APHA PARASITOLOGY GROUP

ANNUAL REVIEW OF LITERATURE & HORIZON SCANNING REPORT

SUMMARY OF PAPERS PUBLISHED IN 2014
## Executive Summary

This is a summary of the literature and horizon scanning report, produced from 270 abstracts and peer and non-peer reviewed papers in parasitology published in 2014, of relevance to Defra, APHA and the wider industry.

### Highlights

The ENHanCed Infectious Diseases database (EID2) is open-access and evidence-based, and describes the pathogens of humans and animals, their host and vector species, and also their global occurrence. Currently, the EID2 evidence suggests that: Within these host species, 793 (30.5%) pathogens were bacteria species, 395 (15.2%) fungi, 705 (27.1%) helminths, 372 (14.3%) protozoa and 332 (12.8%) viruses. The pathogens affecting the greatest number of hosts included: *Escherichia coli*, *Giardia intestinalis*, *Toxoplasma gondii*, *Anaplasma phagocytophilum*, *Cryptosporidium parvum*, *Rabies virus*, *Staphylococcus aureus*, *Neospora caninum* and *Echinococcus granulosus*. There was no attempt made to measure the economic impact of these diseases.

### Gastro-intestinal nematodes of grazing ruminants

- General move to molecular detection methods, but problems remain with integration and interpretation with existing methods
- Use of combined anthelmintic classes in control of nematodes including resistant parasites
- Further work on breeding for resistance/resilience
- *Haemonchus* spp detected with resistance to monepantel
- Risk factors for anthelmintic resistance in cattle (in South America) determined. The results showed that treatment frequency, date of treatment and frequency of treatment in the past with a single drug class were the main risk factors involved.

### Fasciolosis

- Fluke egg count reduction, coproantigen reduction tests and fluke histology were all used to investigate triclabendazole resistance in *Fasciola hepatica*. An egg hatch assay was described to detect albendazole resistance.
- The longitudinal, temporal and micro-spatial distribution of *Galba truncatula*, the intermediate snail host could be greatly enhanced by the use of high resolution cameras on satellites, or aeroplanes that can identify the snail’s breeding areas on farm. Thus providing a more specific prediction of disease on individual farms.

### Vector borne diseases

- The first detection of *Borrelia miyamotoi* in UK *Ixodes ricinus* ticks (which causes relapsing fever in humans) was reported. The three positive *B. miyamotoi* ticks came from three geographically distinct areas, suggesting a widespread distribution, and from two separate years, suggesting some degree of endemicity. This bacterium
has been detected in a number of countries in hard ticks and wildlife. Its importance is as a disease in humans. Its role as a cause of disease in animals is not yet known.

- *Rhipicephalus sanguineus* (a non-native tick) infestation in a home in UK associated with imported dogs was detailed, indicating a risk of zoonotic disease transmission.

- CRIMEAN Congo haemorrhagic fever (CCHF) is a tick-borne zoonotic disease that is non-pathogenic to many animals, but is highly pathogenic to humans and a serious threat to public health. The causative agent is the CCHF virus (CCHFV), which is transmitted to vertebrates by hard ticks of the genus *Hyalomma*. A recent study reported a high prevalence of CCHFV in the tortoise tick *Hyalomma aegyptium* removed from field-caught spur-thighed tortoises (*Testudo graeca*) in south-east Turkey and north-west Syria. Before changes in legislation, *H. aegyptium* specimens were regularly removed from tortoises imported into UK. It must be assumed that illegally imported *T. graeca* continue to be infested with *H. aegyptium* and that the containers used to import them may harbour engorged ticks that drop off the host on completion of their blood meal. This possible route of entry into the UK could potentially expose individuals to infectious agents transmitted by *H. aegyptium*.

- *Besnoitia besnoiti* continues to be detected outside of the known endemic areas in Europe. The development of an indirect immunofluorescence antibody test (IFAT) for the diagnosis of cattle besnoitiosis was reported by French workers. The test appears as a very useful tool to confirm the serological status of samples that exhibited a discrepancy between findings of commercial ELISA kits and WB (gold standard)

**Cestodes**

- A study of *Cysticercus bovis* infection (a zoonotic tapeworm) detected at meat inspection in cattle in France determined the true prevalence of cattle with at least one viable cysticercus was 0.113% (0.076-0.189). Taking into account these results and those of a previous study on the prevalence of human cysticercosis in France, they estimated that one carcass could infest an average of 8-20 individuals.

- New molecular tests for *E. granulosus* (important zoonotic cestode, present in UK) and *E. multilocularis* (important zoonotic cestode not present in UK) in dog/fox faeces were described from workers in China.

- New serological test for echinococcosis in beavers was described by workers in Switzerland.

**GASTRO-INTESTINAL (GI) PARASITES OF RUMINANTS- GENERAL**


Global agriculture will be required to intensify production from a shrinking natural resource base. Helminth infections of ruminants are a major constraint on efficient livestock production. The current challenge is to develop diagnostic methods that detect the production impact of helminth infections on farms in order to target control measures and contribute to the global challenge of preserving food security. This paper reviews the effects of helminth infections and control practices on productivity and the diagnostic tools that can inform this. By combining advances in helminth laboratory diagnostics and animal health economics, sustainable management of helminth infections can be integrated into the whole-farm economic context.


The free-living third-stage larvae (L3) of gastrointestinal nematodes are able to tolerate extreme weather conditions such as desiccation, but little is known about the consequent effects this has on their fitness. This study explored how the desiccation of *Haemonchus contortus* L3 larvae affected their absolute fitness by examining their success at consequent life cycle stages for a complete generation, and comparing them against a control. The results show that while desiccation greatly reduced the survival of the L3 prior to infection in sheep, their absolute fitness was not negatively impacted. Instead, it appears desiccation slightly augmented *H. contortus* fitness by triggering increases in fecundity. The study further explored what influence different gastrointestinal nematode (GIN) species (*H. contortus, Trichostrongylus colubriformis, Teladorsagia circumcincta*), isolates and age of L3 had on their capacity to revive following various periods of desiccation. The results showed desiccation tolerance varied as a function of each of these variables. The greatest L3 survival was found in *Te. circumcincta* followed by *Tr. colubriformis* and finally *H. contortus*. Significant variation was observed between individual species isolates and as a function of age. The results of this study carry important practical implications for the epidemiological understanding of gastrointestinal nematode species of economic

The abomasal nematode *Haemonchus contortus* causes severe disease and production loss in small ruminants in warmer regions and is also an emerging threat in many temperate climates. Specific knowledge of the effects of climate on the epidemiology of *H. contortus* is needed to effectively apply sustainable control strategies, which rely on prediction of infection risk. Although the effects of temperature and rainfall on larval development in this species have been characterised, much less is known about migration out of faeces and onto herbage. This is an important deficit in our understanding of the epidemiology of haemonchosis in regions with relatively low and particularly erratic rainfall. Methods were developed to assess the migration of third stage larvae (L3) out of faeces under simulated rainfall in the laboratory. These were applied in a series of experiments, which showed that rainfall is required for migration. However, a single rainfall event was not sufficient for migration from faeces of which the crust has hardened after having been kept in dry conditions. Light and regular rainfall resulted in rapid emergence from moist faeces kept in humid conditions, but much slower emergence from dry faeces in dry conditions. Ambient relative humidity therefore appears to act through faecal moisture content to modify the effect of rainfall on larval migration. Larvae survived well in dry faeces for a number of days, but did not migrate in the absence of rainfall, so sheep faeces could potentially act as a larval reservoir in dry conditions, with peaks of infection following rainfall. Rates of faecal desiccation and rehydration on pasture could therefore be highly relevant to temporal patterns of larval availability.


Meat inspection (MI) is one of the most widely implemented and longest running systems of surveillance. It was primarily introduced to identify meat of animals that is not fit for human consumption. Additionally, MI was progressively recognised as a suitable source of data collection and for monitoring a broad spectrum of diseases and conditions concerning animal health and welfare. For Europe, MI tasks are regulated at the European rather than country level and include a set of activities before and after stunning (ante and post mortem inspection) involving visual inspection, palpation and incisions. Over the last decade, the current MI protocol has been challenged because of its low sensitivity for important public health hazards. We aimed to assess the strengths and weaknesses of current MI protocols with primary focus on its utility in the context of animal health including both notifiable and production diseases and welfare, i.e. its capacity to detect cases with an aim to quantify the frequency of animal disease and welfare cases. The consequences of an alternative inspection protocol using visual-only inspection were also explored. As a first step, a review of grey and published literature was conducted for a selected number of diseases and welfare conditions in seven species or species groups: swine, poultry, bovines, small ruminants, solipeds (horses etc.) and farmed game, represented by red deer, wild boar, rabbits and ostriches. This review highlighted a substantial lack of suitable and accessible published data on the frequency of occurrence of many diseases and conditions affecting food animals in Europe. Additionally, there were very limited data on the detection performance of MI, particularly in relation to specific degrees of severity of clinical signs. Due to the data gaps, a large proportion of input data used in this work was based on expert opinion and general biologic manifestations of the conditions investigated. The probability of case detection was quantified using a scenario tree modelling approach, taking into account the frequency of case presentation and inspection coverage. In general, the performance of MI was highly correlated with the presence of clinical and/or pathological signs in affected animals. Early or subclinical cases were likely to be "non-detectable" at slaughter. Regarding detectable cases, the impact of moving to visual-only inspection was negligible for most notifiable diseases and conditions considered with a few exceptions, primarily detectable cases of tuberculosis. Current MI activities were found to be effective to detect the majority of animal welfare conditions considered by species, predominantly by ante mortem inspection. The effectiveness of MI was also considered for endemic diseases that are not currently subject to systematic control efforts. These included respiratory diseases and parasite infections. It was shown that MI could provide an efficient means of identifying producers in need of animal health advice, provided that information is collected and fed back to veterinarians and livestock farmers. Within an integrated information system, MI could substantially contribute to the control of a considerable range of animal health and welfare issues. Data already collected need to be made available for on-farm decision making. It was also noted that if the slaughter population is strongly affected by international trade, i.e. where a large proportion of animals originate from one country and are slaughtered in another, the usefulness of MI for endemic disease surveillance will be affected by either reduced coverage or bias or both. In conclusion, our results indicate that while ante mortem inspection remains essential for the detection of animal welfare conditions, a move to visual-only post mortem inspection has for the diseases and conditions considered negligible negative impact on disease control. However in countries or regions that are...
not free of TB, special relevance of palpation and cutting of lymph nodes will have to be considered. MI information has considerable potential to inform disease control efforts, but only few countries use it systematically limiting the actual benefit that is achieved by these data. Finally, MI can also provide "back-up" surveillance in a situation where other means of detection fail and may represent the sole means of case detection for certain infections (e.g. liver fluke or cestodes).

DIAGNOSIS/IDENTIFICATION


Gastrointestinal nematode (GIN) parasites pose a significant economic burden particularly in small ruminant production systems. Anthelmintic resistance is a serious concern to the effective control of GIN parasites and has fuelled the focus to design and promote sustainable control of practices of parasite control. Many facets of sustainable GIN parasite control programs rely on the ability to diagnose infection both qualitatively and quantitatively. Diagnostics are required to determine anthelmintic efficacies, for targeted treatment programs and selection of animals for parasite resistant breeding. This review describes much of the research investigated to date to improve the current diagnostic for the above practices which is based on counting the number of parasite eggs in faeces.

Molecular identification


Agricultural ruminants usually harbour mixed infections of gastrointestinal nematodes. A specific diagnosis is important because distinct species can differ significantly in their fecundity and pathogenicity. Haemonchus spp. and Cooperia spp. are the most important gastrointestinal nematodes infecting ruminants in subtropical/tropical environments. In Brazil, C. punctata is more adapted to cattle than sheep. Additionally, C. spatulata appears to be more adapted to cattle, whereas C. curticei is more adapted to sheep. However, infection of sheep with C. punctata is common when cattle and sheep share the same pasture. Although morphological analyses have been widely used to identify nematodes, molecular methods can overcome technical limitations and help improve species-specific diagnoses. Genetic markers in the first and second internal transcribed spacers (ITS-1 and ITS-2, respectively) of nuclear ribosomal DNA (rDNA) have been used successfully to detect helminths. In the present study, the ITS-1 region was analysed and used to design a species-specific oligonucleotide primer pair to identify C. curticei. The polymerase chain reaction (PCR) product was sequenced and showed 97% similarity to C. oncophora partial ITS-1 clones and 99% similarity to the C. curticei sequence JF680982. The specificity of this primer pair was corroborated by the analysis of 17 species of helminths, including C. curticei, C. punctata and C. spatulata. Species-specific diagnosis, which has implications for rapid and reliable identification, can support studies on the biology, ecology and epidemiology of trichostrongyloid nematodes in a particular geographical location.


A multiplex PCR-based method was developed to overcome the limitations of microscopic examination as a means of identifying individual infective larvae from the wide range of strongylid parasite species commonly encountered in sheep in mixed sheep-cattle grazing situations in New Zealand. The strategy employed targeted unique species-specific sequence markers in the second internal transcribed spacer (ITS-2) region of ribosomal DNA of the nematodes and utilises individual larval lysates as reaction templates. The basic assay involves two sets of reactions designed to target the ten strongylid species most often encountered in ovine faecal cultures under New Zealand conditions (viz. Haemonchus contortus, Teladorsagia circumcincta, Trichostrongylus axei, Trichostrongylus colubriformis, Trichostrongylus vitrinus, Cooperia curticei, Cooperia oncophora, Nematodirus spathiger, Chabertia ovina, and Oesophagostomum venulosum). Five species-specific primers, together with a pair of "generic" (conserved) primers, are used in each of the reactions. Two products are generally amplified, one by the generic primer pair regardless of species (providing a positive PCR control) and the other (whose size is indicative of the species) by the appropriate species-specific primer in combination with one or other of the generic primers. If necessary, any larvae not identified by these reactions can subsequently be tested using primers designed specifically to detect those species less frequently encountered in ovine faecal cultures (viz. Ostertagia ostertagi, Ostertagia leptospicularis, Cooperia punctata, Nematodirus filicollis, and Bunostomum trigonocephalum). Results of assays undertaken on >5500 nematode larvae cultured from lambs on 16 different
farms distributed throughout New Zealand indicated that positive identifications were initially obtained for 92.8% of them, while a further 4.4% of reactions gave a generic but no visible specific product and 2.8% gave no discernible PCR products (indicative of insufficient or poor quality DNA template). Of the reactions which yielded only generic products, 91% gave positive identifications in an assay re-run, resulting in a failure rate of just similar to 0.4% for reactions containing amplifiable template. Although the method was developed primarily to provide a reliable way to identify individual strongyloid larvae for downstream molecular applications, it potentially has a variety of other research and practical applications which are not readily achievable at present using other methods.

dos Santos, M. C., et al. (2014). "Differentiation of Haemonchus placei from Haemonchus contortus by PCR and by morphometrics of adult parasites and third stage larvae." Revista Brasileira De Parasitologia Veterinaria 23(4): 495-500. Molecular and morphological methods were evaluated to distinguish between Haemonchus contortus and Haemonchus placei species. A total of 141 H. contortus and 89 H. placei male adult specimens collected from artificially infected lambs were identified individually by PCR analysis, using a species-specific primer pair. These PCR results were used as gold standard for Haemonchus spp. identification. Haemonchus placei presented higher mean spicule and barb lengths than H. contortus (P<0.05). However, some measurements overlapped. For this reason, a discriminate function did not allow the correct identification of 13 H. contortus and one H. placei specimen. The sheath tail length of the third stage larvae (L3), which comprises the distance between the tip of the larval tail and the end of the sheath tail, were measured. Only three of the 485 H. placei larvae (0.619%) had a sheath tail shorter than 85 μm, while only four of the 500 H. contortus larvae (0.8%) presented a sheath tail longer than 85 μm. The results indicated that 6.09% of the male adult specimens would be misclassified based on the discriminate function, while only 0.71% of infective larvae would be misclassified. Therefore, identification of L3 can be used as the first method to indicate the presence of H. placei and/or H. contortus in a population of domestic ruminants.

Melville, L., et al. (2014). "Development of a loop-mediated isothermal amplification (LAMP) assay for the sensitive detection of Haemonchus contortus eggs in ovine faecal samples." Veterinary Parasitology 206(3-4): 308-312. A major constraint on the effective control and management of helmint parasites in livestock is the lack of rapid and reliable diagnostic tests to identify the parasite species responsible for disease and to allow informed treatment decisions to be made. In the present study, we have developed a novel DNA-based assay for the detection of Haemonchus contortus eggs in ovine faecal samples, using loop-mediated isothermal amplification (or LAMP). LAMP allows for rapid detection of H. contortus DNA under isothermal incubation conditions. The robust nature of this assay negates the need for extensive DNA extraction, allowing amplification from relatively crude samples. Preliminary results suggest that LAMP is highly specific, and does not cross-react with DNA from other common co-infecting parasites. The Haemonchus LAMP assay is also highly sensitive, exhibiting a 10 times lower detection limit than the equivalent PCR, allowing detection in a faecal samples containing two Haemonchus eggs per gram. Translation of this assay onto a real-time platform provided rapid results and highlighted its potential as a quantitative assay which could inform treatment decisions in the future.

Roeber, F. and Kahn, L. (2014). "The specific diagnosis of gastrointestinal nematode infections in livestock: Larval culture technique, its limitations and alternative DNA-based approaches." Veterinary Parasitology 205(3-4): 619-628. The specific diagnosis of gastrointestinal nematode infections in ruminants is routinely based on larval culture technique and on the morphological identification of developed third-stage larvae. However, research on the ecology and developmental requirements of different species suggests that environmental conditions (e.g., temperature and humidity) for optimal development to occur vary between the different species. Thus, employing a common culture protocol for all species will favour the development of certain species over others and can cause a biased result in particular when species proportions in a mixed infection are to be determined. Furthermore, the morphological identification of L3 larvae is complicated by a lack of distinctive, obvious features that would allow the identification of all key species. In the present paper we review in detail the potential limitations of larval culture technique and morphological identification and provide account to some modern molecular alternatives to the specific diagnosis of gastrointestinal nematode infection in ruminants.

Non Molecular Diagnosis

Schurer, J., et al. (2014). "Effects of sub-zero storage temperatures on endoparasites in canine and equine feces." Veterinary Parasitology 204(3-4): 310-315. Fecal samples from wild and domestic carnivores are routinely frozen for three days at -80 degrees C to kill eggs of Echinococcus spp., following recommendations from the World Health Organization (WHO) and World
Organization for Animal Health (OIE). This is done to decrease the risk of zoonotic infection with these pathogenic cestodes. In addition, it is often necessary to freeze fecal samples collected for research prior to batch processing by a limited number of personnel, especially large numbers of samples or those collected in remote locations. The effect of freezing on the recovery of endoparasite eggs, cysts and oocysts from fecal samples is not well documented, even in hosts for which veterinary diagnostic submissions are relatively common. In this study, fecal samples from naturally infected dogs and horses were split into four treatment groups: fresh; -80 degrees C for 3 days; -20 degrees C for 30 days; and -80 degrees C for 3 days followed by -20 degrees C for an additional 30 days. Temperatures and times were chosen to simulate diagnostic and research protocols currently in place. Helminth eggs and sporocysts of Sarcocystis spp. were counted using a quantitative double centrifugation sucrose fecal flotation (modified Stoll egg count). Repeated measures ANOVA was used to detect differences in egg/sporocyst counts between the treatment groups for Sarcocystis spp. sporocysts, taeniid eggs (Taenia and/or Echinococcus spp.), ascarid eggs (Parascaris equorum, Toxocara canis, Toxascaris leonina), and strongyloid type eggs (Uncinaria stenocephala, and equine strongylids, most likely cyathostomins). Counts for P. equorum and strongyloid type eggs (both equine and canine) decreased significantly following freezing. Post-freezing, some samples that had been positive on fresh examination became negative for Parascaris and strongyloid eggs. This study suggests that protocols requiring freezing artificially lowers recovery of eggs of Parascaris and strongyloid nematodes in fecal surveys; however, recovery rates for eggs of other helminth species as well as sporocysts of Sarcocystis spp. were relatively robust compared to the freshly tested fecal samples. This is reassuring for large scale fecal surveys needed for fecal egg count reduction analyses following treatment, and for surveillance in wildlife and remote human and animal populations.


Immunoglobulin A (IgA) activity has been associated with reduced growth and fecundity of Teladorsagia circumcincta. IgA is active at the site of infection in the abomasal mucus. However, while IgA activity in abomasal mucus is not easily measured in live animals without invasive methods, IgA activity can be readily detected in the plasma, making it a potentially valuable tool in diagnosis and control. We used a Bayesian statistical analysis to quantify the relationship between mucosal and plasma IgA in sheep deliberately infected with T. circumcincta. The transfer of IgA depends on mucosal IgA activity as well as its interaction with worm number and size; together these account for over 80% of the variation in plasma IgA activity. By quantifying the impact of mucosal IgA and worm number and size on plasma IgA, we provide a tool that can allow more meaningful interpretation of plasma IgA measurements and aid the development of efficient control programmes.


Teladorsagia circumcincta is among the most important gastrointestinal parasites in small ruminants and the predominant species in Southern European goats. Parasite control is largely based on metaphylactic/preventative treatments, which is often seen as non-sustainable anymore. The reasons are increased consumer demand to reduce chemicals in livestock production and anthelmintic resistance against the common drugs. This study aimed at the development of a T. circumcincta-enzyme-linked immunosorbent assay (ELISA) specifically for goats. Samples were obtained from goats raised parasite-free or infected experimentally. Sampling continued during the following pasture season and housing period. The sensitivity for the use in bulk milk samples as an indicator of T. circumcincta infection levels in grazing goats was examined. The ELISA enables clear differentiation of negative and positive animals. With a specificity of 100 % negative cut-off values for serum and milk were 0.294 and 0.228 (sensitivity, 95 %). Positive cut-off values (sensitivity, 90 %) were 0.606 (serum) and 0.419 (milk), while a sensitivity of 95 % resulted in 0.509 and 0.363, respectively. The grey-zone between negative/positive cut-offs was introduced to deal with animals in pre-patency and decreasing antibody levels after infection. There was no cross reactivity for Trichostrongylus colubriformis and Cooperia oncophora while for Haemonchus contortus and Fasciola hepatica it cannot be fully excluded currently. In bulk milk samples, 5 % of the milk had to be contributed from animals infected with T. circumcincta to be detected as positive. The results derived from experimentally and naturally infected as well as parasite naive animals indicate the potential of the ELISA to be used in targeted anthelmintic treatment regimes in goats.

GENERAL GI PARASITE CONTROL

Considering the increasing concern for the development of anthelmintic resistance, the use of pharmacology-based information is critical to design successful strategies for the future of parasite control in livestock. Integrated evaluation of the available knowledge on pharmacological features is required to optimize the activity and to achieve sustainable use of the existing anthelmintic drugs. The assessment of the drug disposition in the host and the comprehension of the mechanisms of drug influx/efflux/detoxification in different target helminths, has signified a relevant progress on the understanding of the pharmacology of anthelmintic drugs in ruminant species. However, additional scientific knowledge on how to improve the use of available and novel molecules is required to avoid/delay resistance development. Different pharmacokinetic-based approaches to enhance parasite exposure and the use of mixtures of drugs from different chemical families have been proposed as valid strategies to delay the development of anthelmintic resistance. The rationale behind using drug combinations is based on the fact that individual worms may have a lower degree of resistance to a multiple component formulation (each chemical with different mode of action/resistance) compared to that observed when a single anthelmintic is used. However, the limited available information is unclear on the potential additive or synergistic effects occurring after co-administration of two (or more) drugs with different mode of action. This review article contributes to the topic with some pharmacology-based data emerging from the assessment of combined anthelmintic preparations. The activity against multi-drug-resistant isolates based on novel modes of action is a highly favorable element to judge the future of some of the recently developed anthelmintic compounds. More specific knowledge on the basic host parasite kinetic behavior as well as a highly responsible use of those novel compounds will be necessary to secure their maximum lifespans. Overall, the outcome from integrated pharmaco–parasitological research approaches has greatly contributed to optimize drug activity, which seems relevant to preserve existing and particularly novel active ingredients as useful tools for parasite control in livestock animals.

