



**VLA PARASITOLOGY GROUP**

**ANNUAL HORIZON SCANNING REPORT**

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ANNUAL HORIZON SCANNING REPORT  
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## INTRODUCTION.

This is a summary of papers published in 2008 in areas of parasitology of interest to workers in VLA.

## CRYPTOSPORIDIUM

### General Papers

#### Genetic Diversity & Zoonotic Potential of *Cryptosporidium parvum* causing foal diarrhoea.

J. Clin. Microbiology (2008) 46 (7) 2396-2398

Grinberg A and others

This paper from New Zealand showed isolates from diarrhoeic foals to be *C. parvum*. The isolates were genetically diverse, markedly similar to human and bovine isolates, and carried GP60 Ila A18G3R1 alleles, indicating a zoonotic potential.

#### Active invasion and/or encapsulation? A reappraisal of host-cell parasitism by *Cryptosporidium*. (REVIEW ARTICLE).

Trends in Parasitology (2008) 24 (11) p509-515

Barowski H. & others

The parasite appears on the surface of cells, residing in a parasitophorous vacuole (PV) between the cytoplasmic membrane and the apical membrane. The exact mode of *C. parvum* attachment to, and penetration of, intestinal mucus is not understood. Host-cell attachment and invasion is mediated by sporozoite receptors.

Sporozoites then initiate host-cell membrane protrusion that encapsulates the parasite. Excysted sporozoites of *Cryptosporidium* exhibit apical organelle discharge and gliding motility to initiate host-cell invasions. In contrast to other apicomplexans, *Cryptosporidium* does not invade the cytoplasm of host-cells but resides within a PV. This presumably allows the parasite to evade the host immune reactions.

Future research will need to develop anti-cryptosporidial drugs that either pass through the apical membrane engulfing the parasite, or via host-cell transport, reach the parasite through the feeder organelle to target intracellular stages. Also, the possibility to prevent extracellular invasive stages of cryptosporidium (merozoites) from host-cell invasion opens a new window into anti-cryptosporidial drug development.

#### Determining the zoonotic significance of *Giardia* and *Cryptosporidium* in Australian dogs and cats.

Vet. Parasit. (2008) 154 p142-147

Palmer C.S. & others.

The *Cryptosporidium* recovered from dogs and cats was identified as *C.canis* and *C.felis* respectively. This finding suggests the growing evidence that *Cryptosporidium* in companion animals probably is of limited public health significance to healthy people.

A hundred-year retrospective on cryptosporidiosis (REVIEW ARTICLE)

Trends in parasitology (2008) 24 (4) p184-189

S.Tzipori and G.Widmer

Much as been learned about *Cryptosporidium* since 1980, but the genus remains enigmatic. For instance, the intracellular, extra-cytoplasmic location is biologically unique, which might explain several characteristics including its resistance to antimicrobial agents.

Little progress has been made in the last 25 years in the development of effective treatments.

The inability to continually passage the parasite in cell culture and the inability to cryopreserve oocysts or intracellular stages are probably the most serious limitations to investigators working in the field.

**Cryptosporidial infection in cattle**

*Cryptosporidium ryanae* n sp (Apicomplexa: Cryptosporidiidae) in cattle (*Bos Taurus*)

Vet Parasit (2008) 156 p191-198

Fayer R & others

This paper describes a new species *C.ryanae*, previously identified as *Cryptosporidium* deer-like genotype. It is reported to be prevalent in cattle worldwide and is found only in *Bos taurus* calves. Identification of *C.bovis* and *C.ryanae* as the primary species of cryptosporidium found in post-weaned cattle, species neither found to infect humans nor to cause illness in livestock, helps clarify aspects of the complicated epidemiological paradigm associated with the genus *Cryptosporidium*.

Other genotypes of *Cryptosporidium* will continue to be referred to as the deer genotype and the cervine genotype.

A longitudinal study of cryptosporidiosis in dairy cattle from birth to 2 years of age.

Vet Parasit. (2008) 155 p15-23

Santin M. & others.

Faecal specimens were collected from 30 calves from birth to 24 months of age at a dairy farm in Maryland and 19.2% of 990 specimens were positive, the highest prevalence of infection was at two weeks of age when 29/30 calves were excreting oocysts. *C.parvum*, *C.bovis*, the *Cryptosporidium* deer-like genotype and *C.andersoni* were all identified with cumulative prevalences of 100%, 80, 60 and 3.3% respectively. *C.parvum* constituted 97% of infections in pre-weaned calves but only 4% and 0% of infection in post-weaned calves (3-12 months) and heifers (12-24 months).

The presence of *C.parvum* oocysts has zoonotic implications but this study demonstrates that it is crucial to know which species and/or genotypes are actually present in *Cryptosporidium*-positive samples.

Effect of *Cryptosporidium parvum* infection on the absorptive capacity and paracellular permeability of the small intestine in neonatal calves.

Vet Parasit. (2008) 152 p53-59

Klein P. & others.

The parasite damages gut epithelium, resulting in loss of villi and microvilli. This leads to a decrease in enzymatic activity and reduction of absorptive surface, leading to maldigestion and malabsorption. This impairment is mainly in the acute phase of the

disease but is still detectable 14 days post-infection. On day 21 pi no alterations in intestinal function were found. However, smaller weight gains were observed up to 21 days pi indicating reduction in performance for up to three weeks after challenge.

A Bayesian evaluation of four immunological assays for the diagnosis of clinical cryptosporidiosis in calves.

The Vet.J. (2008) 176 p400-402

Geurden T. & others.

The assays – immunofluorescence (IFA), two ELISA tests and an immunochromatographic (dipstick) assay were all found to be relatively specific and sensitive. Despite a lower sensitivity the dipstick assay provided a practical alternative to laboratory diagnosis of clinical cryptosporidiosis in calves. Its main advantage is rapid on-site diagnosis and user-friendly technique.

Prevalence and risk factors for *Cryptosporidium spp* infection in young calves.

Vet. Parasit. (2008) 152 Issues 1-2 p46-52

Brook F. and others.

The study was carried out in North West England. They found a higher risk of infection in calves aged 8-21 days compared to those aged 0-7 days. Calves housed in bedding 11-15cm deep were at lower risk of infection than those on beds 0-5cm deep. Consistency of the faeces was not highly correlated with infection which is surprising given the supposed enteropathogenicity of *Cryptosporidium*. The results suggest that intervention strategies should be targeted at calves under 21-days-old. Such calves represent a significant reservoir of infection and may also pose a risk to public health.

Preventive and therapeutic efficacy of halofuginone–lactate against *Cryptosporidium parvum* in spontaneously infected calves: a centralised, randomised, double-blind, placebo-controlled study.

The Vet. J (2008) 177 p429-431

Klein, P.

The results of the study confirmed the anti-cryptosporidial activity of halofuginone-lactate (HFL) in calves, but showed that the outcome of infection following preventive treatment is comparable to that observed in calves treated after the onset of symptoms. The only difference between both treated groups was a delay in oocyst output and the occurrence of diarrhoea, as infection in the preventatively treated group peaked about 10 days later. The benefit of using a preventive treatment with HFL is therefore questionable. Both oocyst excretion and intensity of diarrhoea were significantly reduced when given both preventively and therapeutically.

Distribution of *Cryptosporidium parvum* subtypes in calves in Germany.

Vet. Parasit. (2008) 154 p8-13

Broglia A. & others

*C. parvum* was identified in all samples examined from pre-weaned calves. 53 samples were subtyped by sequence analysis. All isolates belonged to the allele IIa (with seven subtypes), except for one belonging to the allele IIc. Three novel subtypes of the allele IIa were found. This study confirmed the utility of genotyping and subtyping tools in characterising the transmission of *Cryptosporidium spp*.

High prevalence of *Cryptosporidium bovis* and the deer-like genotype in calves compared to mature cows in beef cow-calf operations.

Vet. Parasitology (2008) 151. Issues 2-4 p191-195  
Feltus D.C. & others.

This study examined 98 calves (6-8 months old) and 114 cows (>2 years old) in North Dakota. *C.bovis* and the deer-like genotype were primarily detected in calves, while *C.andersoni* was only detected in cows. *C.parvum* was not identified in any of the samples.

**Cryptosporidial infection in sheep**

Contemporary identification of previously reported novel *Cryptosporidium* isolates reveals *Cryptosporidium bovis* and the cervine genotype in sheep (*Ovis aries*).

Parasitol Res (2008) 102 p1103-1105  
Elwin K and Chalmers R

Molecular characterization was carried out of *Cryptosporidium spp* from sheep (*Ovis aries*), sampled during the investigation of a water-borne outbreak of human cryptosporidiosis caused by *Cryptosporidium parvum*. 22 samples contained *Cryptosporidium-cervine* genotype, five contained *Cryptosporidium bovis* and four samples contained both the cervine genotype and *C.bovis*.

Regarding the zoonotic implications there are no published reports of *C.bovis* in humans, but the cervine genotype, which has a broad host range, has been reported in a small number of patients with diarrhoea.

Distribution of *Cryptosporidium species* in sheep in the UK.

Vet. Parasit. (2008) 154 p214-219.  
Mueller-Doblies D. and others

These studies showed that *C. parvum* is important in neonatal lamb diarrhoea and is widespread in sheep flocks in the UK. *C. bovis* was also found in flocks sampled reactively to a human case of cryptosporidiosis but at a lower frequency. Both *C. bovis* and the cervine genotype were found in orphan lambs at an open farm as part of a screening programme.

Activity of an anti-inflammatory drug against cryptosporidiosis in neonatal lambs.

Vet. Parasit. (2008) 155 p308-313.  
Castro-Hermida J.A. & others.

In neonatal lambs that were receiving a prophylactic/therapeutic treatment with Bobel-24 (2, 4, 6 – triiodophenol) (50mg/kg B.wt) the appearance of diarrhoea, as well as the duration and intensity of oocyst shedding were considerably reduced. Results suggest that the drug may be suitable for use in the early control of cryptosporidiosis in neonatal lambs. Further studies are required to improve drug formulation.

## **Cryptosporidial infection in alpacas**

Cryptosporidiosis in two alpaca (*Lama pacos*) holdings in the South West of England.  
The Vet. J. (2008) 175 p419-422  
Twomey D.F. & others.

This is the first report documenting genetic confirmation of *Cryptosporidium parvum* in alpacas kept in the UK. Subclinical shedding of oocysts was observed suggesting that healthy crias can act as a source of infection. This also has zoonotic implications although no associated human illness was reported on the holdings.

## **CESTODES**

### General

Effects of climate change on animal and zoonotic helminthiasis.

