a better understanding of astigmatid mite systematics and epidemiology, leading to improved surveillance, treatment and control of sheep scab.

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Pets, parasites and changing rules for travel

An introduction to the risks of importation of parasitic diseases in dogs and cats

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One of the outcomes of a UK Advisory Group report on Quarantine for dogs and cats was an identified need to review the potential risks from the importation of pathogenic organisms in the wake of the proposed changes to the UK quarantine regulations. As a consequence, specific recommendations were made for the control of alveolar echinococcosis (*Echinococcus multilocularis*) and tick infestations in dogs and cats as part of the pilot Pet Travel Scheme (PETS) introduced in February 2000.

Subsequent to the review, further consideration has been given to other infectious and parasitic diseases of cats and dogs, and their potential to become established in the British Isles if imported with these animals. The PETS scheme has now been widened to include rabies-free islands, as defined by OIE, effective from April 2001.

Dogs and cats are host to a whole range of disease pathogens found throughout the world. These include a number of protozoan, nematode, cestode, trematode infections as well as arthropod-transmitted diseases. Many of these are not considered to pose a risk or threat due to their diverse and complex life cycles. There remain several parasitic diseases, however, that may require the implementation of preventative control measures because of either a zoonotic risk, or because of welfare implications or disease threats to indigenous dogs and cats.

This introduction is intended to provide a forum for discussion on identifiable parasitic disease threats, and strategies for their diagnosis and control.

DIAGNOSTIC TESTS FOR PARASITES IN IMPORTED DOGS: A SOUTH AFRICAN PERSPECTIVE

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South Africa is in a fortunate position regarding some parasitic diseases of dogs in that *Babesia gibsoni*, *Trypanosoma evansi*, *Leishmania* spp. and *Dirofilaria immitis* do not occur in South Africa. In dogs designated for import, *B. gibsoni* is diagnosed with the use of the IFAT (indirect immunofluorescent antibody test) and a blood smear examination. Cross reactions with *B. canis* which is endemic in South Africa can be differentiated by cell culture and a second IFAT using *B. gibsoni* and *B. canis* in parallel. If the dog is found to be a carrier of *B. canis*, a sterilization treatment is recommended and the dog then discharged from the quarantine facility. *T. evansi* reactions are detected with the use of a CAT (card agglutination test). Positive dogs are treated with a trypanocidal drug and monitored for the presence of parasites or clinical signs before release. For the detection of *Leishmania* spp. a membrane test is used. Although the vector of *Leishmania* is not found in South Africa, reports of direct transmission and the fact that 2 dogs were euthanased in Pretoria due to *Leishmania* spp. infection gives grounds for concern. The diagnosis of *Dirofilaria immitis* is based on the demonstration of microfilariae in the peripheral blood using a membrane filtration technique and positive samples are then processed using an acid phosphatase staining. It is also vital to know the origin of the dog and other countries which may have been visited to indicate the possible source of infection. Screening of all imported dogs into South Africa has the advantage of stopping foreign diseases from entering the country and possibly becoming endemic.

PCR-BASED DIAGNOSTICS FOR ARTHROPOD-BORNE DISEASES IN COMPANION ANIMALS

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The increase in susceptible animals travelling to and from areas endemic for arthropod-borne microparasitic diseases, has necessitated the development of specific and sensitive diagnostic techniques applicable in both early and chronic stages of disease. Polymerase chain reaction analysis (PCR) fulfils these demands and is becoming more accessible and practical. It requires only small samples of blood, tissue or body fluid, does not require species-specific reagents and single samples can be used to screen for multiple agents. The Acarus laboratory has developed a rapid PCR-based diagnostic service for babesiosis, borreliosis, ehrlichiosis, bartonellosis and leishmaniosis in companion animals. The current method of choice is simple PCR targeting of genus- or species-specific regions of rRNA genes (*Ehrlichia* and *Babesia*), housekeeping genes (citrate synthetase and *gltA* in *Bartonella*), virulence factors (*ospA* in *Borrelia*) or multicopy sequences (*epank1* in *Ehrlichia phagocytophila* and kinetoplast sequences in *Leishmania*).

Between mid-December 2000 and May 2001, 103 blood samples from ill dogs, cats and horses in the UK were PCR tested. Eleven were from dogs with a history of travel outside the UK. Of 89 samples tested for *Ehrlichia*, 6 were positive for *E. phagocytophila* (6.7%) and 2 for *E. canis* (2.2%). Sixteen samples were tested for *Babesia* of which 3 were positive (19%) and of 72 tested for *Borrelia*, 7 (9.7%) were positive. Of the 66 samples tested for *Bartonella*, 2 (3%) were positive. One of 15 samples tested for *Leishmania*, one was positive and one co-infection with *Babesia* and *Leishmania* was identified.

PCR not only provides diagnostic support for arthropod-borne infectious diseases considered "exotic" in the UK, but its application has focussed attention on those that are already endemic.

Progress and problems of teaching of Veterinary Parasitology

THE UTRECHT MODEL OF TEACHING VETERINARY MEDICINE AND THE ROLE OF PARASITOLOGY

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Since 1995 a new curriculum for teaching Veterinary Medicine has been implemented at Utrecht University. Major changes to the previous curriculum imply that the new system is more problem oriented and that teaching is more interdisciplinary. Furthermore, the amount of contact hours has been reduced and students are trained to study for themselves and to be able to find relative information rather than learning it by heart. This implies that the number of oral lectures and practicals has been greatly reduced in favour of workgroups for groups of 25 students. The curriculum also has been made more variable by the inclusion of optional courses of 1-2 weeks. Another change is the inclusion of a research assignment of 3 months at the beginning of the fifth year.

The now final year students appear to know less, but are much better able to find relevant information than their predecessors. This new curriculum also implies a large reduction in contact hours for teaching Parasitology. Students can in fact graduate along the ruminant tract without ever having seen a trichostrongylid or a cattle lungworm. However, he/she will be able to give relevant recommendations on the impact of parasitic gastroenteritis and on parasite control.

Next year the curriculum will be changed again. This will include further emphasis on guided self study and on a further reduction of contact hours. Moreover, a further differentiation in 'study tracts' will be implemented. Specialization along the tracts of companion animals, horses, food animals, veterinary public health or research will start from the first year onwards. Since teaching programmes have to be developed yet it is difficult to guess what this will imply for teaching Parasitology.

THE TEACHING OF VETERINARY PARASITOLOGY: THE ITALIAN PERSPECTIVE

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The undergraduate curriculum in Veterinary Medicine in Italy is undergoing important changes, as in the rest of Europe. The 2001 fall semester will mark the beginning of a new format for the degree in Veterinary Medicine, following the guidelines and suggestions of the European Association of Establishment for Veterinary Education. Obviously, these changes will also affect the teaching of veterinary parasitology. In Italy, veterinary parasitology is currently taught in the second year with a disciplinary approach, similar to that described by Euzeby (1996) and Eckert (2000). 100 hours of lectures and 25 hours of laboratory are offered and are usually divided into Parasitology, followed by Parasitic Diseases. A more problem-oriented approach to parasitology is offered to fifth-year students within several professional routes (Large Animal Medicine, Small Animal Medicine, Meat Inspection, etc.). Indeed, in the last year of study, there are less students and it is possible to present clinical cases and orient the students towards team work and critical discussion. The new curriculum starting this year foresees for veterinary parasitology, a reduction in the number of lecture hours and an increase in both laboratory work and personal study, and in most faculties has been placed in the third year of study. Italian veterinary schools are trying to follow the current European trend of a problem-based approach to teaching (even if there are many colleagues who resist!). Unfortunately, there are several problems that will be extremely difficult to overcome in the near future and are likely similar to those in other countries: too many students and too few teachers; too little laboratory space; limited resources for computer-aided learning and personal study (full-time access to computers and to well-furnished libraries). There is also the risk that basic concepts will be less emphasized; one cannot, for example, understand or implement control strategies without a clear understanding of parasite life-cycles.

Prof. Euzeby, while remembering his own teaching experience, stated "...I realized that the more I said, the less students knew." I cannot believe, however, that the opposite would be true.

Wildlife and parasitism

HETEROLOGOUS TRANSMISSION WITH A NOVEL *DICTYOCAULUS* SPECIES FROM ROE DEER TO CATTLE

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Background: Because recent studies showed that a novel *Dictyocaulus* species occurs in Swedish roe deer and moose, this study was done to determine whether this lungworm species is infective to cattle or if it can induce seroconversion in cattle as measured with an ELISA for *D. viviparus* infection.

Method: Eight lungworm-free Swedish Red Breed Cattle, aged about 2 months old, were used in the study. Four calves were drenched with a Swedish isolate of *Dictyocaulus viviparus* (Dviv-Se) L3 from cattle. The other 4 calves were drenched with *Dictyocaulus* sp. L3 from roe deer. Two of the calves, which were given *Dictyocaulus* sp. L3, were challenged with Dviv-Se L3. All calves were monitored for larval excretion and seroconversion then later necropsied.

Results and Conclusion: All 4 calves that were given Dviv-Se L3 became patently infected and seropositive whereas the other 4 calves that were given roe deer *Dictyocaulus* L3 did not lead to patency, and were also seronegative, neither after challenged with Dviv-Se larvae. This result indicates that the roe *Dictyocaulus* sp. is not infective to cattle under the conditions of this study.

SURVIVAL STRATEGY OF BROWN HARE INTESTINAL COCCIDIA

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Background: The aim of the study is to highlight the survival strategy of intestinal coccidia (*Eimeria* spp.) in a high-density Brown Hare (*Lepus europaeus*) population (>100 heads/kmsq). In particular parasite distribution within population, seasonal pattern of oocysts emission (OE) and the relationship with host ecology were analysed.

Method: The population inhabited a 35 ha fenced area, located in the Po Valley (Northern Italy). From Aug. '93 to Jul. '94, 10 faecal specimens were collected fortnightly, and examined in order to obtain qualitative and quantitative data. Pooled positive faeces were used in order to sporulate the oocysts and then identifying the coccidia. Non-parametric statistical tests were applied. Since the total annual output is a mixture of the oocysts fortnightly distribution, a maximum likelihood estimation of grouped data was used in order to analyse OE emission by time.

Results: Three main peaks of OE emission were recognised; 3 grouped sub-peaks (Dec. 7th, Jan. 5th and Feb. 16th) composed the major one, while two minor peaks were observed respectively at the end of April and at the beginning of July. Oocysts from 3 *Eimeria* species were observed. Parasites fitted Poisson distribution when OE was examined fortnightly, while they fitted a Negative Binomial distribution when the entire year emission was analysed.

Conclusion: *Eimeria* survival strategy seems mainly linked to seasonal and environmental characteristics rather than to Brown Hare ecology.

ANTIBODIES TO NEOSPORA CANINUM IN CHAMOIS FROM A SUBALPINE AREA OF LOMBARDY, ITALY

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Background: Neosporosis is a parasitic disease that affect mostly cattle and dogs, with clinical signs of abortion and neuromuscular disease, respectively. Its aetiological agent, the protozoan *Neospora* (*N.*) *caninum*, can also infect wildlife // wild animals, as suggested by a few published reports of the occurrence of seropositivity, either in ruminants, either in canids. We investigated the seropositivity to *N. caninum* in a population of chamois (*Rupicapra rupicapra*).

Method: Sera (n=97) were examined for antibodies to *N. caninum* with an indirect fluorescent antibody test (IFAT), employing the NC-1 strain of the protozoan, isolated by Dubey in 1987. An agglutination test was concurrently employed to detect antibodies to *Toxoplasma* (*T.*) *gondii*.

Results: Antibodies to *N. caninum* were detected in 14 sera at different titers (8 at 1:640; 3 at 1:1280; 3 at $\geq 1:2560$). No sera tested positive for *T. gondii*.

Conclusion: The antibodies anti-*N. caninum* prevalence values, even at high titers, suggests the infection is not sporadic infection among chamois in the study area.

EPIDEMIOLOGICAL STUDIES ON HELMINTH PARASITES OF RED FOXES (*VULPES VULPES*) IN DENMARK

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Background: although the red fox population has increased in this area over recent years, only a few studies on their helminthes have been conducted in Denmark and in a limited area and number. Therefore, the aim of this study was to assess the epidemiology of red fox helminthes.

Methods: A total of 450 red foxes collected from various localities in Denmark during 1997-2000. Digestive tract and associated organs, trachea, lungs, heart, kidney, urinary bladder and front biceps were examined for helminthes.

