APHA Parasitology Group
Annual Review of Literature and Horizon Scanning Report 2016
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This is a summary of the literature, produced from peer and non-peer reviewed papers in parasitology published in 2016 of relevance to Defra, APHA and the wider industry.

**Highlights**

**Gastro-intestinal nematodes of grazing ruminants - small ruminants**

- Effects of regular, every 4 week anthelmintic treatment of lambs in the UK situation reduced nematode species diversity so that only *Teladorsagia* sp. produced eggs (Melville et al (2016) Parasites & Vectors 9: 199.)

- Molecular studies on *T. circumcincta* - with aim to inform vaccine development

- Number of published papers on *Haemonchus contortus* this year. This nematode is of major importance in the tropical and subtropical areas of the world and a major limitation to sheep production.
  - Molecular studies have aimed to identify targets for new anthelmintics, (Harder, A. (2016). Haemonchus Contortus and Haemonchosis - Past, Present and Future Trends, 93: 69-94) and future vaccine development

- The association between the gastrointestinal tract microbiota and *H. contortus* resistance in sheep investigated for the first time (Tirabassi, A. H., et al. (2016) Semina-Ciencias Agrarias 37(6): 4097-4107) as well as the effects of *H. contortus* infection on abomasal bacteria.

**Gastro-intestinal nematodes of grazing ruminants - cattle**


- *Cooperia oncophora* is one of the most common intestinal nematodes in cattle. It is also the dose-limiting species for the most frequently used anthelmintics, and consequently, the species usually involved in reports of anthelmintic resistance. A systematic review and meta-analysis provides estimates for key life history traits for future models of the parasitic phase of *C. oncophora* (Verschave, S. H., et al. (2016). Veterinary Parasitology 223: 111-114).
• Average liveweight gain was deemed the best method to select calves for targeted selective treatment (TST) by modelling. All monitoring strategies (P. pepsinogen, FECs and Live WtGain) were better at preserving susceptible parasites and maintaining growth when compared with whole-herd treatment at 3, 8, 13 weeks post-turnout. (Berk, Z., et al. (2016). International Journal for Parasitology-Drugs and Drug Resistance 6(3): 258-271)

GI Parasite Control-Sheep
• Modelling targeted selective treatment in sheep suggests that liveweight gain is the most useful in determining animals to treat, with greatest benefit to preserving susceptible genotypes (Laurenson, Y. C. S. M., et al. (2016). Veterinary Parasitology 226: 174-188).
• Work continues into genetic markers in sheep for resistance to GI nematodes A review was published (Sweeney, T., et al. (2016). Parasite Immunology 38(9): 569-586).
• Work into tannin rich plants and their anthelmintic effects continue but one study identified interactions with anthelmintic pharmacokinetics (reducing ivermectin uptake and efficacy when fed a tannin rich diet). This needs further work. (Gaudin, E., et al. (2016). Veterinary Parasitology 227: 122-129)

Antelmintic resistance – sheep
• Resistance of Haemonchus sp to monepantel and reduced efficacy of a derquantel/abamectin combination confirmed in sheep in NSW, Australia. (Sales, N. and S. Love (2016). Veterinary Parasitology 228: 193-196)
• Use of SCOPS principles on UK sheep farms and the effect on anthelmintic resistance development was published (Learmount, J., et al. (2016) Veterinary Parasitology 229: 9-14.

Gi parasite control – cattle
• Work continues on O. ostertagi vaccine for cattle,
• A review was published on recent insights in the farm-specific economic impact of helminth infections on dairy cattle farms as well as in farmer attitudes and behaviour regarding helminth control. Combining better economic impact assessments of helminth infections together with a deeper understanding of the non-economic factors that drive a farmer's animal health decisions should result in more effective control strategies and increased farmer satisfaction (Charlier, J., et al. (2016). Irish Veterinary Journal 69)
- Study on tannin rich plants (pelleted sainfoin) in calves showed the effect of sainfoin on abomasal nematodes, which corroborates results from studies with small ruminants and encourages further investigations of the use of this crop for control of cattle nematodes. (Desrus, O., et al. (2016). Parasites & Vectors 9) and also chicory against Ostertagia sp. nematodes (Pena-Espinoza, M., et al. (2016). Parasitology 143(10): 1279-1293)

**Antelmintic resistance in cattle**

Confirmation of ivermectin resistance in Ostertagia ostertagi in cattle in New Zealand, after carrying out FECRTs was published. (Waghorn, T. S., et al. (2016). Veterinary Parasitology 229: 139-143)

**Parasite diagnostics**

- Studies looking a limited number of parasites from different hosts suggested that eggs of C. oncophora from cattle should be kept vacuum packed at room temperature for the highest larval yield. (Sengupta, M. E., et al. (2016). Veterinary Parasitology 217: 21-24).
- Studies looking at PCR identification and semi-quantification of O ostertagi have shown that storage of faeces is important as embryonation could alter the result. Hence, vacuum packing is an optimal storage strategy prior to molecular diagnostic analyses. Alternatively, aerobic storage at 4 degrees C for up to 72 h can be used. (Drag, M., et al. (2016). Parasites & Vectors 9).
- Pasture larval counts- a review article brings together some of the field applications and difficulties to run and interpret this technique, encouraging its use as a diagnostic procedure. (Molento, M. B., et al. (2016). Livestock Science 192: 48-54).
- Studies on faecal worm egg counts showed that at storage at 4°C, the rate of reduction of the faecal nematode egg counts starts to be significant lower than the ones performed with fresh samples, for both sheep and goats, after the third week of storage. The percentage of the gastrointestinal nematode larvae developing to the infective third-stage alters significantly for the Haemonchus genus, soon after the first week of storage (Drimtzia, A. and E. Papadopoulos (2016). Journal of the Hellenic Veterinary Medical Society 67(3): 177-182)

**Fasciola hepatica**

- A study into the histological changes observed in Fasciola hepatica: following closantel treatment means that potentially this could be used as one way to assess closantel resistance. (Scarcella, S., et al. (2016). Veterinary Parasitology 215: 38-47)
Cestodes