Current knowledge of animal and zoonotic helminthiasis in which effects of climate change have been detected was reviewed. Climate variables are able to affect the prevalence, intensity and geographical distribution of helminths, directly influencing free-living larval stages and indirectly influencing mainly invertebrate, but also vertebrate, hosts. The impact of climate change appears to be more pronounced in trematodes, and is mainly shown by increased cercarial production and emergence associated with global warming. Fascioliasis, schistosomiasis (*S. japonicum*) and cercarial dermatitis caused by avian schistosomes have been the focus of study. **Alveolar echinococcosis** is currently the only cestode disease that climate change has been found to influence. Nematodiasis, including heterakiasis, different trichostrongyliases and protostrongyliases, ancylostomiasis and dirofilariases, are the helminth diseases most intensively analysed with regard to climate change. The authors concluded that helminth diseases should be listed among the infectious diseases with which care should be taken because of climate change in the future, especially in temperate and colder northern latitudes and in areas of high altitude. [Mas-Coma, S., *et al* (2008) *Revue Scientifique et Technique-Office International Des Epizooties*,27,443]

### ***Echinococcus granulosus***

#### ***E. granulosus* vaccine development.**

The elimination of hydatid disease is difficult with current control options, but reducing egg production by *Echinococcus granulosus* in canines will help to reduce transmission in areas where the parasite is endemic. The authors review recently obtained data using recombinant protein-based and live attenuated *Salmonella* vaccines. These are preliminary but encouraging and auger well for the future development of an effective dog vaccine against *E. granulosus*, although much work is required before this becomes a reality. Important additional gains should be expected if the efficacy of the dog vaccines is confirmed and leads to their incorporation into future control options.

#### ***E. granulosus*: The establishment of the metacestode is associated with control of complement-mediated early inflammation.**

This work studied the evolution of early inflammation, complement activation and parasite survival / death along the establishment phase of the *Echinococcus granulosus*

metacestode. They examined cell infiltration and C3 deposition on individual parasites during their development from protoscoleces to cystic forms. The intensity of the initial inflammation decreased around undamaged but not around damaged parasites. At 43 days post infection undamaged parasites were mostly associated with poor / mild inflammation, while damaged parasites with strong inflammation. In addition, whereas complement activation participated in the induction of early inflammation, the deposition of C3 on undamaged parasites progressively diminished. Overall, these results indicated that the establishment and survival of the hydatid cyst is associated with the control of complement and, consequently, of local inflammation at the initial phases of infection.

[Breijo, M. *et al* (2008) *Experimental Parasitology*, **118**, 188]

### ***E. granulosus* epidemiology**

#### **The role of cattle in the epidemiology of *Echinococcus granulosus* in an endemic area of southern Italy.**

An epidemiological and molecular survey was conducted to investigate the role of cattle in the transmission chain of cystic echinococcosis (CE) in the Campania region of southern Italy. Out of a total of 434 cattle examined for CE, 45 (10.4%) were found infected. A total of 363 cysts were collected from the infected animals: 239 in the liver and 124 in the lungs. The cysts were either sterile (42.7%) or calcified / caseous (57.3%); no fertile cysts were found. Most of the cysts had sizes <3 cm (77.1%) and were unilocular (78.8%). The results did not show any significant correlation between the age of infected cattle and the number of cysts. The sequencing of 40 hydatid cysts showed 100% identity with the common sheep G1 (n = 21 cysts), the Tasmanian sheep G2 (n = 2 cysts), and the buffalo G3 (n = 17 cysts) strains, which constitute the species *Echinococcus granulosus* sensu stricto. The findings show that CE is widespread in cattle bred in the Campania region of southern Italy. However, the absence of fertile cysts and of the cattle strain (G5, *E. ortleppi*) suggests that cattle would not have any role in the persistence of this zoonosis but rather a role as indicators of CE infection in this endemic area.

[Rinaldi, L. *et al* (2008) *Parasitology Research* **102**, 815]

#### **Reintroduction of *E. granulosus* by import of cows in the Netherlands.**

This paper highlights the risk of the introduction of parasites in imported animals describing evidence of *E. granulosus* in imported cows in the Netherlands. Since East European countries joined the EU, the import of both dairy and beef cows from these countries has increased considerably. In the period from May to December 2007 about 200 cows per month were imported from Romania. These animals were either slaughtered immediately or in autumn. In autumn, cysts were noticed both in slaughtered cows during meat inspection and in deceased animals (originated from Romania) during post-mortem investigation performed by the Animal Health Service; these were confirmed as *Echinococcus granulosus* hydatid cysts.

[Aalten, M. *et al* (2008) *Tijdschrift Voor Diergeneeskunde*, **133** (21): 898-902]

#### **Cystic echinococcosis in equids in Italy.**

Between March 2003 and February 2007, the livers and the lungs of 2,231 horses from various Italian regions were examined for cystic echinococcosis at the time of slaughter.

Hydatid cysts were found in six horses (four from Sardinia, one from Sicily, and one from Tuscany). Hydatid materials obtained from positive animals were identified as *Echinococcus granulosus* (old G1, sheep strain) and *Echinococcus equinus* (old G4, horse strain). This allowed the presence of *E. equinus* to be recorded for the first time in Italy with molecular tools and also to report new data on the epidemiological situation in Italy.

[Varcasia, A. *et al* (2008) *Parasitology Research* **102**, 815]

### **Detection of *Echinococcus granulosus* coproantigens in dogs from Antakya Province, Turkey.**

Relevant epidemiological note from this study of established risk factors:

A significant difference was detected between unrestrained (or free roaming) and indoor or chained dogs ( $p < 0.05$ ). Although the prevalence of the *E. granulosus* infection in owned dogs in Antakya province is low, the incidence was quite high in the unrestrained and under-wormed dogs.

[Guzel, M. *et al* (2008) *Helminthologia*, **45** (3): 150-153]

### ***Echinococcus multilocularis***

Cost-effective method of DNA extraction from taeniid eggs.

A new cost-effective method using silicon dioxide- and guanidine isothiocyanate-containing buffers, after previous alkaline lysis, was established for the DNA extraction from taeniid eggs isolated from canine faeces. The purified DNA can be used to amplify the species-specific 12S mitochondrial DNA of *Echinococcus multilocularis* in direct and nested polymerase chain reaction in order to differentiate between *E. multilocularis* and *Taenia* spp. This provides an important tool for the investigation of the epidemiology of these species.

[Dyachenko, V. *et al* (2008) *Parasitology Research* **102**, 811]

### **Reduced egg production of *Echinococcus multilocularis* in experimentally infected and re-infected red foxes (*Vulpes vulpes*).**

Previously, the dynamics of the egg excretion of infected carnivores have been studied only where the host animals have been exposed to a single experimental infection. In nature, foxes are most likely repeatedly infected. To study the effect of repeated exposure, 21 foxes were inoculated with a high dose of *E. multilocularis* protoscoleces three times over a 1-month period. For comparative purposes, three groups of 21 foxes were respectively inoculated with low, medium, or high single dose of protoscoleces. For each group, worm number and morphology were analyzed after necropsy of seven foxes at 1, 2, and 4 months after last inoculation. The establishment of intestinal worms was very low in all foxes, and surprisingly, most of the worms did not produce eggs. Although most reproductive structures were detectable, the genital pore and the cirrus pouch often had abnormal enlargements that spread internally, most likely preventing the reproductive function. The reason for this abnormality could not be determined, but the preparation and storage conditions of the inoculated protoscoleces may have contributed to the stunted development. Physical stress of *E. multilocularis* at the larval stage in rodents may later adversely affect the reproductive success of the adult tapeworm in the carnivore definitive host.

[Al-Sabi, M.N.S. *et al* (2008) *Veterinary Parasitology* **155**, 59]

## ***Echinococcus multilocularis* - Epidemiology in Europe**

### **Fears, attitudes and opinions of suburban residents with regards to their urban foxes - A case study in a suburb of Munich.**

Foxes have taken over urban areas as their habitat throughout Central Europe. In Southern Germany, these foxes are also carriers of *Echinococcus multilocularis*. This survey was carried out in a suburb of the city of Munich. A postal questionnaire was used to analyse the attitudes, opinions and fears of the participants towards urban foxes and the background to these attitudes. Questionnaires were sent to all households with gardens; 779 (31%) of questionnaires were returned. Only a few people are afraid of the fox itself; however, 55% were afraid of the fox tapeworm. Worming the animals is the preferred counter-measure, with 81% in favour. The majority of inhabitants are pleased to see a fox in the community and feel the animals have a right to live. People are afraid of the tapeworm either because they have children in the household; because of increased knowledge of the subject; or because it has increasingly become an issue. On the basis of the results of this study, it is to be expected that radical solutions such as killing the foxes are unlikely to be accepted among the population. Worming of the foxes does, however, meet with general approval.

[Konig, A. (2008) European Journal of Wildlife Research **54**, 101]

### **Integrated-baiting concept against *E. multilocularis* in foxes is successful in southern Bavaria, Germany.**

This paper describes the design and the preliminary evaluation of an integrated approach to the control of *Echinococcus multilocularis* in foxes using praziquantel bait. Air distribution of bait in agricultural and recreational areas was combined with distribution of bait by hand in towns and villages to cover the entire fox population in the 213-km<sup>2</sup> baiting area. Bait distribution density was 50/km<sup>2</sup> and bait was distributed once every 4 weeks. Pre-baiting prevalence was 35%. During a 1-year period following the first 4 months of bait distribution, only one positive fox was found. No significant change had occurred in the unbaited control area. This prevalence decline is far more pronounced than in previous fox-baiting studies, which is likely to be due to the increased bait distribution density and baiting frequency, and the inclusion of the 'urban' fox population.