**CATTLE GI PARASITES**


Emerging parasitic diseases detected by scanning surveillance by the AHVLA from 2006 to 2013 were described. The possibilities of new parasites being introduced or changing incidence of endemic parasites is likely to continue due to cattle movements, changing weather patterns, changes in cattle management and drug usage. This paper focuses on four parasites that are emerging in cattle in Great Britain (GB). The incidence of paramphistomiasis (rumen fluke) infections has been influenced by the wet summers of 2007-2012. Disease associated with acute larval paramphistomiasis appears to be rare but when it occurs is a significant cause of disease and mortality. The impact on health and production of moderate and heavy burdens of adult paramphistomes in beef and dairy cattle has not been evaluated. *Trichuris* spp are parasites of the large intestine that can complete their life cycle indoors and may cause diarrhoea and ill-thrift in housed calves. Importation of animals from abroad is likely to have introduced *Toxocara vitulorum* and psoroptic mange to GB. There is a continued risk of spread of these two parasites within the country, and also further introductions through importation. Finally the threat of *Besnoitia besnoiti* introduction is highlighted.


Predictive models of parasite life cycles increase our understanding of how parasite epidemiology is influenced by global changes and can be used to support decisions for more targeted worm control. Estimates of parasite population dynamics are needed to parameterize such models. The aim of this study was to quantify the main life history traits of *Ostertagia ostertagi*, economically the most important nematode of cattle in temperate regions. The main parameters determining parasite density during the parasitic phase of *O. ostertagi* are: (i) the larval establishment rate, (ii) hypobiosis rate, (iii) adult mortality and (iv) female fecundity (number of eggs laid per day per female). A systematic review was performed covering studies from 1962 to 2007, in which helminth-calve calves were artificially infected with *O. ostertagi*. The database was further extended with results of unpublished trials conducted at the Laboratory for Parasitology of Ghent University, Belgium. Overall inverse variance weighted estimates were computed for each of the traits through random effects models. The establishment rate declined when infection dose increased and was lower in younger animals. The proportion of ingested larvae that went into hypobiosis was higher in animals that received concomitant infections with nematode species other than *O. ostertagi* (mixed infections). Adult mortality was positively correlated with infection dose. The average female sex ratio of *O. ostertagi* based on individual animal data (n = 75) from six different studies was estimated to be 0.55. In conclusion, this meta-analysis provides novel estimates for the parameterization of life cycle-based transmission models, explicitly reports measures of variance around these estimates, gives evidence for density dependence of larval establishment and adult mortality, shows that host
age affects larval establishment and, to our knowledge, provides the first evidence for O. ostertagi of a female-biased sex ratio.


Gastrointestinal nematodes are an important cause of reduced performance in cattle. Previous studies in Europe showed that after anthelmintic treatment an average gain in milk production of around 1 kg per day/cow can be expected. However, (1) these studies have mainly evaluated group-based anthelmintic treatments during the grazing season or at housing and (2) little is known about parameters affecting variations in the treatment response amongst cows. A better knowledge of such parameters could help to select animals that benefit most from treatment and thus lead to a more rational use of anthelmintics. Therefore, a randomized, non-blinded, controlled clinical trial was performed on 11 commercial dairy farms (477 animals) in Belgium, aiming (1) to study the effect of eprinomectin treatment at calving on milk production and (2) to investigate whether the milk yield response was related to non-invasive animal parameters such that these could be used to inform targeted selective treatment decisions. Results: Analyses show that eprinomectin treatment around calving resulted in an average (+/- standard error) increase of 0.97 (+/- 0.41) kg in daily milk yield that was followed up over 274 days on average. Milk yield responses were higher in multiparous compared to primiparous cows and in cows with a high (4th quartile) anti-O. ostertagi antibody level in a milk sample from the previous lactation. Nonetheless, high responses were also seen in animals with a low (1st quartile) anti-O. ostertagi antibody level. In addition, positive treatment responses were associated with higher faecal egg counts and a moderate body condition score at calving (2nd quartile). In conclusion, this study provides novel insights into the production response after anthelmintic treatment at calving and factors which influence this. The data could be used to support the development of evidence-based targeted selective anthelmintic treatment strategies in dairy cattle.


Haemonchus placei is an abomasal parasite of cattle, primarily in tropical and subtropical areas of the world. In Australia, this nematode can be extremely pathogenic in summer rainfall areas, particularly in the hot, sub-tropical Kimberley region, in the far north of the state of Western Australia (WA). Although cattle are occasionally transferred to southern parts of WA, it was believed that H. placei did not occur in southern regions of WA, as it is less cold-adapted than Haemonchus contortus, and the free-living stages would not develop during the cold winter and dry summer periods. Here, we show that, although H. contortus is found in cattle in the temperate southern region of WA, it appears that H. placei also occurs in southern WA. While investigating the prevalence of anthelmintic resistance in nematodes of cattle in WA, the existence of H. placei was suspected on a range of participating farms, following the morphological examination of third-stage larvae cultured from faeces, and of adult worms recovered from sheep experimentally infected with these larvae. Genomic DNAs from individual worms as well as eggs from pooled faecal samples from seven farms in southern WA were subjected to PCR-based mutation scanning and sequence analyses of the second internal transcribed spacer (ITS-2) of nuclear ribosomal DNA. The results showed that both H. contortus and H. placei were harboured by cattle. This first record of H. placei in cattle in southern WA raises questions as to the prevalence and distribution of this parasite in other temperate and cool climatic regions of Australia. Although clinical disease due to H. placei has not yet been seen in southern WA, global, climatic trends might suggest an increased importance of this parasite in the longer term.

GI PARASITE CONTROL IN CATTLE


Dictyocaulus viviparus, Ostertagia ostertagi (nematode parasites), and Fasciola hepatica (trematode parasite) result in productivity losses on dairy farms and impact on animal health through clinical and sub-clinical disease. Parasite control in livestock systems is largely based on the use of chemoprophylactic agents (anthelmintics), grazing management, or a combination of both. The objective of this study was to document current parasite control measures employed by Irish dairy farmers in a predominantly pasture-based livestock system. A questionnaire survey of 312 geographically representative farmers was completed in 2009 with a follow up survey completed in 2011. Statistical analysis highlighted significant differences in chemoprophylactic usage between 2009 and 2011. In particular, an increase in the use of albendazole for both trematode (19% in 2009 to 36% in 2011) and nematode (30% in 2009 to 58% in 2011) control was observed. This was most likely due to flukicide restrictions introduced in the Republic of Ireland in 2010 for dairy animals. Logistic regression
highlighted regional differences in chemoprophylactic use. Farmers in southern parts of Ireland, an area with good quality soil, less rainfall, and a higher density of dairy farms than other regions, were approximately half as likely to dose for *F. hepatica* and were more likely (OR > 2.0) to use albendazole for both nematode and fluke control. Approximately 30% of respondents who used a chemoprophylactic treatment for nematodes, used a product which was ‘unsuitable for purpose’ (e.g. ivermectin for the treatment of *F. hepatica*), highlighting the need for increased awareness, continuing research, and regionally targeted education tools regarding optimal parasite control.


As anthelmintic resistance is increasingly being reported in cattle worldwide, there is a need to explore alternative approaches to gastrointestinal nematode control in cattle. A novel approach is the use of targeted selective treatments (TST) where only individual animals are treated instead of the entire group. The study objective was to determine if anthelmintic usage could be reduced using a TST-based approach in rotationally grazed first-grazing season suckler beef calves without affecting calf performance. Eighty-eight spring-born suckler beef calves, naïve to anthelmintics, with an initial mean (s.d.) age and live weight of 159 (22.4) days and 221 (42.4) kg, respectively, were used. All calves were vaccinated at pasture against dictyocaulosis at 8 and 12 weeks old. On August 9th 2013 (Week 0), when the trial began, calves were randomised by age, weight, sex, dam breed and sire breed to one of two treatments: (1) standard treatment (positive control) (n = 44) and (2) TST (n = 44). Samples collected one week prior to the start of the study were used as baseline covariates. Each treatment group was replicated once. All calves in the control groups were treated subcutaneously with levamisole on Week 0 and on Week 6. Individual calves in the TST groups were only eligible for treatment at pasture with the same product if predetermined thresholds were reached [plasma pepsinogen > = 2.0 international units of tyrosine/litre and faecal egg count > = 200 eggs per gram of faeces]. The trial concluded at housing on Week 13. Data were analysed using repeated measures mixed models ANOVA (PROC MIXED) (SAS 93). No calves in the TST groups were treated for gastrointestinal nematodes during the study period as they did not reach pre-determined treatment thresholds. Mean (sem) calf daily live weight gain for control and TST groups was 0.90 (+/- 0.04) and 0.92 (+/- 0.03) kg, respectively (P = 0.68). Using an ELISA to detect antibodies to *Dictyocaulus viviparus* at Week 11.81% of calves were seropositive. Gastrointestinal nematode challenge in spring-born suckler beef calves under these conditions can potentially be controlled with minimal anthelmintic treatments whilst not significantly impairing calf performance, provided appropriate control measures are taken to prevent dictyocaulosis from occurring.


To investigate future tools for targeted selective treatment against gastrointestinal nematodes (GIN) in adult dairy cows, we evaluated herd and individual cow factors associated with the post-treatment milk production (MP) response over time. A field trial involving 20 pasturing dairy herds in Western France was conducted in autumn 2010 and autumn 2011. In each herd, lactating cows were randomly allocated to a treatment group (fenbendazole) (623 cows), or a control group (631 cows). Daily cow MP was recorded from 2 weeks before until 10 to 14 weeks after treatment. Individual serum anti-Ostertagia antibody levels (expressed as ODR), pepsinogen levels, faecal egg count (FEC), and bulk tank milk ODR were measured at the time of treatment. Moreover, in each herd, information regarding heifers’ grazing and treatment history was collected to assess the Time of Effective Contact (TEC, expressed in months) with GIN infective larvae before the first calving. TEC was expected to reflect the development of immunity against GIN, and TEC=8 months was a cautious threshold over which the resistance to re-infection was expected to be established. Daily MP averaged by week was analyzed using linear mixed models with three nested random effects (cow within herd and herd within year). The overall treatment effect was significant but slight (maximum = +0.85 kg/d on week 6 after treatment), and the evolution of treated cows’ MP differed significantly according to several factors. At the herd level, cows from low-TEC herds responded better than cows from high-TEC (>= 8 months) herds; cows from herds in which the percentage of positive FEC was >22.6% (median value) responded better than those from herds where it was lower. At the individual cow level, primiparous cows, cows with days in milk (DIM) < or = 100 at the time of treatment, and cows with low individual ODR (< or = 0.38) responded better than multiparous cows, cows with DIM > 100, and cows with higher ODR, respectively. These results highlight the variability of the treatment response, suggesting that whole herd anthelmintic treatment are not always appropriate, and propose promising key criteria for targeted selective treatment for GIN in dairy cows. Particularly, the TEC is an original criterion which lends support for a simultaneous on-farm qualitative analysis of grazing management factors.
GI PARASITE CONTROL SHEEP AND GOATS


The objective of this study was to investigate the effect of vitamin E supplementation on an experimental Haemonchus contortus infection in lambs. Twenty lambs were stratified into two treatment groups based on fecal egg count. Worm-free lambs, 28-32 weeks of age, were supplemented with vitamin E (D-alpha-tocopherol) for 12 weeks following the recommendations of the National Research Council for the minimum daily requirement or the requirement for optimal immune function. Five weeks following initiation of vitamin E supplementation, lambs were infected with 10,000 H. contortus third stage larvae. Samples were taken weekly to quantify serum alpha-tocopherol, serum total non-specific immunoglobulin (Ig)G, whole worm antigen specific IgG, packed cell volume (PCV), and fecal egg count (FEC). Expression of cytokine genes IFN-lambda and IL-4 were measured in peripheral blood collected prior to slaughter. Lambs were necropsied six weeks after infection and the alpha-tocopherol concentration of liver, muscle and lymph node were measured as well as abomasal worm burden and histologic evaluation of the abomasum for inflammation and enumeration of eosinophils and globule leukocytes. The livers of VE10 lambs contained slightly more alpha-tocopherol than control lambs. No differences were observed in serum, muscle or lymph node alpha-tocopherol concentration, serum IgG or peripheral mRNA expression of IL-4 or IFN-lambda between control and VE10 lambs. However, lambs supplemented at 10 IU/kg BW/d had a lower PCV reduction, FEC and worm burden 49% less than control lambs. Worm burden was negatively correlated with eosinophil (-0.720, P < 0.05) and globule leukocyte count (-0.867, P < 0.05). Strong positive correlations were observed within the inflammatory cell response in VE10 lambs that was absent in control lambs. These data indicate that additional vitamin E supplementation resulted in lower worm burden and greater recruitment of innate effector cells to the site of infection. Further studies are necessary to elucidate the mechanism by which vitamin E affects greater recruitment of innate effector cells to the abomasum during gastrointestinal nematode infection of lambs.


This study was conducted to determine whether targeted anthelmintic treatment of peri-parturient ewes lambing in the winter, spring and/or autumn would suppress the peri-parturient egg rise (PPER) and improve 50-day lamb weights. Three farms in Ontario, Canada, that practiced out-of-season lambing were enrolled in 2010 and sampled for three consecutive lambing seasons (winter, spring and autumn). For each lambing season, all farms were visited three times. On the first visit, all ewes due to lamb that season were randomly allocated to treatment with ivermectin, fenbendazole or levamisole at the recommended dosage, or left untreated. Among these treated ewes, 40-60 animals (10-15 ewes per treatment group) were randomly selected for fecal sampling during the 3 sampling visits and processed individually to measure gastro-intestinal nematode (GIN) fecal egg counts (FECs). Ewe and lamb productivity data, including approximate 50-day lamb weights, were collected for all ewes lambing in each season, where available. A Fecal Egg Count Reduction Test was performed on all three farms to determine the ivermectin, fenbendazole and levamisole resistance status. Both farms A and B had fenbendazole resistance, while farm C had ivermectin and fenbendazole resistance; levamisole was effective on all three farms. The effect of targeted treatment on the subsequent PPER depended on the farm, possibly a partial surrogate variable for the different anthelmintic resistance levels on each farm, lambing season and sampling time-point. On farm A, during the winter and autumn lambing seasons, ivermectin and levamisole were more effective at reducing the FECs, compared to fenbendazole. In contrast, during the spring lambing season, treatment of ewes with ivermectin, fenbendazole or levamisole had no effect on the FECs. On farm B, all anthelmintic treatments were associated with a reduction in the FECs during the spring lambing season, while no reduction was observed during the winter and autumn lambing seasons. On farm C, the FECs decreased in ewes treated with levamisole in both the winter and spring lambing seasons, while ivermectin only reduced the FECs in ewes treated in the winter lambing season. Litter size was positively associated with FECs. Anthelmintic treatment was not associated with approximate 50-day lamb weights, although the power to detect significant difference was lower than anticipated due to only having relevant weight data from farm A. These results suggest that the efficacy of targeted treatment for the suppression of the PPER depends on the anthelmintics’ efficacy and time of treatment in relation to the grazing period.


Monepantel (MOP), a new anthelmintic drug from a group of amino-acetonitrile derivatives, has been
intensively studied during last years. Many authors examined this new drug from different perspectives, e.g. efficacy against different species and stages of parasites, mode of action, metabolism, pharmacokinetics, toxicity, resistance, ecotoxicity, etc. MOP is an anthelmintic for livestock (currently only sheep and goats), with molecular mode of action which is different to all other anthelmintics. MOP has a broad-spectrum of activity against gastrointestinal nematodes of sheep, including adults and L-4 larvae of the most important species. The key feature of MOP is its full effectiveness against strains of nematodes resistant to benzimidazoles, levamisole, macrocyclic lactones and closantel. After oral administration, MOP is quickly absorbed into the bloodstream and quickly metabolized to MOP sulfone that has a similar efficacy as the parent molecule. Several other MOP metabolites formed in ovine hepatocytes were described. MOP and its metabolites are considered to be non-toxic to environment and its components, such as soil microflora, aquatic organisms, dung organisms, vegetation, etc. The aim of the presented review was not to collect all reported data but to bring an overview of various approaches in the study of MOP and to evaluate their principal results.


The aim of this study was to compare the liveweight gain of lambs, infected by multidrug-resistant nematodes, treated by conventional schemes of helminth control or using a schedule based on faecal egg count reduction test (FECRT). The flock was selected after a FECRT (experiment 1) which revealed a parasite population resistant to benzimidazoles, imidazothiazoles, macrocyclic lactones (ivermectin), salicylanilides, nitrophenols, and organophosphates. Despite the parasite resistance to ivermectin (an avermectin), the moxidectin (a milbemycin) was effective against the gastrointestinal nematodes (PR > 90 %). In experiment 2, 48 suckling lambs were distributed in four randomized blocks (G1, G2, G3, and G4) by previous body weighings. G1 was kept as untreated control; G2 was treated following a FECRT-based schedule with drugs chosen based on faecal analysis (first drench with moxidectin, second drench with a combination of moxidectin and levamisole, and third drench with praziquantel, an anti-cestode drug); G3 and G4 received three drenches with ivermectin or disophenol, respectively. Body weighings and faecal analysis of these lambs were performed every 2 weeks over a 98-day period. An effective control of gastrointestinal nematodes was obtained with two nematicidal drenches following the FECRT-based schedule of treatments. On the other hand, eggs per gram of feces (EPG) counts were no different among untreated control, G3, and G4. Lambs treated using the FECRT-based schedule had the greatest liveweight gain among the groups tested. Additionally, liveweight gain was no different among the groups G3, G4, and G1. The FECRT-based schedule of anthelmintic treatments was beneficial regarding productivity and sustainability of helminth control in lambs infected by multidrug-resistant nematodes.


Pharmacokinetics and anthelmintic activity of topical eprinomectin in goats prevented from physical contact to others and self-grooming were studied. Sixteen approximately 7 months old male castrated German White Noble goats harbouring induced infections of gastrointestinal nematode parasites were included in the study. They were blocked based on pre-treatment body weight (range 22.4 to 36.4 kg) and then randomly allocated to the untreated control group or the group treated with topical 0.5 % w/v eprinomectin (EPRINEXA (R) Pour-on, Merial) at 1 mg/kg body weight. Plasma samples were collected prior to and at intervals up to 14 days following treatment and analyzed to determine the concentrations of eprinomectin (B1a component). Parasites were recovered, identified, and counted following necropsy 14 days after treatment. Goats treated with topical eprinomectin had significantly fewer (a parts per thousand yen99 % reduction, p < 0.01) adult Trichostrongylus colubriformis infected than the untreated control group. Goats treated with topical eprinomectin had significantly fewer (a parts per thousand yen99 % reduction, p < 0.01) adult Trichostrongylus colubriformis infected than the untreated control group. Goats treated with topical eprinomectin had significantly fewer (a parts per thousand yen99 % reduction, p < 0.01) adult Trichostrongylus colubriformis infected than the untreated control group. Goats treated with topical eprinomectin had significantly fewer (a parts per thousand yen99 % reduction, p < 0.01) adult Trichostrongylus colubriformis infected than the untreated control group. Goats treated with topical eprinomectin had significantly fewer (a parts per thousand yen99 % reduction, p < 0.01) adult Trichostrongylus colubriformis infected than the untreated control group. Goats treated with topical eprinomectin had significantly fewer (a parts per thousand yen99 % reduction, p < 0.01) adult Trichostrongylus colubriformis infected than the untreated control group.


The goals of the current trial were (a) to characterize the plasma disposition kinetics of levamisole (LEV), albendazole (ABZ) and ivermectin (IVM), each administered either alone (single active ingredient) or as a combined formulation to lambs; (b) to compare the clinical anthelmintic efficacy of the same drugs given either separately or co-administered to lambs infected with resistant nematodes. Fifty Corriedale lambs naturally infected with multiple resistant gastrointestinal nematodes were involved in the following experimental trials: (a) "Pharmacokinetic trial": the animals were allocated into five groups (n = 10 each) and intraruminally treated with
either LEV (8 mg/kg), ABZ (5 mg/kg), IVM (0.2 mg/kg), or with a LEV + ABZ + NM combined formulation, where each active ingredient was administered at the same dose. Blood samples were collected over 15 days post-treatment and drug plasma concentrations measured by HPLC. (b) "Efficacy trial": the same treated groups plus an untreated control group were used to assess the comparative anthelmintic efficacy by the faecal egg count reduction test (FECRT). Although the overall LEV disposition kinetics was unaffected, significantly lower (61%) ABZ-sulphoxide and higher (71%) NM systemic availabilities were obtained after administration of the combined formulation in comparison to those obtained after treatment with each drug alone. A multiple drug resistance situation was observed for Haemonchus spp. The observed efficacies were 52% (LEV), 72% (ABZ), 80% (NM) and 87% (triple combined formulation). The results reported here contribute to the pharma-therapeutic knowledge on drug combinations. This type of research is crucial before further development of combined anthelmintic preparations reaches the market to deal with resistant nematode control. The co-administration of LEV + ABZ + NM did not result in a significant advantageous anthelmintic effect compared to the treatment with NM alone. The simultaneous/combined administration of LEV, ABZ and IVM may account for a drug-drug pharmacological interaction in infected lambs. The pharmacokinetic interaction accounted for a reduced ABZ-sulphoxide and enhanced NM systemic exposure following the combined treatment.

Lifschitz, A., et al. (2014). "Accumulation of monepantel and its sulphone derivative in tissues of nematode location in sheep: Pharmacokinetic support to its excellent nematocidal activity." Veterinary Parasitology 203(1-2): 120-126. The amino-acetonitrile derivatives (AADs) are a new class of anthelmintic molecules active against a wide range of sheep gastrointestinal (GI) nematodes including those that are resistant to other anthelmintic families. The plasma disposition of monepantel (MNP) has been previously characterized in sheep. However, information on drug concentration profiles attained at tissues of parasite location is necessary to fully understand the pharmacological action of this novel compound. The current work aimed to study the relationship between the concentrations of MNP parent drug and its main metabolite monepantel sulphone (MNPSO2), measured in the bloodstream and in different GI tissues of parasite location in sheep. Twenty two (22) uninfected healthy Romney Marsh lambs received MNP (Zolvix (R), Novartis Animal Health) orally administered at 2.5 mg/kg. Blood samples were collected from six animals between 0 and 14 days post-treatment to characterize the drug/metabolite plasma disposition kinetics. Additionally, 16 lambs were sacrificed at 8, 24, 48 and 96 h post-administration to assess the drug concentrations in the GI fluid contents and tissues. MNP and MNPSO2 concentrations were determined by HPLC. MNP parent compound was rapidly oxidized into MNPSO2. MNP systemic availability was significantly lower than that observed for MNPSO2. The peak plasma concentrations were 15.1 (MNP) and 61.4 ng/ml (MNPSO2). The MNPSO2 to MNP plasma concentration profile ratio (values expressed in AUC) reached a value of 12. Markedly higher concentrations of MNP and MNPSO2 were measured in both abomasal and duodenal fluid contents, and mucosal tissues compared to those recovered from the bloodstream. A great MNP availability was measured in the abomasal content with concentration values ranging between 2000 and 4000 ng/g during the first 48 h post-treatment. Interestingly, the metabolite MNPSO2 was also recovered in abomasal content but its concentrations were significantly lower compared to MNP. The parent drug and its sulphone metabolite were detected in the different segments of the sheep intestine. MNPSO2 concentrations in the different intestine sections sampled were significantly higher compared to those measured in the abomasum. Although MNP is metabolized to MNPSO2 in the liver, the large concentrations of both anthelmintically active molecules recovered during the first 48 h post-treatment from the abomasum and small intestine may greatly contribute to the well-established pharmacological activity of MNP against GI nematodes.