Results: An epidemiological study of helminth parasites revealed the total of 21 helminth species were found at autopsy: nine nematodes- *Capillaria plica* (prevalence 91.1%), *Capillaria aerophila* (68.9%), *Crenosoma vulpis* (14.9%), *Angiostrongylus vasorum* (20%), *Toxocara canis* (65.1%), *Toxascaris leonina* (2.7%), *Uncinaria stenocephala* (82%), *Ancylostoma caninum* (0.2%) and *Trichuris vulpis* (0.4%); seven cestodes- *Mesocostoides lineatus* (46.7%), *Taenia pisiformis* (8%), *Taenia hydatigena* (5.8%), *Taenia taeniaformis* (7.8%), *Taenia crassiceps* (1.1%), *Taenia* spp. (6.4%) and *Echinococcus multilocularis* (0.7%); four trematodes- *Alaria alata* (11.6%), *Cryptocotyle lingua* (27.1%), *Pseudoamphystomum truncatum* (1.6%) and *Echinochasmus perfoliatus* (0.7%); one acanthocephala- *Polymorphus* spp. (0.7%). *Echinococcus multilocularis* and *Echinochasmus perfoliatus* are first records in Denmark. No significant differences were found between helminthes prevalence in male and female or between juvenile and adult, however, with *Toxocara canis*, males were more infected than females and juveniles more than adults, but there was significant differences in prevalence of some helminthes between different geographic localities. The average number of each helminth is tabulated.

Conclusion: *Echinococcus multilocularis* and *Echinochasmus perfoliatus* are first records in Denmark.

Corporate Symposia

BAYER ANIMAL HEALTH

Current topics in veterinary parasitology

THE EFFICACY OF 4 ANTHELMINTICS AGAINST ASCARIDS AND HOOKWORMS IN NATURALLY INFESTED DOGS.

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Purpose: A trial was performed to establish the comparative efficacy of 4 different products (A. Febantel + pyrantel embonate + praziquantel, B. Oxantel pamoate + pyrantel pamoate + praziquantel, C. Fenbendazole + praziquantel, and D. Selamectin) against natural infestations of ascarids and hookworms in dogs

Method: [redacted] of 2 - 4 months old were obtained from an area where it is known that there is a fairly high challenge environment. The infection was confirmed by faecal egg counts conducted prior to inclusion of each individual animal into the trial. The products were used at their recommended dosage rates and individual doses were specifically tailored to the exact weight of each animal by fractionating the tablet (products A, B, C) or withdrawing the required volume from the ampoule for topical application (product D). Faeces were collected daily after treatment and all excreted worms were recovered and identified. Seven days after treatment the [redacted] were euthanased and the small intestines processed for worm recovery. Efficacy was calculated by the critical method.

Results: Efficacy means per product in %

	A FEB/PYR/PZQ	B OXA/PYR/PZQ	C FBZ/PZQ	D SEN
Toxocara spp.	97	73	49	30
Ancylostoma spp.	95	72	40	1

Acknowledgement: We wish to thank L van Schalkwyk, JM Marakalale, LM de Visser and H Cilliers for assistance in the conduct of the trial.

AN INTERNATIONAL FLEA SUSCEPTIBILITY MONITORING INITIATIVE

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Background: Although resistance to a range of chemical groups is known in some cat flea (*Ctenocephalides felis felis*) populations, little ongoing research is aimed at anticipating, elucidating or managing potential problems with newer compounds. Consequently, an international team was formed to develop methodology for monitoring flea susceptibility to one such compound, imidacloprid.

Method: A standardised bioassay in which freshly hatched larvae are exposed to imidacloprid-impregnated rearing media was developed and evaluated using defined laboratory flea strains. The proportion of larvae developing to adults was recorded and dose-mortality responses calculated by probit analysis. Field isolates are being tested to investigate the range of values occurring in normal populations.

Results: Causes of variability were identified and the technique adapted accordingly. At each laboratory, the four laboratory strains responded very similarly with little more than a two-fold difference in LC₅₀ values.

Conclusion: The refined bioassay is robust and provides reproducible results. Data accumulating from its use should allow selection of a single diagnostic imidacloprid concentration for susceptibility monitoring.

TREATMENT OF EQUINE PROTOZOAL MYELOENCEPHALITIS (EPM) WITH PONAURIL 15% PASTE

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Naturally occurring clinical cases of equine protozoal myeloencephalitis (EPM) caused by *Sarcocystis neurona* were well characterized by signalment and laboratory diagnosis, randomly allotted to one of two treatment doses of ponazuril paste, treated for a period of 28 days, then evaluated for clinical changes through 90 days post treatment. Disease was characterized by a standardized neurological examination, with serum and cerebrospinal fluid (CSF) *S. neurona* IgG levels determined by Western Blot (WB).

A positive response was defined as a negative CSF WB and clinical improvement of at least one grade by 90 days post-treatment (by physical examination and videotape analysis) or clinical improvement of at least one grade but CSF WB positive.

After treatment with ponazuril paste at 5 mg/kg (2.27 mg/lb.) for 28 days, 28 of 47 horses (60%) improved at least one grade by Day 118 by investigator's evaluation, with 75% corroborated using blinded videotape assessment. At 10 mg/kg (4.54 mg/lb.) for 28 days, 32 of 55 animals (58%) improved at least one grade by Day 118 with 56% corroborated using videotape assessment.

PREVALENCE, DIAGNOSTICS AND CONTROL OF ISOSPORA SUI: CURRENT UNDERSTANDING

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Coccidiosis due to infection with *Isospora suis* is increasingly being acknowledged as a major cause of diarrhoea in suckling piglets. Recent work with *I. suis* is summarized, including aspects of incidence, diagnostics, awareness, pathogenesis, mode of action of antitocoidals and control.

I. suis is far more common on pig-rearing farms throughout the world than has widely been assumed. However, the awareness of its occurrence and its significance still varies. If piglet coccidiosis is suspected, the pathogen must be identified. An overview is given of the various possibilities for diagnosis including autofluorescence microscopy.

The pathogenesis shows the clinical signs (diarrhoea) to be the result of lesions in the mucosa of the small intestine (jejunum and ileum). The development of the parasite as well as the changes in the target tissue (villous structure, necrotic inflammatory processes etc.) are demonstrated under model infection conditions. Effective control includes considerations of the epidemiology of the parasite, management practice and appropriate anticoccidial therapy where necessary.

FORT DODGE ANIMAL HEALTH

Moxidectin: ten years of experience in the world

CONTROL OF EQUINE CYATHOSTOMIASIS BY A SINGLE DOSE OF MOXIDECTIN 2% GEL

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2. FDAH Limited, Southampton, SO30 4QH, UK

Sixteen ponies with naturally acquired Cyathostome and large strongyle infections were housed for 8 weeks prior to a single oral treatment with Moxidectin 2% gel at a dose rate of 0.4mg/kg bodyweight. The animals were kept indoors for a further 8 weeks prior to necropsy. Worm burdens of the treated animals compared to those of the controls indicated a 100% efficacy against adult and luminal L4 cyathostomes with 99.9% and 90.8% reductions in developing and early third stage larvae, respectively. A 100% efficacy was also recorded against adult *Strongylus* and *Tridontophorus* species together with an overall reduction in faecal egg output of between 96 and 100 %. This high efficacy rate, together with its persistent activity profile and a demonstrable efficacy against benzimidazole-resistant cyathostomes, makes Moxidectin 2% oral gel one of the preparations of choice for the treatment of equine cyathostomiasis.

ENDOPARASITE CONTROL IN HORSES WITH A GEL CONTAINING MOXIDECTIN AND PRAZIQUANTEL

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Moxidectin 2% equine gel is used for control of endo-parasites in horses world wide. By adding praziquantel to the formulation, the spectrum of control has been expanded to include tapeworm (*Anoplocephala perfoliata*) in horses. Two studies in Australia tested the efficacy and safety of an anthelmintic for horses combining moxidectin 2% (0.4 mg/kg) with praziquantel 12.5% (2.5 mg/kg). A two part field study included a total of 43 horses for the nematode part (I) and another 8 horses for the tape worm part (II). Part I: Group A (N=26) was treated with the moxidectin/praziquantel gel, Group B (N=10) received an abamectin/praziquantel paste and 9 horses in Group C remained as an untreated control group. The efficacy was monitored by faecal egg per gram (epg) measurement: the average challenge in the Group C ranged from 773.3 to 2928.9 epg. When following a common recommendation to treat when 50% of horses in the group have epg $\geq$ 200, the horses in Group B would have had to be treated between week 6 and 8, when 6 out of 8 horses sampled had epg $\geq$ 200. Group A horses did not warrant a treatment at any time during the 14 week study period. Group C had to be treated at week 12 for animal welfare reasons. Efficacy against tapeworm was 100%. A safety study in 14 young foals from 3 weeks to 17 weeks of age showed moxidectin 2% / praziquantel 12.5% horse gel to be safe when applied up to 3 times recommended dose rate.

MANAGING MACROCYCLIC LACTONE RESISTANCE IN NEMATODE PARASITES OF SHEEP

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Background: With widespread and intense resistance already apparent to the benzimidazole and levamisole families of anthelmintics, it is vital to delay the evolution of resistance to the macrocyclic lactones for as long as possible. The fact that the macrocyclic lactones are the last generally effective broad-spectrum anthelmintic class increases their usage and intensifies their selection pressure for resistance.

Method: The scientific literature shows startling differences in efficacy between the avermectins and milbemycins against macrocyclic lactone resistant strains of sheep nematodes. At the current stage of evolution of resistance, avermectins typically show efficacies of around 40%, while moxidectin's efficacy is still close to 100%. Because of moxidectin's greater potency, its LD₅₀ against resistant worms is still below its recommended dose rate. In contrast, the LD₅₀ for avermectins greatly exceeds their recommended dose rate against resistant *Haemonchus* and *Ostertagia* in sheep. Computer simulation studies indicate that the greater efficacy of moxidectin against resistant worms will more than compensate for the selective effects of its greater persistence, and these results are consistent with field evidence that moxidectin selects more slowly for resistance than do the avermectins.

Conclusion: Moxidectin is the preferred macrocyclic lactone for use against nematode parasites of sheep, as it selects more slowly for resistance than do the avermectins.

12 MONTHS HEARTWORM PREVENTION IN DOGS - MOXIDECTIN 10% SLOW RELEASE INJECTION.

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Three studies were conducted in Australia using a unique moxidectin 10% (0.5mg/kg) slow release (SR) formulation for heartworm prevention in dogs to evaluate yearly treatment for the prevention of heartworm disease. A critical study included heartworm negative dogs in 2 groups of 6, one untreated control group and one group receiving moxidectin SR. The dogs were kept in a heartworm endemic area for 12 months, at which point they were all artificially infected with *D. immitis* L3 stage. 4 months later at necropsy, all control dogs were found infected. None in the treatment group was infected. A field study including a total of 241 dogs injected with moxidectin SR was conducted in 6 endemic areas of Australia. 12 months post-treatment the dogs received conventional heartworm prophylaxis. The dogs were monitored by antigen tests and microfilaria tests for 18 months in total. Moxidectin SR was found safe and effective in preventing heartworm disease for 12 months. A safety study conducted included 14 puppies of different breeds and 12 weeks of age at the time of treatment. One group (N=3) received moxidectin SR at the recommended dose rate, another group (N=8) received moxidectin SR at 3X recommended dose rate and the third group (N=3) remained untreated controls. At no time did any of the puppies exhibit any generalized adverse reaction to the treatments. All 3 studies showed moxidectin 10% SR to be safe and effective in preventing heartworm disease in dogs of different ages, sex and breeds for 12 months.

INJECTABLE, SUSTAINED-RELEASE MOXIDECTIN FORMULATION PROVIDES SIX MONTHS OF PROPHYLACTIC EFFICACY AND RETROACTIVE ACTIVITY AGAINST *DIROFILARIA IMMITIS* IN DOGS

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To evaluate the 6-month prophylactic efficacy against heartworm larvae of a moxidectin canine injectable, sustained-release formulation (MOX-SRI), 2 similar dose confirmation studies were conducted. Groups of 8 dogs received 0, 0.06, 0.17, or 0.50 mg moxidectin/kg b.wt. of MOX-SRI 6 mos. prior to inoculation with 50 *D. immitis* L₃. To evaluate retroactive efficacy, 3 gps of 5 dogs received MOX-SRI at 4 mos. PI, MOX-SRI at 6 mos. PI, and saline, respectively, and were necropsied at 12 mos. PI; 3 gps of 5 dogs received MOX-SRI at 4 and 10 mos. PI, MOX-SRI at 6, 12, and 18 mos. PI, and saline, respectively, and were necropsied at 23 mos. PI. Ante- and postmortem Injection Site Reactions (ISRs) and serial microfilarial (mf) counts and antigen (Ag) scores were monitored. Only 1 (0.06 mg/kg gp) of the 48 dogs treated with MOX-SRI in the 2 dose confirmation studies had worms at necropsy; controls had averages of 25 and 26 worms, respectively. With few exceptions, injection-site swellings in the 0.06 and 0.17 mg/kg gps were palpable only during the first 13 days after treatment. Retroactive efficacies were 85.9% for 4-mo.-PI and 24.7% for 6-mo.-PI injections; all controls had worms (average, 32). Efficacies of 2 and 3 injections were 97.2% and 5.2%, respectively; 4 of 5 controls had worms (mean 25.6). No mf were seen in any treated dogs; all controls with worms had mf. Mean Ag scores generally reflected the live worm burdens. No systemic or ISRs were noted.