- Risk factors for *Taenia saginata* cysticercus infection in cattle in the United Kingdom was published Marshall, L. R., et al. (2016). Preventive Veterinary Medicine 135: 1-8

Besnoitia besnoiti


Sheep and goat gastro-intestinal parasites

UK studies


Background: Refugia based anthelmintic protocols aim to reduce the rate of development of anthelmintic resistance in gastrointestinal nematodes (GIN). Previous studies have illustrated the impact of different drenching regimes on drug efficacy and animal growth; however, the impact on nematode populations has yet to be characterised within natural infections. This study investigated the changes in species composition of GIN throughout the grazing season, following implementation of four different ivermectin drenching regimes over six years: neo-suppressive monthly treatment (NST), targeted selective treatment (TST), strategic prophylactic treatment (SPT) and treatment upon observation of clinical signs (MT).

Methods: Lambs were grazed on one of eight replicate paddocks each grazing season following treatment regimes assigned in year 1. Faecal samples were collected fortnightly from all animals and hatched to first stage larvae (L1). DNA was extracted from individual L-1 and a multiplex PCR assay targeting the internal transcribed spacer 2 (ITS2) region of Teladorsagia circumcincta, Trichostrongylus spp. and Haemonchus contortus conducted. Other species were identified using species-specific PCR. Worm-naive tracer lambs were grazed on the paddocks at the start and end of each grazing season and adult worms recovered at post mortem to investigate the parasite population on pasture.

Results: Results showed an overall decrease in species diversity in egg output from the NST group which occurred within a single grazing season and was consistent throughout the experiment. Species diversity was protected over six years in groups implementing TST, SPT and MT treatment regimes, designed to offer refugia. The expected shift in species prevalence throughout the season from Teladorsagia to Trichostrongylus was observed in all but the NST group where only Teladorsagia spp. were recovered from trial lambs by the end of the experiment. Worm burdens indicated the presence of several species at relatively low abundance on pasture in the NST group in 2011. However, these species were not represented in egg output from trial lambs, probably due to the frequent anthelmintic treatment administered throughout the grazing season.

Conclusion: The molecular methods utilised here worked well. The comparable results of the three refugia-based treatment regimes suggest that nematode diversity can be maintained using part or whole group
treatments if a rich supra-population of parasites are available to re-infect animals post treatment.

Sinclair, R., et al. (2016). "Gastrointestinal nematode species diversity in Soay sheep kept in a natural environment without active parasite control." Veterinary Parasitology 227: 1-7. Molecular methods based on ITS2 sequence analysis were used to identify strongyloid parasites and describe their diversity in a management intervention and anthelmintic drug treatment-free sheep flock. Fourteen different nematode parasite species were identified in the flock and the results showed a greater level of nematode species diversity than is normally reported in managed farmed flocks, with the presence of parasites such as Bunostomum trigonocephalum, Ostertagia leptosicularis, Spiculopteragia houdemerii and Trichostrongylus retortaeformis that are considered to be absent or rare in sheep kept in comparable localities. The implied prevalences of Haemonchus contortus in lambs, and of Trichostrongylus axei in lambs, ewes and rams, were higher than those in farmed sheep kept in similar regions, while those of Teladorsagia circumcincta and Trichostrongylus vitrinus were lower. Comparison of the patterns of nematode parasite infection between the summer and autumn sampling periods showed differences from the scenarios that are commonplace in comparable managed flocks; with T. vitrinus burdens of the lambs being higher in the summer than in the winter, and Oesophagostomum venulosum being the predominant nematode species in the adult sheep during the summer, while more-or-less absent from these groups during the winter. Rams played an important role in the epidemiology of certain parasitic nematode species. The relatively non-pathogenic O. venulosum was the only parasitic nematode species to predominate in any group during the study. This preliminary characterisation of the nematode parasite burdens of sheep extensively grazed on diverse unimproved pastures will aid in the understanding of the parasitological consequences of intensive grazing management and of the manner in which modern agriculture upsets the equilibrium between parasites and their hosts. These factors must be accounted for when defining the concept of sustainable parasite control and informing sustainability with reference to commercially efficient sheep farming.

**Haemonchus contortus**


Different life cycle stages of Haemonchus contortus adapt to different ecosystems. This adaptation is accompanied by alterations in gene transcription and expression associated with the energy, amino acid, nitrogen, lipid and/or nucleic acid metabolism of the respective stages. For example, the aerobic metabolism of larvae depends on an efficient citric acid cycle, whereas the anaerobic metabolism of adults requires glycolysis, resulting in the production of volatile fatty acids, such as acetic acid and propionic acid. There are only few anthelmintics targeting nematode energy metabolism. In addition, H. contortus has reduced pathways for amino acid metabolism, polyamine metabolism and nitrogen excretion pathways. Moreover, nucleic acid metabolism comprising purine and pyrimidine salvage pathways as well as lipid metabolism are reduced. In addition, nematodes possess a particular composition of their cuticle. Energy production of adult worms is mainly linked to egg production and complex regulation of the neuromuscular system in both
females and males. In this context, microtubules consisting of alpha- and beta-tubulin heterodimers play a crucial role in the presynaptic vesicle transport. Due to the significant distinction of its quaternary structure in nematodes in comparison to other organisms, beta-tubulin was identified as a major target for benzimidazoles used for anthelmintic treatment. Concerning the function of the neuromuscular system, acetylcholine, a ligand of the nicotinic acetylcholine receptor (nAChR), is the major excitatory neurotransmitter in H. contortus. In contrast, glutamate-gated chloride channels, calcium-and voltage-dependent potassium channels as well as gamma-aminobutyric acid (GABA) A and its receptors act as inhibitory neurotransmitters and thus opponents to nAChR. For example, the calcium-and voltage-dependent potassium channel SLO-1 is an important target of emodepside, which is involved in the sensitive regulation of activatory and inhibitory receptors of the nervous system. Most of the modern anthelmintics target these different neuromuscular receptors. The mechanisms of resistance to anthelmintics, either specific or non-specific, are associated with changes in the molecular targets of the drugs, changes in metabolism of the drug (inactivation, removal or prevention of its activation) and/or increased efflux systems. The biochemical and molecular analyses of key developmental, metabolic and structural process of H. contortus still require substantial efforts. The nAChR, glutamate-gated chloride channel and calcium-and voltage-dependent potassium channel SLO-1 have long been known as being essential for nematode survival. Therefore, future research should be intensified to fully resolve the three-dimensional structures of these receptors, as has already been started for glutamate-gated chloride channel. With this knowledge, it should be possible to design new anthelmintics, which possess improved binding capacities to corresponding receptors.