HOST RESISTANCE TO GI PARASITISM

Andronicos, N. M., et al. (2014). "A one shot blood phenotype can identify sheep that resist Haemonchus contortus challenge." Veterinary Parasitology 205(3-4): 595-605. Gastrointestinal nematodes remain a major limitation to the productivity of livestock systems. Selective breeding to produce populations that have an enhanced ability to resist infection is a viable and ongoing option to reduce this impact. The development of new phenotypes that facilitate this process is therefore of great interest. For this reason we explored relationships between haematological parameters and the ability of sheep to resist nematode infection. A multivariate analytical approach was used to define algorithms based on the blood parameters that can be used to rank the ability of sheep to resist nematode infection in a single blood sample and can be applied independent of infection status. The algorithms were shown to classify susceptible sheep with a 100% accuracy and resistant sheep with 80% accuracy. Further development of this platform approach may be an important advance for small ruminant production systems worldwide and might also be applied to other diseases of livestock or even environmental stressors such as heat.

The objective of this study was to identify Scottish Blackface lambs that were at the extremes of the spectrum of resistance to gastrointestinal nematodes and characterise their response to an experimental nematode challenge. Lambs (n = 90) were monitored for faecal egg count (FEC) (2 samples from each of 2 independent natural infections). The most resistant (n = 10) and susceptible (n = 10) individuals were selected and challenged with 30,000 *Teladorsagia circumcincta* larvae (13) at 9 months of age. Response to infection was monitored by measuring FEC, plasma pepsinogen, serum antibodies against nematode larval antigens and haematology profile, until necropsy at 71 days post infection. Worm burden, worm fecundity and the level of anti-nematode antibodies in abomasal mucosa were determined at necropsy. FEC was consistently higher in susceptible animals (P < 0.05), validating the selection method. Worm fecundity was significantly reduced in resistant animals (P = 0.03). There was also a significant correlation (r = 0.88; P < 0.001) between the number of adult worms and FEC at slaughter. There was no effect of phenotype (resistance/susceptibility) on plasma pepsinogen or on haematology profile. Phenotype had a significant effect on the level of antinematode IgA antibodies in serum (P < 0.01), reflecting a higher peak in resistant animals at day 7 post infection. It is concluded that significant variation in the response to gastrointestinal nematode challenge exists within the Scottish Blackface population with resistant animals displaying significantly lower FEC, lower worm fecundity and higher concentration of anti-nematode IgA antibodies in serum.

**VACCINATION AGAINST GI PARASITES**


A vaccine containing integral membrane glycoproteins from the intestine of *Haemonchus contortus* was evaluated in three groups of grazing sheep each containing 13 ewes and their 16 lambs naturally infected with gastrointestinal nematodes. Two groups were vaccinated with either 5 or 50 μg of the antigen per immunisation, while the third, the control group, received adjuvant alone. The sheep were immunised six times at 3 week intervals, partly because the vaccine antigens are hidden and thus no immunological boost would be delivered by subsequent infection and partly because the level of *Haemonchus* spp. challenge was expected to be high. The vaccinated ewes, first immunised approximately 1 month before lambing, showed a circulating antibody response but no signs of reduced anaemia or *Haemonchus* spp. egg counts, compared with control ewes. Several ewes with severe haemonchosis in all three groups had to be given precautionary treatment with anthelmintic drugs. In contrast, vaccinating their lambs with either 5 or 50 μg of the antigen per immunisation resulted in 10 fold higher antibody titres. In the case of the lower antigen dose this was associated with significantly less anaemia, 72% reduction in the overall number of *Haemonchus* spp. eggs produced and significantly fewer worms compared with control lambs. It is hypothesised that the heavily pregnant or lactating ewes did not have sufficient physiological reserves to mount a protective response following vaccination in the tropical weather and high challenge conditions that prevailed. Nevertheless, the vaccine could afford useful protection for lambs against *H. contortus*.


A vaccine containing integral membrane glycoproteins from the intestine of *Haemonchus contortus* was evaluated in three groups of eight 5 month old grazing calves, naturally infected by *Haemonchus similis, Haemonchus placei* and other gastrointestinal nematodes. Vaccinated calves received 5 or 50 μg of the antigen and 1 mg of saponin adjuvant, while the controls received adjuvant alone, initially three times, 3 weeks apart and then four more times at 6 weeks intervals. Three weeks after the last immunisation all of the calves were euthanised for worm counts. Immunisation stimulated high titre antibodies against the vaccine antigens, reduced the egg output of *Haemonchus* spp. by 85% and the numbers of *H. placei* and *H. similis* by 63% and 32%, respectively, compared with control calves. It was concluded that vaccination with intestinal membrane glycoproteins from *H. contortus* could substantially reduce the transmission of *H. placei* and *H. similis*, thus providing protective benefit downstream. This appears to be the first known successful demonstration of a vaccine protective for cattle naturally exposed to infection with any gastrointestinal nematode parasite.


Helminth parasites infect over one fourth of the human population and are highly prevalent in livestock
worldwide. In model systems, parasites are strongly immunomodulatory, but the immune system can be driven to expel them by prior vaccination. However, no vaccines are currently available for human use. Recent advances in vaccination with recombinant helminth antigens have been successful against cestode infections of livestock and new vaccines are being tested against nematode parasites of animals. Numerous vaccine antigens are being defined for a wide range of helminth parasite species, but greater understanding is needed to define the mechanisms of vaccine-induced immunity, to lay a rational platform for new vaccines and their optimal design. With human trials underway for hookworm and schistosomiasis vaccines, a greater integration between veterinary and human studies will highlight the common molecular and mechanistic pathways, and accelerate progress towards reducing the global health burden of helminth infection.

ANTHELMINTIC RESISTANCE GENERAL


Gastrointestinal (GI) nematodes are among the most important causes of production loss in farmed ruminants, and anthelmintic resistance is emerging globally. We hypothesized that wild deer could potentially act as reservoirs of anthelmintic-resistant GI nematodes between livestock farms. Adult abomasal nematodes and faecal samples were collected from fallow (n = 24), red (n = 14) and roe deer (n = 10) from venison farms and areas of extensive or intensive livestock farming. Principal components analysis of abomasal nematode species composition revealed differences between wild roe deer grazing in the areas of intensive livestock farming, and fallow and red deer in all environments. Alleles for benzimidazole (BZ) resistance were identified in beta-tubulin of Haemonchus contortus of roe deer and phenotypic resistance confirmed in vitro by an egg hatch test (EC50 = 0.149 μg ml(-1) +/− 0.13 μg ml(-1)) on H. contortus eggs from experimentally infected sheep. This BZ-resistant H. contortus isolate also infected a calf experimentally. We present the first account of in vitro BZ resistance in wild roe deer, but further experiments should firmly establish the presence of phenotypic BZ resistance in vivo. Comprehensive in-field studies should assess whether nematode cross-transmission between deer and livestock occurs and contributes, in any way, to the development of resistance on livestock farms.


Anthelmintic resistance has a great impact on livestock production systems worldwide, is an emerging concern in companion animal medicine, and represents a threat to our ongoing ability to control human soil-transmitted helminths. The Consortium for Anthelmintic Resistance and Susceptibility (CARS) provides a forum for scientists to meet and discuss the latest developments in the search for molecular markers of anthelmintic resistance. Such markers are important for detecting drug resistant worm populations, and indicating the likely impact of the resistance on drug efficacy. The molecular basis of resistance is also important for understanding how anthelmintics work, and how drug resistant populations arise. Changes to target receptors, drug efflux and other biological processes can be involved. This paper reports on the CARS group meeting held in August 2013 in Perth, Australia. The latest knowledge on the development of molecular markers for resistance to each of the principal classes of anthelmintics is reviewed. The molecular basis of resistance is best understood for the benzimidazole group of compounds, and we examine recent work to translate this knowledge into useful diagnostics for field use. We examine recent candidate-gene and whole-genome approaches to understanding anthelmintic resistance and identify markers. We also look at drug transporters in terms of providing both useful markers for resistance, as well as opportunities to overcome resistance through the targeting of the transporters themselves with inhibitors. Finally, we describe the tools available for the application of the newest high-throughput sequencing technologies to the study of anthelmintic resistance.


The present study used in vitro assays to determine the relative potency of commercial macrocyclic lactone (ML) anthelmintics against larvae of drug-susceptible and drug-resistant Australian isolates of important parasites of sheep and cattle, Haemonchus contortus and Haemonchus placei, respectively. Cattle pour-on products containing abamectin, ivermectin, eprinomectin, doramectin or moxidectin were diluted in DMSO and used in larval development assays. Abamectin was the most potent chemical (lowest IC50 value) towards the drug-susceptible H. contortus Kirby isolate. The abamectin IC50 was approximately 2-fold lower than those for ivermectin, moxidectin, eprinomectin and doramectin. Moxidectin and abamectin were the most potent chemicals towards the resistant H. contortus Wallangra isolate. This isolate showed resistance ratios up to 70-
fold towards eprinomectin. The resistance ratio for this species was lowest with moxidectin (ratio of 4.0-fold). Abamectin was also the most potent chemical towards both drug-susceptible (Bremner) and drug-resistant (Dayboro) *H. placei* isolates. The larval development assay only showed low levels of resistance for the drug-resistant *H. placei*, with resistance ratios ranging from 1.7 to 2.0 fold for moxidectin and abamectin, up to 3.3-fold for eprinomectin. This study examined the readily-accessible larval life stages of these parasites in *in vitro* assays, and, hence, the relationship between our findings and relative drug efficacies *in vivo* remains to be determined. Despite this, the study accords with some evidence from the use of these anthelmintics in the field in demonstrating the potency of moxidectin and abamectin against ML-resistant *H. contortus*. The study also highlights the usefulness of eprinomectin as a readily-available compound which is a more sensitive marker for ML resistance in *in vitro* larval development assays than the other commercial ML compounds examined.


The *in vivo* faecal egg count reduction test (FECRT) is the most commonly used test to detect anthelmintic resistance (AR) in gastrointestinal nematodes (GIN) of ruminants in pasture based systems. However, there are several variations on the method, some more appropriate than others in specific circumstances. While in some cases labour and time can be saved by just collecting post-drench faecal worm egg counts (FEC) of treatment groups with controls, or pre- and post-drench FEC of a treatment group with no controls, there are circumstances when pre- and post-drench FEC of an untreated control group as well as from the treatment groups are necessary. Computer simulation techniques were used to determine the most appropriate of several methods for calculating AR when there is continuing larval development during the testing period, as often occurs when anthelmintic treatments against genera of GIN with high biotic potential or high re-infection rates, such as *Haemonchus contortus* of sheep and *Cooperia punctata* of cattle, are less than 100% efficacious. Three field FECRT experimental designs were investigated: (I) post-drench FEC of treatment and controls groups, (II) pre- and post-drench FEC of a treatment group only and (III) pre- and post-drench FEC of treatment and control groups. To investigate the performance of methods of indicating AR for each of these designs, simulated animal FEC were generated from negative binominal distributions with subsequent sampling from the binomial distributions to account for drench effect, with varying parameters for worm burden, larval development and drench resistance. Calculations of percent reductions and confidence limits were based on those of the Standing Committee for Agriculture (SCA) guidelines. For the two field methods with pre-drench FEC, confidence limits were also determined from cumulative inverse Beta distributions of FEC, for eggs per gram (epg) and the number of eggs counted at detection levels of 50 and 25. Two rules for determining AR: (1) %reduction (%R) < 95% and lower confidence limit <90%; and (2) upper confidence limit <95%, were also assessed. For each combination of worm burden, larval development and drench resistance parameters, 1000 simulations were run to determine the number of times the theoretical percent reduction fell within the estimated confidence limits and the number of times resistance would have been declared. When continuing larval development occurs during the testing period of the FECRT, the simulations showed AR should be calculated from pre- and post-drench worm egg counts of an untreated control group as well as from the treatment group. If the widely used resistance rule 1 is used to assess resistance, rule 2 should also be applied, especially when %R is in the range 90 to 95% and resistance is suspected.


The seemingly straightforward task of analysing faecal egg counts resulting from laboratory procedures such as the McMaster technique has, in reality, a number of complexities. These include Poisson errors in the counting technique which result from eggs being randomly distributed in well mixed faecal samples. In addition, counts between animals in a single experimental or observational group are nearly always over-dispersed. We describe the R package “eggCounts” that we have developed that incorporates both sampling error and over-dispersion between animals to calculate the true egg counts in samples of faeces, the probability distribution of the true counts and summary statistics such as the 95% uncertainty intervals. Based on a hierarchical Bayesian framework, the software will also rigorously estimate the percentage reduction of faecal egg counts and the 95% uncertainty intervals of data generated by a faecal egg count reduction test. We have also developed a user friendly web interface that can be used by those with limited knowledge of the R statistical computing environment. We illustrate the package with three simulated data sets of faecal egg count reduction experiments.
ANTHELMINTIC RESISTANCE IN SHEEP


The mechanism of anthelmintic resistance against the widely used macrocyclic lactones (MLs) is still not fully understood. Pharyngeal, somatic body muscles and the ovjector have been proposed as putative sites of action as well as resistance. In the present study the effects of three avermectins and three milbemycins on adult parasitic nematodes were evaluated *in vitro.* The Muscle Transducer system was used to investigate the effects of MLs on muscle contraction in female *Haemonchus contortus* and effects on motility were measured in *Ostertagia (Teladorsagia) circumcincta* using the Micromotility Meter. Concentration-response curves for all substances in both systems shifted to the right in the resistant isolates. Resistance was present to ivermectin (IVM) and its components IVM B1a and IVM B1b, suggesting that both components are involved in the mode of action and resistance. No consistent patterns of potency and resistance of the substances were observed except that milbemycins generally showed lower resistance ratios (RRs) than IVM. IVM and IVM B1b were the most potent inhibitors of contraction and motility in both susceptible isolates and also showed the highest RR in both species. Low RRs for milbemycins recorded *in vitro* for highly resistant isolates *in vivo* suggest that other factors such as pharmacokinetics influence drug potency *in vivo.*


A levamisole-sensitive acetylcholine receptor has recently been described in the parasitic nematode *Haemonchus contortus.* The pentameric receptor is produced from different subunit proteins, one of which is Hco-ACR-8. A truncated transcript, Hco-acr-8b, has been identified in six levamisole-resistant *H. contortus* isolates and was found to be absent in four levamisole-susceptible isolates, indicating Hco-acr-8b could be a potential marker for levamisole resistance. The Hco-acr-8b transcript contains exons 1 and 2 and terminates with 347 bp from within the intron 2. In this work, we investigated genomic DNA sequences of the Hco-acr-8 gene, in a region including exon 2 and exon 3, from a wide range of levamisole-susceptible and resistant *H. contortus* isolates. Sequences potentially involved in generating the truncated splice variant within the second intron were analysed from individuals and pools of parasites. We found an insertion/deletion (indel) of 63 bp located just downstream from the splice acceptor site for the alternative third exon. The sequence of the indel, when present, was similar in the 12 isolates examined. The presence or absence of this indel was statistically (Chi(2) test) correlated with levamisole resistance status. A correlation was also demonstrated between the absence of the indel and the expression of the Hco-acr-8b transcript. We believe this is the first report of a putative DNA marker for levamisole resistance detection. Using this new knowledge, we have developed a novel DNA-based assay for the detection and monitoring of levamisole resistance in parasitic nematodes of animals.


Because we became an unintended world leader in the development of multiple anthelmintic resistance, South Africa has had to find, embrace and implement more sustainable and holistic methods of managing helminths in sheep. The problem has been to wean farmers off a heavy reliance on anthelmintics and to use a wide array of measures that require more management, in the face of inertia and attempts by some unscrupulous drug purveyors to block the needed changes. Packaging the available and proven measures into five practical sections helps to make the change more palatable and attractive to farmers. These sections are termed “The Big Five” and consist of firstly, host resistance and resilience: in the past we have concentrated too much on anthelmintic resistance (AR) and not enough on SR (sheep resistance). The motto must be “Stop Selecting Sissy Sheep!” To achieve this we can apply selection of rams by faecal egg counts (FEC) or FAMACHA, culling of ewes based on targeted selective treatment (TST) results, good nutrition, especially protein and trace elements needed to support immunity, enough exposure to worms for immunity to develop, and control of other diseases. Secondly, reducing parasite load: since the outcome of parasitosis is largely determined by numbers, this needs ongoing attention. This can be achieved by reducing the length of stay in a pasture, reducing the grazing pressure if this is not possible, increasing the time of absence from a pasture, especially at danger times, alternation with non-susceptible grazing species where possible, avoiding worn “hot spots” like grassed pens and leaking water troughs. Thirdly, evaluate pasture factors: the farmer and advisor have to consider the height (length) of grazing as it affects its risk for parasite transfer, the type (pasture species) as it will influence risk, the pasture slope affecting run-off and thus the suitability for larval survival, and the aspect (direction facing) should also be used to assess risk. Fourth, monitoring the situation: farm situations can be assessed by regular (monthly or bimonthly) pooled flock FECs, AR assessments using FEC reduction tests (FECRTs) or other measures,
measures using TST like the 5 POINT CHECK, a weather watch to predict conditions favourable for larval development (rain, humidity, temperature) and grazing monitoring to assess developing dangers. Fifth, optimise drugs to be used: drug usage must be rationalised and minimised. Implement TST and TT (targeted treatment); read the label, follow instructions and check the spectrum covered; weigh the sheep, set dose according to the heaviest in the group and check the gun for accuracy and repeatability; target the most vulnerable (lambs and lactating or heavily pregnant ewes) for special attention; do not buy on cost alone and do not formulate (mix) own farm mixtures. By emphasising the "Big Five" we can encourage and enable farmers to implement four or more items in each section, and thus minimise the long-term effects of internal gut parasites.


Our objective was to determine if the resistance mechanism to moxidectin (MOX) is similar of that to ivermectin (IVM) and involves P-glycoproteins (PGPs). Several Caenorhabditis elegans strains were used: an IVM and MOX sensitive strain, 13 PGP deletion strains and the IVM-R strain which shows synthetic resistance to IVM (by creation of three point mutations in genes coding for alpha-subunits of glutamate gated chloride channels [GluCls]) and cross-resistance to MOX. These strains were used to compare expression of PGP genes, measure motility and pharyngeal pumping phenotypes and evaluate the ability of compounds that inhibit PGP function to potentiate sensitivity or reverse resistance to MOX. The results suggest that C. elegans may use regulation of PGPs as a response mechanism to MOX. This was indicated by the over-expression of several PGPs in both drug sensitive and IVM-R strains and the significant changes in phenotype in the IVM-R strain in the presence of PGP inhibitors. However, as the inhibitors did not completely disrupt expression of the phenotypic traits in the IVM-R strain, this suggests that there likely are multiple avenues for MOX action that may include receptors other than GluCls. If MOX resistance was mediated solely by GluCls then exposure of the IVM-R strain to PGP inhibitors should not have affected sensitivity to MOX. Targeted gene deletions showed that protection of C. elegans against MOX involves complex mechanisms and depends on the PGP gene family, particularly PGP-6. While the results presented are similar to others using IVM, there were some important differences observed with respect to PGPs which may play a role in the disparities seen in the characteristics of resistance to IVM and MOX. The similarities are of concern as parasites resistant to IVM show some degree but not complete cross-resistance to MOX; this could impact nematodes that are resistant to IVM.


The egg hatch assay (EHA) is one of the main in vitro methods for detection of benzimidazole resistance in nematode parasites of small ruminants. However, although the EHA has been standardised at the laboratory level, the diagnostic performance of this method has not been fully characterised for field screenings. In the present work, monthly variation of benzimidazole resistance estimated by EHA was surveyed over two years in three sheep flocks and in one goat and an additional sheep flock sharing the same pastures. Resistance was measured by calculating both the effective dose of thiabendazole (TBZ) that inhibited hatching of > 50% of parasite eggs (ED50) and the proportion (P-dd) of eggs hatching at a discriminating dose of 0.1 μg/mL TBZ. P-dd exhibited higher variability than ED50, in agreement with the higher sensitivity of Pdd to changes in resistance levels. Both resistance parameters, however, were highly correlated, and their variation was similarly related to the same factors. Resistance levels differed among sheep flocks, and the resistance level of the goat flock was higher than that measured for the sheep flock sharing the same pasture. Moreover, monthly variation of resistance in goats did not mirror that recorded in sheep. Resistance levels varied seasonally, with the highest values recorded in the spring, and they were inversely related to the number of days that samples were stored under anaerobic conditions. In addition, they were directly associated with the relative abundance of Teladorsagia spp. but inversely related to the relative abundance of Haemonchus spp. After controlling for the effects of these identified factors for variation, inter-monthly sampling variation due to unknown factors was the main source of variability, accounting for more than 60-70% of variance for both resistance parameters and yielding absolute estimation errors higher than 0.06 for ED50 or 0.2 for P-dd when resistance was estimated from a single sampling. Optimum sample size, estimated from variance components, suggested that at least 4-5 samplings would be needed to halve this absolute error, whereas additional samplings would slightly increase precision but at the cost of substantially increasing sampling effort. More research is needed to identify the main factors involved in this inter-sampling variation to standardise the implementation of EHA under field conditions.

Anthelmintic drugs have been widely used in sheep as a cost-effective means for gastro-intestinal nematode (GIN) control. However, growing anthelmintic resistance (AHR) has created a compelling need to identify evidence-based management recommendations that reduce the risk of further development and impact of AHR. Objective: To identify, critically assess, and synthesize available data from primary research on factors associated with AHR in sheep. Publications reporting original observational or experimental research on selected factors associated with AHR in sheep GINs and published after 1974, were identified through two processes. Three electronic databases (PubMed, Agricola, CAB) and Web of Science (a collection of databases) were searched for potentially relevant publications. Additional publications were identified through consultation with experts, manual search of references of included publications and conference proceedings, and information solicited from small ruminant practitioner lists. Two independent investigators screened abstracts for relevance. Relevant publications were assessed for risk of systematic bias. Where sufficient data were available, random-effects Meta-Analyses (MAs) were performed to estimate the pooled Odds Ratio (OR) and 95% Confidence Intervals (CIs) of AHR for factors reported in \( n \geq 2 \) publications. Of the 1712 abstracts screened for eligibility, 131 were deemed relevant for full publication review. Thirty publications describing 25 individual studies (15 observational studies, 7 challenge trials, and 3 controlled trials) were included in the qualitative synthesis and assessed for systematic bias. Unclear (i.e. not reported, or unable to assess) or high risk of selection bias and confounding bias was found in 93% (14/15) and 60% (9/15) of the observational studies, respectively, while unclear risk of selection bias was identified in all of the trials. Ten independent studies were included in the quantitative synthesis, and MAs were performed for five factors. Only high frequency of treatment was a significant risk factor (OR= 4.39; 95% CI= 1.59, 12.14), while the remaining 4 variables were marginally significant: mixed-species grazing (OR= 1.63; 95% CI= 0.66,4.07); flock size (OR= 1.02; 95% CI= 0.97, 1.07); use of long-acting drug formulations (OR= 2.85; 95% CI= 0.79, 10.24); and drench-and-shift pasture management (OR= 4.08; 95% CI = 0.75, 22.16). While there is abundant literature on the topic of AHR in sheep GINs, few studies have explicitly investigated the association between putative risk or protective factors and AHR. Consequently, several of the current recommendations on parasite management are not evidence-based. Moreover, many of the studies included in this review had a high or unclear risk of systematic bias, highlighting the need to improve study design and/or reporting of future research carried out in this field.


Anthelmintic resistance (AR) in ovine gastro-intestinal nematodes has been reported to affect the health and productivity of sheep globally. The objective of the present study was to evaluate the efficacy of commonly used oral drenches in sheep in France, Greece and Italy. In each country, 10 farms were selected. On each farm, 50 animals were blocked based on the pre-treatment faecal egg count (FEC). Within each block, animals were randomly allocated to one of 5 treatment groups. In addition to an untreated control group, there were 4 groups treated per oral route: moxidectin (MOX) and ivermectin (IVM), both at 0.2 mg/kg bodyweight, levamisole (LEV; at 7.5 mg/kg bodyweight) and a benzimidazole (BZ; at 3.75-5 mg/kg bodyweight). In France, animals were not treated with LEV, but with netobimin (NET; at 7.5 mg/kg bodyweight). The FEC was monitored using a modified McMaster technique. Two weeks after treatment, individual faecal samples were taken from all animals and efficacy was calculated as the difference between arithmetic mean FEC of the control group versus each respective treatment group. The results of the present study indicate the high efficacy of treatment with oral formulations of MOX (99-100%) and IVM (98-100%) on all farms, except on 1 farm in Greece. On this farm, multi drug resistance (MDR) was identified involving 4 anthelmintics (efficacy MOX: 91%; IVM: 0%; BZ: 58% and LEV: 87%). In Greece and Italy, AR against LEV and BZ was observed on some farms, with MDR involving both anthelmintics on 3 farms in Greece and on 2 farms in Italy. In Greece, AR against BZ and NET was observed on all 10 farms included. In all countries, Teladorsagia sp. was the most common nematode larva identified after treatment, followed by Haemonchus sp. and Trichostrongylus sp., with differences among farms and treatments. The current study confirms the high efficacy of oral treatments with MOX and IVM, even on farms with worm populations resistant to BZ, LEV or NET. This study also reports MDR against 4 anthelmintics on one farm in Greece.