MODE OF ACTION AND MECHANISMS OF RESISTANCE: MOXIDECTIN AND THE MACROCYCLIC LACTONES

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Moxidectin (MOX) is a potent milbemycin member of the broad spectrum macrocyclic lactone (ML) endectocides. It shares many similarities with the avermectin (AVM) members of the ML endectocides in terms of spectrum, safety, pharmacokinetics, mode of action, and expression and mechanisms of resistance; but its properties are not identical to those of the AVMs. This work reviews the similarities and differences between MOX and ivermectin (IVM) mode of action and mechanisms of resistance. Both MOX and IVM act on glutamate-gated chloride channels (GluCl) in nematodes. However, differences are seen in the relative response to glutamate potentiation of IVM and MOX binding to nematode GluCl subunit protein and glutamate modulation of MOX or IVM inhibition of pharyngeal pumping. Furthermore, IVM resistance leads to an increase in glutamate specific-binding compared with unselected nematodes. Selection of *Haemonchus contortus* with IVM or MOX exerts selection pressure for specific alleles of GluCl V-subunit and for alleles of P-glycoproteins (Pgps). Pgps may play a role in regulating the concentration of MLs by lowering the concentration of MLs at the ligand-gated chloride channel sites of action. However, different MLs, due to differences in lipophilicity, are likely to respond differently to Pgp transport out of the cell membrane. These subtle differences in mode of action and mechanism of resistance, together with differences in pharmacokinetics, are likely to be responsible for differences between different members of the ML class of endectocides.

EFFICACY OF A COMBINATION 2% MOXIDECTIN AND 12.5% PRAZIQUANTEL EQUINE GEL AGAINST BOTS AND TAPEWORMS IN HORSES

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Background: Moxidectin is a macrocyclic lactone of the milbemycin family with endectocidal activity against a broad spectrum of endo- and ecto-parasites of livestock. In horses, the dose-limiting species are *Gasterophilus* spp. Praziquantel is an anthelmintic used for the treatment of tapeworm infections both in humans and animals.

Method: In a dose confirmation study, 24 horses naturally infested with the tapeworms *Anoplocephala perfoliata* and *Paranoplocephala mamillana* and the horse bots *Gasterophilus intestinalis* and *Gasterophilus nasalis*, were allocated to two groups of 12 according to bodyweight. One group was treated with moxidectin/praziquantel equine gel at 0.4mg moxidectin and 2.5mg praziquantel/kg bodyweight and the other group remained as untreated controls. Horses were housed for 13/14 days prior to necropsy.

Results: Parasite burdens of the treated animals compared to the controls showed >99% efficacy against *A. perfoliata* and *P. mamillana* and 97-99% efficacy against *Gasterophilus* spp. Strongyle faecal egg output was reduced by 99.9%.

Conclusion: The results confirm that the moxidectin and praziquantel combination gel is highly effective against equine bots and tapeworms at a dosage of 0.4mg/kg and 2.5mg/kg bodyweight, respectively.

MOXIDECTIN: A UNIQUE PRODUCT FOR THE CONTROL OF PARASITES IN COMPANION AND FOOD-PRODUCING ANIMALS

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Moxidectin is a potent broad-spectrum macrocyclic lactone endectocide which is a semi-synthetic methoxime derivative of a natural fermentation product of *Streptomyces cyaneogriseus* subsp. *noncyanogenus*. This organism was isolated in 1983 from an Australian soil sample. Moxidectin is a 16-member, second generation pentacyclic lactone of the milbemycin class of compounds and is approved globally in various formulations for use in companion and food-producing animals. Though similar in structure to members of the avermectin class of compounds, moxidectin is a unique compound which possesses superior performance versus various parasite species, efficacy versus parasites not affected by other macrocyclic lactones, extended periods of protection from subsequent parasite infections, control of parasite species resistant to other classes of anthelmintics and endectocides, and superior environmental safety.

**FIELD EFFICACY OF MOXIDECTIN
FORMULATIONS FOR THE CONTROL OF *Dirofilaria immitis*
AND *Dirofilaria (Nochtiella) repens***

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Two field studies at three veterinary practitioners in the Piedmont region of NW Italy were conducted to assess the efficacy and safety of moxidectin tablets and moxidectin SR injectable against *Dirofilaria immitis* and *Dirofilaria (Nochtiella) repens* in dogs. In the first study, 82 dogs of different breeds that had been determined to be negative for both *D. immitis* and *D. repens* were treated monthly with moxidectin tablets at the minimum dose of 3 mcg moxidectin/kg BW from April-May to September (5 treatments). Dogs were examined for circulating microfilariae on day 180 and 390. A group of 25 dogs which had received no treatment were observed as controls. In study 2, a group of 85 dogs were administered 2 doses of the moxidectin SR formulation (0.17 mg moxidectin/kg BW) 6 months apart during the mosquito season of 1999. A group of 32 dogs received moxidectin tablets and 24 untreated dogs were observed as controls. Moxidectin tablets and the SR injectable formulation were 100% effective as a preventative for dogs naturally exposed to a *D. immitis* and *D. repens* challenge in an enzootic area of Italy. The drug was well tolerated with no adverse reactions observed.

INTERVET

Prevention of Leishmaniosis in dog and man

DELTAMETHRIN-IMPREGNATED DOG COLLARS PREVENT *LUTZOMYIA* SAND FLY FEEDING

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Background: Deltamethrin-impregnated PVC dog collars were tested to assess if they were as effective in protecting dogs from sand fly bites of *Lutzomyia longipalpis* and *Lutzomyia migonei* as they were against *Phlebotomus perniciosus* as shown by Killick-Kendrick *et al.* (1997).

Method: Four dogs wearing deltamethrin collars and three dogs wearing placebo collars (not impregnated with deltamethrin) were kept in separate kennels for over 8 months in a village on the outskirts of Fortaleza in Ceará, Brazil. Periodically, a dog from each group was sedated, placed in a net cage for 2 hr in which 150 female sand flies had been released 10-15 minutes before. *Lu. longipalpis* were used 4, 8, 12, 16, 22, 27, and 35 weeks after the attachment of the collars. *Lu. migonei* were used 3, 7, 11, 15, 22, 26, and 36 weeks after attachment.

Results: During 35 weeks, only 4.1% (81 of 2022) *Lu. longipalpis* recovered from the nets with the deltamethrin collared dogs were engorged, an anti-feeding effect averaging 96%. During this time the average mortality was 68% with half of the sand flies dying in the first 2 hr. In contrast, 83% of the 2094 *Lu. longipalpis* recovered from the nets containing the placebo collared dogs were engorged and the average mortality was 8.6% with 1.1% dying in the first 2 hr. Similar findings were found with *Lu. migonei*: of 2034 sand flies recovered over this period, the anti feeding effect was 96.6% and the average mortality was 70.4% compared to engorgement of 79% and a mortality of only 12.1% in the controls.

Conclusion: These results show that deltamethrin impregnated collars can protect dogs against *Lutzomyia* species sand flies for up to 8 months. Thus, they should be useful in a program to control human and canine visceral leishmaniasis. Progress of the current field trials using these collars in Ceara and Bahia, Brazil will be reported.

FIRST FIELD TRIAL OF THE EFFECT OF DELTAMETHRIN-IMPREGNATED DOG COLLARS (SCALIBOR® PROTECTOR BAND®) ON CANINE LEISHMANIASIS: EVIDENCE FOR AN IMPACT ON THE INFECTION INCIDENCE.

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In southern Europe, where canine leishmaniasis (CanL) is widespread, killing seropositive dogs is considered unacceptable and drug treatment has low efficacy in preventing transmission. We made a field evaluation of the efficacy of deltamethrin dog collars in a leishmaniasis focus of southern Italy by assessing their impact on the incidence of CanL. During two consecutive transmission seasons collars were fitted to 350 (1998) and 354 (1999) dogs of an intervention town (70% of the canine population). Control dogs (371 and 264 in the two years, respectively) were from four towns of the same area. Before each transmission season, the CanL seroprevalence in the intervention and control towns was evaluated by cross-sectional surveys and found similar to each other (about 15% in 1998 and 10% in 1999, respectively). After each transmission period, incidence rates of seroconversions were determined in adult dogs that were serologically negative before the season under evaluation, and in puppies. After the 1998 season, 2.7% of the dogs in the intervention town seroconverted compared to 5.4% in the control towns (50% protection, $P = 0.15$). After the 1999 season, 3.5% of collared dogs seroconverted compared to 25.8% of control dogs (86% protection, $P < 0.001$). The increase in seroconversion rates recorded in control dogs suggests an increase in the *Leishmania* force of infection in the canine reservoir during the 1999 sandfly season, as supported by the concomitant increase of human cases in control towns and in the whole Campania region. Our results suggest that the impact of mass use of deltamethrin-impregnated dog collars on the incidence of CanL may be negligible during low transmission seasons or, probably, in low endemic foci, but can be very strong when the force of transmission is high.

IMPACT OF INSECTICIDE IMPREGNATED DOG COLLARS ON THE INCIDENCE OF ZOONOTIC VISCERAL LEISHMANIASIS IN DOGS AND CHILDREN

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A matched community intervention trial was carried out in north-west Iran to assess the effect of deltamethrin-impregnated dog collars on the transmission rate of *Leishmania infantum*. In 9 villages all domestic dogs were provided with collars (the "intervention villages"), and there were 9 control villages matched by pre-intervention seroprevalence data (using the Direct Agglutination Test, DAT). The outcome measurements of the trial were DAT conversion rate in both dogs and children (<10 years old) and the leishmanin skin test (LST) conversion rate in children. Pre-intervention surveys carried out from January-April 2000 found a mean seroprevalence in intervention villages of 10.7% in dogs and 7.8% in children, compared to 10.9% and 7.4%, respectively, in control villages. Collars were applied to dogs in March-April 2000, prior to the sandfly season (from July-September). Spare collars were left with the village health workers for replacing on dogs who had lost the collars and for applying to new dogs. The post-intervention surveys, carried out from January-March 2001, demonstrated a significant reduction in the seroconversion rate amongst collared dogs: Odds Ratio = 0.46 [95% CI: 0.30 - 0.70] and a strong indication that the incidence rate in children from intervention villages was reduced as a result.

MERIAL

Innovations in the Diagnosis and treatment of Parasitic Diseases

EVALUATING AN ELISA FOR MONITORING PARASITE BURDENS IN LACTATING DAIRY CATTLE

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Background: Evaluation of any diagnostic test is best accomplished by comparison of the test results with a "gold standard". Use of total worm counts in slaughtered cows as a gold standard for evaluating a parasite ELISA is limited by the expense of the test and the distinctly different seasonal patterns in worm counts and ELISA optical densities (ODs).

Results: Four alternative approaches to evaluating the ELISA (used on milk samples) have been used in a series of studies carried out in Eastern Canada. (1) The correlation between ODs and fecal egg counts range from 0.15 to 0.7 depending on the level of aggregation of the counts. (2) Herd management factors that are known to influence parasite burdens also affect ODs in a predictable manner. (3) There are significant negative associations between ODs and a variety of milk production parameters, including late summer/fall production and the amount of seasonal variation in production. (4) Late lactation ODs appear ($P=0.07$) to be predictive of the magnitude of the response to eprinomectin treatment at the following calving with low OD cows having very little response and high OD cows experiencing a gain in production of 2.9 kg/day.

Conclusion: The ELISA appears to be a useful measure for monitoring parasite burdens in adult dairy cattle.

PARASITE CONTROL OF THE LACTATING DAIRY HERD - IS THERE A REAL NEED ?