[Konig, A. *et al* (2008) European Journal of Wildlife Research **54**, 439]

### **Infection of red foxes with *E. multilocularis* in western Switzerland.**

In the Jura mountains, Plateau and Alps of western Switzerland important variations in the prevalence of *Echinococcus multilocularis* infection in red foxes were observed between geographical areas from 1990 to 1995. The Jura mountains and the Plateau had higher mean prevalence levels than the Alps with 30.6, 32.4 and 18.8%, respectively. The highest rate was recorded in the Plateau in the canton of Fribourg with a prevalence of 52.3%. The prevalence of *E. multilocularis* infection in foxes in the alpine canton of Valais was the lowest (7.1%). Juvenile foxes were found to be more susceptible to *E. multilocularis* than adults. Adult foxes were less heavily infected in summer and autumn, while the prevalence in juveniles (less than 1 year old) increased between the spring and winter, when they are more than 6 months old. The retrospective data relate to the beginning of the 1990s, since when a drastic prevalence increase of *E. multilocularis* infection in foxes has occurred in several regions of Europe. Nevertheless, the study is a major contribution to the epidemiological situation of *E.*

*multilocularis* in central Europe, in that it contains valuable information on spatial distribution and seasonal differences in different age groups of foxes. [Brossard, M. *et al* (2008) *Journal of Helminthology*, **81**, 369]

### ***E. multilocularis* infections in domestic dogs and cats from Germany and other European countries.**

A cross-sectional survey was conducted to estimate the prevalence of *Echinococcus multilocularis* and *E. granulosus* infections in domestic dogs and cats from Germany and other European countries. Faecal samples of 21,588 dogs and 10,650 cats routinely submitted to a private veterinary laboratory between June 2004 and June 2005 were examined. *E. granulosus* DNA was not detected in any sample, while, *E. multilocularis*-positive samples were detected in dogs from Germany only, those of cats originated from Germany, Denmark and The Netherlands. The prevalence of *E. multilocularis* egg-positive canine samples was significantly higher in southern (0.35%) than in northern Germany (0.13%). In contrast, no significant regional difference was observed in cats from Germany. Taeniid eggs from *Echinococcus*-negative samples and from a few samples with macroscopically detected *Taenia* sp. proglottids were identified as eggs of *T. crassiceps* ( $n = 8$ ), *T. martis*, *T. serialis*, *T. polyacantha*, *T. taeniaeformis* and *T. pisiformis* in dogs ( $n = 1$  of each) and *T. taeniaeformis* ( $n = 11$ ) in cats. The spectrum of cestodes detected in domestic dogs and cats indicate the consumption of small rodents as infection source. The high proportion of *E. multilocularis*-positive samples, suggest domestic dogs and cats as a possible source of *E. multilocularis* infection for humans. [Dyachenko, V. *et al* (2008) *Veterinary Parasitology* **157**, 244-253]

### **Emergence of *E. multilocularis* Germany 1991-2005.**

This study investigated the spatiotemporal distribution of *Echinococcus multilocularis* in Red Foxes. The infection status of 8459 foxes from 43 regions in the north German province of Lower Saxony over three investigation periods 1991–1994, 1994–1997 and 2003–2005 was investigated.

The average prevalence risk for Lower Saxony increased from about 12 to 20% during 1991–2005. Specifically the increases from first to second and to third study periods were estimated as 3.3% and 8.5% respectively. Infections in foxes were clustering and a location stable disease cluster was detected in the south of the province. This study is the first showing evidence for steady emergence of *Echinococcus multilocularis* in Red Foxes. The first cases of human Alveolar Echinococcosis were recorded recently in the region and it was noted that the long latency in humans of 5-15 years may mask a higher level of human infection.

[Berke, O. *et al* (2008) *Veterinary Parasitology* **155**, 319.]

### ***E. multilocularis* in Belgium: Prevalence in red foxes (*Vulpes vulpes*) and in different species of potential intermediate host.**

*Echinococcus multilocularis* is known to be endemic in foxes in several countries of Western and Central Europe. In Western Europe, the common vole (*Microtus arvalis*) and the water vole (*Arvicola terrestris*) are considered to be the most important intermediate host species whereas the red fox is by far the most important final host. This study provided data on the prevalences in Wallonia (Southern part of Belgium) both in the red fox and in different potential intermediate hosts.

A total of 990 red foxes were examined between January 2003 and December 2004. The average prevalence was 24.55% (22.38–27.87). Out of 1249 rodents or

insectivores belonging to the species *Apodemus sylvaticus*, *Arvicola terrestris*, *Clethrionomys glareolus*, *Microtus arvalis*, *Microtus agrestis* and *Sorex araneus*, only one *M. arvalis* (out of 914–0.11%) and one *C. glareolus* (out of 23–4.3%) were found to be infected. However, the muskrat (*Ondatra zibethicus*) seemed to be a good intermediate host as 192 of 1718 (11.2%) were found to be infected. A positive correlation was found between the prevalence in foxes and in muskrats in each of the different regions. This study indicates that the muskrat is highly sensitive to this zoonotic tapeworm and could perhaps represent a good bioindicator when studying the epidemiology of this parasitic infection in Belgium and in other countries where the muskrat is present. The authors noted that the muskrat would not normally be predated upon by foxes, but that muskrats killed by hunters or as part of control programmes may be scavenged by foxes.

[Hanossset, R. *et al* (2008) *Veterinary Parasitology* **151**,212]

### **Infection of red foxes (*Vulpes vulpes*) with *E. multilocularis* during the years 2001-2004 in Poland.**

During the period of 2001-2004, 1,514 red foxes from the north and the southeast of Poland were sampled and examined for infections with *Echinococcus multilocularis*. A mean of 23.8% were found infected in the whole study area. The highest prevalence (50.0-62.9%) was found in some counties in the north-eastern (Warmia-Mazuria) and the south-eastern (Carpathian Foothill) part of the country. During the study period, significant differences in the prevalence were found on a regional level. Based on the results obtained to date, the paper presents an overview of the current epidemiological situation of *E. multilocularis* in the fox population in Poland.

[Malczewski, A. *et al* (2008) *Parasitology Research* **103**, 501]

### **Infection of foxes by *E. multilocularis* in urban and suburban areas of Nancy, France: Influence of feeding habits and environment.**

This study evaluated the impact of biological and environmental factors on the infection of red foxes (*Vulpes vulpes*) by *Echinococcus multilocularis* in an endemic area of north-east France. From January 2004 to April 2006, 127 foxes were examined for *E. multilocularis* and their stomach contents analysed. The effect of year, season, age, sex and urbanisation level on *E. multilocularis* presence was estimated using a logistic regression. Urbanisation level was the only influencing factor, with a decreasing gradient from rural (54 %) to peri-urban (31%) and urban area (4 %). The consumption of *Arvicola terrestris* and *Microtus* sp., grassland vole species, the main presumed intermediate hosts of *E. multilocularis*, was studied by the same approach. The two species were consumed less in the urban area and more in autumn than in spring. Anthropogenic food consumption was linked to urbanisation and to age. The frequency of anthropogenic food consumption decreased in the rural area. A global model explaining the presence of *E. multilocularis* and including urbanisation level and diet was then elaborated. Independently of urbanisation, there was a suggestion of less *E. multilocularis* infection with anthropogenic food consumption. Red foxes consuming *Microtus* sp. and *A. terrestris* had higher worm burden than those that did not. The results suggest that the decreasing gradient observed from rural to urban area is linked to behaviour and feeding habits.

[Robardet, E. *et al* (2008) *Journal de la Societe Francaise de Parasitologie*, **15** 77-85]

### **Ecological and biological factors involved in the transmission of *E. multilocularis* in the French Ardennes.**

In order to identify the respective importance of the ecological and biological factors involved in the transmission of *Echinococcus multilocularis*, the authors estimated grassland vole intermediate host (*Microtus* sp. and *Arvicola terrestris*) population densities, in relation to the diet of the definitive host (red fox, *Vulpes vulpes*) and with the prevalence of *E. multilocularis* in the fox population. The study was conducted in the Ardennes, north-eastern France, which is an area with a high incidence of alveolar echinococcosis. Surface index methods showed that *Microtus* was the most abundant intermediate host in the area. Furthermore, *Microtus* was present in one-third of the 144 faeces and 98 stomach content samples examined and represented more than two-thirds of the rodent occurrences. Red fox predation on *Microtus* was significantly correlated with *Microtus* relative abundance. In contrast, the relative abundance of *A. terrestris* was very low. This species, as well as *Clethrionomys glareolus* and *Apodemus* sp., was little consumed. *E. multilocularis* prevalence in foxes was determined from carcasses and reached 53%. Intensity of infection varied from 2 to 73,380 worms per fox, with 72% of the sampled worm burden harboured by 8% of the sampled foxes. The selected explanatory variables (sex, year, age class, health and nutritional condition, and season) failed to predict prevalence rate and worm burden. The high prevalence rate in foxes indicates the possibility of intense *E. multilocularis* transmission, favourable to large population outbreaks in grassland rodents.

[Guislain, M.H. *et al* (2008) *Journal of Helminthology* **82**, 143]

### ***Taenia* species**

#### **Case-control study to identify risk factors for bovine cysticercosis on farms in Switzerland.**

*Taenia saginata* cysticercosis causes financial losses to the beef industry and farmers, and represents a significant source for human infection in many countries. A case-control study was conducted to identify risk factors for bovine cysticercosis on farms in Switzerland. The case group (n = 119) consisted of farms with infected cattle identified at slaughter in 2005 and 2006. Infections were confirmed by morphological or molecular diagnosis. The control group (n=66) comprised randomly selected farms with cattle slaughtered in the same period but with no evidence or history of infection. In interviews with the farmers, information regarding local surroundings and farm management was collected. Logistic regression revealed the following 5 factors as being positively associated with the occurrence of bovine cysticercosis: the presence of a railway line or a car park close to areas grazed by cattle, leisure activities around these areas, use of purchased roughage and organized public activities on farms attracting visitors. This information is considered useful for government authorities to direct control strategies as well as for farmers to take measures tailored to local situations.

[Flutsch, F. *et al* (2008) *Parasitology* **135**,641]

#### **Vaccination with recombinant oncosphere antigens reduces the susceptibility of sheep to infection with *Taenia multiceps*.**

The larval stage of *Taenia multiceps* encysts in the brain of sheep, goats and sometimes cattle causing an often fatal condition. The parasite also causes rare zoonotic infections in humans. Homologues of the recombinant oncosphere vaccine antigens from *Taenia ovis* and other *Taenia* species were identified in *T. multiceps*. Recombinant proteins

were successfully expressed in *Escherichia coli* as fusion proteins. The antigens were tested in a vaccine trial in sheep. The antigens stimulated immunity in sheep against challenge infection with *T. multiceps* eggs. Five of nine control sheep died due to a challenge infection with *T. multiceps* whereas none of 20 vaccinated animals died as a result of the parasite challenge ( $P = 0.001$ ). In addition, vaccination induced significant protection against the number of parasites encysting in the brain as a result of the challenge infection ( $P = 0.023$ ,  $P = 0.015$ , respectively). No clear relationship was apparent between the level of specific serum antibody in vaccinated animals and either the presence or absence of parasites or the number of parasites that occurred in some of the vaccinated animals. The recombinant oncosphere antigens identified may allow development of effective vaccination strategies against *T. multiceps* infection in sheep. They also raise the potential for the development of a combined vaccine with the *Echinococcus granulosus* EG95 antigen for prevention of *T. multiceps* as well as preventing the transmission of cystic hydatid disease.

[Gauci, C. *et al* (2008) International Journal for Parasitology **38**, 1041]

## ECTOPARASITES

### Cattle ectoparasites

Psoroptic mange in cattle is a recently introduced disease to GB with outbreaks confined at present to farms in South West Wales. The initial focus of infestation was two beef farms, with purchased market cattle the likely source. The outbreak was diagnosed during winter 2007/8. Psoroptic mange is a notable problem in beef cattle in mainland Europe and this was the likely source. Initial Welsh cases did not respond to treatment with injectable macrocyclic lactones, but permethrin 4% pour given on three occasions at two weekly intervals produced a response. Within a twelve month period there has been known spread to 5 other farms, one being a dairy farm. This is a likely under diagnosis. Of concern is the apparent resistance to MLs in all cases and resistance to synthetic pyrethroids in one outbreak.