Objective To establish the prevalence of anthelmintic resistance in ovine gastrointestinal nematodes in southern Queensland. An observational parasitological study using the faecal egg count reduction test. Sheep farms \( n = 20 \) enrolled in this study met the twin criteria of using worm testing for drench decisions and having concerns
about anthelmintic efficacy. On each farm, 105 sheep were randomly allocated to one of six treatment groups or an untreated control group. Faecal samples were collected on day 0 and days 10–14 for worm egg counts and larval differentiation. Single- and multi-combination anthelmintics, persistent and non-persistent, oral liquid or capsule, pour-on and injectable formulations were tested. Monepantel was not tested. Farmers also responded to a questionnaire on drenching practices. Haemonchus contortus was the predominant species. Efficacy <95% was recorded on 85% of farms for one or more anthelmintics and on 10% of farms for six anthelmintics. No resistance was identified on three farms. The 4-way combination product was efficacious (n = 4 farms). Naphthalophos resistance was detected on one farm only. Resistance to levamisole (42% of farms), moxidectin injection (50% of farms) and the closantel/abamectin combination (67% of farms) was identified. Moxidectin oral was efficacious against Trichostrongyulus colubriformis, which was predominant on only one farm. Of the farms tested, 55% ran meat breeds, 60% dosed more than the recommended dose rate and 70% always, mostly or when possible practised a drench and move’ strategy. This level of anthelmintic resistance in southern Queensland will severely compromise worm control and force increased use of monepantel.


On two farms it was noted that after routine treatment with monepantel, faecal egg counts failed to drop. This was accompanied by lambs mortality due to Haemonchus contortus infection. The aim of this work was to evaluate the efficacy of monepantel to control gastrointestinal nematodes (GIN) in two sheep farms, in Uruguay. A Fecal Egg Count Reduction Test (FECRT) was subsequently performed at the Experimental Stations Glencoe of INIA Tacuarena (Farm 1) and Sheep Unit of INIA La Estanzuela (Farm 2) using the World Association for the Advancement of Veterinary Parasitology guidelines. On Farm 1 the FECRT was performed using 6-8 month old Corriedale or Merino Dohne * Corriedale male lambs naturally infected with GIN. On day 0 pre-treatment, three groups of 15 lambs each were selected, blocked by fecal egg count level (FEC) and randomly assigned to one of the following: Group 0 = untreated control, Group 1 = treated with monepantel (Zolvix, Novartis Animal Health Inc.) from stock previously purchased; Group 2 = treated with monepantel from stock provided by the supplier, at the recommended dose of 2.5 mg/kg of body weight. Faecal samples were collected directly from the rectum of each lamb on day 0 and on day 9 post-treatment. On Farm 2, the FECRT was conducted on a group of 8 month old male lambs Milchschaff * Finn. At this farm, 10 lambs were randomly allocated to be treated with monepantel (Group 1) and 10 lambs were randomly allocated to remain as untreated control (Group 0) using the same protocols as Farm 1. Results and conclusions: On farm 1 the FECR was 0.0% (95% CI=0.0-49.0) and 42.0% (95% CI=0.0-75.0) for Group 1 and Group 2 respectively. For Farm 2, the FECR was 82.1% (95% CI=36.0-99.0). Poor efficacy of monepantel in treating GIN parasites was demonstrated on both farms and Haemonchus spp. was the resistant genus.


Eprinomectin (EPN) is a member of the avermectin class of compounds and the only anthelmintic registered for goats in Switzerland with a zero milk withdrawal period. The aim of the present study was to identify the actual efficacy of EPN in an area with a higher density of goat enterprises. Forty-three randomly chosen farms from canton Berne were investigated. At least eight goats were investigated on every farm. Conditions for inclusion in the study were the absence of anthelmintic treatment during the previous six weeks and a pooled faecal sample showing a mean faecal egg count (FEC) higher than 600 epg faeces. Pre- and 14-16 days post-treatment samples were individually collected directly from the rectum. Animals were treated with the recommended dose of EPN (1 mg/kg body weight) after taking the pre-treatment samples. Efficacy of EPN was tested with the faecal egg count reduction test (FECRT) and faecal cultures were performed on every farm from pooled faeces samples before and after treatment. Additionally the farmers completed a questionnaire. None of the gastrointestinal nematode populations of the 43 investigated farms were susceptible to EPN at the required level. The mean egg count reduction was 40%. None of the typical risk factors, such as production type, stocking rate, animal traffic and quarantine measures showed an association with the level of eprinomectin resistance. It can be concluded with 80% certainty that the prevalence of EPN resistance on goat farms is at least 95% in canton Berne.


While there is some evidence that changes in nicotinic acetylcholine receptor (nAChR) subunits confer resistance to levamisole in gastrointestinal helminth parasites, the exact nature of the resistance mechanism(s) is unclear. We utilised the presence of a resistant fraction within the Wallangra 2003 isolate of *Haemonchus contortus*

Heavy reliance on macrocyclic lactones to treat parasitic nematodes has resulted in the evolution of widespread drug resistance that threatens human and animal health. Management strategies have been proposed that would slow the rise of resistance, however testing these strategies has been hampered by the lack of identified strongly resistant parasite markers of resistance. We show that the *Caenorhabditis elegans* gene Cel_dyf-7, necessary for amphid sensory neuron development, also confers macrocyclic lactone sensitivity. In the sheep parasite *Haemonchus contortus*: (i) strains selected for macrocyclic lactone resistance were enriched in, a Hco_dyf-7 haplotype that was rare in the drug-naive population, (ii) the resistant haplotype correlated with the sensory neuron defects, and (iii) the resistant haplotype was associated with decreased Hco_dyf-7 expression. Resistant field isolates of *H. contortus* from five continents were enriched for the resistant haplotype, demonstrating the relevance of the Hco_dyf-7 haplotype to practise and indicating that it is a locus of strong effect. Hemizygosity resulting from sex linkage of dyf-7 likely contributes to the rise of resistance in treated populations.


AIM: To determine the distribution of the three common *Trichostrongylus* spp. infecting sheep and their resistance status on farms throughout New Zealand, using PCR. Cultures were prepared from faecal samples from 70 farms while conducting faecal egg count reduction tests (FECRT) in lambs between 2010 and 2012. *Trichostrongylus*-type infective stage larvae (L3) were recovered from cultures, derived from untreated control and albendazole-, levamisole- and ivermectin-treated groups of lambs on each of the farms involved, and these were identified to species using PCR analysis of the second internal transcribed spacer region of ribosomal DNA. The species composition of the larvae present in cultures from the untreated control groups was examined across all farms to assess any potential differences in geographical distribution. In addition, the species composition of larvae cultured from the untreated and anthelmintic-treated lamb groups were compared to determine which species exhibited resistance to each of the anthelmintics used in the FECRT. Of 67 farms with *Trichostrongylus* spp. present, 42 (63%) cultures from the untreated control groups contained all three *Trichostrongylus* spp. and no significant geographical patterns in their distribution were detected. Seven samples contained only one species. Irrespective of the anthelmintic efficacy levels, *Trichostrongylus colubriformis* dominated cultures prepared from lambs following treatment with albendazole (99.1% (95% CI = 97-100)% of larvae) or levamisole (81.6% (95%CI = 75.3-87.9)% of larvae), indicating the presence of widespread resistance in this species. In cultures prepared from levamisole-treated lambs, small numbers of *T. axei* larvae were also frequently present (5.4% (95% CI = 1.3-12.4)% of larvae). Resistance to ivermectin was not found in any of the three *Trichostrongylus* spp. after PCR identification. Although larvae were identified, based on length, as being *Trichostrongylus* spp., for 24 of the 48 samples cultured following treatment with ivermectin, 100% of the larvae present were identified as *Teladorsagia circumcincta*. As in previous surveys, all three *Trichostrongylus* spp. were common throughout New Zealand and no geographical patterns were detected in the current study. On all farms where resistance to albendazole and/or levamisole was indicated (i.e. efficacy <95%), the species identified as being resistant was *T. colubriformis*. Even where efficacies were >95%, *T. colubriformis* still tended to dominate in post-treatment cultures. While this could reflect a lower susceptibility of *T. colubriformis* to these...
Anthelmintics, it seems more likely to indicate the presence of resistant genotypes in these populations. Similarly, T. axei also tended to be present after treatment with levamisole, which likely reflects a known lower susceptibility of this species to these anthelmintics.

**ANTHELMINTIC RESISTANCE IN CATTLE**


Effects of the cytochrome P450 inhibitor piperonyl butoxide and the P-glycoprotein inhibitor verapamil on the efficacy of ivermectin and thiabendazole were studied in vitro in susceptible and resistant isolates of the cattle parasitic nematodes Cooperia oncophora and Ostertagia ostertagi. The effects of combined use of drug and piperonyl butoxide/verapamil, respectively, were investigated in the Egg Hatch Assay, the Larval Development Assay and the Larval Migration Inhibition Assay. The effects of piperonyl butoxide and verapamil as inhibitors of thiabendazole and ivermectin responses were particularly marked for larval development, where both inhibitors were able to completely eliminate all differences between susceptible and resistant isolates. Even the lowest concentrations of anthelmintics used in combination with inhibitors caused complete inhibition of development. Differences and/or similarities among responses in different isolates were only obtained in the two other assays: in the Egg Hatch Assay piperonyl butoxide caused a shift in concentration-response curves obtained with thiabendazole to the left for all isolates tested, changing relative differences between isolates. In contrast, an effect of verapamil in the Egg Hatch Assay was only apparent for benzimidazole-resistant isolates. In the Larval Migration Inhibition Assay only ivermectin was tested and piperonyl butoxide shifted the concentration-response curves for all isolates to the left, again eliminating differences in EC50 values between susceptible and resistant isolates. This was not the case using verapamil as an inhibitor, where curves for both susceptible and benzimidazole-resistant isolates shifted to the left in Ostertagia isolates. In Cooperia the picture was more complex with ivermectin-resistant isolates showing a larger shift than the susceptible isolate. Single nucleotide polymorphisms in the beta-tubulin isotype I gene were investigated. Significantly increased frequencies of resistance-associated alleles were observed for the codons 167 and 200 in one benzimidazole-resistant isolate but not in an isolate selected for benzimidazole resistance at an early stage of selection.


Anthelmintic resistance (AR) is an increasing problem for the ruminant livestock sector worldwide. However, the extent of the problem is still relatively unknown, especially for parasitic nematodes of cattle. The effect of ivermectin (IVM) (ivomec inj., Merial) was investigated in Swedish isolates of gastrointestinal nematode (GIN) populations showing signs of AR in the field to further characterise the AR status by a range of in vivo and in vitro methods. Three groups, each of 11 calves, were infected with an equal mixture of third stage larvae (L3) of Cooperia oncophora and Ostertagia ostertagi. Group A was inoculated with an IVM-responsive laboratory isolate and groups B and C with isolates originating from 'resistant' cattle farms. Faecal egg counts (FEC) were monitored from 0 to 45 days post infection (d.p.i.), and L3 were harvested continuously for larval migration inhibition testing (LMIT) and species-specific PCR (IT52). At 31 d.p.i., one calf from each group was necropsied and adult worms were recovered pre-treatment. At 35 d.p.i., calves from all groups were injected with IVM at the recommended dose (0.2 mg/kg bodyweight). At 45 d.p.i., another two animals from each group were sacrificed and established gastrointestinal worms were collected and counted. A few animals in all three groups were still excreting eggs (50-150 per g faeces) 10 days post IVM injection. However, there was no significant difference in the FEC reductions in groups A (95%; 95% CI 81-99), B (98%; 92-100) and C (99%; 97-100) between 35 and 44 d.p.i. Furthermore, LMIT showed no significant difference between the three groups. Approximately 100 adult O. ostertagi were found in the abomasum of one calf (group B), whereas low to moderate numbers (400-12 200) of C. oncophora remained in the small intestine of the calves in all three groups at 45 d.p.i. PCR on L3 harvested from faecal samples up to 10 days post treatment showed a ratio of 100% C. oncophora in the calves inoculated with isolates A and B, whereas C also had 8% O. ostertagi. Overall, this experiment showed that the animals were successfully treated according to the Faecal egg count reduction test (FECRT) standard (≥95% reduction). However, several adult worms of the dose-limiting species C. oncophora demonstrably survived the IVM treatment.


The occurrence of anthelmintic resistance to levamisole, albendazole, ivermectin and moxidectin was investigated in cattle from 10 farms located in Sao Paulo State, Brazil, using two techniques for counting eggs in

The first documented case of macrocyclic lactone resistance in gastrointestinal (GI) nematodes of cattle was seen in the US approximately 10 years ago. Since that time the increase incidence of anthelmintic resistance has continued at an alarming rate. Currently parasites of the genera *Cooperia* and/or *Haemonchus* resistant to generic or brand-name macrocyclic lactones have been demonstrated in more than half of all operations examined. Both of these parasite genera are capable of causing economic losses by decreasing food intake and subsequently animal productivity. Currently, there are no easy and quick means to detect anthelmintic resistant GI nematodes. Definitive identification requires killing of cattle. The most commonly used field detection method is the fecal egg count reduction test (FECRT). This method can be adapted for use as a screening agent for Veterinarians and producers to identify less than desired clearance of the parasites after anthelmintic treatment. Further studies can then define the reasons for persistence of the egg counts. The appearance of anthelmintic resistance is largely due to the development of very effective nematode control programs that have significantly improved the productivity of the US cattle industry, but at the same time has placed a high level of selective pressure on the parasite genome. The challenges ahead include the development of programs that control the anthelmintic resistant nematodes but at the same time result in more sustainable parasite control. The goal is to maintain high levels of productivity but to exert less selective pressures on the parasites. One of the most effective means to slow the development of drug resistance is through the simultaneous use of multiple classes of anthelmintics, each of which has a different mode of action. Reduction of the selective pressure on the parasites can be attained through a more targeted approach to drug treatments where the producer’s needs are met by selective treatment of different classes of animals and not by blanket treatment at a location. The implementation of such programs will vary by the sector of the industry and the individual site. In general, the feedlot will be the easiest sector for developing of programs, while stocker/backgrounder operations will provide the most challenging problems. A major question that must be addressed is whether it is
Risk factors for anthelmintic resistance (AR) on bovine ranches were studied. Data were derived from a survey made to 50 ranch owners, who had conducted a faecal egg-count-reduction test. The questionnaire contained descriptors of bovine ranch management and nematode control. A case-control design study was undertaken and AR cases were present in 26 herds. Associations between the binary outcome variable (AR versus not AR) and risk factors recorded in the questionnaire were evaluated. Variables associated with the presence of AR at P<0.15 and/or odds ratio (OR) >2 were subjected to a multivariable logistic regression model. The main effects contributing to general AR (avermectin AVM and/or benzimidazole) in the final model were total number of annual treatments (OR 7.68; 95% CI 2.4 to 28.3) and use of more than 75% of AVM in the past (OR=18.6; 95% CI 1.3 to 97.3), whereas for AVM resistance alone were total number of AVM annual treatments (OR=11.5; 95% CI 2.9 to 45.5) and number of AVM Nov-Jan treatments (OR=5.8; 95% CI 1.71 to 47.9). The results showed that treatment frequency, date of treatment and frequency of treatment in the past with a single drug were the main risk factors involved in AR development.


We report the findings of a faecal egg count reduction test (FECRT) conducted at two Irish agricultural research farms (Study A and B). Study A was conducted at Teagasc, Johnstown Castle, Wexford, Co. Wexford, Ireland. Sixty Holstein-Friesian male calves, naive to anthelmintics, were used in this study. Calves were randomised by age, weight and FEC to one of four treatment groups (n=15 per treatment) on July 4, 2013 (day 0) using the recorded calf age, live weight and FEC at day -7; (1) untreated control; (2) ivermectin injection (Ivomec Classic Injection for Cattle and Sheep 10 mg/ml, Merial Animal Health Limited); (3) levamisole injection (Levacide Injection 75 mg/ml, Norbrook Laboratories Limited) and (4) fenbendazole drench (Panacur 10 per cent W/V Oral Suspension, MSD Animal Health Ireland). Mean calf age (sd), live weight (sd) and FEC were 148 (8.4) days, 154 (47.7) kg and 473 (322) eggs per gram (EPG) at day 0, respectively. Calves were individually faecal sampled on days 0, 7 and 14 to determine their FEC.

Study B was conducted at the Animal Grassland Research and Innovation Centre, Teagasc, Grange, Dunsany, Co. Meath, Ireland. Twenty-four Holstein-Friesian male calves, naive to anthelmintics, were used in this study. Calves were vaccinated against dictyocaulosis (Bovilis Huskvac, MSD Animal Health Ireland) at two and six weeks before turnout on July 4, 2013. Calves were randomised by age, live weight and FEC to one of two treatment groups (n=12 per treatment) on August 12, 2013 (day 0) using the recorded calf age, live weight and FEC at day -4; (1) untreated control and (2) ivermectin injection (Ivomec Classic Injection for Cattle and Sheep 10 mg/ml, Merial Animal Health Limited). Mean calf age (sd), live weight (sd) and faecal egg count (FEC) were 252 (43.6) days, 154 (47.7) kg and 473 (322) EPG at day 0, respectively. Calves were individually faecal sampled on days 0 and 14 to determine FEC. Individual calf FECs in both studies were determined using the McMaster method with a sensitivity of 50 EPG (Urquhart and others 1996). Composite faecal cultures were performed on each sampling occasion to determine the composition of nematode genera in each treatment group. Data were analysed using the RESO FECRTv4 programme and the ‘eggCounts’ package. Cooperia was the only nematode genus recovered in post-treatment culture on day 14 in ivermectin-treated calves in both studies, according to WAAVP guidelines, resistance to ivermectin was detected on both farms in the present study. As Cooperia is the dose-limiting nematode for macrocyclic lactones in cattle, resistance of this genus to ivermectin is always likely to develop. However, as injectable macrocyclic lactones achieve higher concentrations in abomasal mucosa than in intestinal mucosa this may be another factor that explains their poorer efficacy towards intestinal dwelling nematodes such as Cooperia. Although Cooperia species are regarded as less pathogenic compared to Ostertagia, and as such diagnosis of Cooperia-associated AR may not be as clinically significant, Cooperia infection can still result in production losses. Levamisole and fenbendazole demonstrated high efficacy in treating GIN challenge. However, the results of the FECRT conducted in Study A demonstrate the necessity to always sample levamisole-treated calves on day 7 post-treatment and use these FEC values for determining levamisole efficacy. This is due to the modest efficacy of levamisole against juvenile stages of common GIN some of which may begin producing nematode eggs by day 14 post-treatment leading to misleading results for levamisole efficacy. Both software programmes used to determine FECR demonstrated a high level of agreement in both studies.

Routine investigation into an ill-thrift situation with grazing cattle led to the discovery of the first reported case of macrocyclic lactone (ML) resistance in cattle in the USA. Research revealed that resistant parasites were originating on pastures in southeastern USA and were not an anomalous resident population on Wisconsin pastures. Prior to using anthelmintics in combination, ML-resistant *Cooperia* and *Haemonchus* spp. were shown to survive treatment with single-active MLs and were being transported in shipped cattle and seeding summer grazing pastures. Treatment and management strategies implemented in 2011 and 2012 suggested that ML-surviving parasites were introduced into the conditioning facility and surviving treatment with ML Data also demonstrated the use of combination ML+oral levamisole was highly effective in minimizing the transport of ML-surviving parasites from southeastern USA to Wisconsin pastures. The value of fecal egg count monitoring and PCR evaluation of nematode species under production conditions are confirmed.


A major hindrance to evaluating nematode populations for anthelmintic resistance, as well as for screening existing drugs, new compounds, or bioactive plant extracts for anthelmintic properties, is the lack of an efficient, objective, and reproducible in vitro assay that is adaptable to multiple life stages and parasite genera. To address this need we have developed the "Worminator" system, which objectively and quantitatively measures the motility of microscopic stages of parasitic nematodes. The system is built around the computer application "WormAssay", developed at the Center for Discovery and Innovation in Parasitic Diseases at the University of California, San Francisco. WormAssay was designed to assess motility of microscopic parasites for the purpose of high throughput screening of potential anthelmintic compounds, utilizing high definition video as an input to assess motion of adult stage (macroscopic) parasites (e. g. *Brugia malayi*). We adapted this assay for use with microscopic parasites by modifying the software to support a full frame analysis mode that applies the motion algorithm to the entire video frame. Thus, the motility of all parasites in a given well are recorded and measured simultaneously. Assays performed on third-stage larvae (L3) of the bovine intestinal nematode *Cooperia* spp., as well as microfilariae (mf) of the filarial nematodes *B. malayi* and *Dirofilaria immitis*, yielded reproducible dose responses using the macrocyclic lactones ivermectin, doramectin, and moxidectin, as well as the nicotinic agonists, pyrantel, oxantel, morantel, and tribendimidine. This new computer based-assay is simple to use, requires minimal new investment in equipment, is robust across nematode genera and developmental stage, and does not require subjective scoring of motility by an observer. Thus, the "Worminator" provides a relatively low-cost platform for developing genera- and stage-specific assays with high efficiency and reproducibility, low labor input, and yields objective motility data that is not subject to scorer bias.

**DICTYOCALUS VIVIPARUS**


Lungworm antibody ELISAs developed in Germany (DE) and The Netherlands (NL) were compared using four sets of serum (S) and bulk-tank milk (BTM) samples from adult dairy cows. The samples originated from 37 farms with or without a suspected clinical lungworm infection during August-October 2010 (Dataset 1), from cows excreting lungworm larvae or not during August-October 2010 (n =59) or May-June 2011 (n=164) (Dataset 2), from 305 farms in a national survey during October 2010 (Dataset 3), and 14 zero-grazing farms during February-April 2011 (Dataset 4). During August-October 2010, covering the second half of the grazing season, the NL-S and NL-BTM ELISA outperformed the DE-S and DE-BTM ELISAs in terms of sensitivity. For at least the NL-S and DE-S ELISA the opposite was found during May-June 2011, covering the end of the winter housing period and the early grazing season. Of the 305 farms in the survey 62.6% were found positive with the NL-BTM ELISA, whereas 2.6% was found positive with the DE-BTM ELISA. ODR values for the zero-grazing farms indicated that a cut-off value of 30% for the DE-BTM ELISA might be more appropriate than the currently used 24%. Results suggest that the NL ELISAs also respond to lungworm antigens other than Major Sperm Protein as used in the DE ELISAs. It is concluded that the generally higher sensitivity of the NL-BTM ELISA makes it better suited for large-scale prevalence studies and herd health monitoring programmes than the DE-BTM ELISA, positivity of which is more associated with the presence of clinical lungworm infection. Reducing the cut-off value of the DE-BTM ELISA from the original 49.3% to the current 41% or the possibly more appropriate 30% increased its sensitivity for detecting lungworm infections, but did not lead to similar sensitivity estimates as found for the NL-BTM ELISA. (C) 2013 Elsevier B.V. All rights reserved.
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FASCIOLA HEPATICA.

Therapeutics

Brockwell investigated resistance to TCBZ on dairy and beef farms in southern Australia. Using FEC and a coproantigen reduction test (CRT) to measure the efficacy of treatment, he demonstrated that there was resistance on these farms, and therefore it was likely to be widespread in this region.