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There continues to be debate about the real impact of parasite infection in dairy cattle. Recent parasitological monitoring studies conducted in different regions with large numbers of lactating dairy cows, were able to detect the presence of gastrointestinal and pulmonary nematodes, mange, particularly chorioptic, and hypodermosis. Results from a study conducted in Switzerland suggest a strong link between management conditions, particularly the feeding management and the parasite control strategy adopted in the herd, and the levels of nematode infection. In most of the evaluated herds, internal and external parasite challenges were low. Low parasite burdens were also detected in productivity studies recently developed in Switzerland (Pfister et al. 2001), Italy (Kramer et al. 2000), Germany (Schönberg et al., 2000), Canada (Nødtvedt et al., 2001), New Zealand (McPherson et al., 2001), and Australia (Little et al., 2000). Despite the low challenges, these trials detected positive responses to treatment with the endectocide eprinomectin. Considering these results, dairy cattle herds should be integrated in a herd health monitoring program in order to establish the risk of external and internal parasite contamination and infection. The real issue regarding parasite control of the adult dairy herd therefore, is to establish a reliable monitoring system in order to design programs tailored to the different management practices.

PCR IN PRACTICE: DIAGNOSIS OF ARTHROPOD-BORNE DISEASES IN COMPANION ANIMALS Susan E. Shaw

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The emergence and re-emergence of arthropod-transmitted diseases is a challenge for both human and veterinary medicine. A complex interaction of factors is involved: climate change, changes in land/water management, movements of pets...Advantages of PCR include its sensitivity and specificity. It can be applied to blood, tissue aspirates, body fluids and tissue samples. Single diagnostic samples can be screened for several pathogens. The presence of microbial DNA demonstrates a current infection. In anticipation of increased recognition of both endemic and imported arthropod-borne infections in dogs and cats, a rapid PCR-based diagnostic service for babesiosis, borreliosis, ehrlichiosis, bartonellosis and leishmaniosis in pets has been developed at the Acarus laboratory (University of Bristol). Current methods include simple PCR targeting of genus- or species-specific regions of *rRNA* genes (*Ehrlichia* and *Babesia*), housekeeping genes (citrate synthetase and *gltA* in *Bartonella*), virulence factors (*ospA* in *Borrelia*) or multicopy sequences (*epank1* in *Ehrlichia phagocytophila*) and kinetoplast sequences in *Leishmania*. Analysis of samples submitted to the PCR diagnostic service over a 3-month period, revealed that 20% (55% from imported animals) were positive for at least one arthropod-borne pathogen were positive. In addition ACARUS has been used to carry out epidemiological survey. PCR-based surveys have been undertaken to obtain a preliminary picture of arthropod-borne infections in northern Europe. From July to October 2000, blood samples from 572 dogs and 163 cats from 52 danish veterinary clinics were tested for *Borrelia*, *Ehrlichia phagocytophila* and *Bartonella* DNA. Bacterial DNA was detected in 4.2% of dogs and 5.5% of cats; both healthy and clinically ill animals were infected. In the second study, blood samples from 180 ill animals (120 dogs and 60 cats) from 41 UK or Irish practices were collected during September-October, 2000 and tested. No animals were positive for *Babesia*, *Leishmania* or *Ehrlichia canis*. However, DNA of endemic arthropod-borne pathogens was detected in 6.6% of sick dogs and 5% of sick cats. These studies suggest considerable exposure of companion animals and possibly their owners, to infected arthropod vectors.

NOVARTIS ANIMAL HEALTH

Parasite vaccines

DEVELOPMENT OF A HORN FLY VACCINE: TARGETING SALIVARY FACTORS AS ANTIGENS Cupp, E. W., Cupp, M. S., & Zhang, D.

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Background: To better understand molecular mechanisms associated with blood-feeding by horn flies (*Haematobia irritans irritans*), we determined that saliva from this insect lacks apyrase and a vasodilator, instead disrupting coagulation using a unique anti-thrombin peptide "thrombostasin" - TS).

Methods: Thrombostasin was purified from saliva of field-collected flies using HPLC and a corresponding cDNA sequence was cloned from a *H. i. i.* salivary gland cDNA library. TS clones from this library ($n=11$) and genomic DNA ($n=38$) from field (Alabama) and laboratory populations (Texas) were analyzed. Horn flies collected from several locations in South America, a subspecies from Australia (*H. i. exigua* - buffalo fly), and *Stomoxys calcitrans* (stable fly) were also examined using PCR to determine the presence/absence of thrombostasin.

Results: A total of 10 TS protein isoforms were identified from field/lab populations in the USA. Molecular masses of these isoforms ranged from 9,183 to 9,274 Da; isoelectric points varied from 4.19 to 4.05. All populations of *H. i. i.* were PCR positive for TS as was *H. i. exigua*. *S. calcitrans* was PCR negative for TS and did not show anti-thrombin activity in its saliva as determined by the recalcification assay.

Conclusion: Thrombostasin is produced from a polymorphic gene that yields several major isoforms. This gene also occurs in buffalo flies but not in *S. calcitrans*. TS could serve as a vaccine candidate for *Haematobia irritans sensu lato*.

Acknowledgement: Supported by Novartis Animal Health and USDA/NRICGP Awards #9601791 and #9802578.

IMMUNOGENICITY OF RECOMBINANT LEISHMANIA ANTIGENS IN DOGS Frank, G.R., Johnson, C.M. and Sellins, K.S.

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Background: Canine leishmaniosis is a clinically significant disease for the dog and a reservoir for human infections. Treatment of dogs usually results only in temporary remission, and there is no preventative for dogs other than sandfly avoidance. An effective vaccine would be a public health asset.

Methods: Antigens to be evaluated consisted of a mixture of three *Leishmania* antigens (MAPS, M15, LeIF) and a fusion protein of all three (MML) and were cloned and characterized by the Corixa Corporation. Each antigen was expressed as a recombinant protein in *E. coli* and was purified. Each antigen set then was formulated with two adjuvants, a β -glucan (Adjuprime™, Pierce) and a stable emulsion of monophosphoryl lipid A (MPL®-SE, Corixa™). Forty-two male beagles were assigned to 7 groups of 6 dogs each: untreated, 2 adjuvant control groups, 2 adjuvanted antigen mixture groups and 2 adjuvanted MML groups. Three immunizations were given at 4 week intervals followed by intravenous infection 4 weeks later with *L. chagasi* amastigotes. Total IgG responses and PBMC proliferative responses to the individual antigens and the fusion, MML, were monitored during the immunization period and after challenge. Infection status is being monitored by K39 ELISA, culture, PCR and clinical evaluation.

Results: All antigens were immunogenic in dogs. The MPL®-SE adjuvant induced greater IgG responses as measured by ELISA, and also induced proliferative responses in more dogs than did Adjuprime™. The MML fusion protein was able to induce immune responses to the 3 individual proteins. IFN γ and IL-4 responses in stimulated cells as well as infection status are being monitored.

Conclusion: MPL®-SE adjuvanted MML represents a candidate vaccine for canine leishmaniosis.

Acknowledgments: Sponsored in part by Novartis Animal Health.

EVALUATION OF RECOMBINANT H11 VACCINES AGAINST HAEMONCHUS CONTORTUS

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Background: H11 is a well-known 'hidden' antigen derived from the intestinal microvillar membrane of adult *H. contortus*.

Results and Conclusion: Native H11 (nH11) has been tested in many trials and on average, vaccination with nH11 reduces faecal egg counts (FEC) and worm burdens by >90% and >75%, respectively. As with other concealed antigens, levels of protection are correlated with circulating specific antibody titres. cDNAs encoding 3 isoforms of H11, designated H11-1, -2 and -3, have been cloned, and database searches revealed similarity to mammalian microsomal aminopeptidases (Ap). Both nH11 and baculovirus-expressed recombinant H11 (rH11) possess Ap activity, and the level of protection in sheep immunised with nH11 is correlated with the degree to which antisera can inhibit Ap activity *in vitro*. However although sheep immunised with baculovirus rH11 raise antibodies which strongly inhibit Ap activity, these sheep are not significantly protected against *H. contortus* challenge. Active site domains (longDOM and DOM5) have been expressed in *E. coli*, and sheep immunised with these domain constructs from H11-1 show reduced FEC and worm burdens. While this reduction is not high nor statistically significant, there does appear to be a correlation between the level of antibodies induced which cross-react with nH11 and the level of protection. These results suggest that inhibition of activity *per se* may not be the mechanism of immunity, and that expression strategies which induce higher levels of anti-nH11 antibodies are required.

TOWARDS A BROAD SPECTRUM ANTI-TICK VACCINE

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Background: The difficulty in identifying suitable antigenic tick targets is reflected by just a single subunit vaccine on the market, TickGARD™ (Australia) / GAVAC™ (South America), containing the 'concealed' antigen, Bm86, a midgut membrane-bound protein of the cattle tick, *Boophilus microplus*. Bm86-induced antibody-mediated immunity causes midgut cell damage, killing the ticks or reducing their fecundity. As concealed antigen-induced protection is short-lived, an ideal ectoparasite vaccine should also induce an anamnestic response.

Methods: Our 'dual action' approach to recombinant anti-tick vaccines applied secreted ('exposed') antigens to develop a host immune response against concealed antigens, resulting in damage to the attached parasite, while allowing natural tick infestations to boost and maintain high levels of immunity. We used truncated versions (64trps) of a tick cement protein (64P), expressed in *E. coli* as soluble and denatured antigens, from adults of the African brown ear tick, *Rhipicephalus appendiculatus*.

Results: Ticks (*R. appendiculatus* and *R. sanguineus* adults and nymphs) feeding on guinea pigs vaccinated with above antigens induced a pronounced, transient cutaneous inflammatory response, and engorged ticks turned black and died, indicating damage to their midgut. Similar results were observed with *Ixodes ricinus* nymphal ticks feeding on immunised hamsters. Antibody titres were boosted by tick infestation. The vaccine effect correlated with cross-reactivity between anti-64trps antibodies and tick tissue extracts.

Conclusions: The 64trp vaccines contain immuno-protective antigens effective against both adult and immature tick stages. The dual action at the site of tick feeding and in the midgut offers the prospect of tick control that is self-sustaining through the booster effect of natural infestations. Moreover, the cross-species anti-tick effects observed with *R. sanguineus* and *Ixodes ricinus* offer opportunities to develop a broad-spectrum anti-tick vaccine.

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PFIZER ANIMAL HEALTH

Latest developments on selamectin use and mode of action

SELALECTIN AND FLEA CONTROL: ACTIVITY AGAINST ADULT AND IMMATURE STAGES OF *Ctenocephalides felis felis*

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Background: The activity of selamectin against adult and immature stages of *Ctenocephalides felis felis* was evaluated in 5 studies.

Method: In 3 laboratory studies, cats were randomly allocated to topical treatment with selamectin (6 mg/kg) or a placebo. To evaluate speed of kill of newly acquired fleas, 50-100 adult fleas were applied to cats 24-72h after treatment. After exposure for 1, 2, 4, 8, 12, or 24h, all fleas were removed from cats by combing and from collection pans beneath the cages. The numbers of live and dead fleas were determined. To assess ovicidal activity, normal flea eggs were placed on the back of each cat 1 day after treatment and, thereafter, at weekly intervals for 1 mo. Eggs were collected after exposure to the cats for 0-2h, 2-6h, and 6-12h, and were evaluated for hatchability and development to adults. In 2 field studies, conducted in 42 households in the Tampa area of Florida, flea-infested cats and dogs were treated with selamectin, fipronil, or imidacloprid. Each treatment was administered once monthly for 3 mo.

Results: Adult flea mortality was proportional to exposure time on selamectin-treated cats, with 95% mortality within 12h after placement on cats treated 1d previously. Selamectin administered once to cats on day 0, was highly effective ($\geq 98\%$) at preventing development of flea eggs deposited on days 1, 7, 14, 21, and 28, with similar reductions for all egg collection periods. Field studies showed no significant differences between selamectin, fipronil, and imidacloprid. Fleas on pets were reduced by $>90\%$ by 7 d after the first selamectin treatment and by 98-100% by 40-45 days. Environmental flea populations were reduced by 93% for selamectin treatment by day 21 and $>98\%$ for all treatments by day 40.

SAFETY AND EFFICACY OF SELAMECTIN IN DOGS WITH *Dirofilaria immitis* INFECTION

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Purpose: To determine the safety and efficacy of selamectin in dogs with high microfilaremia and its prophylactic effect in dogs inoculated with *Dirofilaria immitis* larvae 3 mo. before treatment. **Methods:** Selamectin was administered: for 3 mo. to dogs with high microfilaremia (mean count, $>10,000$ mf/ml; study A); for 18 mo. to dogs with patent mature adult *D. immitis* infections (study B); or for 12 mo., beginning 3 mo. after infection (study C). In each study, 16 Beagles were treated topically with selamectin (6 mg/kg) or placebo at 30-day intervals, beginning on day 0. Dogs were inoculated with 20 adult *D. immitis* on day -222 (study A) or day -30 (study B); or 50 *D. immitis* L₃ larvae on day -90 (study C). Serial blood samples were collected for microfilarial counts and/or adult heartworm antigen determination. Adult worm counts were performed at necropsy.