Recent climate change has resulted in changes to the phenology and distribution of invertebrates worldwide. Where invertebrates are associated with disease, climate variability and changes in climate may also affect the spatio-temporal dynamics of disease. Due to its significant impact on sheep production and welfare, the recent increase in diagnoses of ovine haemonchosis caused by the nematode Haemonchus contortus in some temperate regions is particularly concerning. This study is the first to evaluate the impact of climate change on H. contortus at a continental scale. A model of the basic reproductive quotient of macroparasites, Q(0), adapted to H. contortus and extended to incorporate environmental stochasticity and parasite behaviour, was used to simulate Pan-European spatio-temporal changes in H. contortus infection pressure under scenarios of climate change. Baseline Q(0) simulations, using historic climate observations, reflected the current distribution of H. contortus in Europe. In northern Europe, the distribution of H. contortus is currently limited by temperatures falling below the development threshold during the winter months and within-host arrested development is necessary for population persistence over winter. In southern Europe, H. contortus infection pressure is limited during the summer months by increased temperature and decreased moisture. Compared with this baseline, Q(0) simulations driven by a climate model ensemble predicted an increase in H. contortus infection pressure by the 2080s. In northern Europe, a temporal range expansion was predicted as the mean period of transmission increased by 2-3 months. A bimodal seasonal pattern of infection pressure, similar to that currently observed in southern Europe, emerges in northern Europe due to increasing summer temperatures and
decreasing moisture. The predicted patterns of change could alter the epidemiology of H. contortus in Europe, affect the future sustainability of contemporary control strategies, and potentially drive local adaptation to climate change in parasite populations.


Haemonchus contortus is a highly pathogenic, blood-feeding nematode of small ruminants, and a significant cause of mortalities worldwide. Haemonchosis is a particularly significant threat in tropical, subtropical and warm temperate regions, where warm and moist conditions favour the free-living stages, but periodic outbreaks occur more widely during periods of transient environmental favourability. The clinical diagnosis of haemonchosis is based mostly on the detection of anaemia in association with a characteristic epidemiological picture, and confirmed at postmortem by the finding of large numbers of H. contortus in the abomasum. The detection of impending haemonchosis relies chiefly on periodic monitoring for anaemia, including through the 'FAMACHA' conjunctival-colour index, or through faecal worm egg counts and other laboratory procedures. A range of anthelmintics for use against H. contortus is available, but in most endemic situations anthelmintic resistance significantly limits the available treatment options. Effective preventative programmes vary depending on environments and enterprise types, and according to the scale of the haemonchosis risk and the local epidemiology of infections, but should aim to prevent disease outbreaks while maintaining anthelmintic efficacy.

Appropriate strategies include animal management programmes to avoid excessive H. contortus challenge, genetic and nutritional approaches to enhance resistance and resilience to infection, and the monitoring of H. contortus infection on an individual animal or flock basis. Specific strategies to manage anthelmintic resistance centre on the appropriate use of effective anthelmintics, and refugia-based treatment schedules. Alternative approaches, such as biological control, may also prove useful, and vaccination against H. contortus appears to have significant potential in control programmes.


Infection with the abomasal nematode Haemonchus contortus is responsible for considerable production loss in small ruminants globally, and especially in warm, summer-rainfall regions. Previous attempts to predict infection levels have followed the traditional framework for macroparasite models, i.e. tracking parasite population sizes as a function of host and climatic factors. Targeted treatment strategies, in which patho-physiological indices are used to identify the individuals most affected by parasites, could provide a foundation for alternative, incidence-based epidemiological models. In this paper, an elaboration of the classic susceptible-infected-recovered (SIR) model framework for microparasites was adapted to haemonchosis and used to predict disease in Merino sheep on a commercial farm in South Africa. Incidence was monitored over a single grazing season using the FAMACHA scoring system for conjunctival mucosal coloration, which indicates high burdens of H. contortus, and used to fit the model by estimating transmission parameters. The model predicted force of infection (FOI) between sequential FAMACHA monitoring events in groups of dry, pregnant and lactating ewes, and
related FOI to factors including climate (temperature, rainfall and rainfall entropy), using a random effects model with reproductive status group as the cluster variable. Temperature and rainfall in the seven days prior to monitoring significantly predicted the interval FOI ($p <= 0.002$), while rainfall entropy did not ($p = 0.289$). Differences across the three groups accounted for approximately 90% of the variability in the interval FOI over the period of investigation. Maintained FOI during targeted treatment of cases of haemonchosis suggests strong underlying transmission from sub-clinically infected animals, and/or limited impact on pre-existing pasture contamination by removal of clinical worm burdens later in the grazing season. The model has the potential to contribute to the sustainable management of H. contortus by predicting periods of heightened risk, and hence to focus and optimise limited resources for monitoring and treatment. SIR-type model frameworks are an alternative to classic abundance-based compartmental models of macroparasite epidemiology, and could be useful where incidence data are available. Significant challenges remain, however, in the ability to calibrate such models to field data at spatial and temporal scales that are useful for decision support at farm level.