Psoroptic mange in cattle in south Wales. Jones and others ( 2008) The Veterinary Record 162: 460.

The susceptibility of cattle to psoroptic mange and the response to treatment with macrocyclic lactones varies between breeds. In Europe, especially Italy, it is a recognised constraint to beef cattle production. MLs are noted to be efficacious against this form of mange and are used prophylactically as well as therapeutically. A comparison of the efficacy of two injectable formulations of ivermectin, Ivomec, the reference product and Ivogell, a generic formulation was carried out. Briefly, a group of 22 affected feedlot cattle were divided into 2 groups of 11 animals. Group 1 was treated with Ivomec and group 2 with Ivogell. Animals were treated on day 0 and day 8. By day 25 all cattle in group 1 had recovered clinically and parasitologically while group 2 cattle still had mange lesions and 2 animals had live mites. In terms of live weight gains, cattle in group 1 weighed 20kg more than those in group 2. In conclusion, generally generic compounds have a lower efficacy and persistence when compared to the original reference formulation, despite very similar pharmacokinetic profiles. This has implications in the choice of drug for treatment of an infestation.

Comparative evaluation of two ivermectin injectable formulations against psoroptic mange in feedlot cattle.

Genchi and others ( 2008) Veterinary Parasitology 158: 110-116.

Despite many European countries becoming free from hypodermosis, France had difficulty achieving this status. Up to the nineties, after various attempts at control the prevalence remained variable with some regions reporting 50%. The paper reviews the background to the problem in France with related information given on other countries. The success or failure of control programmes was evaluated which identified the main success factors as: an awareness of economic effects; specific legislation or government commitment; organisation at national level of compulsory treatment and surveillance to maintain freedom from disease. These factors seem relevant to many diseases, not just Warble Fly. Interestingly, in the present climate where government is stepping back from the industry, the identified factors point to a need for a body to take control and enforce measures which will allow eradication to be an option.

An initial pilot control programme had eradicated disease in the 32 study herds within 2 years resulting in larger regional control schemes being introduced with success. A national control programme was implemented in 1998, managed and enforced at a regional level. The essential measures were: monitoring of the programme; concentric control zones progressively included within a maximum of three years; compulsory winter treatment until herd prevalence fell to 5%. After this tactical treatment was used and control measures put in place to restrict any spread. The treatment used was a micro dose of Ivomec (2µg/kg). Studies on micro doses had previously been undertaken considering milk and meat withdrawal, ecological factors and potential resistance. No withdrawal period was required, excretion in dung was not considered a problem and it was concluded that resistance was unlikely.

There are pockets of hypodermosis on the borders with Spain and Belgium which remain infected. Since 2006 it has become a notifiable disease in France.

A successful, sustainable and low cost control-programme for bovine hypodermosis in France.

Boulard and others (2008) *Veterinary Parasitology* 158; 1-10.

The authors of this paper report four groups of cattle, infested with *Hypoderma lineatum* after either a primary infestation or in the absence of previous challenge. As an introduction to the study, they discussed the potential benefits of alternative control methods besides the current use of organophosphorus or macrocyclic lactones. Two considered methods are vaccination and sterile insects. The later has proved impractical and the former requires investigation. The larvae release a protein hypodermin C and an antigen capture ELISA was used to examine the host response to infection.

The anti-hypodermin C antibody profiles revealed an amnastic response which was not influenced by whether animals had been treated post primary infestation. In the previously uninfested group an antibody response was much slower. There was no difference in larval survival. The conclusion being that previous infestation did not confer protective immunity when rechallenged.

Impact of previous infestation on dynamics of circulating hypodermin C in cattle artificially infested with *Hypoderma lineatum*.

Colwell and others (2008) *Veterinary Parasitology* 154; 114-121.

Using an indirect ELISA to hypodermin C antigen, 634 samples of cattle sera from eastern and southeastern provinces of Turkey were examined. The results reported a prevalence of 26%. The authors suggest further testing to obtain national prevalence followed by a national eradication programme.

Seroprevalence of hypodermosis in cattle in some provinces of Turkey. Simsek and others (2008) *Research in Veterinary Science* 84; 246-249.

## **FASCIOLA HEPATICA**

This review has been sub-divided into the following titles, diagnostics, immunology, epidemiology, human infection, pathology, therapeutics, cell biology and vaccinology

### **Diagnostics**

There were two papers of note this year, the first by Charlier and others evaluated coprological and serological tests for the detection of *F. hepatica* in field conditions. They compared the sedimentation technique for detecting fluke eggs with a copro-antigen ELISA. Two different serum antibody ELISA's were also compared. The degree of infection was measured by taking faeces, blood and examining the livers of infected animals at abattoirs.

Weak and moderate correlations were observed between the serum ELISA's and the copro-antigen ELISA's.

A similar evaluation of a serum ELISA compared to intensity of infection was carried out by Salimi – Bejestani and others. Two hundred and ninety four blood samples were collected from infected cattle at a local abattoir and compared to the level of infection in the livers of these animals. The results indicated that there was a significant correlation between the ELISA value and intensity of infection. Values between 15 and 28% of a positive control indicated a low intensity of infection and 28-50% as a medium intensity of infection and values above 50% indicated a high intensity of infection.

*Charlier, J., L. De Meulemeester, et al. (2008). "Qualitative and quantitative evaluation of coprological and serological techniques for the diagnosis of fasciolosis in cattle." Veterinary Parasitology 153(1-2): 44-51.*

*Salimi-Bejestani, M. R., P. Cripps, et al. (2008). "Evaluation of an ELISA to assess the intensity of Fasciola hepatica infection in cattle." Veterinary Record 162(4): 109-111.*

### **Immunology**

Garfias and others studied the affect of injecting excretory secretory proteins of the parasite intra-peritoneally into mice. They concluded that *F. hepatica* excretory secretory (ES) products injected into mice caused immunosuppression to thymus dependent antigens in the mice.

A further paper by these workers continued this work by assessing the number of cells producing antibodies against sheep erythrocytes that were generated by NIH mice following intraperitoneal injection of (ES) products. Similar observations were made with the ES proteins inducing immuno-suppression to thymus dependent antigens in the mice.

Flynn and others studied the roles of interleukin and transforming growth factor (TGF) in controlling interleukin and gamma interferon production in experimentally infected *F. hepatica*. They concluded that suppression of gamma interferon by the parasite

molecules occurs during infection and it is possible that this suppression mediates parasite survival in the host.

Garfias, C. R. and A. Lebrija Rodriguez (2008). "The *Fasciola hepatica* excretory-secretory products reduce the production of cells producing antibodies against thymus-dependent antigens in mice." *Veterinaria Mexico* 39(4): 429-433.

Garfias, C. R. B. and A. L. Rodriguez (2008). "The *Fasciola hepatica* excretory-secretory products reduce the production of cells producing antibodies against thymus-dependent antigens in mice." *Veterinaria Mexico* 39(4): 429-433.

Flynn, R. J. and G. Mulcahy (2008). "The roles of IL-10 and TGF-beta in controlling IL-4 and IFN-gamma production during experimental *Fasciola hepatica* infection." *International Journal for Parasitology* 38(14): 1673-1680.

## **Epidemiology**

Alasaad and others (2008) collected 483 samples of *F. hepatica* from naturally infected host species at sixteen localities in Spain. They identified a novel genetic marker in order to look at genetic variability of the parasite in Spain. They concluded that this marker was very useful in revealing sufficient polymorphism in *F. hepatica* samples and that this marker could be useful in studying population genetics of the parasite in Spain. A similar study was undertaken by Ali and others (2008) in the Niger Delta. They used a different genetic marker and were able to identify both *F. hepatica* and *F. gigantica* in the Niger Delta.

Rapsch and others (2008) looked at the snail intermediate host of *F. hepatica* in Switzerland. They concluded there was a mean prevalence of bovine fasciolosis in Switzerland of between 8.4 and 21.4%. They constructed an interactive map in order to demonstrate the relative risk of transmission by modelling environmental conditions that promote survival and reproduction of the intermediate stages of the parasite.

Alasaad, S., Q. Y. Li, et al. (2008). "Genetic variability among *Fasciola hepatica* samples from different host species and geographical localities in Spain revealed by the novel SRAP marker." *Parasitology Research* 103(1): 181-186.

Ali, H., L. Ai, et al. (2008). "Genetic characterisation of *Fasciola* samples from different host species and geographical localities revealed the existence of *F. hepatica* and *F. gigantica* in Niger." *Parasitology Research* 102(5): 1021-1024.

Rapsch, C., T. Dahinden, et al. (2008). "An interactive map to assess the potential spread of *Lymnaea truncatula* and the free-living stages of *Fasciola hepatica* in Switzerland." *Veterinary Parasitology* 154(3-4): 242-249.

## **Human Fasciolosis**

Alatoom and others (2008) reviewed *F. hepatica* infection in the United States. They used an ELISA test to look for antibodies in blood of infected patients and also examined faeces for fluke eggs. They also used radiography to look at infected livers of patients. They concluded that fasciolosis is under diagnosed in humans and that physicians should consider this infection in patients with abnormal liver function and a peripheral eosinophilia, particularly in immigrant populations.

Le and others (2008) studied human Fasciolosis in sub-tropical regions of eastern Asia. Hybrids between *F. hepatica* and *F. gigantica* were detected by sequencing the DNA of both parasites. Samples of the parasites were obtained from domestic stock at slaughter and also from human patients. They concluded that Fasciolosis was an important zoonosis in this region.

Periago (2008) also demonstrated a phenotype of a hybrid between *Fasciola hepatica* and *Fasciola gigantica* in the Nile Delta.

Zhou and others (2008) described the pathological lesions of ectopic Fasciolosis in a human patient. Lesions were found in the brain and the eye. Treatment with a flukicide was successful in curing the patient.

*Alatoom, A., D. Cavuoti, et al. (2008). "Fasciola hepatica infection in the United States." Labmedicine 39(7): 425-428.*

*Le, T. H., N. Van De, et al. (2008). "Human fascioliasis and the presence of hybrid/introgressed forms of Fasciola hepatica and Fasciola gigantica in Vietnam." International Journal for Parasitology 38(6): 725-730.*

*Periago, M. V., M. A. Valero, et al. (2008). "First phenotypic description of Fasciola hepatica Fasciola gigantica intermediate forms from the human endemic area of the Nile Delta, Egypt." Infection Genetics and Evolution 8(1): 51-58.*

*Zhou, L. X., L. L. Luo, et al. (2008). "Multiple brain hemorrhages and hematomas associated with ectopic fascioliasis in brain and eye." Surgical Neurology 69(5): 516-521.*

## **Pathology**

Mohri and others (2008) studied the relationship between haematology profiles and hepatic enzyme activities in cattle infected with *F. hepatica*. They found significant increases in AST, GGT and ALP in infected cattle compared to uninfected cattle. The results were statically significant. They also concluded that these animals had a normocytic, hypochromic anaemia caused by chronic blood loss due to the blood sucking activities of adult flukes.