Canevari investigated resistance to albendazole (ABZ) by an egg hatch assay, in different strains of *F hepatica* from Northern Ireland, Peru, Uruguay and Argentina. Two of the isolates were resistant, those from Peru and Uruguay. This method of EHA would be useful to test for resistance in other parts of the world.

Chowdhury investigated the efficacy of two different flukicides containing nitroxynil. He monitored weight gains and FEC post treatment and found no difference between the two products. Again using nitroxynil with TCBZ, Forbes looked for evidence of synergy between the two flukicides when given at the therapeutic dose to sheep infected with early immature fluke larvae (four weeks old). The fluke larvae were either TCBZ sensitive or resistant, and efficacy was measured by slaughtering the sheep at different times post treatment and counting the flukes present. The results showed that there was no synergy to the flukicides either given separately or in combination to TCBZ-R flukes.

A further study by Forbes looked at performance of cattle following a housing treatment with either clorsulon, TCBZ or nitroxynil. No fluke eggs were recovered post treatment with TCBZ whereas fluke eggs reappeared eight weeks after treatment with clorsulon and 13 weeks with nitroxynil. Despite differences in the efficacy profiles among the flukicide-treated groups, there were no significant differences (P>0.05) in growth rates among any of the four treatment groups. There was, however, a significant negative association (P<0.001) between fluke positivity at housing and subsequent growth performance, irrespective of treatment group.

Hannah studied the effects of treatment with TCBZ, nitroxynil and closantel on upland sheep farms in Northern Ireland. He compared FEC reduction, a CRT and examined flukes recovered from infected sheep histologically. The study highlighted the high level of penetration of TCBZ resistance throughout *F. hepatica* populations in areas of intensively managed sheep production, where there was a high level of fluke challenge. It emphasized the importance of preemptive chemotherapeutic action against chronic fasciolosis, using flukicides effective against the egg-producing adult flukes to minimize pasture contamination for the next season’s lamb crop. The FECRT, CRT and fluke histology were all useful in confirming fluke drug resistance.

Kolodziejczyk looked at the effect of saprophytic fungi on fluke eggs. Similar studies were reported in last year’s literature review. Six different soil borne fungi were incubated with fluke eggs, and egg embryonation and hatching was observed. The three fungi *Trichothecium roseum*, *Penicillium clinosogenum* (R-3) and *P. commune* had the strongest ovistatic effect on fluke eggs. The study showed no morphological damage to the shells of the *F. hepatica* eggs which may suggest a biochemical basis of antagonistic interactions by the fungi associated with the activity of fungal enzymes, mycotoxins and antibiotics.

Martinez-Perez has a long history in research on fasciolosis. The first of two papers last year looked at the effect of flaxseed oil or vitamin E dietary supplementation on fluke infection in sheep. At post mortem at the end of the study, those given the flaxseed oil diet had a lower worm burden compared to a control group. There was no effect on the level or gene expression of IFN-gamma and IL-4 in the flaxseed oil supplemented group. On the other hand, the supplementation with VE led to a reduction in adult fluke burden (97.7 +/- 39.9) and lower lipid oxidation in the liver. A second paper by this author examined the effect of lipopolysaccharides from the bacterium *Ochrobactrum intermedium* in sheep with fasciolosis. It was reported previously that treatment with LPS of this bacterium lowered fecal egg counts and fluke burdens. He concluded that the TH type 2 response induced in infected sheep enhanced the action of the LPS of this bacterium and the effects observed in infected sheep.

Martinez-Valladares examined the effect of a combination of flukicides albendazole (ABZ) and clorsulon (CLOR), in five sheep flocks in Spain. One flock was identified where there was resistance to CLOR and one with resistance to ABZ. A combination of the drugs given at full therapeutic dose rate was effective at eliminating infection on each of these farms, but was not effective when given at half the recommended dose rate.
References


Diagnostic tests

A study to compare different diagnostic tests in a region of Turkey was carried out by Avcioglu. He selected the coproantigen ELISA as the gold standard compared to the sedimentation FEC and serum antibody ELISA. Using samples from cattle over a three month period he assessed the sensitivity and specificity of the FEC and AB ELISA as 100% and 59.3%, and 93% and 100% respectively for these tests. He concluded that that fasciolosis prevalence can greatly vary by the diagnostic methods and should be cautiously interpreted as they reflect disease status at different stages.

Caban-Hernandez developed two sandwich ELISA tests to detect Fasciola antibodies in human infection. The antigens used were a ferritin and a tegument associated protein. The conclusion was that they would be useful for mass screening of serum samples in endemic areas for this parasite.

Charlier reviewed current diagnostic tests, stating that the intensity of infection could also be estimated by current techniques and the impact on carcass weight and milk yield in modern cattle production systems. He stated that there were still major drawbacks in predicting levels of infection because of difficulties with treatment on dairy farms (because of milk withdrawal periods of most flukicides). He also stated that recent research had focused on understanding spatial risk and delivering region-specific spatial distribution maps, and that further advances in epidemiological and economic research are expected to deliver farm-specific economic assessments of disease impact.

Palmer evaluated a commercial coproantigen ELISA kit for use as a screening test for infection in imported cattle, sheep and horses to Australia. The recommended cut offs were not as accurate as lower ones that were described in this paper. The modified cut-off was described as providing a useful screening test for cattle and sheep but was not of use in horses.

Robles-Perez described the development of an egg hatch assay to assess resistance to the flukicide albendazole. The EHA was confirmed as being useful to discriminate between ABZ-R and ABZ-S strains of the liver fluke.
Trivilin described the histological examination of livers infected with liver fluke. A classification based on the intensity of fibrosis and inflammatory response was proposed, from Category 1 – the least amount of damage, to 3, the most damage.

References


Epidemiology

Knubben-Schweizer suggested that control of fasciolosis could be based on the location of Galba truncatula habitats on farms. She concluded that when a control strategy is tailored according to the specific epidemiology found on the individual farm, egg shedding and F. hepatica-seroprevalence can be reduced significantly. This approach supported the responsible use of the available flukicides.

Bennema investigated the prevalence of F. hepatica in Brazil using data from condemnation of bovine livers in several states. The observed prevalence and the kriged prevalence maps presented in this paper could assist both animal and human health workers in estimating the risk of infection in their state or municipality.

Caron assessed the epidemiological role of different lymnaeid snails as intermediate hosts of the liver fluke Fasciola hepatica in Belgium and Luxembourg. The conclusion was that the Radix balthica snail could also be infected in certain habitats in addition to G. truncatula, the usual recognized intermediate host.

Charlier studied the longitudinal and temporal and micro-spatial distribution of Galba truncatula in four farms in Belgium as a base for small-scale risk mapping of Fasciola hepatica. He concluded that farm-level predictions of G. truncatula risk and subsequent risk for F. hepatica occurrence would require a rainfall, soil type (representing the agricultural region) and SWB layer in a geographic information system. While rainfall and soil type information is easily accessible, the recent advances in very high spatial resolution cameras carried on board of satellites, planes or drones should allow the delineation of SWBs in the future.

Elliott reported evidence for high genetic diversity of NAD1 and COX1 mitochondrial haplotypes among triclabendazole resistant and susceptible populations and field isolates of Fasciola hepatica in Australia. Analysis of TCBZ-resistant infrapopulations from 3 individual cattle grazing one property revealed considerable sequence parasite diversity between cattle. Analysis of parasite TCBZ-resistant infrapopulations from sheep and cattle revealed haplotypes unique to each host, but no significant difference between parasite populations. Fst analysis of fluke populations revealed little differentiation between the resistant and field populations. This study revealed a high level of diversity in field and drug resistant flukes in South-Eastern Australia.

Dreyfuss observed a decrease in prevalence of natural infection in habitats colonized by Galba truncatula and Lymnaea glabra in both F. hepatica and Paramphistomum daubneyi. The numbers of snails for each population was significantly lower in a lymnaeid community than in a monospecific population, while the shell height of overwintering snails in March did not show any significant difference. The greater density of snails in the overlapping areas of habitats colonized for both species, however, suggests a competitive situation.
by a lymnaeid community and the role of *L. glabra* as an intermediate host of both digeneans (only susceptible in its first weeks of life) might be the cause of this decrease in prevalence of natural infection.

Rondelaud is another French researcher who has studied snail intermediate hosts and their susceptibility to *F. hepatica* infection. In this paper he studied the adaptation of *Lymnaea fuscus* and *Radix balthica* to *F. hepatica* through the experimental infection of several successive snail generations. He concluded that *F. hepatica* infection of several successive snail generations, coming from parents infected with this parasite, resulted in a progressive increase in prevalence and intensity of snail infection. This may explain high prevalence of fasciolosis noted in several cattle breeding farms when the common snail host of this digenean, *G. truncatula*, is lacking.

An important and relevant paper to the epidemiology of *F. hepatica* infection in the UK was published by Selemetas from studies in Ireland. The conclusion was that, among the 127 herds that provided two monthly milk samples, 83 herds were exposed to *F. hepatica* and 82 increased their *F. hepatica* antibody levels at the later sampling time (P<0.01). The findings of this study confirm the high prevalence of *F. hepatica* antibodies in Irish dairy herds and show the rise in antibody levels during autumn. This study is the first step towards assessing the spatiotemporal pattern of fasciolosis in dairy herds in Ireland.

Vignoles looked at the effect of the natural light level on cercarial emergence from temperature-challenged *Galba truncatula*. The study showed that contrary to the intensity of artificial light, which did not influence cercarial emergence, the natural light level had a significant effect on this process when *F. hepatica*-infected snails were subjected to a regular thermal shock during the patent period.

References


Immunology and Molecular Studies

In this section, advances in molecular techniques are highlighted, and their application particularly to immunological
Adams studied the M2 subset of host macrophages and concluded that tegumental antigens FhTeg indirectly induce an M2 macrophage-like phenotype in vivo. The study highlighted the important role of FhTeg as an immune-modulatory source during F. hepatica infection and sheds further light on helminth-macrophage interactions.

Alba explored the antigenic features of F. hepatica rediae through the evaluation of different antigenic candidates for monoclonal antibody generation. The study found a high immunogenicity of the crude extract of rediae (third antigen), mainly related to parasite's specific epitopes. The rediae homogenate was the most promising antigen from those evaluated, for monoclonal antibody development with potential for detecting F. hepatica infected snails.

A further paper by Alba described ‘A novel monoclonal antibody-based immunoenzymatic assay for epidemiological surveillance of the vector snails of F. hepatica. The ELISA enabled detection of the infection from day 8 p.i. in G. cubensis, while in P. columella it was noted as early as day 4. ‘To our knowledge no previous immunoassays method have been reported to detect helminth-infected snails and the developed sandwich ELISA therefore suggested for infection status validation in natural populations of lymnaeid snails’.

Aldemir wrote a paper entitled ‘Differential diagnosis of F. hepatica from different animal hosts by PCR’. The aim was the differential diagnosis of Fasciola hepatica from sheep and goat by PCR. The results showed that different primers gave different bands (fragments) and allowed the identification of genetic variations of F. hepatica. The bands were species-specific to F. hepatica from goat and sheep. Conclusions: The RAPD-PCR method can be useful for the differential diagnosis of F. hepatica of sheep and goat.

Elliott investigated the T687 G SNP in a P-glycoprotein gene of F. hepatica as a marker for triclabendazole in two resistant Australian populations. Their conclusion was that the T687 G SNP was not observed in either of the resistant or susceptible populations, and that the T687 G SNP in this Pgp gene was not associated with TCBZ resistance in these Australian F. hepatica populations and therefore unlikely to be a universal molecular marker for TCBZ resistance.

Falcon looked at the F. hepatica Kunitz Type Molecule to investigate whether it decreased dendritic cell activation and the ability to induce inflammatory responses. He demonstrated that a Kunitz type molecule (Fh-KTM), present in F<10 kDa, was responsible for suppressing pro-inflammatory cytokine production in LPS-activated DC, by printing tolerogenic features on DC that impaired their ability to induce inflammatory responses. These results suggest a modulatory role for this protein, which may be involved in the immune evasion mechanisms of the parasite.

Hacariz produced a number of papers in 2013. One notable paper this year was entitled ‘Generating a detailed protein profile of F. hepatica during the chronic stage of infection in cattle’. The impressive results were that of a total of 776 identified proteins, 206 and 332 were specific to the surface and internal layers of the parasite, respectively. Furthermore, 238 proteins were common to both layers, with comparative differences of 172 proteins detected. Specific proteins not previously identified in F. hepatica, but shown to be immunomodulatory or potential drug targets for other parasites, are discussed in the paper.

Robles-Pérez analyzed the genetic variability of F. hepatica populations from different geographical locations by ISSR-PCR. Parasites were from Spain UK, Ireland and Mexico. The genetic relationships between populations and individuals were shown by a UPGMA dendrogram and a principal coordinate analysis. The lowest level of diversity was observed between UK and Ireland.

References


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Human Infection

This section reports on human infection with *F. hepatica*. Human fasciolosis is now well recognized and the section includes case reports and other aspects of infection.

Behzad, described "Finding of biliary fascioliasis by endoscopic ultrasonography in a patient with eosinophilic liver abscess." The patient had no eosinophilia with negative stool examinations, so was initially treated with antibiotics for liver abscess. Her clinical condition as well as follow-up imagings showed appropriate response after antibiotic therapy. Finally, endoscopic ultrasonography revealed *F. hepatica*, which was then extracted with endoscopic retrograde cholangiopancreatography.\[57x72\]

Carlos Gil described finding resistance to the fasciolicide triclabendazole in four patients in Chile. The patients were diagnosed by direct visualization of parasitic eggs in stool examination, by positive testing for *F. hepatica* antigens in stools, or by direct observation of parasites by endoscopic retrograde cholangiopancreatography or surgery.

Gabrielli, described a family outbreak of human fasciolosis in Italy. In 2010, three children who were born in a Romanian cattle farm family went to Italy to join their mother. One of them was admitted to an Italian pediatric hospital for severe anemia that, when she was in her country, had been treated with blood transfusion. Blood tests and an abdominal ultrasound study triggered the suspicion of biliary parasitosis. The child underwent a cholangiopancreatography that caused the release of parasitic material microscopically identified as *F. hepatica*. Anamnesis, parasite biology, and genetic data strongly suggested that the sisters became infected in Romania. Gabrielli concluded that human fasciolosis is an emerging sanitary problem, favored by climate changes and global drivers; therefore, it deserves more attention on behalf of physicians working in both developing and developed countries.

Mas-Coma is one of the world's foremost authorities on fasciolosis. He wrote a paper entitled "Diagnosis of human fasciolosis by stool and blood techniques: update for the present global scenario." He concluded that there were pronounced difficulties in diagnosing fasciolosis given the different infection phases and parasite migration capacities, the clinical heterogeneity, immunological complexity and different epidemiological situations and transmission patterns. Also the lack of a diagnostic technique covering all needs and situations, and the advisability for a combined use of different techniques, at least including a stool technique and a blood technique.

References


RUMEN FLUKE (PARAMPHISTOMES).

This review covers the papers relevant to paramphistomosis in the UK. Paramphistomes are regarded as more pathogenic in tropical climates and there are a larger number of papers referring to these parasites from India, Iran and other countries where buffaloes are of greater importance. Two of these papers are included below.

Carmen Ferreras reported the prevalence paramphistomes in cattle slaughtered in the Castilla y Leon region (Spain) over a 3 year-period. The overall prevalence of positive animals was 6.20%. The parasite burden per animal ranged from 8 to 8005 (median = 144) and the ruminal atrium had the highest parasite burden whereas the ruminal dorsal sac the lowest. The prevalence and parasite burden increased with age while these parameters were lower in cattle under intensive management. Calicophoron daubneyi was the only paramphistome species identified using morphoanatomical, histological and molecular methods in the studied animals.

Chourasia described the histopathology of rumen flukes from water buffaloes in India. The aim of the study was to find histopathological changes of affected rumen of buffalo to species level. Key morphological findings were as follows: affected areas of rumen looked whitish in color with complete denudation of rumen papillae. Papillae appeared anaemic, atrophied and their tips showed degeneration and sloughing.

A second paper by Chourasia, described the surface structures and internal anatomy of rumen flukes from reindeer, as seen by scanning electron microscopy (SEM) and in histological sections under light microscopy. The aim of the study was to find morphological information to enable identification of rumen flukes in buffalo to species level. Key morphological findings were as follows: The acetabulum of the rumen flukes was of paramphistomum type, the pharynx of liorchis type, and the genital atrium of leydeni type. Both winter and summer flukes shared these morphological features. Based on these findings, it was concluded that rumen flukes of buffalo in Gujarat belonged to the species Paramphistomum cervi.

O'Toole tried to determine the role of deer as reservoirs for rumen fluke infections in livestock in Ireland. A total of 144 deer faecal samples (88 from fallow deer, 32 from red deer and 24 samples from sika, sika/red deer hybrids) were screened for the presence of fluke eggs. Based on the ITS-2 rDNA locus plus flanking 5.8S and 28S sequences (ITS-2+), fluke eggs were identified to species level. The results indicated that, of the 3 deer species, fallow deer had the highest fluke infection rates. Two rumen fluke species, Calicophoron daubneyi and Paramphistomum leydeni, with morphologically distinct eggs, were identified. Concurrent infections of the two paramphistome species and liver fluke, Fasciola hepatica, were common. Considering the comparatively low egg burdens observed in this study, it is unlikely that deer represent a significant source of infection for Irish livestock.

Rondelaud conducted several experiments on the breeding of trematode-infected Galba truncatula for obtaining and packaging Fasciola hepatica and Paramphistomum daubneyi metacercariae, to determine the more convenient methods to use for commercial production of these infective stages. Compared to the breeding of infected snails in aquaterraria, the use of 14-cm Petri dishes allowed a greater prevalence of snail infection and a higher number of metacercariae. The production of these larvae was still 2.3-3.4 times greater if infected snails were dissected during the patent period. The aspiration of these metacercariae at the extremity of a Pasteur pipette significantly shortens the time necessary for their transfer from Petri dishes to Eppendorf tubes. Using 14-cm Petri dishes, snail dissection and metacercarial aspiration for their transfer strongly reduce the cost price for metacercarial production of the trematodes Fasciola hepatica and Paramphistomum daubneyi.

Sanabria assessed the efficacy of oxyclozanide (OCZ) against Paramphistomum leydeni in naturally infected adult sheep. OCZ concentrations in blood stream and gastrointestinal fluids collected from treated animals were also measured. Fifteen P. leydeni naturally infected sheep were randomly divided into two groups: untreated control (n=5) and treated (n=10). The treated group was orally drenched with OCZ (20 mg/kg, day 0). A second dose was administered 72 h later. Faecal samples were taken at days 0, +3 and +5. Five sheep from both groups were slaughtered at day +5. At necropsies, rumen, abomasum and small intestine were examined for adult and immature flukes. All recovered flukes were counted and the treatment efficacy was estimated. Additionally, serum and gastrointestinal fluid content (ruminal, abomasal and small intestine) samples, obtained from five treated animals at day +5, were analyzed by HPLC to measure OCZ.
concentrations. OCZ showed high efficacy (99%) against mature *P. leydeni*. The post-treatment egg reduction was also high after the first dose with values ranging from 98.4% (day +3) to 99.5% (day +5). The highest OCZ concentrations were measured in serum followed by the small intestinal fluid. Very low OCZ concentrations were measured in ruminal and abomasal fluids. OCZ administered to sheep twice (20 mg/kg) by the oral route was highly efficacious against mature stages of *P. leydeni* in naturally infected sheep. Despite a high drug concentration at the intestinal fluid, OCZ efficacy against immature stages could not be assessed.

Zheng produced an important paper characterizing the complete nuclear ribosomal DNA sequences of *Paramphistomum cervi*. The phylogenetic position of *P. cervi* in Paramphistomatidae was analyzed based on the 18S rDNA sequences and should aid in studying the intra- and interspecific variation of the Paramphistomatidae and the systematics and phylogenetics of Digenea.

Zintl produced the most interesting and relevant paper on paramphistomes entitled ‘Bovine paramphistomes in Ireland’. During this study, historical diagnostic laboratory records were interrogated for recent changes in the incidence of rumen fluke in Ireland. Three cattle herds were monitored for the presence of paramphistome eggs using coprological analysis over a period of 2 months (in the case of a group of housed steers) and 14 months (in the case of two extensively operated farms), respectively. Adult rumen fluke collected following slaughter were weighed and typed in two loci. *Calicophoron daubneyi* was the most common if not only paramphistome species present in Ireland, and infections in cattle were now much more prevalent than five or six years ago. The phylogenetic relationship of the isolates to the only published sequence and to *C. daubneyi* isolates from Northern Ireland was analysed. Genetic heterogeneity was similar all over the island and comparable to that of *F. hepatica*, a fact that may have implications for the parasite’s ability to develop resistance to the very limited number of drugs currently available for treatment. The same haplotypes predominated throughout the island. He concluded that clinical significance of *C. daubneyi* is still uncertain, but considering the apparent pervasiveness of the parasite, rumen fluke should be considered a differential diagnosis when treating scour or ill-thrift in young calves, and goats and sheep of any age.

References


PARASITES OF PIGS


Two single nucleotide polymorphisms (SNP TXNIP and SNP ARNT), both on chromosome 4, have been reported to be associated with roundworm (Ascaris suum) burden in pigs. In the present study, we selected pigs with two
EIMERIA IN POULTRY

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Comparative evaluation of probiotic and salinomycin effects on performance and coccidiosis control in broiler chickens.

Poultry Science 93 (12) 3002-3008

The annual financial loss to the poultry industry as a result of coccidiosis has been estimated at about US $3 billion. The objective of this study was to evaluate and compare the effects of probiotics and salinomycin as feed additives on performance and coccidiosis control in male broilers raised to 42 d of age. The study consisted of 360 Cobb male broiler chickens randomly allocated to 4 groups each with 3 replicates. Group 1: untreated, unchallenged negative control group (NC); group 2: untreated, challenged positive control group (PC); group 3: negative control supplemented with salinomycin 66 mg/kg, challenged group (Sal); and group 4: negative control supplemented with probiotics, challenged (Prob mix). On d 15, all birds (except group 1) were challenged with approximately 75,000, 25,000, and 75,000 of Eimeria acervulina, Eimeria maxima, and Eimeria tenella oocysts, respectively that were mixed into the feed. Feed conversion ratio and mortality were recorded throughout the experiment. On d 21 and 42, intestinal lesions and litter conditions were scored. On d 7, 14, 21, 28, 35, and 42, oocyst counts were determined from 10 freshly collected fecal samples per pen. The results showed that mortality, litter, and lesion scores at d 21 and 42, and oocyst shedding at d 21 did not differ significantly between the Prob mix and the Sal groups. However on d 28, oocyst shedding was significantly lower in the Sal group than in the PC group but insignificantly lower than the Prob mix group. Body weights of the Prob mix group at d 42 were significantly lower than the Sal group; however, the feed conversion ratio values were similar between the 2 groups. The results of this study showed that probiotics supplementation could be considered as a potential strategy to control coccidiosis in broiler chickens.

Jenkins MC; O'Brien CN; Fuller L; Mathis GF; Fetterer R (2014)
A rapid method for determining salinomycin and monensin sensitivity in Eimeria tenella.