Results: In study A, no adverse effects were observed in dogs with high microfilarial counts. In study B, dogs receiving long-term treatment with selamectin had significantly fewer microfilaria (95.8-100% reduction) than placebo from day 59 onward. The reduction (36.4%) in adult worms was not significant. In study C, selamectin had a 3-mo. 'reachback' effect, with a reduction in live adult *D. immitis* of 98.5%, compared with placebo. Dogs treated with selamectin had no microfilaria.

Conclusion: Selamectin demonstrated exceptional safety in heartworm-positive dogs and provided robust 'reachback' activity.

STRATEGIC USE OF SELAMECTIN TO BLOCK VERTICAL TRANSMISSION OF ROUNDWORMS (*Toxocara* spp.) IN DOGS AND CATS

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Background: Transmission of *Toxocara canis* and *T. cati* from the dam to the neonate is due to infection by somatic larvae *in utero* (for *T. canis*) or during suckling (for both species) as well as by ingestion of infective eggs. A series of 3 studies was conducted to determine whether selamectin administered to pregnant dogs and cats would prevent *Toxocara* spp. infections in their offspring, and to investigate the effect of selamectin against somatic *T. canis* larvae.

Method: Animals were treated with selamectin (6 mg/kg) or a placebo. Dogs had naturally acquired infections of *T. canis*. Cats were inoculated with *T. cati* ova. In 2 studies, pregnant dogs and cats were treated at least twice at a 30-day interval pre-partum and again 10 and 38 days post-partum. Efficacy was assessed by fecal worm egg counts and intestinal worm counts in pups and kittens at necropsy. In the third study, at 28 days after a single treatment, tissues were collected from dogs at necropsy for recovery of somatic larvae by digestion.

Results: Mean fecal egg counts in pups from selamectin-treated dams were reduced by $\geq 96\%$ on days 24 and 34 post-partum ($P = 0.0001$). At necropsy, adult worm counts for offspring of selamectin-treated dams, compared with placebo, were reduced by 98.2% for pups and by 100% for kittens ($P \leq 0.0018$). The reduction in somatic larvae recovered from selamectin-treated dogs, compared with that for placebo, was 95.2% ($P = 0.0004$). The results suggest that selamectin treatment of bitches and queens significantly reduces transmission of *Toxocara* spp. to their offspring.

Author Index

Abbott E.M. 123
 Abdalla A.L. 127
 Abner S.R. 38
 Acardi HH 95
 Acero G. 35
 Adams Z.J.O. 191
 Adibpour M. 68
 Afify M.A. 150
 Agergaard N. 32
 Aghede R.I.S. 84
 Agostini A. 159, 202
 Agoulon A. 98
 Ahren D. 122
 Ajanusi J.O. 57
 Ajanusi O.J. 59, 64, 153
 Akyol Ç.V. 59
 Alak J.I.B. 5
 Alberdi J. 130
 Alhassan A.K. 54
 Almería S. 18
 Aluja A.S. de 35
 Alva R. 151
 Alvarez I. 35
 Álvarez-Sánchez M.A. 149, 155
 Alves L.C. 67
 Alvinerie M. 110, 148, 160, 181
 Anderson D.L. 5
 Anderson L.J. 122
 Andreoletti O. 191
 Andrews F. 199
 Anisimova E. 60
 Anjos D.H.S. 187
 Anthonissens E. 161
 Anziani O.S. 159
 Apsari IAP 66
 Araújo J.V. 129
 Ard M.B. 206
 Ardana I.B.K. 168
 Arias M. 37
 Armas A. 166
 Asatrian A. 75, 157, 176
 Assmann G. 85
 Ataeian A. 176
 Atanásio A. 121
 Athanasiadou S. 117, 122
 Attili A.R. 5
 Atwell R. 200
 Audho J. 73
 Aue A. 177
 Aulicino F. 109
 Aumont G. 148
 Avenant-Oldewage A. 45, 124
 Awaad M.H. 150
 Ayuya J.M. 56

 Bach Knudsen K.E. 39
 Bach T. 199

 Bach U. 23
 Bacigalupe D. 20, 38, 62
 Baggesen D. L. 32
 Bain F. 199
 Bairden K. 200, 201
 Baker D.J. 123
 Baker L. 148
 Baker R.L. 73
 Ballet J. 120
 Bandi C. 141
 Banerjee D. P. 35
 Barbosa C.L. 67
 Barbosa O.F. 118
 Barbosa R.G. 154
 Barchetti A. 196
 Bareev R. 47
 Barger I.A. 200
 Barlett P.C. 156
 Barley J.P. 123
 Baroncini L. 41
 Barral B. 35
 Barrick R.A. 150
 Barroga E.F. 131
 Barron R. 143
 Bartley D. 73, 117
 Bartley D.J. 55
 Bartley P. 16
 Bartnik C. 60
 Barutski D. 8, 159
 Basso N. 67, 134
 Basso W. 20, 38, 62
 Bates P.G. 192
 Baudena M.A. 32
 Bauer C. 8, 59, 64, 66, 75, 168, 169, 190
 Bavia M. 124
 Bazzocchi C. 61, 141
 Bazzoli S. 135
 Bazzoni G. 132
 Behan P. 141
 Behnke J.M. 37
 Bekish V. J. 73
 Bell N.J. 54, 166
 Bellotti M. 25, 196
 Beltrán C. 35
 Beninati T. 88
 Benítez R. 58
 Bennison J.J. 126
 Bent Mohamed A. 58
 Benzimra P. 65
 Bergeaud J.P. 147, 191
 Bergeaud JP 37, 126
 Bergquist R. 124
 Berkvens D. 83
 Bernard W. 199
 Bernardi M. 124
 Berrag B. 120
 Bertolino S. 21

Besier R.B. 120
 Bessonov A. 97
 Bettelli G. 196
 Betti A. 40, 67
 Bettinelli L. 55
 Beugnet F. 67, 81
 Bezerra H.S. 203
 Beziade O. 40, 82
 Birtles R.J. 79, 83
 Bishop S. 73
 Bisset S. 149
 Bjørn B. 183
 Blagburn B.L. 199
 Blaha T. 54
 Blaxter M. 113
 Bledsoe D.L. 199
 Boa M. E. 7
 Boag P. 105
 Boersema J. 170
 Boes J. 32, 139
 Bompani A. 132
 Boni M. 81
 Boomker J. 121
 Borda T. 64, 65
 Bordin E.L. 118
 Boshoff H.M. 128
 Bosi G. 45
 Bourdeau P. 10, 40, 65, 66, 82, 101
 Bourdoiseau G. 67
 Bowman D.D. 92
 Boyd P.C. 108
 Boyezuk D. 38
 Braca G. 41
 Branco F. 170
 Brandt J. 6, 10, 83, 93
 Brandt K.S. 109
 Braun M. 165
 Braune G. 177
 Breborowicz D. 8
 Bresciane K.D.S. 160
 Bret-Bennis L. 191
 Breton C. 101
 Brewer M.M. 165, 166
 Briceño L. 52
 Brochier B. 10, 176
 Brooks A. 193
 Broom D.M. 192
 Broqua C. 126
 Brown D. 193
 Bruni R. 134
 Buchmann K. 45
 Buckle T. 170
 Bulman G.M. 95, 132, 133
 Bulman M. 118
 Buret A.G. 9, 149
 Burkindine S. 206
 Buxton D. 16

Byars D. 199
 Cabaj W. 18, 19, 34
 Cabaret J. 96, 120
 Cabral D.D. 21
 Cacciò S. 109, 186
 Cadiergues M.C. 66, 93, 102, 160
 Cafarchia C. 161
 Campbell J. 141
 Campero C. 20
 Campo R. 37
 Campos A.K. 129
 Canestri Trotti G. 21
 Cannas A. 17
 Cantaloube B. 93
 Capelli G. 19, 31, 52
 Capucchio M.T. 190
 Carabin H. 7
 Cárdenas C. 52
 Cardillo N. 67
 Carelle M.S. 46
 Carelli G. 80
 Carfagna G. 57
 Cargill C. 121, 150
 Carlson A.C. 54
 Casiraghi M. 61, 91, 93
 Castagnolli C. K. 152
 Castagnolli K.C. 21, 160
 Castellà J. 18
 Castillo R. 157
 Catley A. 63
 Cattadori I. 84
 Catton G. 199
 Ceci L. 80
 Cecilian F. 141
 Ceri H. 33
 Chaka G. 83
 Chandrawathani P. 19, 122
 Chang Reissig E. 61
 Chapman M. 31, 165
 Chapman M.R. 32
 Charleston W.A.G. 166
 Chartier C. 120, 126
 Chauvin A. 36, 85, 98
 Cheah T. S. 19
 Chick B. 131, 170
 Chiejina S.N. 37, 74
 Chirico J. 123
 Chomicz L. 101, 177
 Choromanski L. 16, 18, 19
 Chowdhury E. H. 150
 Christensson D.A. 183
 Church S. 200
 Cielecka D. 9
 Çirak V.Y. 59
 Citroni D. 83, 102
 Citroni H.D. 87

Citterio C. 25
 Citterio C. V. 61
 Claerebout E. 183
 Coadwell W.J. 105
 Coati N. 100
 Coelho L.H. 58
 Colantonio M. 152, 154
 Cole s. 29
 Coles G. C. 161, 166, 167, 168, 169, 171
 Coles G.C. 57, 61, 132, 158, 167, 169
 Colwell D. D. 35, 190
 Comer K. 166
 Conder G. 124
 Cook V. 106
 Cooke D. 99, 139
 Coop R.L. 30, 55, 117, 122
 Corba J. 171
 Corke M.J. 192
 Corona S. 91
 Corwin A.L. 68
 Costa A.J. 21, 22, 118, 128, 152, 154
 Costa G.H.N. 21, 160
 Couthier A. 105
 Couto L.B. 154
 Craig H. 29
 Cramer L.G. 59
 Cringoli G. 52, 81
 Crosa 46
 Croubels S. 147, 153
 Cruel J.N. 54
 Cruickshank I. 191
 Cruthers L. 123
 Cruz J. 125
 Csizsmárová G. 68, 98
 Cunningham L.D. 38
 Cupp E. 102, 109
 Cupp E.W. 205
 Cupp M. 109
 Cupp M. S. 102, 205
 Curioni V. 88
 Czaplińska U. 62
 Czeszczyszyn T. 133

 Dabara V.A. 84
 Dadhich H. 38
 D'Agostino B. 83, 87
 D'Agostino B.I. 102
 Dalik M. 167
 Dalton J.P. 141
 Damriyasa I M 8, 64, 66, 168, 169
 Das P. M. 53
 Daugschies A. 11, 23, 33, 93, 142, 199
 David J.R. 203
 Davidson J. 168
 Davoust B. 81
 Davydov O. 47
 Dawson D.A. 193

 Dawson K. 200
 Day M.J. 79, 83
 De Backer P. 147, 153
 De Bont J. 33
 De Bruin C. 87
 De Giacomo M. 109
 De Laurentis N. 161
 De Meerschman F. 19
 De Souza W. 94, 100
 De Torres R. 10, 40
 de Vries E. 108
 De Vries E. 108, 111
 Degorce F. 40, 101
 Degregorio O. 10
 Dellepiane M. 135
 Deloffre P. 66
 Delorn A.L. 160
 Demiaszkiewicz A.W. 57
 Deng K.D. 127
 Denholm I. 199
 Deplazes P. 5, 175, 192
 DeRisio L. 25
 Devignon C. 94
 Dharmawan N.S. 64
 Di Domenico D. 132
 Di Giulio G. 81
 Di Marco V. 190
 Díaz A. 35
 Díaz-Hernandez A. 122
 Diez-Banos P. 35, 190
 Dimander S.O. 55
 Ding X. 24
 Diop C. 58
 Divina B. P. 54, 131, 196
 Dobson R.J. 120
 Dohoo I.R. 204
 Doligalska M. 34
 Domeneghini C. 45
 Domingo D. 206
 Donald D. 97, 105, 106
 Donnelly C. 169
 D'Orazi A. 203
 Dorchies P. 37, 190, 191
 Dorchies Ph 126, 127, 147, 190
 Dorny O. 125
 Dorny P. 6, 7, 10, 183
 Drag M. 110
 Dreyer F. H. 134
 Drózd J. 57
 Dryden M.W. 199, 206
 Du Plessis A. 124
 Dubey I.P. 141
 Dubinsky P. 66, 175
 Duchene A. 10
 Duffield T. 15, 20
 Dugast E. 10
 Duncan R. 74