*Mohri, et al. (2008). "The relationship between normocytic, hypochromic anaemia and iron concentration together with hepatic enzyme activities in cattle infected with Fasciola hepatica." Journal of Helminthology 82(1): 85-88.*

## **Therapeutics**

Borgsteede and others looked at the efficacy of an Avermectin/ Closantel injection against the experimentally induced infections with gastro-intestinal nematodes and *F. hepatica*. The efficacy of the treatment was calculated based on post mortem fluke and nematode counts and also faecal egg output in field samples. The efficacy of the product against *F. hepatica* was 99.2% for 9-week old flukes and it was 100% effective against *Ostertagia* worms. Details of efficacy against other roundworms were recorded.

Braga and others evaluated the usefulness of Nematophagous fungi on *F. hepatica* eggs. Four of these fungi were used in this study. One of these fungi *Pochonia chlamydosporia* was effective in reducing the viability of *F. hepatica* larvae hatched from

eggs. They concluded that this maybe considered as the potential biological control for this parasite.

Halferty and others studied the tegumental surface changes in juvenile *F. hepatica* in response to treatment with the flukicide Triclabendazole. They used different strains of *F. hepatica* and scanning electron microscopy to study the damage caused to the tegument by this flukicide. The tegument was grossly disrupted by 96 hours.

Keiser and others investigated tegumental damage in *F. hepatica* following experimental administration in vitro and in vivo of the experimental flukicide Oz78. They concluded that the mechanism of action was on the tegument, which showed severe damage and loss of spines particularly in the tail region. Further work by this group assessed the usefulness of two other experimental flukicides, Artesunate and Artemether. Again they used scanning electron microscopy to look at the damage caused to the tegument. Both of these compounds appeared to be potent flukicidal drugs.

McConville looked at another potential flukicide, Compound Alpha. Using scanning electron microscopy, there was extensive tegumental loss and substantial degeneration of cells within the tegument between 48 and 72-hours. There was also damage to muscular layers within the parasite.

Mezo and others assessed the usefulness of another potential flukicidal product Genistein. They concluded that this product caused severe disruption to the tegument and the muscular layers below the tegument in in-vitro experiments where the flukes were incubated with the product.

Webster and others compared the time taken to administer three different flukicide products and worming products on commercial beef cattle farms at housing. They concluded that to administer Ivermectin and Clorsulon by single injection was significantly less time than injecting Ivermectin and Closantel, and this was again significantly less time than administering another product containing Levamisole and Triclabendazole. The information would aid beef farmers if they had to treat large numbers of animals at housing at time, particularly when labour costs were increasing.

*Borgsteede, F. H. M., S. M. Taylor, et al. (2008). "The efficacy of an ivermectin/closantel injection against experimentally induced infections and field infections with gastrointestinal nematodes and liver fluke in cattle." Veterinary Parasitology 155(3-4): 235-241.*

*Braga, F. R., J. V. Araujo, et al. (2008). "In vitro evaluation of the action of the nematophagous fungi Duddingtonia flagrans, Monacrosporium sinense and Pochonia chlamydosporia on Fasciola hepatica eggs." World Journal of Microbiology & Biotechnology 24(8): 1559-1564.*

*Halferty, L., G. P. Brennan, et al. (2008). "Tegumental surface changes in juvenile Fasciola hepatica in response to treatment in vivo with triclabendazole." Veterinary Parasitology 155(1-2): 49-58.*

*Keiser, J. and G. Morson (2008). "Fasciola hepatica: Surface tegumental responses to in vitro and in vivo treatment with the experimental fasciolicide OZ78." Experimental Parasitology 119(1): 87-93.*

Keiser, J. and G. Morson (2008). "*Fasciola hepatica*: Tegumental alterations in adult flukes following *in vitro* and *in vivo* administration of artesunate and artemether." *Experimental Parasitology* 118(2): 228-237.

McConville, A., G. P. Brennan, et al. (2008). "Surface and internal tegumental changes in juvenile *Fasciola hepatica* following treatment *in vivo* with the experimental fasciolicide, compound alpha." *Veterinary Parasitology* 153(1-2): 52-64.

Mezo, M., M. Gonzalez-Warleta, et al. (2008). "Evaluation of the flukicide treatment policy for dairy cattle in Galicia (NW Spain)." *Veterinary Parasitology* 157(3-4): 235-243.

Toner, E., G. P. Brennan, et al. (2008). "Physiological and morphological effects of genistein against the liver fluke, *Fasciola hepatica*." *Parasitology* 135(10): 1189-1203.

Webster, R., K. Knox, et al. (2008). "Comparison of the time required to administer three different fluke and worm combination products to commercial beef cattle at housing." *Veterinary Therapeutics* 9(1): 45-52.

## Cell Biology

An extensive review of the cathepsins was undertaken by Cancela and others. They described the cathepsins released by *F. hepatica* at different ages. Cathepsins are important in facilitating invasion and migration of the parasite through the tissues of the host. Some of these enzymes would be relevant for potential vaccines and this is discussed.

Hannah and others undertook histological examination of egg producing *F. hepatica* adults of different strains. Some of these were laboratory grown strains of the parasite and others were derived from field strains. Incidentally field strains from an outbreak in West Wales was supplied by VLA Carmarthen, and described in this article.

Rioux and others detected serum-bio markers expressed during the first twelve weeks of *F. hepatica* infection in sheep. These bio-markers could be useful to provide an insight into the mechanisms of pathogenesis and the immune response to *F. hepatica* by the host. They described the interaction between the host and *F. hepatica* as being complex with bio-markers changing as the parasite develops and grows within the host.

An important paper by Robinson and Dalton described *F. hepatica* as a significant and emerging human pathogen. They stated that the parasite expresses the largest range of cathepsin L cysteine protease enzymes yet described for a parasite. Recent phylogenetic, biochemical and structural studies indicate that this family contains five separate clades, which exhibit overlapping but distinct substrate specificities created by a process of gene duplication followed by subtle residue divergence within the protease active site. The expression of these protease enzymes changes as it passes through the host tissues and encounters different host macro molecules.

Robinson and others undertook phylogenetic analysis of the cathepsin protease enzymes of *F. hepatica*. They identified five clades by proteomic and phylogenetic analysis and that these clades were expressed differently in immature and mature parasites. Diversification of these clades produces a repertoire of enzymes with overlapping and complimentary specificities that cleave host macro molecules more efficiently.

Ryan and others also undertook phylogenetic analysis of the alpha and beta tubulin isotypes of *F. hepatica*. These tubulins are integral structures of the parasite's tegument or outer layer. The studies indicated that two of the alpha and two of the beta tubulins were distinctly divergent from those of other trematode and nematode tubulin sequences.

Stack and others examined the structural and functional relationships in the virulence-associated cathepsin L proteases of *F. hepatica*. The study of the enzymes indicated that specialised collagenolytic function in *F. hepatica* contributes to the success of this tissue-invasive parasite.

Cancela, M., D. Acosta, et al. (2008). "A distinctive repertoire of cathepsins is expressed by juvenile invasive *Fasciola hepatica*." *Biochimie* 90(10): 1461-1475.

Hanna, R. E. B., H. Edgar, et al. (2008). "*Fasciola hepatica*: Histology of the testis in egg-producing adults of several laboratory-maintained isolates of flukes grown to maturity in cattle and sheep and in flukes from naturally infected hosts." *Veterinary Parasitology* 157(3-4): 222-234.

Rioux, M. C., C. Carmona, et al. (2008). "Discovery and validation of serum biomarkers expressed over the first twelve weeks of *Fasciola hepatica* infection in sheep." *International Journal for Parasitology* 38(1): 123-136.

Robinson, M. W., J. P. Dalton, et al. (2008). "Helminth pathogen cathepsin proteases: it's a family affair." *Trends in Biochemical Sciences* 33(12): 601-608.

Robinson, M. W., J. F. Tort, et al. (2008). "Proteomics and phylogenetic analysis of the cathepsin L protease family of the helminth pathogen *Fasciola hepatica*." *Molecular & Cellular Proteomics* 7(6): 1111-1123.

Ryan, L. A., E. Hoey, et al. (2008). "*Fasciola hepatica* expresses multiple alpha- and beta-tubulin isotypes." *Molecular and Biochemical Parasitology* 159(1): 73-78.

Stack, C. M., C. R. Caffrey, et al. (2008). "Structural and functional relationships in the virulence-associated cathepsin L proteases of the parasitic liver fluke, *Fasciola hepatica*." *Journal of Biological Chemistry* 283(15): 9896-9908.

## **Vaccinology**

Acosta and others considered leucylaminopeptidases as good candidates for vaccine manufacture. They cloned a full length section of cDNA from adult *F. hepatica* including a member of this enzyme family that was able to express the enzyme. They undertook vaccine trials using this enzyme in rabbits and showed a strong IgG response and significant level of protection after experimental infection with *F. hepatica*.

Lopez – Aban and others looked at fatty acid binding proteins as vaccine candidates. They also used an adjuvant called Adad for experimental inoculations in mice and sheep. This adjuvant was said to improve protection of experimentally infected sheep against *F. hepatica* infection.

Villa-Mancera and others looked at cathepsin proteases as vaccine candidates. They described a significant reduction in worm size and burden in sheep immunised with one

particular clone of cathepsins referred to as clone 1. They obtained this clone from a display library. Another clone, clone 20, produced a significant reduction in egg output and egg viability when used as an experimental vaccine in sheep. Also, clones specific antibodies were produced and were boosted after challenge with metacercariae of *F. hepatica*.

Acosta, D., M. Cancela, et al. (2008). "*Fasciola hepatica* leucine aminopeptidase, a promising candidate for vaccination against ruminant fasciolosis." *Molecular and Biochemical Parasitology* 158(1): 52-64.

Lopez-Aban, J., J. J. Nogal-Ruiz, et al. (2008). "The addition of a new immunomodulator with the adjuvant adaptation ADAD system using fatty acid binding proteins increases the protection against *Fasciola hepatica*." *Veterinary Parasitology* 153(1-2): 176-181.

Villa-Mancera, A., H. Quiroz-Romero, et al. (2008). "Induction of immunity in sheep to *Fasciola hepatica* with mimotopes of cathepsin L selected from a phage display library." *Parasitology* 135(12): 1437-1445.