Haemonchus contortus (Barber’s pole worm or “BPW”) is the nematode "nemesis" of small ruminant production systems in tropical and subtropical regions of the world. Its reputation derives from a combination of high fecundity and a short generational interval that provides an enviable developmental plasticity for adaptation or resistance to control measures. This review critically examines the historical and current literature on the host-parasite-environment interaction for H. contortus, particularly in sheep, to highlight changes in parasite distribution and ecology on pasture, changes to the seasonal inhibition of fourth stage larvae and the most appropriate models to identify protective responses and assess vaccines. The review also proposes pathways to bring host genetics to fruition and avenues where advances in the parasite genome may complement control measures.


The disease caused by Haemonchus contortus, a blood-feeding nematode of small ruminants, is of major economic importance worldwide. The infective third-stage larva (L3) of this nematode is enclosed in a second cuticle. Once the L3 is ingested by the host, the outer cuticle undergoes an exsheathment process that marks the transition from the free-living stage to the parasitic stage. This study explored the changes in protein expression relative to this transition. Proteins extracted from free living L3 and exsheathed L3 (xL3) were analyzed by two dimensional differential gel electrophoresis (2D-DIGE). More than 2200 protein spots were recognized, and 124 of them was found to be differentially expressed (average ratio of xL3/L3 >1.5 or xL3/L3 < -1.5, $p < 0.05$). Of these, 83 spots were up-regulated and 41 spots were down-regulated in xL3 when compared with L3. These differentially expressed spots were analyzed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOFMS) or MALDI-TOF-MS/MS and 40 proteins were identified. To predict the functions of these identified proteins, they were assigned for gene ontology (GO) annotation. Results showed that the proteins may be involved in biological processes of reproduction, cellular organization or biogenesis, multi-cellular organismal processes, single-organism processes, metabolic
processes, signaling, biological regulation, response to stimulus, cellular processes, biological adhesion, growth, locomotion, localization, developmental processes and multi-organism processes. Kyoto Encyclopedia of Genes and Genomes (KEGG) annotations were also performed, which was useful for exploring the process of metabolism and signal transduction pathways. This study indicated that some key alterations taking place, during the transition from L3 to xL3 may be interesting antiparasite targets, and some of the proteins involved in this process might be candidate antigens for vaccine development.


Background: The impact of drug selection pressure on the overall genetic diversity of parasitic nematode populations in the field is poorly understood. In this study, we address this issue for the small ruminant parasite Haemonchus contortus in the Punjab, Pakistan. This region provides an opportunity to compare H. contortus populations that have been subjected to a prolonged period of frequent benzimidazole drug treatments on government farms with parasite populations that have been exposed to little or no drug treatment in neighbouring pastoral herds.

Methods: Adult H. contortus worms were collected from the abomasum of small ruminants from three government farms frequently using benzimidazole drugs, and closed to animal movement, for over 30 years and also from from eighteen pastoral herds subject to minimal drug selection. The frequency of three known benzimidazole resistance associated mutations was determined in each parasite population. For the seven parasite populations in which resistance mutations were found, the diversity, geographical distribution and phylogenetic relationships of isotype-1 beta-tubulin benzimidazole resistance haplotypes were determined. In addition, the genetic diversity of the parasite populations on the three government farms were compared with those from four pastoral herds. Results: The F200Y (TAC) resistance mutation was present at a very high frequency in H. contortus populations from government herds, but not from pastoral herds, consistent with their respective drug selection histories. Population genetic analysis, using a panel of microsatellite markers, revealed that there was little genetic differentiation among the parasite populations with no significant difference in the overall genetic diversity between government and pastoral herds. In addition, sequence analysis of the isotype-1 beta-tubulin locus revealed multiple F200Y (TAC) haplotypes demonstrating soft selective sweeps even in government herds with little or no contemporary parasite migration. Conclusions: The results suggest that, although the frequent drug treatment used on government farms has selected for a high frequency of benzimidazole resistance mutations, there has been little or no reduction in the overall genetic diversity of the selected parasite populations.


The widespread occurrence of anthelmintic-resistant gastrointestinal nematodes (GINs), particularly Haemonchus contortus, in sheep production systems has magnified the need to identify and develop alternative control strategies. Strategies include the selection of genetically GIN-resistant sheep and the implementation of biological parasite control to reduce dependence on anthelmintic drugs. In this study, we aimed to establish the molecular identity of bacterial communities present
in the abomasum of sheep classified as resistant or susceptible to H. contortus. Thirty-eight sheep were experimentally infected with L3 Haemonchus contortus and analyzed for fecal egg count (FEC), and hematocrit (Ht) to establish haemonchosis resistance or susceptibility. Four resistant sheep (RS) and four susceptible sheep (SS) were selected for microbial sampling and subsequent phylogenetic analysis. Molecular identification of the bacteria was based on amplification of the bacterial 16S rRNA gene, construction of a 16S rDNA clone library, and subsequent gene sequencing. Significant differences (p = 0.05) were observed in the occurrence of different phyla identified in RS and SS libraries: Firmicutes (61.4% and 37.2%, respectively), Proteobacteria (10.2% and 37.2%, respectively), Bacteroidetes (12.8% and 5.8%, respectively), and unclassified bacteria (12.8% and 17%, respectively). Differences between the proportions of bacterial communities present in the RS and SS pool samples were observed, contributing as a first step toward the assessment of the association between the gastrointestinal tract microbiota and nematode resistance in sheep.


Background: Studying genetic variation within and among Haemonchus contortus populations can inform some aspects of this parasite's population genetics and epidemiology. However, almost nothing is known about such variation in China.

Methods: Adult males of H. contortus (n = 184) representing seven distinct populations in China were collected, and genetic variation within and among these populations was explored using eight distinct microsatellite markers.

Results: Genetic parameters, such as heterozygosity and inbreeding coefficient (F-IS) indicated that all eight microsatellites were highly polymorphic. Various analyses (AMOVA, F-ST, phylogenetic, structure, mantel test and population dynamics) revealed high within-population variation, low population genetic differentiation and high gene flow for H. contortus in China. Conclusions: This study provides a first snapshot of the genetic substructuring of H. contortus populations in China using polymorphic markers, and might provide a starting point for assessing genetic changes over space and time during or following the implementation of particular treatment or control strategies, or changes as a consequence of environmental, management and climatic factors.