Veterinary Parasitology 206 (3-4) 153-158

Standard methods of determining the ionophore sensitivity of Eimeria rely on infecting chickens with an isolate or a mixture of Eimeria spp. oocysts in the presence of different anti-coccidial drugs. The purpose of this study was to develop a rapid in vitro method for assessing salinomycin and monensin sensitivity in Eimeria tenella. Cultures of MDBK cells were grown to 85% confluency, and then inoculated with excysted E. tenella laboratory strain (APU-1) sporozoites in the presence of different concentrations of salinomycin or monensin. At various timepoints, the monolayers were fixed for counting intracellular sporozoites, or were subjected to DNA extraction, followed by molecular analysis using quantitative (qPCR) or semi-quantitative PCR (sqPCR). Preliminary experiments showed that 24 h was the optimum time for harvesting the E. tenella-infected cell cultures. The average number of E. tenella sporozoites relative to untreated controls displayed a linear decrease between 0.3 and 33.0 μg/ml salinomycin and between 0.3 and 3.3 μg/ml monensin. A similar pattern was observed in the relative amount of E. tenella DNA as measured by sqPCR. A linear decrease in the relative amount of E. tenella DNA was observed over the entire range of salinomycin and monensin concentrations as measured by qPCR possibly reflecting the greater sensitivity of this assay. Comparison of sporozoite counting, sqPCR, and qPCR signals using a criterion of 50% inhibition in sporozoite numbers or level of PCR amplification product showed good agreement.
between the three assays. E. tenella field isolates (FS-1 and FS-2) displaying resistance to salinomycin and monensin were evaluated in the in vitro assay using qPCR and sqPCR. Compared to E. tenella APU-1, the E. tenella FS-1 and FS-2 isolates showed higher levels of E. tenella DNA at 24 h by both qPCR and sqPCR. This in vitro assay represents a significant advance in developing rapid, cost-effective methods for assessing ionophore sensitivity in E. tenella.

Frolich S; Shahparee A; Wasinger VC; Wallach M (2014) In vivo localization of antibodies raised against Eimeria maxima wall forming bodies during sexual intracellular development. Parasitology 141 (13) 1726-1735

Apicomplexan parasites cause devastating diseases in humans and livestock. Previously we demonstrated that antibodies targeting transmissible forms of the apicomplexan parasite, Eimeria, are effective at reducing parasite shedding thus preventing the transmission of the disease. However, the mechanisms responsible have not been fully defined. Moreover, there is no direct evidence that the parasite-specific IgG antibodies can reach the parasite developing in the enterocytes of the infected chicken host. This study summarizes our efforts using host immunity, parasite proteomics and 3D microscopy to provide a step forward in our understanding of how this immune response works. Eimeria maxima is an important pathogen of poultry and used as a surrogate for a number of human pathogens including Toxoplasma and Plasmodium. Our studies demonstrate that immunization with the purified wall forming bodies (WFBs) results in a production of parasite-specific IgG antibodies, which have the ability to reach in situ gametocytes in the intestinal lumen and permeate the enterocyte/parasite membranes in order to bind to the cytoplasmic Type 1 and Type 2 WFBs. This raises the intriguing possibility that via this process antibodies block the development of Eimeria maxima in vivo.

Fetterer RH; Jenkins MC; Miska KB; Barfield RC (2014) Evaluation of an experimental irradiated oocyst vaccine to protect broiler chicks against avian coccidiosis. Avian Diseases 58 (3) 391-397

The current study investigates the use of irradiated oocysts to protect broiler chicks, raised on litter, from infection with multiple species of Eimeria. In order to determine the optimum radiation dose for each Eimeria species, 1-day-old chicks were immunized with oocysts of Eimeria maxima, Eimeria acervulina, or Eimeria tenella exposed to gamma radiation ranging from 0–500 Gy. The litter oocyst counts at 7 days post immunization, and the effect on weight gain following a challenge infection, decreased with an optimum dose between 150–200 Gy. Based on this finding, broiler chicks were immunized with a mixture of E.maxima, E. acervulina, and E. tenella that had been exposed to 150 or 200 Gy. This resulted in more than a 100-fold reduction in litter oocyst counts and significant protection from a challenge infection, as measured by improved weight gain and feed conversion ratio (FCR). Immunization of birds with oocysts receiving 200 Gy was less effective in providing protection from a challenge infection. An additional formulation of vaccines containing two different oocyst doses of the three species that had been irradiated with 150 Gy were evaluated in their ability to attenuate oocyst output and convey protection to challenge. Results were similar with both high and low numbers of irradiated oocysts. Immunized chicks shed less oocysts at 7 days post immunization and were protected from negative effects of challenge infection as measured by FCR, changes in weight gain, lesion scores, and measurement of body composition. However, the level of protection was somewhat less than that achieved by immunization with non-irradiated oocysts. The overall conclusion is that an irradiated oocyst vaccine developed in this study can effectively protect chicks that are raised on litter from challenge infection with multiple species of Eimeria, comparable to vaccines with virulent or precocious strains.

Wu LL; Lin RQ; Sun MF; Liu LD; Duan WF; Zou SS; Yuan ZG; Weng YB (2014) Biological characteristics of Chinese precocious strain of Eimeria acervulina and its immune efficacy against different field strains. Avian Diseases 58 (3) 367-372

In this study, the biologic characteristics of one experimental precocious strain of Eimeria acervulina and seven field isolates from different geographic locations in China were compared, and the immune efficacy of two precocious strains against coccidiosis in chickens was assessed to explore their potential use as coccidiosis vaccines. All the different strains were purified by single oocyst separation and their monospecificity was confirmed using E. acervulina-specific PCR assays. The average sizes of E. acervulina oocysts were 18.28–20.19 μm at 14.09–14.79 mm and the shape indexes were from 1.28 to 1.40. The prepatent periods ranged from 93 to 115 hr, except for the Heyuan precocious strain (HYP; 75 hr). Chickens infected with Huadu field strain (GHD) produced the highest oocyst output whereas HYP induced the lowest
level. When inoculated with 50,000 sporulated oocysts or more, the average weight gains of infected chickens were reduced, with apparent clinical symptoms. To assess the immunogenicity of precocious strains HYP and Baoding (BDP), birds were orally immunized and challenged with seven different field strains of *E. acervulina*. Body weight gain, fecal oocyst output, and gut lesion scores were compared to evaluate their vaccine potential. The results showed that the average body weight gains of chickens in all the vaccinated and challenged groups were higher than those of non-vaccinated and challenged groups. In general, oocyst shedding was reduced 34.39%–95.31% and gut lesion scores decreased 31.03%–86.21% compared with unvaccinated and challenged control chickens. In summary, this study indicated that the precocious strains of *E. acervulina* could induce a protective immune effect with various responses against coccidiosis caused by different field strains.

Shah MAA; Umar S; Iqbal MF; Rehman F; Qadri I; He N (2014)
Recent developments in DNA vaccination approaches against poultry coccidiosis and its future endeavours.
*World's Poultry Science Journal* 70 (2) 315-328

In 1948 the first research paper was published about the treatment of coccidiosis. After 60 years the attention has focused on DNA vaccination especially as certain anticoccidials have failed due to resistance and residues. Thus far vaccination is partially successful but there are disadvantages e.g. instability, inferior control, cost effectiveness and inefficacy due to large numbers of coccidial strains. Developments whereby genetically engineered DNA can be administered in vaccine form to provoke immune responses have led to a huge development in the practical application in this field. The DNA fragments taken from all the four important species, *Eimeria tenella*, *Eimeria necatrix*, *Eimeria maxima* and *Eimeria acervulina* were able to provoke appropriate immune responses against challenging infections with homologous species however most of them were not able to provoke a response with heterologous infection. The shared DNA antigen in *Eimeria tenella* and Eimeria acervulina was able to produce sufficient immune responses not only against these species but also against *Eimeria necatrix*. *Eimeria maxima* is the biggest and most complex of all the seven species and it is a challenge for DNA vaccine researchers.

Rathinam T; Gadde U; Chapman HD (2014)
Sericea lespedeza has no anticoccidial effect when included in the diet of chickens infected with three species of Eimeria.
*Veterinary Parasitology* 202 (3-4) 265-269

Anticoccidial effects of *Sericea Lespedeza* (SL) included in the diet at different levels were evaluated in chickens following oral infection with sporulated oocysts of either *Eimeria acervulina*, *Eimeria maxima* or *Eimeria tenella*. A series of experiments were conducted to determine the effect of SL upon the ability of the parasites to multiply in the intestine, and the effect on bodyweight gain, feed intake, and feed conversion following infection. Chicks infected with a low dose of oocysts (500 oocysts/bird) of *E. acervulina* or *E. maxima* did not show differences in the numbers of oocysts produced in the feces whether they were given 0, 1, 2, or 4% SL in the diet. There was no significant difference in the weight gain, feed intake, or FCR of birds infected with high doses of *E. acervulina* or *E. maxima* (200,000 or 100,000 oocysts/bird respectively) whether 0, 1, 2, or 4% SL was included in the feed. No significant difference in the numbers of oocysts in the feces, weight gain, feed intake, and FCR of birds infected with *E. tenella* (low dose of 500 oocysts; high dose of 50,000 oocysts per bird) whether 4% SL was included in the feed. The results of this study indicate that SL has no anticoccidial activity against *Eimeria* species in the chicken.

Su S; Miska KB; Fetterer RH; Jenkins MC; Wong EA (2014)
Expression of digestive enzymes and nutrient transporters in Eimeria acervulina challenged layers and broilers.
*Poultry Science* 93 (5) 1217-1226

Avian coccidiosis is a disease caused by intestinal protozoa in the genus *Eimeria*. Clinical signs of coccidiosis include intestinal lesions and reduced feed efficiency and BW gain. This growth reduction may be due to changes in expression of digestive enzymes and nutrient transporters in the intestine. The objective of this study was to examine the differential expression of digestive enzymes, transporters of amino acids, peptides, sugars, and minerals, and an antimicrobial peptide in the small intestine of *Eimeria acervulina*-infected broilers and layers. Uninfected broilers and layers, in general, expressed these genes at comparable levels. Some differences included 3-fold and 2-fold greater expression of the peptide transporter PepT1 and the antimicrobial peptide LEAP2 (liver expressed antimicrobial peptide 2), respectively, in
the jejunum of layers compared with broilers and 17-fold greater expression of LEAP2 in the duodenum of broilers compared with layers. In the duodenum of *Eimeria*-infected broilers and layers, there was downregulation of aminopeptidase N; sucrase-isomaltase; the neutral, cationic, and anionic amino acid transporters b^0^\^AT/rBAT, b^0^\^AT, CAT2, and EAAT3; the sugar transporter GLUT2; the zinc transporter ZnT1; and LEAP2. In the jejunum of infected layers there was downregulation of many of the same genes as in the duodenum plus downregulation of PepT1, b^6^\^AT/rBAT, and the y’L system amino acid transporters y’LAT1 and y’LAT2. In the ileum of infected layers there was downregulation of CAT2, y’LAT1, the L type amino acid transporter LAT1, and the sugar transporter GLUT1, and upregulation of APN, PepT1, the sodium glucose transporter SGLT4, and LEAP2. In *E. acervulina*-infected broilers, there were no gene expression changes in the jejunum and ileum. These changes in intestinal digestive enzyme and nutrient transporter gene expression may result in a decrease in the efficiency of protein digestion, uptake of important amino acids and sugars, and disruption of mineral balance that may affect intestinal cell metabolism and *Eimeria* replication.

Del Cacho E; Gallego M; Lillehoj H; Quilez J; Lilhehoj EP; Ramo A; Sanchez-Acedo C (2014)
**IL-17A regulates *Eimeria tenella* schizont maturation and migration in avian coccidiosis.**
*Veterinary Research* 45:25

Although IL17A is associated with the immunological control of various infectious diseases, its role in host response to *Eimeria* infections is not well understood. In an effort to better dissect the role of IL17A in host-pathogen interactions in avian coccidiosis, a neutralizing antibody (Ab) to chicken IL17A was used to counteract IL17A bioactivity in vivo. Chickens infected with *Eimeria tenella* and treated intravenously with IL17A Ab, exhibited reduced intracellular schizont and merozoite development, diminished lesion score, compared with untreated controls. Immunohistological evaluation of cecal lesions in the parasitized tissues indicated reduced migration and maturation of second-generation schizonts and reduced lesions in lamina propria and submucosa. In contrast, untreated and infected chickens had epithelial cells harboring second-generation schizonts, which extend into the submucosa through muscularis mucosa disruptions, maturing into second generation merozoites. Furthermore, IL17A Ab treatment was associated with increased parameters of Th1 immunity (IL2- and IFNγ-producing cells), reduced levels of reactive oxygen species (ROS), and diminished levels of serum matrix metalloproteinase-9 (MMP-9). Finally, schizonts from untreated and infected chickens expressed S100, Wiskott-Aldrich syndrome protein family member 3 (WASF3), and heat shock protein-70 (HSP70) proteins as merozoites matured, whereas the expression of these proteins was absent in IL17A Ab-treated chickens. These results provide the first evidence that the administration of an IL17A neutralizing Ab to *E. tenella*-infected chickens inhibits the migration of parasitized epithelial cells, markedly reduces the production of ROS and MMP-9, and decreases cecal lesions, suggesting that IL17A might be a potential therapeutic target for coccidiosis control.

Almeida GFD; Thamsborg SM; Madeira A; Ferreira JFS; Magalhaes PM; Dematt (2014)
**The effects of combining Artemisia annua and Curcuma longa ethanolic extracts in broilers challenged with infective oocysts of *Eimeria acervulina* and *E. maxima*.** *Parasitology* 141 (3) 347-355

Due to an increasing demand for natural products to control coccidiosis in broilers, we investigated the effects of supplementing a combination of ethanolic extracts of *Artemisia annua* and *Curcuma longa* in drinking water. Three different dosages of this herbal mixture were compared with a negative control (uninfected), a positive control (infected and untreated), chemical coccidiostats (nicarbazin+narazin, and, later, salinomycin), vaccination, and a product based on oregano. Differences in performance (weight gain, feed intake, and feed conversion rate), mortality, gross intestinal lesions and oocyst excretion were investigated. Broilers given chemical coccidiostats performed better than all other groups. Broilers given the two highest dosages of the herbal mixture had intermediate lesion scores caused by *Eimeria acervulina*, which was higher than in broilers given coccidiostats, but less than in broilers given vaccination, oregano and in negative controls. There was a trend for lower mortality (*P* = 0.08) in the later stage of the growing period (23–43 days) in broilers given the highest dosage of herbal mixture compared with broilers given chemical coccidiostats. In conclusion, the delivery strategy of the herbal extracts is easy to implement at farm level, but further studies on dose levels and modes of action are needed.

Sun H; Wang L; Wang T; Zhang J; Liu Q; Chen P; Chen Z; Wang F; Li H; Xiao Y; Zhao X (2014)
**Display of *Eimeria tenella* EtMic2 protein on the surface of Saccharomyces cerevisiae as a potential oral vaccine against chicken coccidiosis.**
*Vaccine* 32 (16) 1869-1876
S. cerevisiae is generally regarded as safe and benign organism and its surface display system may be used as a unique eukaryotic expression system that is suitable for expressing eukaryotic antigen. In addition to the convenience of vaccine delivery, the yeast cell wall has been shown to enhance the innate immunity when immunized with the yeast live oral vaccine. In the present study, we expressed the chicken coccidian E. tenella EtMic2, a microneme protein, on the surface of the S. cerevisiae and evaluated it as a potential oral vaccine for chicken against E. tenella challenge. The protective efficacy against a homologous challenge was evaluated by body weight gains, lesion scores and fecal oocyst shedding. The results showed that the live oral vaccine can improve weight gains, reduced cecal pathology and lower oocyst fecal shedding compared with non-immunized controls. In addition, the yeast oral vaccine could stimulate humoral as well as cell mediate immune responses. These results suggested that EtMic2 displayed on the cell surface of S. cerevisiae could be used as potential live vaccine against chicken coccidiosis.

VECTORS


Tabanids are nuisance pests for people and livestock because of their painful and irritating bite, persistent biting behavior, and blood ingestion. About 4400 tabanid species have been described; they are seasonally present in all kinds of landscapes, latitudes, and altitudes. High populations have a significant economic impact on outdoor activities, tourism, and livestock production. Tabanids are also vectors of animal disease agents, including viruses, bacteria and parasites. However, tabanids have received little attention in comparison with other hematophagous Diptera. Here, we highlight the many direct and indirect impacts of tabanids and provide a brief summary of tabanid morphology, biology, and life cycle. Impacts include pathogen transmission, parasite transportation (Dermatobia hominis), biological transmission (Loa loa), and mechanical transmission of viruses, such as equine infectious anemia virus, protozoa, such as Trypanosoma evansi and Besnoitia besnoiti, and bacteria, such as Bacillus anthracis and Anaplasma marginale. We discuss parameters of mechanical transmission and its mathematical modeling. Control methods for tabanid populations are also summarized; these include trapping, the use of insecticides, repellents, and livestock protection. Lastly recommendations are provided for the direction of future research.

VECTOR BORNE DISEASES

Anaplasma phagocytophilum infection in a horse from Switzerland with severe neurological symptoms
Gussmann, K; Czech, C; Hermann, M; Schaarschmidt-Kiener, D; von Loewenich, FD
SCHWEIZER ARCHIV FUR TIERHEILKUNDE, 156 (7):345-348

A 22-year old mare from Switzerland was admitted to an equine clinic in May 2011. She presented with fever, lethargy, icteric mucous membranes, reduced alertness, an unsteady gait and ataxia. An Anaplasma phagocytophilum infection was confirmed by blood smear and PCR. The mare was treated with oxytetracycline and recovered rapidly, but she still suffered from a slight atactic gait disturbance at 3 weeks post infection.

Tick-borne infections of animals and humans: a common ground
Baneth, G.INTERNATIONAL JOURNAL FOR PARASITOLOGY, 44 (9):591-596

A wide variety of pathogens is transmitted from ticks to vertebrates including viruses, bacteria, protozoa and helminths, of which most have a life cycle that requires passage through the vertebrate host. Tick-borne infections of humans, farm and companion animals are essentially associated with wildlife animal reservoirs. While some insect-borne diseases of humans such as malaria, filariasis and Kala Azar caused by Leishmania donovani target people as their main host, major tick-borne infections of humans, although potentially causing disease in large numbers of individuals, are typically an infringement of a circulation between wildlife animal reservoirs and tick vectors. While new tick-borne infectious agents are frequently recognised, emerging agents of human tick-borne infections were probably circulating among wildlife animal and tick populations long before being recognised as clinical causes of human disease as has been shown for Borrelia burgdorferi sensu lato. Co-infection with more than one tick-borne infection is common and can enhance pathogenic processes and augment disease severity as found in B. burgdorferi and Anaplasma phagocytophilum co-infection. The role of wild animal reservoirs in co-infection of human hosts appears to be central, further linking human and animal tick-borne infections. Although transmission of most tick-borne infections is through the tick saliva, additional routes of transmission, shown mostly in animals, include infection by oral uptake of infected ticks, by carnivorism, animal
bites and transplacentally. Additionally, artificial infection via blood transfusion is a growing threat in both human and veterinary medicine. Due to the close association between human and animal tick-borne infections, control programs for these diseases require integration of data from veterinary and human reporting systems, surveillance in wildlife and tick populations, and combined teams of experts from several scientific disciplines such as entomology, epidemiology, medicine, public health and veterinary medicine.

The ecology of ticks and epidemiology of tick-borne viral diseases
Estrada-Peña, A; de la Fuente, J. ANTIMOVIRAL RESEARCH, 108:104-128

A number of tick-borne diseases of humans have increased in incidence and geographic range over the past few decades, and there is concern that they will pose an even greater threat to public health in future. Although global warming is often cited as the underlying mechanism favouring the spread of tick-borne diseases, climate is just one of many factors that determine which tick species are found in a given geographic region, their population density, the likelihood that they will be infected with microbes pathogenic for humans and the frequency of tick human contact. This article provides basic information needed for microbiologists to understand the many factors that affect the geographic range and population density of ticks and the risk of human exposure to infected ticks. It first briefly summarizes the life cycle and basic ecology of ticks and how ticks and vertebrate hosts interact, then reviews current understanding of the role of climate, sociodemographic factors, agricultural development and changes in human behaviour that affect the incidence of tick-borne diseases. These concepts are then illustrated in specific discussions of tick-borne encephalitis and Crimean-Congo haemorrhagic fever.

Probable transfusion-transmission of Anaplasma phagocytophilum by leukoreduced platelets
Townsend, RL; Moritz, ED; Fialkow, LB; Berardi, V; Stramer, SL. TRANSFUSION, 54 (11):2828-2832

Background A. phagocytophilum, a tick-borne obligate intracellular bacterium, causes human granulocytic anaplasmosis (HGA) and has been implicated in seven transfusion-transmitted (TT)-HGA cases associated with red blood cells (RBCs). Here we report the first probable case of TT-HGA involving leukoreduced platelets (PLTs).

A hospitalized male received 25 blood components (November 2012) before his death from trauma. Hospital testing confirmed HGA by peripheral blood smears; samples were also tested for A. phagocytophilum using PCR and IgM and IgG enzyme immunoassay. All 12 potentially transmitting donors provided follow-up samples. Recipient smears progressed from negative to predominantly positive 16 days post transfusion; PCR was positive on Day 22 and IgM and IgG negative 14 to 23 days post transfusion. The recipient had no known risk factors for tick borne disease. One of 12 donors of RBCs or PLTs (leukoreduced 5-day-old PLTs) provided six follow-up samples; all were strongly IgG positive and IgM negative; one was PCR-positive. The IgG-positive donor was a 52-year-old female from Hudson Valley, New York, an area endemic for human anaplasmosis. She reported tick bites in September to October 2012 with no travel outside New York. The donor remained asymptomatic and received no treatment. The cocomponent PLT unit was transfused to a 78-year-old male who died of causes unrelated to human anaplasmosis. This eighth case of probable TT-HGA indicates that leukoreduced PLTs may be infectious. An antibody- and PCR-positive donor having prior tick exposure living in an endemic area was identified. PCR positivity and elevated IgG levels, which continue to exceed the assay’s detectable range even in the absence of IgM, indicate active donor infection.

Development and Validation of a Sensitive and Specific sodB-Based Quantitative PCR Assay for Molecular Detection of Ehrlichia Species
Qurollo, BA; Riggins, D; Comyn, A; Zewde, MT; Breitschwerdt, EB
JOURNAL OF CLINICAL MICROBIOLOGY, 58 (11):4030-4032

This paper describes the development of a sensitive and specific sodB-based quantitative PCR assay to detect Ehrlichia spp. The assay’s limit of detection was 5 copies/reaction, and it did not amplify nonspecific DNA. Compared with a 16S rRNA gene PCR target, the sodB target may offer an improved molecular diagnostic assay to detect Ehrlichia spp.

Using open-access taxonomic and spatial information to create a comprehensive database for the study of Mammalian and avian livestock and pet infections
McIntyre, KM; Setzkorn, C; Wardeh, M; Hepworth, PJ; Radford, AD; Baylis, M
PREVENTIVE VETERINARY MEDICINE, 116 (3):325-335
Only 10 years ago, the first comprehensive pathogen list was compiled for humans; we still have no equivalent for animals. This paper describes the creation of a novel pathogen database, and present outputs from the database that demonstrate its value.

The ENHanCED Infectious Diseases database (EID2) is open-access and evidence-based, and describes the pathogens of humans and animals, their host and vector species, and also their global occurrence. The EID2 systematically collates information on pathogens into a single resource using evidence from the NCBI Taxonomy database, the NCBI MeSH (Medical Subject Headings) library and PubMed. Information about pathogens is assigned using data-mining of meta-data and semi-automated literature searches. The authors focus on 47 mammalian and avian hosts, including humans and animals commonly used in Europe as food or kept as pets. Currently, the EID2 evidence suggests that: Within these host species, 793 (30.5%) pathogens were bacteria species, 395 (15.2%) fungi, 705 (27.1%) helminths, 372 (14.3%) protozoa and 332 (12.8%) viruses. The odds of pathogens being emerging compared to not emerging differed by taxonomic division, and increased when pathogens had greater numbers of host species associated with them, and were zoonotic rather than non-zoonotic. The odds of pathogens being zoonotic compared to non-zoonotic differed by taxonomic division and also increased when associated with greater host numbers. Comparing pathogens which are zoonotic (n = 829) with those non-zoonotic (n = 527) indicated that the odds of a pathogen being zoonotic were 16 times higher if they were helminth pathogens, more than twice as high if they were protozoans and nearly four times higher if they were viruses, all compared to bacteria. Further, the odds of pathogens being zoonotic were more than three times higher if they had more than two compared to two host species associated with them, and 70% lower if they had only one host compared to two host species associated with them. Pathogenicity did not significantly affect the odds of a pathogen being zoonotic.