## **PARASITIC GASTRO-ENTERITIS (PGE)**

### Parasitic gastro-enteritis in sheep and goats

Analysis of VLA and SAC diagnostic data since 1975 was carried out for a paper in 2008. Over the past 5-10 years, highly significant increases in the overall rate of diagnosis of PGE were observed for all species categories. The effect of climate change on parasite epidemiology proved the most likely explanation for the observed patterns. It was thought that fewer larvae of *Teladorsagia* and *Trichostrongylus* species survive the winter and spring at pasture, while the windows of transmission of these species, and of *Haemonchus contortus*, have extended into the autumn. Nematodirosis showed a pronounced peak in spring and early summer in Scotland while in the Southwest, where fewer diagnoses were made, it also appeared regularly at other times of year (van Dijk et al., 2008).

Studying faecal egg counts (FEC) in ewes post lambing was part of a paper published in 2008. In this, oral moxidectin was used at lambing which was effective at reducing FEC, but there was a post treatment rise in FEC 9-11 weeks post lambing. Older ewes had a significantly higher post-treatment rise than gimmers. 3-4 year olds had the lowest rise (Wilson et al., 2008).

The reduction in food intake that is known to occur in lambs parasitized with gastrointestinal (GI) nematodes was the subject of a paper published in 2008. In the study they showed that the anorexia was associated with parasitism of a susceptible (as determined by FECs) breed (Suffolk x Greyface) but not in a more resistant breed (Scottish Blackface). Plasma leptin concentrations were measured throughout and were higher in infected lambs than in uninfected controls but did not explain the difference between the anorexia shown by the different breeds (Zaralis et al., 2008).

Studies on an unmanaged population of Soay sheep and interactions with bodyweight indicated that in lambs, the intensity of strongyle eggs was positively correlated with that of *Nematodirus* spp. eggs, while in yearlings and adults, strongyle eggs and coccidia oocysts were positively correlated. In lambs and yearlings, of the parasite taxa tested, only strongyle eggs were significantly and negatively associated with host weight.

However, in adult hosts, strongyles and coccidia were independently and negatively associated with host weight (Craig et al., 2008).

The immune response of sheep to gastro-intestinal parasites and the costs involved were summarised in papers published in 2008 (Colditz, 2008) and (Greer, 2008). The possibility manipulating host immunity to improve nematode parasite control was also published in 2008 (Sykes, 2008).

A review published in 2008 explained how dietary mineral intake may affect the immune system, with particular reference to gastrointestinal nematode infestations of livestock and highlighted further areas for research (McClure, 2008).

### *Haemonchus contortus*

Studies of *H. contortus* infection in sheep, found that the number of eggs present in individual adult females was significantly correlated with worm size (consistent with other workers). Also, a significant inverse correlation was found between host blood lymphocyte counts and worm fecundity. This led to the hypothesis that egg production in *H. contortus* is limited by immune regulation of worm size and presumably growth. Mean worm size and fecundity declined as sheep received more prolonged trickle infections before necropsy, confirming previous reports that immune responses to adult worms are enhanced by ongoing larval challenge (Rowe et al., 2008).

Studies on the humoral and cell mediated responses in the abomasal mucosa and lymph nodes in goats infected with mainly *H. contortus* was published. There were marked increases of T cells, (particularly CD4(+)),  $\gamma$  delta(+) lymphocytes, B cells and IgG(+) plasma cells 10 and 13 days post infection. However this rapid host response was unable to induce larval expulsion (Perez et al., 2008).

### *Nematodirus battus*

In this paper the temperatures for egg development, hatching and larval survival of *N. battus* are described. *N. battus* eggs were able to develop between 11.5 and 27 °C (but more successfully at lower temperatures). Embryonated eggs did not hatch below 11°C or above 17 °C . This is the first report of an upper threshold for hatching. In contrast to established thought, substantial proportions of eggs were able to hatch without chilling. Larval death rates increased at temperatures towards the upper threshold for hatching. Eggs resulting from spring infections will mainly be fully developed by the autumn. A proportion will hatch then if the temperature is within the hatching range. Eggs deposited in the autumn, are unlikely to be fully developed until the following autumn and may hatch then or the following spring (van Dijk and Morgan, 2008).

### Diagnosis of PGE

A summary of advances to date of DNA-based methods of diagnosis of nematode infections and the characterisation of the nematodes involved was published (Gasser et al., 2008). In this the authors suggest that for laboratory based assays a quantitative or semi-quantitative real-time PCR employing genus- or species-specific primer sets, combined with HRM(high resolution melting)analysis to confirm the specificity of amplicons following PCR, would be currently the best option. Development of field tests should also be considered.

Also published in 2008 was a technique for the detection and semi-quantification of *Strongylus vulgaris* DNA from equine faeces by real time quantitative PCR (Nielsen et al., 2008). This technique may be applicable to other diagnostic faeces samples.

It is known that there is a high degree of genetic diversity in *Haemonchus contortus*. A paper published in 2008 detailed a way using molecular techniques to fingerprint the isolates used for experimental work around the world (Redman et al., 2008).

A test for occult blood in faeces was studied and proved to be useful by detection of blood in faeces during heavy *H. contortus* infections prior to the emergence of high worm egg counts (WECs). However the possibility of positive test results from other conditions such as fasciolosis, coccidiosis and some bacterial enteric infections was acknowledged (Colditz and Le Jambre, 2008).

Two papers were published where an excretory/secretory (ES) product from *H. contortus* was suggested as a possible marker for infection (Du et al., 2008) and (Mir et al., 2008). They concluded that the ELISA test using excretory-secretory antigens in sheep serum had a diagnostic sensitivity of 87.5% and 92.02% specificity. No cross reactions were found to *Teladorsagia circumcincta*, *Bunostomum trigonocephalum* and *Trichuris ovis*.

An unusual and alternative method of diagnosis was the subject of a paper in 2008 where dogs were trained to differentiate between nematode-infected and uninfected sheep faeces. Two German shepherd bitches were trained for scent detection over a 6-month period. Over 80 trials the dog had a mean success rate of greater than 80% in the detection of *T. circumcincta*- or *Trichostrongylus vitrinus*-infected faeces. *H. contortus*-infected faeces was detected with a slightly lower reliability of 76%, but mixed infections were detected at 92% reliability (Richards et al., 2008).

A comparison of two worm counting techniques was published this year. In it the author demonstrated that cutting open the intestine was unnecessary if it had already been washed out by introducing water into it. The size of sieve to be used should be determined by the purpose of the examination. If adult worms are to be counted then a 250µm sieve is adequate, if larval stages then 38µm sieve-or the samples should be sequentially sieved through both sizes of sieve with each fraction being examined separately (McKenna, 2008a).

#### Chemical (anthelmintic) control of gastro-intestinal nematodes

The discovery, synthesis and biological results of amino-acetonitrile derivatives (AAD), a new class of synthetic anthelmintic compounds, was described in 2008 (Ducray et al., 2008; Kaminsky et al., 2008a). The identification of an AAD compound, monepantel, for use in sheep and cattle was also described. (Kaminsky et al., 2008b). A paper was also published that described dose determination studies for the oral use of monepantel in sheep. 2.5mg/kg was chosen as a suitable minimum dose rate, providing effective control against L4 stages of *Haemonchus*, *Trichostrongylus*, *Nematodirus*, *Teladorsagia*, *Cooperia*, *Chabertia* and *Oesophagostomum* spp (Hosking et al., 2008)

The pharmacokinetics of another potential anthelmintic, paraherquamide was studied. There is rapid absorption in sheep from oral administration and metabolism results in products that generally retained antiparasitic activity. In dogs this was not the case. Inactive metabolites were generated and with more rapid excretion in the dog than in the

sheep, which may explain why the drug does not have anthelmintic activity in that host species (Aloysius et al., 2008).

A series of novel 2-alkoxy- and 2-aryloxyiminoalkyl trifluoromethanesulfonamide derivatives have shown significant *in vitro* parasitocidal activity against the ectoparasites *Ctenocephalides felis* (cat flea) and *Rhipicephalus sanguineus* (brown dog tick). A number of these compounds also displayed significant *in vitro* endoparasite activity against the nematode *H. contortus* (Ali et al., 2008).

The possibility of increasing the efficacy of macrocyclic lactones as anthelmintics in sheep by the use of blockers that inhibit multidrug resistance transporters (MDRs) such as P-glycoproteins was discussed in a paper published in 2008. Theoretically, the inhibition of these transporters should result in an increase of the drug concentration within the parasites and higher treatment efficiency (Lespine et al., 2008).

The efficacy of a combination ivermectin/closantel injection in cattle was published. In experimental studies, Activity was >94% for 9 week old *Fasciola hepatica* and >98% for 12 week old *F. hepatica*. In field studies, faecal examinations 14 days after treatment confirmed the post-mortem results with 100% reduction of egg output for *Ostertagia ostertagi*, *Cooperia punctata*, *Trichostrongylus* spp. and *Trichuris* spp. and low egg output of *Cooperia oncophora* and *Nematodirus helvetianus* (Borgsteede et al., 2008).

Albendazole and ivermectin given separately or in combination to lambs infected with parasites resistant to both classes were studied in 2008. In one paper no synergism was detected between the two classes, but no negative interactions either (Entrocasso et al., 2008).

The use of eprinomectin pour-on in goats experimentally infected with *H. contortus* showed that no eggs were shed for 10-17 days after a single treatment. (Molina et al., 2008).

#### Anthelmintic resistance in sheep

A summary of anthelmintic resistance and alternative methods of control was published in 2008 (Papadopoulos, 2008).

A study on a single sheep farm in New Zealand detected ivermectin resistance in *Teladorsagia*, *Trichostrongylus* and *Haemonchus* spp, and BZ/LEV combination resistant *Teladorsagia* and *Trichostrongylus* spp were also present. This confirmed multiple, multi-generic anthelmintic resistance including the first confirmed case of ivermectin resistance in *T. colubriformis* from sheep in New Zealand (Sutherland et al., 2008).

A paper published in New Zealand in 2008 where the results of a random survey for anthelmintic resistance published in 2006 was compared with the results of tests sent into diagnostic laboratories. There was considerable similarities between the two sources of data, leading the author to conclude that examination of FECRT case submissions to veterinary laboratories may offer a useful source of information regarding changes in the prevalence of anthelmintic-resistant sheep nematodes in New Zealand (McKenna, 2008b).

## Managing anthelmintic resistance

A study in New Zealand set out to measure progress of anthelmintic resistance where animals were treated and moved to pasture with low contamination compared to leaving 10 or 20% of animals untreated prior to the move. The results demonstrate that creating a reservoir of unselected parasites (i.e. in untreated animals) slows the development of anthelmintic resistance, and emphasises the risk of treating all animals prior to a shift on to low-contamination pasture. However, higher levels of pasture contamination, resulting from untreated animals, indicate the difficulty in managing both worm control and resistance (Waghorn et al., 2008).