Haemonchus contortus is arguably the most injurious helminth parasite for small ruminants. We characterized the impact of H. contortus infection on the caprine abomasal microbiome. Fourteen parasite naive goats were inoculated with 5,000 H. contortus infective larvae and followed for 50 days. Six age-matched naive goats served as uninfected controls. Reduced bodyweight gain and a significant increase in the abomasal pH was observed in infected goats compared to uninfected controls. Infection also increased the bacterial load while reducing the abundance of the Archaea in the abomasum but did not appear to affect microbial diversity. Nevertheless, the infection altered the abundance of approximately 19% of the 432 species-level operational taxonomic units (OTU) detected per sample. A total of 30 taxa displayed a significantly different abundance between control and infected goats. Furthermore, the infection resulted in a distinct difference in the microbiome structure. As many as 8 KEGG pathways were predicted to be significantly affected...
by infection. In addition, H. contortus-induced changes in butyrate producing bacteria could regulate mucosal inflammation and tissue repair. Our results provided insight into physiological consequences of helminth infection in small ruminants and could facilitate the development of novel control strategies to improve animal and human health.

Other parasites


The hypothesis tested in this experiment was that Trichostrongylus colubriformis infection would reduce growth rates of grazing meat-breed lambs; however production loss would be reduced by suppression of the host immune response. The experiment had a 3 x 2 factorial design using 6-7 month old meat breed lambs which remained uninfected or infected (IF gamma) with 2000 or 4000 T. colubriformis L3/week for 12 weeks and were immunosuppressed (SUPY) using methylprednisolone acetate once weekly or remained non-immunosuppressed (SUPN). Immunosuppression increased worm egg counts (WEC) of infected lambs (SUPY 2421 eggs per gram (epg), SUPN 1154 epg on day 84, p < 0.05) and T. colubriformis burdens (p < 0.05-0.10) and reduced circulating eosinophils (p < 0.05 on days 11, 42, 56 and 84) and intestinal total antibody titres (p < 0.02). There was a significant (p < 0.05) interaction between the main effects of infection and immunosuppression with infection having a larger negative effect on the liveweight of non-immunosuppressed lambs. The immunological response of the host to T. colubriformis infection accounted for 75% of the overall cost of infection (3.1 kg) with the majority of this cost occurring during the first 35 days of infection. In contrast, most of the cost associated with the direct effect of infection occurred after day 35. These results confirm in grazing meat-breed lambs that the host's immunological response to T. colubriformis infection is the major component of production loss.


Teladorsagia circumcincta is a major cause of ovine parasitic gastroenteritis in temperate climatic regions. The development of high levels of anthelmintic resistance in this nematode species challenges its future control. Recent research indicates that many parasite species release extracellular vesicles into their environment, many of which have been classified as endocytic in origin, termed exosomes. These vesicles are considered to play important roles in the intercellular communication between parasites and their hosts, and thus represent potentially useful targets for novel control strategies. Here, we demonstrate that exosome-like extracellular vesicles can be isolated from excretory-secretory (ES) products released by T. circumcincta fourth stage larvae (Tci-L4ES). Furthermore, we perform a comparative proteomic analysis of vesicle-enriched and vesicle-free Tci-L4ES. Approximately 73% of the proteins identified in the vesicle-enriched fraction were unique to this fraction, whilst the remaining 27% were present in both vesicle-enriched and vesicle-free fraction. These unique proteins included structural proteins, nuclear proteins, metabolic proteins, proteolytic enzymes and activation-associated secreted proteins. Finally, we demonstrate that molecules present within
the vesicles-enriched material are targets of the IgA and IgG response in T. circumcincta infected sheep, and could potentially represent useful targets for future vaccine intervention studies.


Sheep display considerable variation in both the timing and magnitude of development of immunity to gastrointestinal nematodes (GIN). Onset of immunity is dependent on a number of factors, including antigenic stimulus, nutrition supply, age and size of the animals, the latter of which are confounded. Here, we review the factors associated with the development of immunity to GIN in sheep, particularly in the context of the role that relative maturity may have through applying the rules of genetic size scaling based on examples from published literature. Comparing animals based on their metabolic age, rather than chronological age, may provide an explanation for the timing of immune development and may reduce the variation in immune development that frequently is observed both between and within breeds. Further, this approach may help explain the phenotypic differences in animal performance between animals of varying immunological capacity to GIN through influences on mature body weight. As such, when considering factors influencing immune development to GIN, physiological age or relative maturity may be considered an overlooked paradigm. We propose it may be worthwhile to consider metabolic age when comparing the immune competence of animals to ensure the subjects are at an analogous stage of physiological development.

**Cattle gastro-intestinal parasites**


It has been well documented that cattle raised on pasture are slow in weight gain when compared to those fed with grain. Inflammation in the digestive system commonly caused by pasture-transmitted gastrointestinal (GI) nematode parasites that could negatively impact feed conversion has never been compared in cattle raised with no pasture exposure (NPE, uninfected), limited pasture exposure (LPE, exposure until weaning), or continuous pasture exposure (CPE, life time exposure). In the present study, the abomasal mucosa} immune responses and inflammation of LPE and CPE cattle were investigated. Our results indicate that CPE cattle displayed inflamed abomasa with enlarged draining lymph nodes, the presence of Ostertagia ostertagi larvae and higher levels of Ostertagia-specific antibodies in circulation. The level of B cells was elevated in the abomasal mucosa in the presence (nodular) or absence (non-nodular) of Ostertagia-specific pathology, where B cells were 4-fold higher in the nodular mucosa. Foxp3(+) CD4T cells were also noticeably elevated in both the abomasal mucosa and blood, but were only slightly higher in non-nodular mucosa than in the nodular mucosa of CPE animals. In contrast, LPE animals presented no enlargement of abomasal draining lymph nodes and exhibited little to no immune cell infiltration in the abomasal mucosa. Further, CPE animals had higher numbers of mucosal mast cells when compared to LPE animals, though mucosal mast cells were high in all animals. Overall, CPE cattle displayed significantly higher levels of inflammation and pathology in their
abomasa and may explain in part slowed weight gain relative to LPE animals. The results of this study emphasize the need for GI nematode parasite control in CPE animals and development and application of vaccines which are compatible with the organic cattle production system.