The pathogens affecting the greatest number of hosts included: Escherichia coli, Giardia intestinalis, Toxoplasma gondii, Anaplasma phagocytophilum, Cryptosporidium parvum, Rabies virus, Staphylococcus aureus, Neospora caninum and Echinococcus granulosus. The pathogens of humans and domestic animal hosts are characterised by 4223 interactions between pathogen and host species, with the greatest number found in: humans, sheep/goats, cattle, small mammals, pigs, dogs and equids. The number of pathogen species varied by European country. The odds of a pathogen being found in Europe compared to the rest of the world differed by taxonomic division, and increased if they were emerging compared to not emerging, or had a larger number of host species associated with them.

Changing incidence of bovine babesiosis in Ireland
Zintl, A; McGrath, G; O'Grady, L; Fanning, J; Downing, K; Roche, D; Casey, M; Gray, J.
IRISH VETERINARY JOURNAL, 67 19-19

In Ireland, bovine babesiosis is caused by the tick-borne blood parasite Babesia divergens. A survey of veterinary practitioners and farmers in the 1980's revealed an annual incidence of 1.7% associated with considerable economic losses. However, two subsequent surveys in the 1990's indicated a decline in clinical babesiosis. Recent evidence from continental Europe suggests that, probably due to climate change, the distribution of Ixodes ricinus the tick vector of B. divergens, is extending to more northerly regions and higher altitudes. In addition, milder winters are thought to widen the window of tick activity. In order to determine whether any such changes have affected the incidence of bovine babesiosis in Ireland, a questionnaire survey of farmers and veterinarians was carried out and compared with data from previous surveys. The survey indicates that while the incidence of clinical disease has continued to decline, cases can occur at any time of year. In contrast to previous surveys, affected farms were the same size as unaffected ones. There was no correlation between disease risk and the presence of deer on the land. Disease severity and mortality rates were increased because many infections were advanced by the time they were detected and treated. While the precise reasons for the decline in the incidence of redwater are unknown, changes in agricultural practice are likely to be of importance. A reversal of the trend could be devastating, as vigilance among farmers and veterinarians is flagging and the national herd is losing its protective immunity to disease.

Ixodes ricinus (Ixodidae), an occasional phoront on necrophagous and coprophagous beetles in Europe
Salona-Bordas, M; de la Puebla, PB; Martin, BD; Sumner, J; Perotti, MA
EXPERIMENTAL AND APPLIED ACAROLOGY, 65 (2):243-248

For ticks, phoretic behaviour using insects associated with vertebrates might offer an alternative strategy to host-seeking. The authors report, for the first time, the presence of immature stages of the most widespread tick species in Western Europe, Ixodes ricinus (Acar: Ixodidae), on three beetle species belonging to families Silphidae and Geotrupidae (Coleoptera). Specimens were collected while performing fieldwork surveys on insect diversity during the peak of tick's questing behaviour, in July and August of 2009 and 2010. The collections took place in two Natural Parks, the Aiako
Climate change and Ixodes tick-borne diseases of humans

Ostfeld, RS; Brunner, JL

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B-BIOLOGICAL SCIENCES, 370 (1665):40051-40051

The evidence that climate warming is changing the distribution of Ixodes ticks and the pathogens they transmit is reviewed and evaluated. The primary approaches are either phenomenological, which typically assume that climate alone limits current and future distributions, or mechanistic, asking which tick-demographic parameters are affected by specific abiotic conditions. Both approaches have promise but are severely limited when applied separately. For instance, phenomenological approaches (e.g. climate envelope models) often select abiotic variables arbitrarily and produce results that can be hard to interpret biologically. On the other hand, although laboratory studies demonstrate strict temperature and humidity thresholds for tick survival, these limits rarely apply to field situations. Similarly, no studies address the influence of abiotic conditions on more than a few life stages, transitions or demographic processes, preventing comprehensive assessments. Nevertheless, despite their divergent approaches, both mechanistic and phenomenological models suggest dramatic range expansions of Ixodes ticks and tick-borne disease as the climate warms. The predicted distributions, however, vary strongly with the models’ assumptions, which are rarely tested against reasonable alternatives. These inconsistencies, limited data about key tick-demographic and climatic processes and only limited incorporation of non-climatic processes have weakened the application of this rich area of research to public health policy or actions. The authors urge further investigation of the influence of climate on vertebrate hosts and tick-borne pathogen dynamics. In addition, testing model assumptions and mechanisms in a range of natural contexts and comparing their relative importance as competing models in a rigorous statistical framework will significantly advance understanding of how climate change will alter the distribution, dynamics and risk of tick-borne disease.

Borrelia miyamotoi in host-seeking Ixodes ricinus ticks in England

Hansford, KM; Fonville, M; Jahfari, S; Sprong, H; Medlock, JM

EPIDEMIOLOGY AND INFECTION, 143 (5):1079-1087

This paper reports the first detection of Borrelia miyamotoi in UK Ixodes ricinus ticks which causes relapsing fever as opposed to Lyme disease. It also reports on the presence and infection rates of I. ricinus for a number of other tick-borne pathogens of public health importance. Ticks from seven regions in southern England were screened for B. miyamotoi, Borrelia burgdorferi sensu lato (s.l.), Anaplasma phagocytophilum and Neoehrlichia mikurensis using qPCR. A total of 954 I. ricinus ticks were tested, 40 were positive for B. burgdorferi s.l., 22 positive for A. phagocytophilum and three positive for B. miyamotoi, with no N. mikurensis detected. The three positive B. miyamotoi ticks came from three geographically distinct areas, suggesting a widespread distribution, and from two separate years, suggesting some degree of endemicity. Understanding the prevalence of Borrelia and other tick-borne pathogens in ticks is crucial for locating high-risk areas of disease transmission.

Further thoughts on the taxonomy and vector role of Rhipicephalus sanguineus group ticks

Dantas-Torres, F; Otranto, D

VETERINARY PARASITOLOGY, 208 (1-2):9-13

Rhipicephalus sanguineus is a tick species described in 1806 by Latreille, based on specimens probably collected in France. However, this is a taxon with uncertain morphological definition and recent studies have gathered irrefutable evidence supporting the existence of a cryptic species-complex under the name R. sanguineus, whose number of sibling species around the world has yet to be ascertained. This fact is of great medical and veterinary concern, also considering that ticks currently identified as R. sanguineus have been regarded as proven or putative vectors of several pathogens infecting dogs and humans. Differences in the distribution and prevalence of some of these microorganisms (e.g., Ehrlichia canis and Hepatozoon canis) further support the existence of distinct species under the name R. sanguineus and suggest that the vector competence of these tick species may vary. This article provides an account on the taxonomy and the vector role of ticks belonging to the R. sanguineus group in the light of recent research.

Exposure to Anaplasma phagocytophilum in two dogs in Belgium

Khatat, SE; Defauw, P; Marynissen, S; Van de Maele, I; van Dongen, A; Daminet, S

VLAAMS DIERGENEESKUNDIG TIJDSCHRIFT, 84 (1):39-46
ANNUAL PARASITOLOGY HORIZON SCANNING REPORT 2014

In this report, two dogs are described, which were exposed to *Anaplasma phagocytophilum* in Belgium. The first case was presented for acute weakness and collapse, and was diagnosed with immune mediated haemolytic anaemia. Vector-borne serology panel revealed a positive antibody titre for *A. phagocytophilum*, and the dog recovered during doxycycline therapy. The second patient suffered from an immune mediated glomerulopathy, and concurrently had a highly increased antibody titre for *A. phagocytophilum*. The relationship between canine granulocytic anaplasmosis and both IMHA and protein-losing nephropathy is unclear in these cases. However, it is suspected that *A. phagocytophilum* could be associated with kidney injury, as it is described in the second case.

**Genome mining offers a new starting point for parasitology research**

Lv, ZY; Wu, ZD; Zhang, LM; Ji, PY; Cai, YF; Luo, SQ; Wang, HX; Li, H
PARASITOLOGY RESEARCH, 114 (2):399-409

Parasites including helminths, protozoa, and arthropod vectors are a major cause of global infectious diseases, affecting one-sixth of the world’s population, which are responsible for enormous levels of morbidity and mortality remain impediments to economic development especially in tropical countries. Prevalent drug resistance, lack of highly effective and practical vaccines, as well as specific and sensitive diagnostic markers are proving to be challenging problems in parasitic disease control in most parts of the world. The impressive progress recently made in genome-wide analysis of parasites of medical importance, including trematode species *Clonorchis sinensis*, *Opisthorchis viverrini*, *Schistosoma haematobium*, *S. japonicum*, and *S. mansoni*; nematodes *Brugia malayi*, *Loa loa*, *Necator americanus*, *Trichinella spiralis*, and *Trichuris suis*; cestodes *Echinococcus granulosus*, *E. multilocularis*, and *Taenia solium*; protozoa *Babesia bovis*, *B. microti*, *Cryptosporidium hominis*, *Eimeria falciformis*, *E. histolytica*, *Giardia intestinalis*, *Leishmania braziliensis*, *L. donovani*, *L. major*, *Plasmodium falciparum*, *P. vivax*, *Trichomonas vaginalis*, *Trypanosoma brucei* and *T. cruzi*; and mosquito vectors *Aedes aegypti*, *Anopheles darlingi*, *A. sinensis*, and *Culex quinquefasciatus*, have been systematically covered in this review for a comprehensive understanding of the genetic information contained in nuclear, mitochondrial, kinetoplast, plastid, or endosymbiotic bacterial genomes of parasites, further valuable insight into parasite-host interactions and development of promising novel drug and vaccine candidates and preferable diagnostic tools, thereby underpinning the prevention and control of parasitic diseases.

**Identification of binding domains on red blood cell glycophrins for Babesia divergens**

Cursino-Santos, JR; Halverson, G; Rodriguez, M; Narla, M; Lobo, CA
TRANSFUSION, 54 (4):982-989

Invasion of red blood cells (RBCs) is one of the critical points in the life cycle of *Babesia*. The parasite does not invade other host cells. Earlier work has shown that GPA and GPB function as putative receptors during parasite invasion. The primary focus of this study was the delineation of parasite-binding domains on GPA and GPB. The assay of choice to validate molecules that participate in invasion is an inhibition of invasion assay, in which changes in parasitaemia are assessed relative to a wild-type assay (no inhibitors). Inhibition of invasion can be achieved by modification of different components of the assay or by the addition of competitors of the molecules that participate in invasion. In this study purified antibody fragments to various domains on GPA and GPB were tested for magnitude of inhibition of different components of the assay or by the addition of competitors of the molecules that participate in invasion. Effects on invasion were monitored by assessment of Giemsa-stained smears every 24 hours. Among 10 selected antibodies directed at various epitopes on GPA and GPB, antibodies directed against GPA(M) epitopes had the most severe effect (up to 35%) on inhibition of invasion, followed by antibodies directed against GPB(S) epitope (up to 24%). This study confirms the role of RBC glycophrins A and B in *B. divergens* invasion and shows that the GPA(M) and GPB(S) epitopes are likely to play an important role in the entry process.

**Brown dog tick infestation of a home in England**

Hansford KM, Pietzsch M, Cull B and Medlock JM
VETERINARY RECORD,129 Letter

Public Health England (PHE) recently investigated a tick infestation in a family home in Essex during September 2014. Specimens removed from two pet dogs and within the property were identified as the brown dog tick, *Rhipicephalus sanguineus* (15 males, 14 females and one nymph), a non-native species. House infestations are rarely documented in the UK (Best and others 1969, Fox and Sykes 1985) but may become more common with the increased importation of *R. sanguineus* via travelling and imported pets. Ticks were noticed on two dogs within the property and approximately 100 ticks were also seen crawling on the walls, furniture and soft furnishings. The following day, the dogs were treated with

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 ticks were also seen crawling on the walls, furniture and soft furnishings. The following day, the dogs were treated with.
Bovine besnoitiosis is caused by the largely unexplored apicomplexan parasite Besnoitia besnoiti. In cows, infection during pregnancy often results in abortion, and chronically infected bulls become infertile. Similar to other apicomplexans *B. besnoiti* has acquired a largely intracellular lifestyle, but its complete life cycle is still unknown, modes of transmission have not been entirely resolved and the definitive host has not been identified.

Outbreaks of bovine besnoitiosis in cattle were described in the 1990s in Portugal and Spain, and later several cases were also detected in France. More cases have been reported recently in hitherto unaffected countries, including Italy, Germany, Switzerland, Hungary and Croatia. To date, there is still no effective pharmaceutical compound available for the treatment of besnoitiosis in cattle, and progress in the identification of novel targets...
for intervention through pharmacological or immunological means is hampered by the lack of molecular data on the genomic and transcriptomic level. In addition, the lack of an appropriate small animal laboratory model, and wide gaps in our knowledge on the host-parasite interplay during the life cycle of this parasite, renders vaccine and drug development a cost- and labour-intensive undertaking.

Gazzonis, A. L., et al. (2014). “Besnoitia besnoiti among cattle in insular and northwestern Italy: endemic infection or isolated outbreaks?” Parasites and Vectors 7(585): (10 December 2014)-(2010 December 2014). Bovine besnoitiosis, caused by the apicomplexan Besnoitia besnoiti, is a chronic and debilitating disease considered as emerging in Europe. In Spain, Portugal and France it is endemic and foci of infection were recorded in Germany, Switzerland, Hungary, Greece and Italy. In Italy, cases of bovine besnoitiosis were registered both in imported and autochthonous cattle, and mostly in central regions; high seroprevalence was also revealed by an epidemiological survey performed in the southern part of the country. Aiming to update information on the disease in northwestern and insular areas of Italy, where data on bovine besnoitiosis were missing, a serosurvey was designed for the present study. Three thousand one hundred and forty bovine blood samples from both dairy and beef farms (n=126) were collected in northwestern regions (Lombardy, Piedmont and Liguria) and in the island of Sardinia. Samples were analyzed by a standardized in-house ELISA and those resulted positive were re-tested by Western Blot (WB) for confirmation. On results obtained by both ELISA and WB, apparent (AP) and true prevalence (TP) were calculated at individual and herd levels. Further, a panel of sera resulted positive to ELISA was analyzed by IFAT. Results: A total of 712 animals (AP=22.7%; TP=18.8%) of 109 farms (AP=86.5%; TP=88.2%) showed a positive reaction in ELISA. Only ten (AP=0.3%; TP=0%) specimens proceeding from five farms (AP=3.9%; TP=1.7%) from Lombardy were confirmed positive to the WB, corresponding to two Holstein Friesian cows and eight beef cattle. IFAT showed a low sensitivity (44.4%) scoring positive in only four samples out of 9 positive to WB. Conclusions: The survey demonstrated that bovine besnoitiosis cannot still be considered endemic in the whole of Italy. In fact, independent foci of infection were registered only in Lombardy region. Therefore, a sanitary strategy aimed to increase control measures and to organize monitoring plans, by adequate diagnostic tools is necessary to avoid overestimation of Besnoitia in Italy.

Hornok, S., et al. (2014). “Bovine besnoitiosis emerging in Central-Eastern Europe, Hungary.” Parasites and Vectors 7(20): (13 January 2014)-(2013 January 2014). Besnoitia besnoiti, the cause of bovine besnoitiosis, is a cyst-forming coccidian parasite that has recently been shown to be spreading in several Western and Southern European countries. Clinical cases of bovine besnoitiosis were confirmed for the first time in Hungary, by histological, serological and PCR analyses. Conclusions: This is the first report of autochthonous bovine besnoitiosis in Central-Eastern Europe. The emergence of bovine besnoitiosis in this region represents a further example, when human activity (i.e. cattle trading) is the main factor involved in the geographical spread of an infectious disease.

Lenfant, F., et al. (2014). “Development of an indirect immunofluorescence antibody test (IFAT) for the diagnosis of cattle besnoitiosis.” Revue De Medecine Veterinaire 165(11-12): 327-333. Bovine besnoitiosis is an emergent disease in Europe and is responsible for heavy economic losses. Most affected animals are asymptomatic and constitute a reservoir that must be identified as quickly as possible. This work presents the development of an indirect immunofluorescence antibody test (IFAT) at the Laboratory of Parasitology in ENV Toulouse. In this study, we evaluated the performance of the IFAT compared to Western Blot (Gold Standard) and to ELISA, through the analysis of 403 sera and using four dilutions (1:100, 1:200, 1:400, 1:800). Considering the positive cut-off at 1:200 serum dilution, the IFAT showed an almost perfect test agreement with the Western Blot; (kappa = 0.940) showing a relative sensitivity of 91.8% and a relative specificity of 100%. The test appears as a very useful tool to confirm the serological status of samples that exhibited a discrepancy between findings of commercial ELISA kits and WB.

CESTODES

Taenia spp

Dupuy, C., et al. (2014). “Prevalence of Taenia saginata cysticercosis in French cattle in 2010.” Veterinary Parasitology 203(1-2): 65-72. Bovine cysticercosis is a foodborne disease caused by the cestode Taenia saginata with cattle as the intermediate host and humans as the final host. This disease is responsible for direct financial losses for farmers.
It is also economically important because human infestation through raw or undercooked meat consumption can have a negative impact on the confidence the consumer has in the food industry. This study aimed to determine the apparent and true prevalence of bovine cysticercosis in France and describe the locations of identified cysticercosis lesions. The study sample included 4,564,065 cattle slaughtered in 2010 in France, among which 6491 were detected as harbouring cysticercosis lesions using the current EU meat inspection process. The overall apparent prevalence (including both viable and degenerated cysticerci) was estimated at 0.142% [0.142-0.143]. The true overall prevalence defined as the estimation of the prevalence after taking into account the sensitivity of meat inspection (detection fraction) was 1.23% [0.83-1.93]. The true prevalence of cattle with at least one viable cysticercus was 0.113% [0.076-0.189]. Taking into account both our results and those of a previous study on the prevalence of human cysticercosis in France, we estimated that one carcass could infest an average of 8-20 individuals. The spatial distribution of viable cysticerci showed that the highest apparent prevalence was found in eastern France. This study, the largest survey ever conducted on bovine cysticercosis in France, indicated a low but spatially heterogeneous prevalence of the parasite among the cattle population. Considering French eating habits, according to which it is not uncommon to consume undercooked meat, the possibility of humans being infested even though viable cysticerci are not detected during meat inspection is high. Increasing the detection sensitivity of meat inspection through the use of a risk-based meat inspection procedure should improve prevention of human infestation.


Cross sectional study using random sampling to determine the prevalence of Cysticercus bovis from May to June 2014 on a total of 439 cattle slaughtered at Shire municipal abattoir (Ethiopia) was conducted. The overall prevalence of the parasite was 5.2%. The distribution of the parasite in adult cattle was (3.8%) where as in old cattle it was 1.8%. The rate of the parasite in old and adult cattle didn't vary significantly (p>0.05). The rate of the parasite in male and female was 4.6% and 0.79% respectively where there is no sex wise significant variation (p>0.05). At the same time, there is significance variation of the parasites in different organs with highest rate of the parasite in liver (4.3%) (p<0.05).


Cysticercosis by Taenia solium metacestode affects pigs, giving ground for meat confiscation. Composting is an alternative disposition method for confiscated carcasses and other animal debris, inactivating and destroying pathogens in the carcasses. In this study, composting was evaluated as a method to inactivate T solium metacestodes. Seven compost cone-shaped piles were built, and three depth-zones were defined within them. Each zone was divided into 4 subzones, and a portion of contaminated meat was introduced into each subzone. Meat was sampled at 24, 36, 48, and 72 h and tested for evagination in vitro. The maximum required time for cysticercus inactivation was 48 h. Meat was incorporated to compost after 7 days. No significant differences were found in cysticercus inactivation among the compost zones (P > 0.05), but significant differences were found with respect to the outside. Therefore, all zones were regarded equally effective to inactivate viable T solium cysticerci.


Cysticercus tenuicollis, the metacestode stage of Taenia hydatigena are responsible for a high degree of morbidity and mortality in livestock. This study was performed in order to investigate the variations of blood parameters (hematological and biochemical) and pathological changes in 50 sheep infected with C. tenuicollis in comparison with 50 non-infected control group. The blood samples were taken from the sheep that were slaughtered in the Kerman slaughterhouse. Blood and sera samples were analyzed for hematology and biochemical parameters and infected livers were transported to the pathology laboratory for further examinations. According to the analyses performed on the animals’ blood, a significant increase was detected in number of white blood cells, activities of AST, ALT and levels of total bilirubin in animals with cysticercosis (p<0.05). Also in infected animals, a significant reduction was observed in number of red blood cells, hemoglobin concentration and hematocrit values (p<0.05). In histopathological examination, hepatocellular degeneration and necrosis, fibrosis, mucus gland and biliary hyperplasia, mild lymphocytic hepatitis, granuloma and telangiectasis were observed. It seems that the increased and reduction of significant blood parameters, may be due to liver failure and pathological changes following larval migration and stimulating of immune responses.
Cestode detection


The study was conducted to establish a loop-mediated isothermal amplification (LAMP) assay for the rapid detection of Echinococcus sp. specific DNA isolated from dog faeces. Four primers which recognized six distinct regions of the NADH dehydrogenase subunit 2 (ND2) gene of E. granulosus were designed and used for LAMP assay. The specificity of LAMP assay was evaluated using DNA extracted from E. granulosus, Taenia saginata, and other dog intestinal parasites. The sensitivity of LAMP assay was compared with conventional PCR using recombinant plasmid carrying E. granulosus ND2 gene fragment as standard template DNA after 104 serial dilution. DNA was extracted from 46 canine faecal samples collected and DNA was tested using LAMP and necropsy method. Results showed that E. granulosus ND2 primer sets could differentiate E. granulosus from E. multilocularis without cross reaction to other parasites detected. The LAMP assay with primer sets to the ND2 gene could detect 4*10 2 copies of the target gene, demonstrating 103 times higher sensitivity than conventional PCR methods. The LAMP assay with primer set to ND2 gene showed good sensitivity and specificity to detect DNA samples extracted from faecal samples of 46 dogs. There was no significant difference between LAMP and necropsy. A sensitive, specific and rapid DNA detection LAMP assay was developed successfully for the diagnosis of dogs infected with E. granulosus. The LAMP assay is a promising new tool for rapid detection of dogs infected with Echinococcus spp. due to its rapidity, simplicity, specificity and sensitivity during field survey or in poorly equipped laboratories.


Bovine cysticercosis is detected during the routine post mortem examination of carcasses by visual inspection (knife and eye method). However, the sensitivity of this procedure is several times lower than immunoassays, even when it is performed by qualified professionals. In the present study, a new generation capture antigens were screened from a phage display peptide library using antibodies from Taenia saginata-infected animals. Eight phage clones were selected, and one, Tsag 3 (VHTSIRPCRQRAITPR), produced similar results to the T. saginata metacestode crude antigen (TsCa) when used as a capture antigen in an ELISA. The phage-displayed peptides competed with TsCa for binding sites, reducing the reactivity by approximately 30 %. Alanine scanning indicated that proline, arginine, and serine are important residues for antibody binding. Tsag 1 (HFYQITWLNPFPAR), the most frequent affinity-selected clone, and Tsag 6 (YRWSTPSASRQATL) shared similarity with highly conserved proteins from the Taeniidae family with known immunogenicity. Due to their epitopic or mimotopic properties, these affinity-selected phages could contribute to the rational design of an ante mortem immunodiagnosis method for bovine cysticercosis, as well as an epitope-based vaccine to interrupt the taeniosis/cysticercosis complex.

A novel species-specific anti-beaver-IgG-alkaline-phosphatase conjugate was synthesized for the development of a new serological test for echinococcosis in beavers. Two different ELISAs conventionally used for human Echinococcus multilocularis serology (Em18-ELISA and Em2-ELISA) yielded diagnostic sensitivities of 0% and 46%, respectively. In contrast, the subsequently developed immunoblotting assay gave an 85% diagnostic sensitivity (11 out of 13 beavers with alveolar echinococcosis were immunoblotting-positive, i.e. showed reactivity with a specific 21 Mr band), and maximal specificity. In conclusion, this immunoblotting assay should be the method of choice for use in serological studies on E. multilocularis in Eurasian beavers, and the test proved suitable to investigate both animals alive and post-mortem.