The beneficial effect of grazing untreated ewes after treated lambs on the effects of anthelmintic resistance (by acting as a source of parasites in refugia) was determined by a study carried out in New Zealand. The study was carried out on paddocks where lambs grazed for 1 week, followed by ewes, then a three week rest before lambs returned. The study also showed that where anthelmintic resistance limited effective parasite control in the lambs, the ewes could suppress the growth of parasite populations. The lambs performed better (live weight gains and body condition scores) when grazed before ewes. As the use of an anthelmintic that only produced faecal egg count reductions of 57-59% was part of this trial, an estimate of the cost in production of this level of anthelmintic resistance was made at 19-24% reduction in growth rate (38-48g/day). The effect on the development of anthelmintic resistance is being studied further (Leathwick et al., 2008).

Targeted selective treatment (TST) i.e. treating only the animals that are more severely parasitized, is now being promoted as a way to control GI tract parasitism without promoting resistance (i.e. by maintaining refugia). In areas where *H. contortus* is the predominant parasite, the FAMACHA © system (where anaemia is detected by comparing conjunctival colour against a chart), has been used to identify the animals for treatment successfully for a number of years. A paper comparing TST using FAMACHA © against suppressive chemical treatment, showed some production losses with the TST system but this was considered small as the suppressive treatment was not sustainable in the longer term. Also it was noted that the degree of improvement in the colour of the ocular mucous membrane from pale to red in individually drenched anaemic animals over a period of 7-14 days could give a good indication of the efficacy of the compound(s) used (Van Wyk, 2008).

## Mechanisms of anthelmintic resistance

Although MLs exert their antiparasitic effect through binding to ligand-gated chloride channels, benzimidazoles interact with beta-tubulin. Molecular studies have indicated a possible link between exposure to ML anthelmintics in *H. contortus* and changes occurring that are associated with benzimidazole resistance. Two papers published in 2008 looked at this possible interaction. In one, genetic evidence of selection at a P-glycoprotein (a cell membrane transport protein) locus during selection for benzimidazole resistance in *H. contortus* was presented. In *H. contortus*, resistance to avermectins has also been correlated with genetic changes at a gene encoding a P-glycoprotein. (Blackhall et al., 2008) In the second, the authors state that ML use may predispose parasitic nematodes to benzimidazole resistance. This has major implications for parasite control programs dependent on MLs/ benzimidazole rotations and on the use of ML/ benzimidazole combination therapy (Mottier and Prichard, 2008).

Over expression of the multidrug resistance associated protein (MRP) is a regulatory mechanism that some organisms use to overcome drug effects and may be reflected as transcriptional up regulation. The objective of the study published in 2008 was to determine the role that MRP plays in protecting nematodes from the macrocyclic lactone drugs ivermectin and moxidectin, using *Caenorhabditis elegans* as the model nematode. It provided evidence for the involvement of MRP in reducing the toxicity of ivermectin and moxidectin in parasites (Ardelli and Prichard, 2008).

A paper published in 2008 stated that anthelmintic resistance offers a good opportunity to apply fundamental evolutionary and ecological principles to the management of a potentially crucial public health problem (Churcher and Basanez, 2008).

#### Alternative (non anthelmintic) methods of parasite control

An excellent review of non-chemical methods of nematode parasite control in sheep and the feasibility of the options was published in 2008. This includes a good summary on the current position on breeding for parasite resistance (Stear, 2008). Another similar study, with more emphasis on goats, was also published. (Torres-Acosta and Hoste, 2008)

The use of copper oxide wire particles (COWP) to control gastro-intestinal parasitism particularly *H. contortus* has been the subject of a number of papers from the USA for the last couple of years (In the UK, likely problems with copper toxicity would have to be overcome). However a paper was published detailing their use in scimitar-horned Oryx [Oryx dams], roan antelope [*Hippotragus equimus*], blackbuck [*Antilope cervicapra*], and blesbok [*Damaliscus pygargus phillipsi*] where good reductions in faecal egg counts were recorded (Fontenot et al., 2008).

The use of multinutritional pellets that contained *Duddingtonia flagrans* (a fungus) fed to sheep showed that good nematode trapping ability towards *H. contortus* was achieved in the treated sheep faeces (Aguilar et al., 2008). A paper detailing the effect of this fungus on *H. contortus* L3 larvae as viewed by electron microscopy was also published this year. Trapping and cuticle penetration of the larvae was observed (Campos et al., 2008). In other papers *in-vitro* studies with nematophagous fungi concluded that *Pochonia chlamydiosporia* is a potential biological control agent of *Ascaris suum* and *Fasciola hepatica* eggs (Araujo et al., 2008; Braga et al., 2008).

The role of *Bacillus thuringiensis*, a soil derived bacterium, in killing *H. contortus* was investigated in a paper this year (Linares et al., 2008).

Numerous studies on the possibility of plants being used for their abilities to control gastro-intestinal (GI) tract nematodes were published in 2008.

Plants containing condensed tannins have been shown to have some effect on GI parasites. In one paper condensed tannins derived from Acacia (Akkari et al., 2008) caused a reduction in faecal egg counts. *In vitro* studies showed tropical plants containing tannins affected exsheathment of *H. contortus* and *T. colubriformis* L3 larvae (Alonso-Diaz et al., 2008a; Alonso-Diaz et al., 2008b)

Also tannin rich plants fed to goats experimentally infected with *H. contortus* and *T. colubriformis* significantly reduced the larval establishment of both nematode species compared to the controls. (Brunet et al., 2008a). However a study in sheep using condensed tannin extracts (CTE) from *Acacia molissima* showed effects against *H. contortus* (FEC and numbers of parasites reduced) but not against *T.*

*colubriformis*.(Minho et al., 2008). Another study in sheep showed that exposure to tannins (from sainfoin) could also affect the establishment, i.e., the tissue association/penetration of the exsheathed *H. contortus* L3s into the digestive mucosae.(Brunet et al., 2008b).

However another study found no effect on feeding condensed tannins (CT) in the form of sainfoin on *H. contortus* infection in sheep. The authors conclude that the lack of effects on resilience to nematode infection might have been the result of the unexpectedly low CT content (3.6% in DM) of the material used. It cannot be excluded that longer term feeding of this batch of sainfoin might have been effective.(Scharenberg et al., 2008).

A summary of tanniferous forage plants and their use in this way was published. Tannin concentrations were highest for *Onobrychis viciifolia*, (sainfoin), followed by *Lotus corniculatus* (birdsfoot trefoil) and very low for *Cichorium intybus* (chicory). Palatability of all tanniferous forages was comparable to that of a ryegrass/clover mixture when fed as dried forage and, when offered as silage, palatability of *O. viciifolia* was clearly superior to that of the respective ryegrass/clover control. Administration of dried or ensiled *O. viciifolia* reduced parasite egg counts in faeces of lambs co-infected with the gastrointestinal nematode species *H. contortus* and *Cooperia curticei*. (Haring et al., 2008).

The effects of other plants were also studied. Essential oils from *Lippia sidoides* (a plant native to south America) were also shown to have an effect against *Haemonchus* and *Trichostrongylus* spp.(Camurca-Vasconcelos et al., 2008) . The anthelmintic activity of *Iris hookeriana* Linn. Rhizome and *Achillea millifolium* was also studied with some encouraging results *in vitro* and *in vivo* (Tariq et al., 2008a) and (Tariq et al., 2008b)

The role of nutritional supplementation and bioactive forages on GI tract nematodes in sheep and possible synergy with other chemical or non-chemical methods of control were summarised in a paper published in 2008 (Athanasidou et al., 2008).

### Parasite vaccines

Work is continuing in this area, with a vaccines available against *H. contortus* being the most likely (LeJambre et al., 2008).

There are difficulties in producing vaccines against all the GI tract parasites that cause PGE in sheep. In one paper antibodies against a carbohydrate larval antigen in *T. colubriformis* L3 were identified as reducing infection significantly while there was much less effect on abomasal parasites *T. circumcincta* and *H. contortus* (Harrison et al., 2008). A paper published in 2008 looked at the possibility of multi-antigen vaccines against parasites, as very few antigens have been identified that achieve a degree of efficacy likely to make them candidates for single-antigen vaccines. More research in this area is needed.(Willadsen, 2008).

### Parasitic gastro-enteritis in cattle

The use of a bulk milk tank *O. ostertagi* ELISA test was further evaluated in several countries in Europe. Variations between different European countries appeared to reflect different husbandry practices, particularly those related to access to pasture. The authors state that when diagnostic values appropriate for different production situations and environments have been further validated, the test will provide an objective, quantitative assessment of the *O. ostertagi* status of a dairy herd and the possible

impact this may have on performance and potential responses to anthelmintic treatment. (Forbes et al., 2008).

#### Alternative methods of parasite control in cattle

A paper was published that showed reduction of faecal egg counts and pasture contamination by *Ostertagia* and *Cooperia* spp parasites in grazing cattle in Brazil by daily feeding of *Duddingtonia flagrans* chlamydiospores. (Jobim et al., 2008).

#### Anthelmintic Resistance in Cattle

*Cooperia punctata* resistant to ivermectin and doramectin was detected in a study on a dairy farm in Brazil. (Cardoso et al., 2008).

#### PGE in new world camelids

*Camelostrongylus metulatus*, (a parasite of the C3), *Trichuris tenuis* (a parasite of the large intestine) and other parasites causing fatal PGE in new world camelids in GB was reported in 2008 (de B Welchman et al., 2008). Zoological collections have recognised *C. mentulatus* for a number of years, with a syndrome similar to type II ostertagiosis seen in blackbuck (*Antilope cervicapra*) in the past (Flach, 2008).

A comparison of methods for examining faeces for nematode eggs and coccidial oocysts from new world camelids was published by workers in the USA. They summarised that a centrifugation/sucrose flotation technique was better at detecting *Eimeria macusaniensis*, *Trichuris*, *Nematodirus* and *Capillaria* spp. While the saline McMaster technique was better at detecting small coccidia (Cebra and Stang, 2008). VLA has taken note of this work and now offers a specific camelid faecal egg count examination.

#### *Heterakis gallinarum* in poultry

The possibility of breeding poultry (White Leghorn and New Hampshire) resistant to *Heterakis gallinarum* was suggested, after studies in both these breeds. (Gauly et al., 2008)

#### Anthelmintic resistance in birds

A paper investigated benzimidazole (BZ) resistance in *Trichostrongylus tenuis*, a nematode parasite of red grouse, *Lagopus lagopus scotica*. BZ anthelmintics had been in use for up to 15 years, without any evidence of resistance. The parasite was screened for the presence of mutations known to confer BZ resistance in other nematodes. None were found.

Alternative mechanisms may be responsible for BZ resistance in this parasite, or the method and timing of treatments may have reduced selection pressure for BZ resistance by creating substantial refugia for susceptible genotypes. (Webster et al., 2008).