Nematode infections are an important economic constraint to cattle farming. Future risk levels and transmission dynamics will be affected by changes in climate and farm management. The prospect of altered parasite epidemiology in combination with anthelmintic resistance requires the adaptation of current control approaches. Mathematical models that simulate disease dynamics under changing climate and farm management can help to guide the optimization of helminth control strategies. Recent efforts have increasingly employed such models to assess the impact of predicted climate scenarios on future infection pressure for gastrointestinal nematodes (GINs) in cattle, and to evaluate possible adaptive control measures. This review aims to consolidate progress in this field to facilitate further modeling and application.

Parasitic infections caused by nematodes are a major problem in bovines that resulting in losses in animal health and production. Thus, the aim of this study was to evaluate alterations in selected serum biochemical analytes in calves naturally infected with gastrointestinal (GI) and pulmonary nematodes without clinical signs. For this, samples of feces and blood of 86 calves were collected. Fecal egg counts (FEC) were determined using the modified McMaster technique with a sensitivity of 50 eggs per gram of feces (EPG). Positive nematode FEC was processed for coproculture using pooled samples to identify Strongylidae infective larvae (L3). First stage-larvae (L1) of Dictyocaulus viviparous were identified by a modified Baermann method. The biochemical analytes determined were: acute phase proteins such as haptoglobin and paraoxonase type 1; the enzymes acetylcholinesterase; butyrylcholinesterase; the lipid profile (triglycerides and total, HDL, and LDL-cholesterol); serum iron profile (iron and unsaturated iron-binding capacity); total protein and albumin; pancreatic profile (amylase and lipase); and minerals (phosphorus and calcium). The calves were divided into four groups according to the results of EPG and the modified Baermann method. Group 1: healthy control animals (n = 16); Group 2: calves with only GI parasites (n = 51): This group was sub-divided into sub-groups according to the EPG threshold: 2a-GI parasites with low EPG (n = 23), and 2b-G1 parasites with high EPG (n = 28). Group 3: animals with only lungworms (n = 5), and Group 4: calves with lung + GI parasites (n = 14). The more prevalent genera in all coprocultures were: Cooperia spp., Haemonchus spp., Oesophagostomum spp., and Trichostrongylus spp. The nonparametric Kruskal-Wallis test was used to compare the groups and Dunn's post-test was used for multiple comparisons as the data was not normally distributed (P<0.05). The haptoglobin concentration increased in calves with GI and pulmonary parasites. A significant increase in acetylcholinesterase was observed in calves infected with lungworms. Cholesterol, triglycerides, HDL, and LDL concentrations decreased but lipase concentration increased in calves with GI parasites. Therefore, this paper provides an overview of the biochemical effects produced by nematode parasites in calves in field conditions. These findings in
calves without any evident clinical signs of disease could provide an indication of GI parasites and lungworm infection, especially in an endemic area for these parasites.


Helminth infections of cattle affect productivity in all classes of stock, and are amongst the most important production-limiting diseases of grazing ruminants. Over the last decade, there has been a shift in focus in the diagnosis of these infections from merely detecting presence/absence of infection towards detecting its impact on production. This has been facilitated by studies observing consistent negative correlations between helminth diagnostic test results and measures of productivity. Veterinarians are increasingly challenged to consider the economic aspects of their work, and the use of these tests should now be integrated in economic evaluation frameworks for improved decision making. In this paper, we review recent insights in the farm-specific economic impact of helminth infections on dairy cattle farms as well as in farmer attitudes and behaviour regarding helminth control. Combining better economic impact assessments of helminth infections together with a deeper understanding of the non-economic factors that drive a farmer's animal health decisions should result in more effective control strategies and increased farmer satisfaction.


Anthelmintic resistance (AR) of gastrointestinal nematodes to macrocyclic lactones is an increasingly common worldwide phenomenon limiting cattle production. This has motivated the search for alternatives, such as new active compounds, added drug synergisms, different doses, and alternate administration routes. The aim of this study was the assessment of moxidectin (MXD) performance in feedlot calves with a history of AR to ivermectin (IVM). Crossbred female calves aged 6-7 months and weighing 163 kg (SD =34 kg) were divided into 3 groups of 35 animals each. They were assigned to the following antiparasitic treatment groups: IVM group (0.2 mg/kg IVM); MXD group (0.2 mg/kg MXD), and ricobendazole + levamisole (RBZ+ LEV) group (7.5 mg/kg RBZ+ 8 mg/kg LEV). On days 0, 26, and 47, fecal samples were taken and the weight of each animal was registered. Anthelmintic efficacy (by fecal egg count reduction), total weight gain (TWG) and average daily weight gain (AWG) were compared between the groups. A mixed SAS procedure was used for statistical analysis. Fecal egg count reduction 26 days post-treatment (PT) was calculated at 28% for the IVM group, 85% for the MXD group, and 99% for the RBZ+ LEV group. AWGs (Standard Error) of 1.095 g (56), 1.264 g (49), and 1.340 g (52) were registered for the IVM, MXD, and RBZ + LEV groups, respectively (p < 0.05). Coprocultures revealed that MXD more effectively reduced Haemonchus spp. and Cooperia spp. egg counts than IVM. This resulted in higher AWGs and TWGs for this group; similar results were seen for the RBZ + LEV group as well. In this study, animals treated with MXD gained about 160 more g/day than animals treated with IVM. This represents a gain of 16 USD per animal over the 47 day trial.