The Enzyme-linked Immunoelectrotransfer Blot (EITB) has been used widely as a screening test for Taenia solium cysticercosis in swine. However, the relation between seropositivity and infection in pig populations from endemic areas has not been well defined. The aim of this study is to relate EITB seropositivity with infection and infection burden, analyse the trade-off between sensitivity and specificity with various cut-off points for the EITB assay, and finally describe the serology changes in a cohort of rural pigs raised under natural conditions. A group of 107 pigs that were used as controls during a vaccination field trial in Peru was our study population. The prevalence of porcine cysticercosis determined by necropsy examination was 16.82% (18/107) in these animals. Using EITB reactivity to >1 band as a cut-off point for the assay, the sensitivity was 88.89% (65.29-98.62, 95% CI) and the specificity was 48.31% (37.59-59.16, 95% CI). Comparing other cut-off points, involving up to as many as 7 reactive bands, a reactivity of >3 bands provided the best trade-offs in sensitivity and specificity. Using this cut-off point for the assay, the sensitivity was 77.77% (52.36-93.59, 95% CI) and the specificity was 76.40% (66.22-84.76, 95% CI). A significant association was found between cyst counts over 100 cysts and reactivity to 23 bands in the EITB assay (Fisher’s exact test, p <0.05). The results of this study suggest that the use of the EITB assay to study porcine cysticercosis may require setting different cut-offs under field and experimental conditions, and depending upon the objective of the screening process.


The dog tapeworm Echinococcus granulosus is the causative agent of cystic hydatid disease in domestic/wild herbivores animals and man. Accurate immunodiagnosis of the infection requires highly specific and sensitive antigens. The aim of this study was to develop and evaluate various immunoassays with principles of precipitation, agglutination and enzyme immunoassays for the identification of sheep infected with hydatid cyst which would allow the monitoring of animals from endemic areas and identifying infected animals prior to slaughter. The immunoassays were developed and validated using hydatid specific, non-cross reactive low molecular weight 8 kDa hydatid cyst fluid protein. Sera used for the assay validations were obtained from 150 sheep infected naturally with hydatid cyst and 150 noninfected sheep. The highest diagnostic sensitivity was obtained in enzyme linked immune electro transfer blot (EITB) at 99.33 per cent followed by latex agglutination test (98.67 per cent) and counter immunoelectrophoresis (94.67 per cent). The study demonstrated that EITB was most sensitive immunological test for the detection of cystic echinococcosis in sheep. It should be useful for the conformation of hydatid cyst infected individual sheep. However, CIEP and LAT methods can be applied to practical use for screening studies.


Echinococcus multilocularis is one of the most relevant zoonotic parasites with about 18,000 human cases per year. Its detection in wild host is crucial for disease prevention. The present study aimed to determine factors affecting the sensitivity of E. multilocularis detection by PCR using DNA extracted from fecal samples of coyotes (Canis latrans). Fecal samples were screened for the presence of Taeniidae eggs through centrifugation and sedimentation. DNA was extracted from fecal samples with and without prior freeze-thawing of the sample and then subjected to PCR targeting a mitochondrial gene (nad1) and a multi-loci microsatellite marker (Ems8). The presence of PCR inhibitors was determined through internal amplification control. Subjecting the sample to repeated freeze-thaw cycles significantly increased the sensitivity of the PCR by 20%. Likewise, egg intensity had a significant effect on PCR, an effect which was more pronounced for samples not subjected to freeze-thawing. Two or more eggs per gram of feces significantly increased the odds for a positive PCR outcome. The presence of PCR inhibitors had no effect on PCR in samples subjected to freeze-thaw cycles, whereas in samples not
subjected to freeze-thaw cycles, the presence of PCR inhibitors was associated with a 0.78 lower odds ratio of positive PCR outcome. Targeting a nuclear versus a mitochondrial gene did not have a significant effect on the sensitivity of PCR. We recommend freeze-thawing samples prior to DNA extraction to become a standard procedure for E. multilocularis detection in canid feces.


Background: Cystic echinococcosis is highly prevalent in northwest China. A cost-effective, easy to operate diagnostic tool with high sensitivity and specificity would greatly facilitate the monitoring of Echinococcus infections in canine definitive hosts. Methods: The primers used in the LAMP assay were based on the mitochondrial nad5 gene of E. granulosus sensu stricto (E. granulosus s.s., or E.g.s.s.) and were designed using Primer Explorer V4 software. The developed LAMP assay was compared with a conventional PCR method, copro-ELISA and microscopy, using the faeces of dogs experimentally infected with E.g.s.s., and field-collected faeces of domestic dogs including 190 from Qinghai province highly endemic for E.g.s.s. and 30 controls from an area in Gansu, where a domestic dog de-worming program was in operation. Results: The positivity rates obtained for the field-collected faecal samples were 12.6%, 1.6% and 2.1% by the LAMP, PCR and copro-ELISA assays, respectively. All samples obtained from the control dogs were negative. Compared with the conventional PCR, the LAMP assay provided 88.8% specificity and 100% sensitivity. The higher sensitivity of the LAMP method was also shown by the fact that it could detect the presence of laboratory challenge dog infections of E. granulosus s.s. four days earlier than the PCR method. Three copro-samples shown positive by the commercial copro-ELISA were all negative by LAMP, PCR and microscopy, which suggests these samples may have originated from another infection rather than E. granulosus s.s., possibly E. shiquicus or E. canadensis, which is also present in China. Conclusions: We have developed a potentially useful surveillance tool for determining the prevalence of canine E. granulosus s.s. infections in the field. The LAMP assay may lead to a more cost-effective and practicable way of tracking Echinococcus infections in canids, especially when combined with the copro-ELISA.


Background: Alveolar echinococcosis, caused by the metacestode larval stage of Echinococcus multilocularis, is a zoonosis of public health significance and is highly prevalent in northwest China. To effectively monitor its transmission, we developed a new rapid and cheap diagnostic assay, based on loop-mediated isothermal amplification (LAMP), to identify canine definitive hosts infected with E. multilocularis. Methods: The primers used in the LAMP assay were based on the mitochondrial nad5 gene of E. multilocularis and were designed using Primer Explorer V4 software. The developed LAMP assay was compared with a conventional PCR assay, using DNA extracted from the feces of dogs experimentally infected with E. multilocularis, on 189 dog fecal samples collected from three E. multilocularis-endemic regions in Qinghai province, the People's Republic of China, and 30 negative control copro-samples from dogs from an area in Gansu province that had been subjected to an intensive de-worming program. Light microscopy was also used to examine the experimentally obtained and field collected dog copro-samples for the presence of E. multilocularis eggs. Results: The E. multilocularis-positivity rates obtained for the field-collected fecal samples were 16.4% and 5.3% by the LAMP and PCR assays, respectively, and all samples obtained from the control dogs were negative. The LAMP assay was able to detect E. multilocularis DNA in the feces of experimentally infected dogs at 12 days post-infection, whereas the PCR assay was positive on the 17th day and eggs were first detectable by light microscopy at day 44 post-challenge. Conclusion: The earlier specific detection of an E. multilocularis infection in dog copro-samples indicates that the LAMP assay we developed is a realistic alternative method for the field surveillance of canines in echinococcosis-endemic areas.


Background: In endemic areas with very low infection prevalence, the frequency and intensity of Echinococcus multilocularis can be extremely low. This necessitates efficient, specific and sensitive molecular tools. We wanted to compare the existing molecular tools, used in the Norwegian national surveillance programme, and compare these with new techniques for detection of this zoonotic pathogen in fox faeces. Here we present the results of screening samples containing a known level of E. multilocularis eggs with two highly sensitive DNA isolation and extraction methods combined with one conventional PCR and three real-time PCR methods for detection.

The oncosphere stage of Echinococcus multilocularis in red fox stools can lead, after ingestion, to the development of alveolar echinococcosis in the intermediate hosts, commonly small mammals and occasionally humans. Monitoring animal infection and environmental contamination is a key issue in public health surveillance. We developed a quantitative real-time PCR technique (qPCR) to detect and quantify E. multilocularis DNA released in fox faeces. A qPCR technique using a hydrolysis probe targeting part of the mitochondrial gene rrnL was assessed on (i) a reference collection of stools from 57 necropsied foxes simultaneously investigated using the segmental sedimentation and counting technique (SSCT) (29 positive for E. multilocularis worms and 28 negative animals for the parasite); (ii) a collection of 114 fox stools sampled in the field: two sets of 50 samples from contrasted endemic regions in France and 14 from an E. multilocularis-free area (Greenland). Of the negative SSCT controls, 26/28 were qPCR-negative and two were weakly positive. Of the positive SSCT foxes, 25/29 samples were found to be positive by qPCR. Of the field samples, qPCR was positive in 21/50(42%) and 5/48(10.4%) stools (2 samples inhibited), originating respectively from high and low endemic areas. In faeces, averages of 0.1 pg/µL of DNA in the Jura area and 0.7 pg/µl in the Saone-et-Loire area were detected. All qPCR-positive samples were confirmed by sequencing. The qPCR technique developed here allowed us to quantify environmental E. multilocularis contamination by fox faeces by studying the infectious agent directly. No previous study had performed this test in a one-step reaction.

Control of cestodes


Canine tapeworms that have sheep as their intermediate host can be responsible for unpredictable significant economic losses for individual farms. In addition, one of these dog-sheep tapeworms, Echinococcus granulosus, is zoonotic, causing cystic echinococcosis in humans, which can be fatal. Given that detection of tapeworm infestation is often only achieved at postmortem abattoir inspection, reactive control measures are limited. The aim of this article is to illustrate how the principles of the Hazard Analysis and Critical Control Points (HACCP) system, which is widely used in the food industry, can be used to identify proactive control measures. The role of the small animal veterinary surgeon in control is also discussed.


Deworming wild foxes by baiting with the anthelmintic praziquantel is being established as a preventive technique against environmental contamination with Echinococcus multilocularis eggs. Improvement of the cost-benefit performance of baiting treatment is required urgently to raise and maintain the efficacy of deworming. We established a spatial model of den site selection by urban red foxes, the definitive host, to specify the optimal micro-habitats for delivering baits in a new modeling approach modified for urban fox populations. The model was established for two cities (Obihiro and Sapporo) in Hokkaido, Japan, in which a sylvatic cycle of E. multilocularis is maintained. The two cities have different degrees of urbanization. The modeling process was designed to detect the best combination of key environmental factors and spatial scale that foxes pay attention to most (here named ‘heeding range’) when they select den sites. All possible models were generated using logistic regression analysis, with “presence” or “absence” of fox den as the objective variable, and nine landscape categories customized for urban environments as predictor variables to detect the best subset of predictors. This procedure was conducted for each of ten sizes of concentric circles from dens and control points to detect the best circle size. Out of all models generated, the most parsimonious model was selected using Akaike's Information Criterion (AIC) inspection. Results: Our models suggest that fox dens in Obihiro are located at the center of a circle with 500 m radius including low percentages of wide roads, narrow roads, and occupied
buildings, but high percentages of green covered areas; the dens in Sapporo within 300 m radius with low percentages of wide roads, occupied buildings, but high percentages of riverbeds and green covered areas. The variation of the models suggests the necessity of accumulating models for various types of cities in order to reveal the patterns of the model. Conclusions: Our denning models indicating suitable sites for delivering baits will improve the cost-benefit performance of the campaign. Our modeling protocol is suitable for the urban landscapes, and for extracting the heeding range when they select the den sites.


Characterizing the force of infection (FOI) is an essential part of planning cost effective control strategies for zoonotic diseases. *Echinococcus multilocularis* is the causative agent of alveolar echinococcosis in humans, a serious disease with a high fatality rate and an increasing global spread. Red foxes are high prevalence hosts of *E. multilocularis*. Through a mathematical modelling approach, using field data collected from in and around the city of Zurich, Switzerland, we find compelling evidence that the FOI is periodic with highly variable amplitude, and, while this amplitude is similar across habitat types, the mean FOI differs markedly between urban and periurban habitats suggesting a considerable risk differential. The FOI, during an annual cycle, ranges from (0.1,0.8) insults (95% CI) in urban habitat in the summer to (9.4, 9.7) (95% CI) in periurban (rural) habitat in winter. Such large temporal and spatial variations in FOI suggest that control strategies are optimal when tailored to local FOI dynamics.

**Cestode infections in humans**


Due to increased globalization, food-borne parasitic infections are becoming more prevalent worldwide, including in countries where these parasites and parasitic diseases had previously been well controlled or eradicated. Improved sanitation, health education, and establishment of appropriate food safety mechanisms can go a long way towards the control of many these infections. However, food-borne parasitic infections are still common diseases in developing countries, especially in rural areas. As many of today's travellers are looking to explore more distant locations and partake in the local cuisine, they may be at greater risk of acquiring a food-borne parasitic infection, including those caused by a number of adult and larval tapeworms. This review discusses fish and meat-borne tapeworms and zoonotic metacestodiases of public health importance to both developing and developed countries, with a focus on infection prevention in travellers.


Cystic hydatid disease is still an important health problem in European Mediterranean areas. In spite of being traditionally considered as a “benign” pathology, cystic echinococcosis is an important cause of morbidity in these areas. Nevertheless, there are few analyses of mortality attributed to human hydatidosis. Objective: To describe the epidemiology, the mortality rate and the causes of mortality due to *E. granulosus* infection in an endemic area. A retrospective study followed up over a period of 14 years (1998-2011). Of the 567 patients diagnosed with hydatid disease over the period 1998-2011, eleven deaths directly related to hydatid disease complications were recorded. Ten patients (90.9%) died due to infectious complications and the remaining one (9.1%) died due to mechanical complications after a massive hemoptysis. We registered a case fatality rate of 1.94% and a mortality rate of 3.1 per 100,000 inhabitants. Hydatidosis is still a frequent parasitic disease that causes a considerable mortality. The main complications in patients with hydatidosis are complications related to the rupture of CE cysts with suppurative collangitis.


Genetic variability in the species group *Echinococcus granulosus sensu lato* is well recognised as affecting intermediate host susceptibility and other biological features of the parasites. Molecular methods have allowed discrimination of different genotypes (G1-10 and the 'lion strain'), some of which are now considered separate species. An accumulation of genotypic analyses undertaken on parasite isolates from human cases of cystic echinococcosis provides the basis upon which an assessment is made here of the relative contribution of the different genotypes to human disease. The allocation of samples to G-numbers becomes increasingly difficult, because much more variability than previously recognised exists in the genotypic clusters G1-3 (=*E. granulosus sensu stricto*) and G6-10 (*Echinococcus canadensis*). To accommodate the heterogeneous criteria used for genotyping in the literature, we restrict ourselves to differentiate between *E. granulosus sensu stricto* (G1-3),
Echinococcus equinus (G4), Echinococcus ortleppi (G5) and E. canadensis (G6-7, G8, G10). The genotype G1 is responsible for the great majority of human cystic echinococcosis worldwide (88.44%), has the most cosmopolitan distribution and is often associated with transmission via sheep as intermediate hosts. The closely related genotypes G6 and G7 cause a significant number of human infections (11.07%). The genotype G6 was found to be responsible for 7.34% of infections worldwide. This strain is known from Africa and Asia, where it is transmitted mainly by camels (and goats), and South America, where it appears to be mainly transmitted by goats. The G7 genotype has been responsible for 3.73% of human cases of cystic echinococcosis in eastern European countries, where the parasite is transmitted by pigs. Some of the samples (11) could not be identified with a single specific genotype belonging to E. canadensis (G6/10). Rare cases of human cystic echinococcosis have been identified as having been caused by the G5, G8 and G10 genotypes. No cases of human infection with G4 have been described. Biological differences between the species and genotypes have potential to affect the transmission dynamics of the parasite, requiring modification of methods used in disease control initiatives. Recent investigations have revealed that the protective vaccine antigen (EG95), developed for the G1 genotype, is immunologically different in the G6 genotype. Further research will be required to determine whether the current EG95 vaccine would be effective against the G6 or G7 genotypes, or whether it will be necessary, and possible, to develop genotype-specific vaccines.


Cystic echinococcosis (CE) is a cosmopolitan disease caused by the dog tapeworm *Echinococcus granulosus*. The disease is difficult to diagnose, treat, and control and is responsible for considerable human morbidity and mortality globally. There is an urgent need for new diagnostic tests and new drugs for treatment of CE and the development of a vaccine against adult worms of *E. granulosus* in dogs. We recently presented a draft genomic sequence for the worm comprising 151.6 Mb encoding 11,325 proteins. We undertook an extensive comparative analysis of the *E. granulosus* transcriptome using representative life stages (protoscoleces, cyst germinal cells and membranes, adult worms, and oncospheres) to explore different aspects of tapeworm biology and parasitism. The genome and transcriptome of *E. granulosus* provide a unique platform for post-genomic research and to facilitate the development of new, effective treatments and interventions for echinococcosis control.


*Taenia saginata*, *T. solium*, and *T. asiatica* are causative agents of taeniasis in humans. The difficulty of morphological identification of human taeniids can lead to misdiagnosis or confusion. To overcome this problem, several molecular methods have been developed, but use of these tends to be time-consuming. Here, a rapid and high-throughput pyrosequencing approach was developed for the identification of three human taeniids originating from various countries. Primers targeting the mitochondrial cytochrome c oxidase subunit 1 (cox1) gene of the three *Taenia* species were designed. Variations in a 26-nucleotide target region were used for identification. The reproducibility and accuracy of the pyrosequencing technology was confirmed by Sanger sequencing. This technique will be a valuable tool to distinguish between sympatric human taeniids that occur in Thailand, Asia and Pacific countries. This method could potentially be used for the molecular identification of the taeniid species that might be associated with suspicious cysts and lesions, or cyst residues in humans or livestock at the slaughterhouse.

**OTHER ZOONOTIC PARASITES**


'Orphan' zoonotic diseases attract disproportionately low scientific and public health attention for the impact that they can have. This article pulls together information on their health burden in the UK from routine and enhanced data sources. These diseases are heterogeneous in nature; some have very low case numbers (e.g. hydatid disease), whilst others affect hundreds of patients each year (e.g. toxoplasmosis). The number of deaths attributed to orphan zoonoses is relatively low, and the majority recorded in this article were caused by toxoplasmosis. There is a clear issue of under-reporting and under-diagnosis in the data sets presented, and further work should be carried out to obtain more accurate estimates of the prevalence of zoonotic infections. Joint human and veterinary studies are especially important for these diseases.
This paper reports on the discussions conducted during the 2012 expert meeting on multi-criteria based ranking for food-borne parasites risk management, involving 24 parasites. The meeting experts defined global criteria for evaluating the parasites and rated each parasite along these criteria which were weighted in terms of their importance. The criteria can be summarized as: (1) number of global illnesses; (2) global distribution; (3) acute morbidity; (4) chronic morbidity; (5) percentage of chronic infections; (6) mortality; (7) increasing illness potential; (8) trade relevance; and, (9) socioeconomic impact. The primary outputs of the expert meeting were the development of the ranking tool and the actual global ranking of food-borne parasites, based on public health concerns, and the identification of foods of greatest concern for the most important parasites. In addition, the paper presents risk management options and considerations for the control of the higher ranked parasites.


The single-dose benzimidazoles used against Trichuris trichiura infections in humans are not satisfactory. Likewise, the benzimidazole, fenbendazole, has varied efficacy against Trichuris suis whereas Oesophagostomum dentatum is highly sensitive to the drug. The reasons for low treatment efficacy of Trichuris spp. infections are not known. We studied the effect of fenbendazole, albendazole and levamisole on the motility of T. suis and O. dentatum and measured concentrations of the parent drug compounds and metabolites of the benzimidazoles within worms in vitro. The motility of T. suis was generally less decreased than the motility of O. dentatum when incubated in benzimidazoles, but was more decreased when incubated in levamisole. The total drug concentrations (pmol/mg dry worm tissue) were significantly lower within T. suis than O. dentatum whether killed or alive when incubated in all tested drugs (except in living worms exposed to fenbendazole). Relatively high proportions of the anthelmintic inactive metabolite fenbendazole sulphone was measured within T. suis as compared to O. dentatum. The general lower sensitivity of T. suis towards BZs in vitro seems to be related to a lower drug uptake. Furthermore, the relatively high occurrence of fenbendazole sulphone suggests a higher detoxifying capacity of T. suis as compared to O. dentatum.


Helminth infections are responsible for a considerable public health burden, yet the current drug armamentarium is small. Given the high cost of drug discovery and development, the high failure rates and the long duration to develop novel treatments, drug repurposing circumvents these obstacles by finding new uses for compounds other than those they were initially intended to treat. In the present review, we summarize in vivo and clinical trial findings testing clinical candidates and marketed drugs against schistosomes, food-borne trematodes, soil-transmitted helminths, Strongyloides stercoralis, the major human filariases lymphatic filariasis and onchocerciasis, taeniasis, neurocysticercosis and echinococcosis. While expanding the applications of broad-spectrum or veterinary anthelmintics continues to fuel alternative treatment options, antimalarials, antibiotics, antiprotozoals and anticancer agents appear to be producing fruitful results as well. The trematodes and nematodes continue to be most investigated, while cestodal drug discovery will need to be accelerated. The most clinically advanced drug candidates include the artemisinins and mefloquine against schistosomiasis, tribendimidine against liver flukes, oxantel pamoate against trichuriasis, and doxycycline against filariasis. Preclinical studies indicate a handful of promising future candidates, and are beginning to elucidate the broad-spectrum activity of some currently used anthelmintics. Challenges and opportunities are further discussed.
Invasive wildlife species have the potential to act as additional host and vector species for infectious diseases. The raccoon dog (Nyctereutes procyonoides), a carnivore species that has its origin in Asia, was taken as an example to demonstrate biological and ecological prerequisites which enables an invasive species to occupy a new habitat permanently. Studies conducted during the last 20 years identified a total of 35 species of endoparasites, five ectoparasites, six bacterial or protozoan species, and five viruses found in the subspecies *Nyctereutes procyonoides ussuriensis* in its original and newly occupied habitat or in *Nyctereutes procyonoides koreensis* in its original habitat, respectively. With reference to raccoon dogs’ impact as vector species and the relevance for human and animal health, we selected *Trichinella* spp., *Echinococcus multilocularis*, *Francisella tularensis*, rabies virus, and canine distemper virus for detailed description. Results of studies from Finland and Germany furthermore showed that biological characteristics of the raccoon dog make this carnivore an ideal host and vector for a variety of pathogens. This may result in a growing importance of this invasive species concerning the epidemiology of some transmissible diseases in Europe, including the hazard that the existence of autochthonous wildlife, particularly small populations, is endangered. Potential adverse effects on human and animal health in the livestock sector must also be taken into account. Especially with regard to its potential as a reservoir for zoonotic diseases, the raccoon dog should receive more attention in disease prevention and eradication strategies.

Foodborne diseases (FBDs) are a major cause of morbidity and mortality in the human population. Accurate information on the burden of FBDs is needed to inform policy makers and allocate appropriate resources for food safety control and intervention. Consequently, in 2006 the WHO launched an initiative to estimate the global burden of FBDs in terms of Disability Adjusted Life Years (DALYs). This review gives an update of the progress on evaluating the burden of foodborne parasitic diseases that has been generated by this study. Results to date indicate that parasitic diseases that can be transmitted through food make a substantial contribution to the global burden of disease.

Pneumonia is the leading killer of children and disproportionately affects developing countries. Vaccination campaigns against Streptococcus pneumoniae, the leading cause of pneumonia, have recently been launched with a new conjugate vaccine in Africa. Using a mouse model, we assessed the potential role that the high burden of helminth infections in the countries targeted for vaccine might have on vaccine effectiveness. Mice vaccinated with either commercial conjugate or purified polysaccharide vaccines had impaired antibody responses if they were chronically infected with *Taenia crassiceps*. This translated to increased susceptibility to pneumococcal pneumonia and high mortality compared to helminth-negative vaccinated animals, which were fully protected from disease and death. Antibodies taken from *Taenia*-infected, vaccinated mice were unable to effectively opsonize *S. pneumoniae* for killing by alveolar macrophages, and did not protect against pneumococcal challenge when adoptively transferred into naive animals. These data may have implications for vaccination programs in countries endemic with helminths.