- Dupuy J. 148
 Durand A. M. 87, 151, 152
 Durand D. 128
 Durantón C. 37, 191
 Duscio B. 106
 Dvoinos G. 133
 Dwinata IM 64, 66
 Dziemian E. 140
 Dzimianski M.T. 101, 161, 206
- Earle C. G. 167
 Echevarria F. 128, 147
 Echeverría J. 152, 154
 Eckert E. 11
 Eddi C. 165, 170
 Edelhofer R. 8, 20
 Edge V. 20
 Edmonds J. 31
 Eduardo S.L. 54
 Edwards G.B. 168
 Egan D. 84
 Eisler M.C. 143
 El Bahy M. 124
 El-Azazy O.M.E. 63
 El-Bahy N.M. 59
 El-Bastawesy R. 59
 El-Ghayish A. 141
 El-Kasaby A. 150
 El-Metenawy T.M. 63
 El-Seify M.A. 59
 Engelen M.A.C.M. 161
 Epe B. 183
 Epe C. 55, 99, 100, 139, 143, 160
 Erhardt G. 75
 Erhart A. 6
 Eriksen L. 32, 53, 86
 Errecalde J.O. 152, 154, 181
 Ersbøll A.K. 53
 Escudero A.
 Esper C.R. 22
 Esposito F. 5
 Estrada Peña A. 83, 87
 Evans L.M. 193
 Evans N.A. 206
 Eydal M. 124, 125
 Eysker M. 30, 51, 108, 111, 187, 195
- Faccini J.L.H. 96
 Facuri-Filho E.J. 126
 Faedo M. 122
 Fain A. 65, 101
 Fair C.J. 61
 Faizal A.C.M. 45
 Fakae B.B. 37
 Falconi F. 192
 Fallah E. 39
 Farkas R. 170
- Favia G. 84, 88, 140
 Fels J.B. 121
 Ferrari N. 84
 Ferrer D. 18
 Ferreras M.C. 37
 Ferro M.I.T. 22
 Ferry F. 95
 Ferry F. R. A. 96
 Fiel C. 55, 56
 Fiel C.A. 129
 Finn N. 169
 Fisher E. 169
 Fitzpatrick C. 141
 Flores S.G. 159
 Floris I. 132
 Focant C. 19, 20
 Fogarty E.A. 92
 Foggin C.M. 91
 Folaranmi D.O. 57
 Forbes L.B. 6
 Fossati A. 134
 Fox M.T. 31
 Fragosó G. 35
 Franc M. 66, 93, 102, 160
 Franceschi A. 41
 Frangipane di Regalbono A. 19, 143
 Frank G.R. 185, 205
 Frappier D. 128
 Fraquelli C. 69, 175
 Frasinelli C. 129
 Frayssinet L. 131, 170
 French D.D. 165
 Fromeaux-Cau C. 101
 Frontera E.M. 38
 Frosio G.D. 196
 Fuentes M. 124
 Fujisaki K. 86, 112, 113, 142
 Fukumoto S. 86, 142
 Fulton L. 97
 Furr M. 199
 Furuta P.I. 21
 Fusé L. 55, 56
 Fusé L.A. 129
- Gaafar N. 141
 Gabai G. 31
 Gabler C. 167
 Gabriél S. 7, 33
 Gabrielli S. 140
 Gaffuri A. 196
 Gaglio G. 53
 Gahlot A.K. 62
 Gaines P.J. 109
 Gajadhar A. 141
 Gajadhar A.A. 6
 Galli P. 46
 Galuppi R. 46

Gamble H. R. 54, 108
 Gamble R. 6
 Garaguso M. 159
 García G. 35
 García P.M. 165
 García-Iglesias M.J. 37
 García-Marín J.F. 37
 Garippa G. 132
 Garossino K. 119
 Gasnier N. 120
 Gasser R. B. 105
 Gatehouse T. 29
 Gatongi P.M. 125
 Gatti S. 5
 Gauly M. 75
 Gaxiola C.S.M. 74
 Gázquez A. 38
 Geerts S. 6, 10
 Genchi C. 25, 84, 88, 91, 93, 134, 135, 140, 141, 184
 Gennari S.M. 127
 Geslani K.B. 131
 Gesta S. 110
 Gevorgian H. 176
 Gevorgian G. 35
 Giangaspero A. 91, 112
 Giannetto S. 53, 57
 Giari L. 45
 Gibbons L.M. 100
 Gibson W.C. 39
 Gierczak A. 9
 Gilleard J.S. 74, 105
 Gimba D. 57
 Gimenez R.A. 134
 Githigia S. 118
 Githiori J. B. 148
 Gloria-Faustino M.A. 67
 Goldová M. 60, 68, 98
 Gomes R.A. 152
 Gómez L. 64, 65
 Gonçalves I. 128
 Gonçalves Ruiz P.M. 98
 Gonchigoogin B. 54
 González-Lanza C. 34
 González-Lanza C. 37
 Gopal R. M. 126
 Gottstein B. 16, 17, 140, 175
 Goudeau C. 126
 Gowing H. 24
 Gradoni L. 203
 Graham M. 105
 Graminha E.B.N. 128, 152
 Gray J. 82, 86
 Gray J.S. 40, 84
 Greco B. 80
 Green L. 132
 Green R.S. 29, 36
 Greenwood C.A. 97
 Greif G. 17
 Grewing M. 159
 Griffiths T. 97, 106
 Griffiths T. 205
 Grillo T. V. 74
 Grimbeek R.J. 139
 Griset C. 147
 Grisi L. 156, 170
 Grønvold J. 92
 Gross S. 118
 Grout E. 171
 Grunder S. 5
 Grytner-Ziecina B. 9, 177
 Grzonka E. 25
 Guberti V. 196
 Guerrero J. 185
 Guglielmone A.A. 159
 Guimaraes M.P. 58, 126
 Gundlach J. 170
 Gunnarsson A. 86
 Guselle N. 17
 Gustafsson M.K.S. 105, 110
 Guthrie A. J. 123
 Gutiérrez J.F. 18
 Guy C. S. 17
 Guy F. 17
 Gyr P. 80
 Habluetzel A. 5
 Hack R. 206
 Hadley P. 132
 Hajjaran H. 39
 Hall M.J.R. 191
 Hallu R. 67
 Ham A.A. 133
 Hamamoto K. 124
 Hamilton C. 16
 Han Q. 113
 Hansen J.W. 139
 Hansen O. 199
 Hanzelová V. 108
 Haralabidis S. 68
 Harder A. 107, 151, 156, 157, 158
 Harrison L.J.S. 143
 Hartman D. 97, 105, 106
 Harutunyan M. 75, 157
 Hasse D. 105, 106
 Havemann A. R. 124
 Hayasaki M. 142
 He B. 111
 Heckeroth A.R. 15
 Heimsath S.F. 133
 Hein W.R. 29
 Heinen K. 123
 Hellmann K. 55, 117
 Hennessy D. 148, 155, 181
 Hennessy D.R. 147

- Hereu C. 87
 Hernández A. 157
 Hernández M. 35
 Herphelin F. 113
 Hertzberg H. 120
 Hetzel N. 167
 Hewakopara A. 45
 Hietala S. 15
 Hilali M. 141, 150
 Hill D. 108
 Hill D.E. 38
 Hillyer M. H. 166, 169
 Himonas C.A. 191
 Hinkle N.C. 199
 Hirata H. 54, 112
 Hobson J. 15, 20
 Hodgkinson J.E. 168, 187
 Hodjati M.H. 203
 Höglund J. 55, 106, 122, 148, 170, 187, 196
 Holdsworth P. 124
 Holland W. 6
 Holmes P.H. 126
 Holm-Martin M. 200
 Honza M. 98
 Hopkins T. 123, 199
 Hopkins T. J. 80
 Horak I. G. 79
 Horohov D. 31
 Hoste H. 37, 126, 127, 189, 191
 Houdeshell L. 206
 Houdijk J.G.M. 30, 188
 Houffschmitt P. 131, 156, 165
 Hourcq P. 93
 Hove T. 25
 Hovnanyan G. 99
 Huang C.C. 23
 Huang W. 111
 Hudson P.J. 79, 84
 Huerta M. 35
 Hughes V. 168
 Huh O. 124
 Huiskes J.H. 121
 Hunter J.S. 151
 Huntley I. 117
 Huntley J.F. 30, 73, 192
 Hutchinson M.J. 131

 Ibarra F. 125, 157
 Ibisch C. 101
 Ibrahim I.-N. 68
 Igarashi I. 54, 86, 112, 113, 142
 Iglesias L. 128, 129
 Ikadai H. 112
 Imperiale F. 148, 154, 155
 Inada T. 96
 Innes E.A. 16
 Inoue N. 112, 142

 Irasola C. 130
 Irion T. 18
 Irion T.C. 24, 25
 Irwin J. 151
 Irwin P. 10
 Isaeva N. 47
 Islam M. K. 53
 Islam M.Z. 166

 Jackson E. 55, 73, 117
 Jackson F. 30, 55, 73, 74, 117, 122
 Jacobs D. E. 100, 131, 199
 Jacobs J. 147, 153
 Jacobson L. S. 81
 Jacquiet P. 37, 58, 191
 Jamali R. 11, 68
 Jammah O. 122
 Janse van Rensburg B. 24
 Jara M. 52
 Jaramillo L. 60
 Järvis T. 9
 Jeannin P. 150, 151
 Jedlina-Panasiuk L. 35
 Jennings A. 168
 Jensen J.R. 36
 Jensen O.L. 7
 Jensen T. K. 32
 Jiang J. 22, 24, 112
 Joachim A. 11, 33, 93, 142
 Johansen M. V. 155
 Johnson A.M. 15
 Johnson C.M. 205
 Johnston K. 55
 Jokela S. 6
 Jones K.M. 156
 Jonsson N.N. 143
 Josh P.F. 122
 Joubert C. 102
 Juárez G. 58
 Juyal P. D. 39, 149

 K sik M. 35
 Kagaruki L. K. 85
 Kahn L.P. 31
 Kalthoff V. 23, 25
 Kalthoft V. 23
 Kamau D.L. 63
 Kamio T. 113
 Kamiya M. 177
 Kang'ethe E.K. 143
 Kanobana K. 30
 Kanyari P.W.N. 5
 Kapel C.M.O. 5, 92, 100, 196
 Karbowiak G. 62
 Karim J. 150
 Kasper L.H. 21
 Kassuku A.A. 7, 51, 75

Kato S. 92
 Katunguka-Rwakishaya E. 126
 Kelton D. 15, 20
 Kennedy T. 24, 25, 199
 Kenny M.J. 79, 83
 Kent J.B. 57
 Kenyon F. 113
 Kervyn T. 176
 Kerwick C. 80
 Ketzis J. 92
 Key E.L. 166
 Keyyu J.D. 7, 51
 Khalaf S. 30, 36
 Khudesta A. 63
 Killick-Kendric R. 203
 Kim D.Y. 22
 Kim J.T. 16, 22, 140
 Kington H. A. 167
 Kirvar E. 16
 Kirwan J.A. 61
 Kiseliene V. 96
 Kitagawa H. 185
 Kitoh K. 185
 Kivaria F.M. 85
 Klei T. 31
 Klei T.R. 32, 165
 Knoppe T. 55, 117
 Knox D.P. 29, 113
 Knox M. R. 122, 127, 188
 Kociecka W. 8
 Kofta W. 33
 Kolodziej-Sobocinska M. 140
 Königová A. 66, 171
 Kooyman F.N.J. 30, 51
 Körber R. 25
 Kordylewska M. 8
 Korneva J. 99
 Kosuth P. 46, 60
 Kotze A.C. 106, 120, 147
 Kowalewski C. 101
 Kraglund H.O. 92
 Krämer F. 143
 Kramer L. 25, 93, 195
 Kramer L. H. 184
 Krecek R. C. 123, 124, 128, 139
 Kristensen T. 124
 Kritzner S. 17
 Kumar R. 35
 Kurowsky L. 47
 Kuzmina T. 127, 133
 Kyriazakis I. 117, 122
 Kyriazakis I. 30
 Kyvsgaard N. 51
 Kyvsgaard N.C. 51, 75