The efficacy of ivermectin (IVM) against gastrointestinal nematodes in Danish cattle was assessed by faecal egg count reduction test (FECRT). Six cattle farms with history of clinical parasitism and avermectin use were included. On the day of treatment (Day 0), 20 naturally infected calves per farm (total n = 120) were stratified by initial faecal egg counts (FEC) and randomly allocated to a treatment group dosed with 0.2 mg IVM kg(-1) body weight s.c. (IVM; n = 10) or an untreated control group (CTL; n = 10). Individual FEC were obtained at Day 0 and Day 14 post-treatment and pooled faeces by group were cultured to isolate L3 for detection of Ostertagia ostertagi and Cooperia oncophora by qPCR. Treatment efficacies were analysed using the recommended WAAVP method and two open-source statistical procedures based on Bayesian modelling: 'eggCounts' and 'Bayescount'. A simulation study evaluated the performance of the different procedures to correctly identify FEC reduction percentages of simulated bovine FEC data representing the observed real data. In the FECRT, reduced IVM efficacy was detected in three farms by all procedures using data from treated animals only, and in one farm according to the procedures including data from treated and untreated cattle. Post-treatment, O. ostertagi and C. oncophora L3 were detected by qPCR in faeces of treated animals from one and three herds with declared reduced IVM efficacy, respectively. Based on the simulation study, all methods showed a reduced performance when FEC aggregation increased post-treatment and suggested that a treatment group of 10 animals is insufficient for the FECRT in cattle. This is the first report of reduced anthelmintic efficacy in Danish cattle and warrants the implementation of larger surveys. Advantages and caveats regarding the use of Bayesian modelling and the relevance of including untreated cattle in the FECRT are discussed.


In grazing cattle, infections with gastrointestinal nematodes pose some of the most important health threats and subclinical infections result in considerable production losses. While there is little doubt that climate change will affect grazing ruminants directly, mean temperature increases of similar to 3 degrees C and longer drought stress periods in summer may also influence the free-living stages of parasitic nematodes. Hostile climatic conditions reduce the number of L3s on pasture and therefore the refugium, which is expected to result in a higher selection pressure, accelerating development of resistance against anthelmintic drugs. The aim of the current experiments was to investigate the effects of drought stress and different temperature/humidity ranges over time on the survival and fitness of Cooperia oncophora L3s and their distribution in grass and soil under controlled conditions using a climate chamber. Grass containers inoculated with L3s were analysed after 1-6 weeks using descriptive statistics as well as linear models. A large proportion of L3s was recovered from soil where fitness was also better preserved than on grass. Numbers and fitness of recovered L3s declined with duration in the climate chamber under both temperature profiles. However, the results of the linear models confirmed that higher temperatures (20-33 degrees C versus 17-22.6 degrees C) significantly impaired survival, distribution and fitness of L3s. Application of drought stress, known as another important factor, had a surprisingly smaller impact than its duration or higher temperatures. The climate chamber enabled exclusion of confounding factors and therefore accurate interpretation of the investigated climatic aspects. The obtained results highlight the relative importance of those factors, and will help to design better models for the population dynamics of L3s on...
pasture in the future. Additionally, the outcomes of these investigations may offer explanations regarding interdependencies of development of anthelmintic resistance and the presence of hot/dry weather conditions.


The present study evaluated the viability and possible effects of Haemonchus contortus infections in experimentally prime infected calves, comparing them to infections by Haemonchus placei. Ten male Holstein newborns were used. All calves were individually weighed for subsequent group formation, in which two animals were kept as a control group, inoculated with water (GI); four animals were inoculated with 10,000 third stage (L3) Haemonchus contortus larvae (GII); and the remaining four calves were inoculated with 10,000 third stage (L3) H. placei larvae (GIII). All experimental animals were necropsied on the 42nd day after inoculation. Based on results obtained by the present study, it can be concluded that bovine calves were susceptible to infections by both Haemonchus species (placei and contortus). H. contortus presented an inferior pre-patent period when compared to H. placei. No significant difference (P > 0.05) was observed between Haemonchus burdens recovered from both infected groups (GII and GIII). Moreover, H. contortus females maintained an egg production rate similar to H. placei females in young animals, which can contribute to pasture contamination by both Haemonchus species. This could possibly lead to negative reflexes on helminth control based on a mixed pasture with bovines and ovines, especially when it involves younglings.


The present study had the primary objective of evaluating clinical, hematological and biochemical parameters, as well as observing anatomical and histopathological characteristics of abomasums, from calves prime-infected with Haemonchus contortus or H. placei. Ten male Holstein newborns were subdivided in three groups (GI placebo; GII infected with H. contortus; GIII inoculated with H. placei). Eye mucosa staining was evaluated. Hematological and biochemical tests were performed on animals. The euthanasia of all ten experimental calves was performed on the 42nd day post-inoculation. Fragments were collected from each of all 10 abomasums for histopathological analysis. Discrete submandibular edema was diagnosed in animals from both infected groups (H. contortus or H. placei). However, there were no significant changes (P > 0.05) in the color of the ocular mucosa of calves from all three experimental groups across the entire experimental period. Hematological and biochemical changes diagnosed on animals could not be linked to infections by species of Haemonchus spp. Regarding histopathological exams, it was possible to diagnose hypertrophy, hyperplasia, binucleated cells, inflammatory infiltrate, multifocal hemorrhage and edema in abomasums from calves of both groups infected with H. placei and H. contortus. It can, thus, be concluded that not only are calves susceptible to infections by both Haemonchus species, but they can also present clinical changes and similar anatomic histopathological lesions independent of being infected by Haemonchus placei or Haemonchus contortus. These results reflect a negative effect on helminth control by mixed grazing between sheep and cattle, especially when using calves.

Cooperia oncophora is one of the most common intestinal nematodes in cattle. It is also the dose-limiting species for the most frequently used anthelmintics, and consequently, the species usually involved in reports of anthelmintic resistance. However, little information is available on its population dynamics, hindering the parameterisation of transmission models to support understanding of the impact of anthelmintic resistance, climate change and alternative control strategies on nematode epidemiology. This systematic review and meta-analysis provides estimates for key life history traits of the parasitic phase of C. oncophora and investigates potential influences of acquired immunity on these traits.