#### Anthelmintic resistance in zoological animals

There was a report of anthelmintic resistance in gemsbok held in a zoological collection in the UK (Hewitt, 2008)

## **DICTYOCAULUS VIVIPARUS**

A summary of the disease caused by this parasite in the UK and its diagnosis was published. In this the factors that predispose to an outbreak of clinical disease were enumerated. These were

- Rainfall. Disease is more common in the wetter west of the country.
- A dry season (low translation of L3 onto pasture leading to reduced immunity) followed by a wet summer (which increases dispersal of larvae from faeces and promotes *Pilobolus* sp fungi). (Matthews, 2008)

The major sperm protein (MSP) of *D. viviparus*, a small and abundant protein used in some diagnostic immunoassays, has been shown to be variable in some nematodes but not others. Almost no sequence variation, and thus no antigenic diversity, was detected in MSP between worms from different sub-populations or in the other *Dictyocaulus* species investigated. A functional test of a recombinant variant of the MSP showed that the expressed protein was recognized by antibodies in sera from infected cattle (Hoglund et al., 2008).

A paper was published which reported the development of an optimised enzyme-linked immunosorbent assay (ELISA) for the detection of *Dictyocaulus viviparus*-specific antibodies using a recombinant MSP antigen. ELISA results showed a calculated specificity and sensitivity as well as positive and negative predictive values of > 99%. No cross-reactions with sera from calves infected with *O. ostertagi* or *C. oncophora* were observed. Lungworm-specific immunoglobulins were first detected from 28 to 35 days post-infection (von Holtum et al., 2008).

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## COCCIDIOSIS

### **Coccidiosis in poultry**

#### **Control**

A paper “Kokzidiose-Impfung von Broilerterntieren über Nippeltränken” (von Steffi Albrecht *et al*, *Tierarztl. Umschau* 63 (2008), 426-431) reported that the vaccination of Broiler chicks with anticoccidial live vaccine (Paracox) *via* nipple drinkers was as effective as administration *via* bell drinkers.

Mark Jenkins and colleagues at USDA, Beltsville published a paper entitled “*Eimeria praecox* infection ameliorates effects of *Eimeria maxima* infection in chickens”, *Veterinary Parasitology* 155 (2008), 10 – 14, in which they reported that chickens infected with a dual infection of *E. praecox* and *E. maxima* performed better than those infected with *E. maxima* as a single species infection.

Sherif Hassan and colleagues from Texas A&M University, USA investigated the use of Guar meal to control *Eimeria tenella* infections in broilers. Results indicated that including 5% Guar meal in the diet reduced oocyst shedding and prevented bloody diarrhoea, but did not affect body weight loss. *Veterinary Parasitology* (2008) 157 (1&2), 133-138.

Another paper from USA reported that there is widespread resistance to ionophorous anticoccidials and that birds treated with Nicarbazin (a non-ionophorous compound) alone performed better than those on a combination of Nicarbazin and the iosophore Narasin. It was suggested that this was because of the increased resistance to ionophores. Bafundo *et al*, *Poultry Science* (2008), 87, 1760-1767.

A comprehensive paper looking at the history of the anticoccidial compound Sulfaquinoxaline charts its beginnings as an anti-malarial human drug in the 1930's to its introduction in 1948 as the world's first anticoccidial drug for chickens. This single-handedly transformed the poultry industry and elevated the humble (but expensive!) chicken to the world's most popular (and cheap) meat food. William C Campbell, *Journal of Parasitology* (2008), 94(4) 934-945.

## Technical

Blake *et al* from IAH Compton reported on “The development and validation of polymerase chain reaction assays specific to four species of *Eimeria*” (Avian Pathology (2008), 37(1), 89-94). They developed and validated quantitative assays for the four most pathogenic species of *Eimeria* in chickens (*E. acervulina*, *E. maxima*, *E. necatrix* and *E. tenella*).

As a consequence of this paper VLA Weybridge is currently investigating the use and refinement of this PCR technique in conjunction with the IAH group. Results so far have been very encouraging and have led to a major pharmaceutical company getting involved.

Another group in Japan also reported on PCR developments, although their assay was not quantitative. “Detection of five avian *Eimeria* species by species-specific real-time polymerase chain reaction”, Kawahara *et al*. Avian Diseases (2008), 52, 652-656.

### Coccidiosis in Cattle

#### The costs of Coccidiosis

Veterinary Practice, August 2008 p28-29

Dr A. Andrews

Quoted some American studies involving experimental infestation showing losses in weight gain which were still present over 10 months later. The negative costs calculated were £24.50 to £59.25 at 2008 prices plus treatment costs.

#### Coccidiosis and cryptosporidiosis – growing performance complications

Veterinary Times (2008) Vol 38 (25) p8-9

Hany Elsheikha

Both diseases are still associated with significant morbidity and mortality. This emphasises the need for sound management practices and to maintain good hygiene standards using effective cleaning and disinfection methods. The role of metaphylaxis as a preventative measure is still unclear in terms of the economic benefit.

#### Cytokine gene expression and NF-kappa B activation following infection of intestinal epithelial cells with *Eimeria bovis* or *Eimeria alabamensis* in vitro

Parasite immunology (2008) 30 (3) p175-179

Alcala-canto Y. Ibarra-Velarde F.

The intensity of host cell responses triggered by these two species could be considered as potential determinants of pathogenicity. *E. bovis* triggers severe intestinal damage while *E. alabamensis* causes minimal damage.

#### Dynamics of *Eimeria* oocyst excretion in dairy calves in the Province of Buenos Aires (Argentina) during their first two months of life.

Veterinary Parasitology (2008) 151 Issues 2-4 p133-138

R O Sanchez & others

This study of dairy calves aged 2-8 weeks showed the peak average of 85% of infection prevalence in the group between 26-30 days old. This trend appeared in every month. Oocysts of *E. ellipsoidalis*, *E. bovis*, *E. zuernii* and *E. auburnensis* appeared in the

highest numbers. The highest prevalence of infection and of oocyst values occurred during the periods with better environmental conditions for sporulation, survival and dispersion of oocysts (spring and autumn) and coincided with the highest stocking densities in the paddocks.

*Eimeria* species in cattle on farms in England and Wales.

Vet Record (2008) 162 (15) p482-483

I D Stewart & others

A total of 1253 samples were collected during 208 visits to 57 farms. *Eimeria spp* were detected in 88 of the samples from 36 farms. Of the 88 positive samples, 44 had four or more species, 60 had three or more and 76 had two or more. The most frequently identified species were *E. bovis* and *E. zuernii*.

Younger calves had a greater prevalence of coccidial infection and there was also a significant association with age in animals showing no clinical signs. This suggests an important epidemiological role for asymptomatic animals. The highest proportion of positive samples was found in the autumn and the highest monthly proportion in June. The authors expressed the need for further work to investigate management practices that may reduce the prevalence of *Eimeria spp* in cattle herds, to reduce the risk of coccidiosis in young calves and the potential production losses.

Impact of sub-clinical coccidiosis in cattle

International Dairy Topics (2008) 7 (5) 9-11.

E. Thomas.

Work on six farms in Belgium, France and Germany concluded that metaphylactic treatment with diclazuril (Vercoxan) is effective in reducing faecal coccidial oocyst shedding in cattle. Treated calves on all farms had a significantly higher liveweight gain of 2.7kg compared to controls.

Fatal intestinal coccidiosis in a 3-week-old buffalo calf (*Bubalus bubalis*).

J. Parasitology (2008) 94 (6) 1289-1294

J. D. Dubey & others.

This describes one of the first confirmed cases of coccidiosis in a water buffalo. It occurred in a 22 day-old calf that died after a 3-4 day illness. Oocysts indistinguishable from *E.bareillyi* were found in the faeces.

Subclinical coccidiosis: continuing major challenge to cattle

Veterinary Times (2008) Vol 38 (8) p10-11

Adam Martin,

The article concludes that as farms get bigger, stocking densities and margins get tighter, coccidiosis, both clinical and subclinical, is likely to continue to represent a major challenge to the cattle industry.

Coccidiosis in calves at grass: An intractable problem?

UK Vet (2008)13, No.2 p29-34

Richard Laven

VIDA data show that the numbers since 1998 show a gradual downwards trend. The article illustrates a particularly difficult problem seen in cows at grass on an organic farm

and follows up with a discussion with a number of UK practitioners on the merits of various licensed treatments currently available. The likely role of infection acquired indoors and then seeding of pastures is also discussed.

### **Coccidiosis in pigs**

#### **Survival of *Isospora suis* oocysts under controlled environmental conditions.**

Veterinary Parasitology (2008) **152** Issues 3-4 p186-193

M Langkjaer and A. Roepstorff

Little knowledge exists on the ecology of the oocysts. Known numbers of oocysts were exposed to all combinations of four relative humidities (RH) and three temperatures. The sporulation rate increased with temperature; however the infective sporocyst stage was reached within 24 hours at all temperatures, while RH did not seem to affect sporulation. There was a rapid reduction in viable oocysts exposed to high temperatures (25°C and 30°C) in combination with low relative humidities (53% and 62%) – oocysts died within 24 hours. Viability was higher when oocysts were exposed to higher relative humidities (75% and 100%) as well as a lower temperature (20°C). The most favourable condition appeared to be 100% RH at 25°C at which the percentage of viable oocysts decreased from 100% to 17% in 96 hours.

So it may be possible to reduce infection presence in modern sow herds by changing the environmental conditions and/or the management within the farrowing pens.

### **Coccidiosis in new world camelids**

An interesting paper describing a case of “Coccidiosis in British alpacas (*Vicugna pacos*)” was published by VLA colleagues in the Veterinary Record (2007) 160, 805 – 806.

### **Coccidiosis in captive animals**

VLA colleagues at Starcross, Lasswade and Aberystwyth reported concurrent coccidiosis and listeriosis in a captive Bennett’s wallaby, and concluded “that listeriosis should be considered in the differential diagnosis when macropods with signs of septicaemia are presented. Coccidiosis is already a recognised differential diagnosis for sudden death and diarrhoea in macropods”.

Twomey *et al*, Veterinary Record (2008), 163, 635-636.

### **Coccidiosis in wildlife**

A paper from Israel “Proliferative visceral *Isospora* (atxoplasmosis) with morbid impact on the Israeli sparrow *Passer domesticus biblicus* Hartert, 1904”, (Gill & Paperna (2008), Parasitology Research 103(3), 493-499) reported that 70% of birds sampled in 3 regions Israel were found to have the infection, and that in one area (the Jordan valley) this caused a severe disease which compromised their survival after capture. This finding confirms observations in the UK by Ralph Marshall at VLA Weybridge that recently captured passerines very quickly succumb to isosporidiosis, probably due to stress.