 La Rosa G. 91, 97
 Labarthe N.V. 67

 Lachowicz J. 19, 57
 Lacinio R. 80
 Lacroux C. 191
 Lamberti J.C. 95, 132, 133
 Lamparska M. 33
 Lanfranchi P. 61, 196
 Lanfredi R.M. 94, 95, 96, 100
 Langholff W.K. 59
 Lanusse C. 148, 154, 155, 181
 Larramde C. 35
 Larsen Enemark H. 183
 Larsen K.S. 79, 83, 160
 Larsen M. 122
 Larsen M.N. 92, 118
 Lartigue E. del C. 129
 Latour S. 81
 Lauro T. 18
 Lawal A.I. 84
 Lazar P. 60
 Le Dinh Cong 6
 Le Pla J. 169
 Lea J.M. 31
 Leathwick D.M. 149
 Leclair D. 6
 Leclipteux T. 19, 20, 94, 113, 131
 Lee J.-K. 140
 Lefrileux Y. 126
 Leignel V. 96, 120
 Leira W. 96
 Lekimme M. 94, 113, 131
 Lerga A.I. 79, 83
 Leslie K. 15
 Letková V. 46, 60, 68, 98
 Letonja T. 124
 Letty B.A. 139
 Lewa A.K. 168
 Lewis T. 206
 L'Hostis M. 85, 98
 Li A. 112
 Li G. 110
 Li J.Y. 113
 Lia R. 81, 159, 161
 Liang Y.S. 158
 Lichtenfels J.R. 187
 Liébano E. 125
 Liebisch A. 85, 130, 160
 Liebisch G. 85, 130
 Lifschitz A. 148, 154, 155
 Lima W. S. 58, 126
 Lindsey M. 200
 Link M. 91
 Lissemore K. 15, 20
 Literák I. 98
 Liu Q. 112
 Ljunggren E. 109
 Lo N. 88
 Lomax M.A. 117

Lomba C. 37
 Lopes C.W.G. 94
 Lopez C. 10
 López C. 35
 Lòpez E.V. 130
 Lopez H.S.R. 74
 Lopez-Rebollar L.M. 24, 194
 Lopez-Sandez C. 190
 Losson B. 19, 20, 94, 113, 131, 176
 Louvandini H. 127
 Louw J.P. 151, 152
 Love S. 187
 Lubega G.W. 57, 110
 Lucas S.F. 54, 131
 Luguru S.M.K.J. 86
 Lukesova D. 128
 Lützel Schwab C. 55, 56, 129
 Lynch A. 169

Machell J. 192
 Machila N. 143
 Machnicka B. 140
 Machuca M. 20
 Maciel A.E. 110, 118
 MacKay R. 199
 MacKellar A. 30
 Madder M. 83, 93
 Maddox Hyttel M. 183
 Maddux J.D. 109
 Madzingira O. 125
 Maeda G.E.M. 7
 Mage C. 18, 58
 Magnino S. 25, 196
 Magona J. W. 143, 153
 Magwisha H.B. 75
 Maia A.S. 128
 Mainar-Jaime R.C. 149, 155, 155
 Maingi N. 119, 168
 Makumi J.N. 39
 Malacco M. 118
 Malakauskas A. 5
 Malandrin L. 85
 Malavasi G. 132
 Maldonado A. JR 95
 Maley S. 16
 Maljutina T.A. 111
 Mallet X. 93
 Malone J. 124
 Mañas F. 18
 Mancebo O.A. 95
 Manfredi M.T. 69, 84, 175
 Manga-González M.Y. 34, 37
 Mansfield L.S. 38, 156
 Mansour A. 142
 Mansour A.E. 161, 206
 Marchington T. 106
 Marcos C. 184

Marcovics A. 68
 Máriássy A.T. 98
 Mariette F. 66
 Markham F. 204
 Maroli M. 203
 Martignoni L. 10
 Martin S.W. 20
 Martínez J.J. 35
 Martín-Gómez S. 149, 155
 Martins S.M. 98
 Martini K. 92
 Marucci G. 91, 97
 Masuku M. 33
 Mathis A. 5
 Mathis G.F. 24, 25
 Matjila P.T. 17
 Matthee S. 123, 134
 Matthews J.B. 168, 187
 Mattiucci S. 108
 Mattos C. 87, 102
 Mattos C.E. 83
 Mattsson J. 106
 Mattsson J.G. 109
 Mavenyengwa M. 36
 Mazloumi Gavgani A. S. 203
 McAllister T.A. 119, 149
 McBean D. 73
 McCall J. 151
 McCall J.W. 93, 101, 131, 142, 161, 184, 201, 206
 McCall S.D. 206
 McCarroll J. 124
 McClure S.J. 106
 McDonnell P.A. 9
 McEwen B. 15
 McGarry J.W. 17
 McKay J. 17
 McLauchlin J. 186
 McTier T.L. 206
 Meana G. 55
 Meditna D. 118
 Mehlhorn H. 159, 199
 Mejer H. 53, 117, 183
 Mejia M. 151
 Mencke N. 10, 123, 131, 160, 199
 Méndez M. 58
 Merchant M. 73
 Mercier P. 131, 156, 165, 170
 Merli M. 175
 Mesquita E.F.M. 94
 Mestorino N. 152, 154
 Meyer A. 120
 Michael L.M. 128
 Mieszczanek J. 29, 33
 Migani M.B. 22
 Mignon B. 176
 Mignone W. 135
 Mikami T. 86, 112, 113, 142

Mikkonen T. 9
 Milillo M.A. 161
 Miller H.R.P. 192
 Miller I. 9
 Milligan D.N. 119
 Milnes A. 132
 Minarik J. 87, 151, 152
 Mineo J.R. 21
 Mineo T.W.P. 21
 Mira G. 40
 Miranda Z.B. 94
 Mirck M. 183
 Mitchell G.B.B. 55
 Miterpáková M. 66, 175
 Mizzoni V. 203
 Moebedi I. 11
 Moen I.C. 149
 Mohebalı M. 39
 Mohite H. 203
 Mojumder S. 84
 Møller K. 32
 Molloy J. 85
 Mondal M. M. H.53
 Monrad J. 36, 51
 Monteagudo-Rodríguez M. 149, 155
 Monzón CM 95
 Moore P. 20
 Moore R. 31
 Moraes Neto A.H.A. 100
 Morchon R. 184
 Morck D.W. 33
 Moreau E. 36
 Morriss C.J. 54, 166
 Morrondo P. 35, 37, 40
 Morrondo-Pelayo M. 190
 Mortarino M. 135
 Moseti F. 73
 Moskwa B. 18, 19, 34
 Moureu A.M. 37
 Mouritsen K.N. 61
 Mousa M. 141
 Movsessian S. 95
 Movsessian S. O. 111
 Mrozewicz B. 8
 Mugambi J.M. 73
 Mukaratirwa S. 25, 36, 56, 91, 125
 Mulcahy G. 24, 82, 141
 Müller N. 140
 Mundt H. C. 23, 25, 142, 199
 Munn E. 106
 Munn E.A. 97, 105, 205
 Munyua W.K. 134, 168
 Murat K. 32
 Murray J. 29
 Murrell K.D. 32, 39
 Musisi G. 153
 Musongong G.A. 37

Muturi K.N. 117
 Mwangi J. 63
 Mwayi W. 52, 80
 Mwazonga M. 73
 Mwendia C.M.T. 39
 Mwendia C.M.T. 63
 My L.N. 6

 Nagasawa H. 86, 112, 113, 142
 Najle R. 55
 Nanteza A.S. 57
 Nascimento A.A. 21, 152
 Nasinyama G. 52, 80
 Näslund K. 109
 Natale A. 196
 Navarrete I. 38
 Navathong P. 54
 Ndung'u J.M. 39, 63
 Neuwirth L. 142, 161
 Newton S. 97, 105, 106, 205
 Ng'ang'a C.J. 119
 Ngatia T.A. 168
 Nginyi J.M. 125
 Ngotho J.M. 39
 Ngotho J.W. 56, 134
 Ngowi H.A. 7
 Nguyen Van De 6
 Nijzink A. 51
 Nikander S. 9
 Nikogossian M.A. 95
 Nikolaou S. 105, 106
 Nilsson O. 187
 Nishikawa Y. 142
 Njiru Z.K. 63
 Njomo S.M. 73
 Njue A.107
 Nnadi P.A. 37
 Nöckler K. 64, 66
 Noh J.U. 16, 22
 Nolan J.V. 127
 Nolan T.J. 185
 Nolsø A. 61
 Norman R. 79
 Novak P. 128
 Ntwasa M. 107
 Nunes R.F. 58
 Nuttall P.A. 205
 Nya F.V. 64
 Nylund M. 9

 O'Brien D. 40, 193
 Obwolo M. 36
 Ochanda H. 143
 Ochola D.O. 110
 Ochs H. 192
 Ogunrinade A. 107
 Oh H.G. 16, 22

O'Handley R.M. 149
 Oivanen L. 6, 9
 Okano K. 124
 Okello-Onen J. 52, 80
 Okomo M. 73
 Olaechea F.V. 61, 130
 Olaho-Mukani W. 153
 Oliveira A. 94
 Oliveira Lima J.W. 203
 Olson M. E. 9, 17, 33, 119, 149
 O'Neill S. 141
 Onyango-Abuje J.A. 143
 Ooi H.K. 23
 Orabi F. 150
 Orsel K. 51
 Ortuño A. 18
 Osterman Lind E. 187
 Otero J. 60
 Otieno R.O. 56
 Otranto D. 81, 91, 112, 143, 159, 161, 190
 Ouma J.O. 63
 Oyajide A.O. 5
 Ozols V.V. 109

Padilha T. 128
 Padilla M.A. 54
 Padungtod P. 156
 Paiva F. 34
 Palmer D.G. 120
 Panadero R. 35, 37, 40
 Panadero-Fontan R. 190
 Pandey V.S. 125
 Paolini V. 127
 Papadopoulos E. 122
 Pape M. 107, 111
 Paquet-Durand I. 143
 Park J.Y. 16, 22
 Parker L. 193, 200, 201
 Parker S. 141
 Parkins J.J. 126
 Parr S. 40
 Parra F. 190
 Parzy D. 81
 Pasotto D. 31
 Passeri B. 25
 Passos L.M. 98
 Pastusiak K. 18, 19, 85
 Patsoula E. 68
 Payne P. 199
 Payne-Johnson M. 206
 Paz A. 35
 Paz-Silva A. 37, 40
 Pearce J.C. 57
 Pedreira J. 37
 Pedrotti L. 69
 Pengo G. 150
 Penzhorn B. 81

Penzhorn B.L. 17
 Peregrine A.S. 15, 20
 Pereira L.R.L. 154
 Pérez C. 37
 Pérez Mata A. 165, 166
 Perez Tort G. 67
 Pérez-García J. 149, 155
 Permin A. 75
 Pernthaner A. 29
 Perotti R.A. 130
 Pesoa J. 152, 154
 Peter R.J. 79, 80
 Petit S. 65
 Petkevicius S. 39
 Petkeviciute R. 95
 Petlele E. 24
 Petry F. 11
 Pettit D. 29
 Pettit J.J. 73
 Pfister K. 118, 204
 Pfukenyi D.M. 56
 Phiri I. K. 7, 33
 Piccolo G. 69
 Pichon B. 84
 Pietrobelli M. 31, 143
 Pineau T. 110
 Pinheiro A. 147
 Pis A. 154
 Pisanu A. 10, 40
 Ploeger H.W. 30, 51, 108
 Plucienniczak A. 35
 Poddubnaya L. 94
 Poglayen G. 53, 57
 Polinas L. 132
 Pollmeier M. 150
 Polozowsky A. 133
 Pomroy W. E. 15, 126, 166
 Popp A. 23
 Pors I. 126
 Poujade A. 40, 101
 Poumay Y. 94, 113
 Povah C. 167
 Pozio E. 9, 91, 97, 109, 186
 Presidente P. 97, 106
 Previsiol K. 175
 Prévot F. 147, 190
 Prevot F. 37, 191
 Prichard R. 182, 201
 Prichard R.K. 107, 110
 Prieto G. 141, 184
 Prieto O. 64, 65
 Prokopowicz D. 140
 Prosl H. 20, 167
 Proudman C.J. 168
 Provin N. 65
 Przemeck S. 30
 Przemeck S.M.C. 36

Puccini V. 81, 159, 161
 Puel O. 110
 Pugliese A. 79
 Putri M. 68
 Pytloch P. 133

 Qian B. Z. 52
 Quintero J. 60
 Quintero M.T. 58, 60
 Quiroz H. 125, 157
 Quiróz RG 95

 Radeloff I. 117
 Rae A. 16
 Rahman M. H. 63, 84
 Rajapaksha G. 45
 Ralston B.J. 119
 Rambags P.G.M. 121
 Ramos E. 125
 Ramsey Y.H. 187
 Rasmussen T. 32
 Reay R. 171
 Rebollo M. 18
 Reche M. 67
 Reed S. 199
 Rees B. 123
 Reggis J. 87
 Rehbein S. 59, 124, 153
 Reichel M.P. 15
 Reid S.W.J. 54, 166
 Reimann U. 160
 Reina D. 38
 Reist M. 118
 Reiterová K. 175
 Ren P. 105
 Rettinger C. 20
 Reynolds G.W. 31, 36
 Rezende P.C.B. 154
 Ribeiro M.F.B. 98, 126
 Ribicich M. 40, 67
 Richter S.H. 124, 125
 Riili S. 190
 Rinaldi L. 52, 81
 Rinder H. 5
 Rinke M. 23
 Ríos A. 125
 Ríos Centeno A. 165
 Ritter L. 54
 Riveau G. 33
 Rizzoli A. 79, 84, 88, 140
 Roberstson I. 10
 Roberts R.E. 161
 Robinson S. 200
 Robles C.A. 61
 Robles L.C. 34
 Rocha J. 97
 Rock D. W. 201

 Rodgers S. 16
 Rodrigues M.D.L. 187
 Rodrigues M.L.A. 91, 96
 Rodríguez E. 55, 128
 Rodríguez I. 206
 Roepstorff A. 32, 53, 92, 117, 139, 183
 Rogan M. T. 176
 Rojo F. 37, 40
 Rojo-Vázquez F.A. 149, 155
 Romano A. 87, 88, 130, 158
 Romano P. L. 87, 88, 130, 158
 Romasanta A. 40
 Romero J.A. 40
 Rosa A. 10, 67
 Rosà R. 79, 84
 Rossanigo C.E. 129
 Rossi G. 41
 Rossi L. 202
 Roulet A. 110
 Roussel E. 134
 Roy A. K. 84
 Rubaire-Akiiki C. 52, 80
 Rugaimukamu E. 7
 Ruggeri L. 132
 Rugutt M.K. 125
 Rust M.K. 199
 Ruttkowski B. 93, 142

 Saalfeld B. 199
 Sacchi L. 91, 93
 Saeed I. 61, 92, 100, 196
 Sager H. 16, 17, 140
 Sala M. 61
 Sallovitz J. 148, 154, 155
 Samson-Himmelstjerna G. v. 139
 Samson-Himmelstjerna G. von 107
 Samson-Himmelstjerna von G. 99, 107, 111
 Sanchez H.F. 18
 Sanchez J. 204
 Sánchez-Andrade Fernández R. 37
 Sánchez-Andrade R. 40
 Sandhu B. S. 39
 Sangster N.C. 147
 Sani R. A. 19, 100
 Sankari S. 6
 Santa Cruz A. 64, 65
 Santamarta D. 93
 Santoni R. 88
 Santos J.M. dos 128
 Sasaki Y. 185
 Sato M.O. 34
 Saumell C. 55, 56, 128, 129
 Savin K. 105, 106
 Sayyaf Dezfuli B. 45
 Sbordi L.C. 87, 88
 Scaglia M. 5
 Scaife J. 117

- Scala A. 53, 57
 Scandrett B.S. 6
 Scarpelli L.C. 22
 Schaper R. 159
 Schares G. 66
 Scheibler N. 64, 65
 Schein E. 25, 60, 66
 Schellenberg R. 6
 Schimmel A. 159
 Schiønning S. 79, 83
 Schlüsche A. 159
 Schmid H. 87
 Schneider T. 100, 107, 111, 143
 Schock A. 16
 Schoeman T. 81
 Schöffel I. 177
 Schuster R. 11, 46, 60, 67, 170, 177
 Schwendenwein I. 167
 Sciutto E. 35
 Scott I. 30, 31, 36, 139
 Scott K. 9
 Sebbag H. 36
 Sellins K.S. 205
 Selnik B. 59
 Seo H.S. 16, 22
 Sereda M.J. 177
 Serrano F. 38
 Serri G. 113, 131
 Sexton J. 106
 Shanks D.J. 206
 Sharma G.D. 38
 Shaw R.J. 29
 Shaw S. E. 79, 83, 194, 204
 Sheriff J.C. 147
 Shimalov V. 65, 67
 Shimalov V.T. 65
 Shuttleworth R. 168
 Siavashi M. R. 8
 Sibeko K. P. 107
 Siddiki A.M.A.M. 150
 Sievers G. 52
 Sigh J. 45
 Signorini A. 83
 Siles-Lucas M. 175
 Silva D.A.O. 21
 Silva E. 130
 Silva G.S. 152
 Silva W.W. 91, 96
 Silva L.V. de Almeida 67
 Silveira D.M. 160
 Silver G.M. 109
 Silvestre A. 120
 Simcock D.C. 139
 Simon F. 141, 184
 Simoncini L. 93, 141
 Simpson H.V. 30, 31, 36, 139
 Singh A.P. 62
 Singh D. K. 62
 Singh D.P. 11, 82
 Singh V. 62
 Singla L. D. 39, 149
 Sinistaj M. 106
 Siragusa C. 203
 Sisson J.W. 68
 Siteo C. 121
 Skerrett H. 82, 86
 Skirnisson K. 124, 125
 Slivinska K. 133
 Slone R. 123
 Slotved H-C 32
 Smekens-Leclercq M. 94
 Smith J. 105
 Smith K. E. 191, 193
 Smith R. 17
 Smith S.K. 29
 Smith T. 105, 106
 Smith W.D. 29, 34
 Snábel V. 108
 Soch M. 128
 Sohns B. 85
 Soll M. 110
 Sommerfelt I.E. 10, 40
 Sotiraki S. 46, 191
 Souza L.M. 21, 152
 Souza P.C.A. 96
 Souza R.N. 203
 Spagnolo V. 135
 Spanakos G. 68
 Speybroeck N. 83
 Spickett A. 194
 Spurlock M. S. 161
 Srinivasarao G. 62
 Stafford K. A. 61, 161, 169, 171
 Stamm L.M. 203
 Stancapiano L. 196
 Staneviciūtė G. 96
 Stanneck D. 160
 Staubach C. 67
 Steenhard N. R. 32
 Steffan P. 55, 129
 Steffan P.E. 56
 Steffens W.L. 206
 Stein P.A. 120
 Stella V. 143
 Steng G. 190
 Stevens J.R. 191, 193
 Stiebling U. 67
 Stosic P. 132
 Strydom T. 79, 80
 Suárez J.L. 37, 40
 Suarez V.H. 156
 Sukura A. 6, 9
 Supakorndej N. 161, 206
 Supakorndej P. 101, 142, 161

Suratma N.A. 64, 66
Sutherland I.A. 149, 188
Sutra J.F. 160
Suzuki N. 54, 112
Swiderski Z. 101, 177
Szabò M.P.J. 160

Tabouret G. 37, 191
Taccini E. 41
Taeymans L. 93
Tagboto S.K. 158
Tagliapietra V. 84
Taherkhani H. 176
Taira K. 100, 196
Taira K. 92
Tait S.A. 61
Tampieri M.P. 46
Tan B. 6
Tanaka T. 86
Tarantino C. 41
Tarimo M. 85
Tassi P. 80
Tauson R. 123
Taylor F. G. R. 169
Taylor H. 31
Taylor M. 192, 194
Taylor M.A. 192
Temnikhanov Y. 47
Tenter A. 64
Tenter A-M. 15
Terenina N. 110, 111
Terenina N.B. 105
Tessier Ph 147
Testini G. 81
Thamsborg S. 51, 118
Thamsborg S.M. 53, 86, 117, 188
Thilakaratne I. D.S.I. 45
Thomas D.G. 36
Thompson A.R.C. 186
Thompson R.C.
Thorin M. 98
Tierney J. 24
Tinar R. 59
Toeh D.A. 9
Toledo A. 35
Tournadre H. 120
Townson S. 158
Traldi G. 5
Traub R.J. 10
Traversa D. 81, 91, 112
Trawford A.F. 54, 166
Trees A.J. 17
Trentini M. 132
Trimnell A.R. 205
Tsuji N. 113
Tunlid A. 122
Turceková L. 175

Turic E. 152, 154
Turilli C. 19
Turkowicz M. 9
Tyrrell K.L. 120

Uggla A. 55, 187
Ulavi B.M. 57, 59, 64, 84, 153
Umoh J.U. 59, 153
Unzaga J.M. 20, 38, 62
Upcroft J.A. 9
Upcroft P. 9
Uras P. 57
Urban J.F. 38, 156
Usman S. 57

Vaarst M. 52, 80
Vakalis N. 68
Valenzuela G. 52
Vamvakopoulos N. 68
van Aarle D. 51
Van den Broek A. 192
Van Der Merwe J. S. 87, 124, 151, 152
van der Merwe J.S. 24
Van der Veer M. 108
Van Leemput L. 147, 153
Van Niekerk F. E. 134
van Schalkwyk P.C. 199
van Strijp M.F. 34
Van V.V. 68
van Wyk J.A. 34
Vangeel I. 23
Várady M. 66, 171, 175
Varandas N.P. 21, 22, 160
Vascellari M. 31
Vásquez P.V.M. 74
Vassalou E. 68
Vassilev G. D. 91, 119
Vatta A. 139
Vatta A. F. 124
Vaughn M.B. 199
Velazquez SM 165
Ven der Linde M.J. 139
Venco L. 184
Veneziano V. 52, 81
Venturini L. 20, 62
Venturini M.C. 20, 38, 62
Vera Y. 157
Vercammen F. 10
Vercruysse J. 6, 7, 33, 124, 183
Verdi JH 95
Verhagen R. 10
Vervaeke M. 10
Vervelde L. 30
Verzberger I. 68
Veys P. 23
Viallard J. 81
Vickers J. A. 167

Vieira Bressan M.C. 111
 Vieira Bressan M.C.R. 34, 36, 108
 Vila R. 35
 Villalobos N. 35
 Villapando R. 54
 Villard I. 169, 170
 Virkel G. 155
 Visser M. 153
 Visser M. 59
 Vizio E. 134
 Volmer R. 8
 von Ancken A.C.B. 21
 Vullo S. 190
 Vuotto I. 102

Wakelin D. 37, 74
 Walkden-Brown S.W. 31
 Wall R. 191, 193
 Waller P. 148
 Waller P.J. 55, 122
 Walton J.K. 15
 Walubengo J. 153
 Wamae L.W. 125
 Wanha K. 20
 Wanjek K. 46
 Wanzala W. 143
 Ward P. 5
 Waruiru R.M. 56, 134
 Wassink G.J. 126
 Webster P. 5
 Weda E.H. 56
 Wedrychowicz H. 29, 33, 35
 West D.M. 126
 Westbrook C. 82, 86
 White C. 170
 White C. R. 170
 Wicaksana P.B. 68
 Widyaningsih C. 68
 Wilder S.R. 38
 Wildeus S. 74
 Wilhelmsson E. 106
 Williams D.J.L. 17
 Williamson N.B. 15
 Willingham A.L. 7, 51
 Willingham L. 51
 Wilson S.T. 5
 Windya Adnyana I.B. 64
 Winoto I.M. 68
 Winter R. 59
 Wiceniowski M. 29
 Wisniewski N. 109
 Wita I. 62
 Witschi U. 140
 Wittstatt U. 60, 177
 Wojdan J. 18
 Wong C.W. 127
 Woodgate R.G. 120

Wright H. B. L. 167
 Wright S.E. 16
 Wüstenberg S. 23

Xie H. 110
 Ximenes T. 82
 Xuan X. 54, 86, 112, 113, 142

Yacob H.T. 37
 Yang C.H. 23
 Yatsuda A.P. 30, 108, 111
 Yilma J.M. 124
 Yokoyama N. 112
 You M. 113
 Youn H.J. 16, 22, 140

Zahner H. 8
 Zajac A. 74
 Zalunardo M. 150
 Zanginyan H. 75
 Zarlenga D. 108
 Zayatiin B. 54
 Zhang D. 102, 109, 205
 Zhang L. 22
 Zhang W.Y. 36
 Zhou X.N. 124
 Zhu X. 110
 Zimmerman J. 16
 Zintl A. 82, 86
 Zonderland J.J. 121
 Zouelfakar S. 150
 Zweygarth E.P. 194