The development of anthelmintic resistance by helminths can be slowed by maintaining refugia on pasture or in untreated hosts. Targeted selective treatments (TST) may achieve this through the treatment only of individuals that would benefit most from anthelmintic, according to certain criteria. However TST consequences on cattle are uncertain, mainly due to difficulties of comparison between alternative strategies. We developed a mathematical model to compare: 1) the most 'beneficial' indicator for treatment selection and 2) the method of selection of calves exposed to Ostertagia ostertagi, i.e. treating a fixed percentage of the population with the lowest (or highest) indicator values versus treating individuals who exceed (or are below) a given indicator threshold. The indicators evaluated were average daily gain (ADG), faecal egg counts (FEC), plasma pepsinogen, combined FEC and plasma pepsinogen, versus random selection of individuals. Treatment success was assessed in terms of benefit per R (BPR), the ratio of average benefit in weight gain to change in frequency of resistance alleles R (relative to an untreated population). The optimal indicator in terms of BPR for fixed percentages of calves treated was plasma pepsinogen and the worst ADG; in the latter case treatment was applied to some individuals who were not in need of treatment. The reverse was found when calves were treated according to threshold criteria, with ADG being the best target indicator for treatment. This was also the most beneficial strategy overall, with a significantly higher BPR value than any other strategy, but its degree of success depended on the chosen threshold of the indicator. The study shows strong support for TST, with all strategies showing improvements on calves treated selectively, compared with whole-herd treatment at 3, 8, 13 weeks post-turnout. The developed model appeared capable of assessing the consequences of other TST strategies on calf populations.


Efficiency analysis is used for assessing links between technical efficiency (TE) of livestock farms and animal diseases. However, previous studies often do not make the link with the allocation of inputs and mainly present average effects that ignore the often huge differences among farms. In this paper, we studied the relationship between exposure to gastrointestinal (GI) nematode infections, the TE and the input allocation on dairy farms. Although the traditional cost allocative efficiency (CAE) indicator adequately measures how a given input allocation differs from the cost-
minimising input allocation, they do not represent the unique input allocation of farms. Similar CAE scores may be obtained for farms with different input allocations. Therefore, we propose an adjusted allocative efficiency index (AAEI) to measure the unique input allocation of farms. Combining this AAEI with the TE score allows determining the unique input-output position of each farm. The method is illustrated by estimating efficiency scores using data envelopment analysis (DEA) on a sample of 152 dairy farms in Flanders for which both accountancy and parasitic monitoring data were available. Three groups of farms with a different input-output position can be distinguished based on cluster analysis: (1) technically inefficient farms, with a relatively low use of concentrates per 1001 milk and a high exposure to infection, (2) farms with an intermediate TE, relatively high use of concentrates per 1001 milk and a low exposure to infection, (3) farms with the highest TE, relatively low roughage use per 1001 milk and a relatively high exposure to infection. Correlation analysis indicates for each group how the level of exposure to GI nematodes is associated or not with improved economic performance. The results suggest that improving both the economic performance and exposure to infection seems only of interest for highly TE farms. The findings indicate that current farm recommendations regarding GI nematode infections could be improved by also accounting for the allocation of inputs on the farm.


A dynamic, deterministic model was developed to investigate the consequences of parasitism with Ostertagia ostertagi, the most prevalent and economically important gastrointestinal parasite of cattle in temperate regions. Interactions between host and parasite were considered to predict the level of parasitism and performance of an infected calf. Key model inputs included calf intrinsic growth rate, feed quality and mode and level of infection. The effects of these varied inputs were simulated on a daily basis for key parasitological (worm burden, total egg output and faecal egg count) and performance outputs (feed intake and bodyweight) over a 6 month grazing period. Data from published literature were used to parameterise the model and its sensitivity was tested for uncertain parameters by a Latin hypercube sensitivity design. For the latter each parameter tested was subject to a 20% coefficient of variation. The model parasitological outputs were most sensitive to the immune rate parameters that affected overall worm burdens. The model predicted the expected larger worm burdens along with disproportionately greater body weight losses with increasing daily infection levels. The model was validated against published literature using graphical and statistical comparisons. Its predictions were quantitatively consistent with the parasitological outputs of published experiments in which calves were subjected to different infection levels. The consequences of model weaknesses are discussed and point towards model improvements. Future work should focus on developing a stochastic model to account for calf variation in performance and immune response; this will ultimately be used to test the effectiveness of different parasite control strategies in naturally infected calf populations.


A survey of gastrointestinal parasites in Saskatchewan beef herds was conducted over the summer of 2014. Fecal samples were collected on 3 occasions during the summer grazing season from beef cows and calves from 14 herds. The mean
number of strongylid eggs per gram of feces recovered from calves increased 9-fold (95% CI: 4.5 to 18) over the summer period, while egg counts in the cows remained constant over the same period. The prevalence and infection intensities of gastrointestinal nematode parasites in cow-calf herds in Saskatchewan were comparable to what is seen in cattle grazing in the northern regions of the United States and for which anthelmintic treatments have resulted in positive production benefits.

Parasite diagnostics


Strongyle eggs of helminths of livestock usually hatch within a few hours or days after deposition with faeces. This poses a problem when faecal sampling is performed in the field. As oxygen is needed for embryonic development, it is recommended to reduce air supply during transport and refrigerate. The present study therefore investigated the combined effect of vacuum packing and temperature on survival of strongyle eggs and their subsequent ability to hatch and develop into L3. Fresh faecal samples were collected from calves infected with Cooperia oncophora, pigs infected with Oesophagostomum dentatum, and horses infected with Strongylus vulgaris and cyathostomins. The samples were allocated into four treatments: vacuum packing and storage at 5 degrees C or 20 degrees C (5 V and 20 V); normal packing in plastic gloves closed with a loose knot and storage at 5 degrees C or 20 degrees C (5 N and 20 N). The number of eggs per gram faeces (EPG) was estimated every fourth day until day 28 post set up (p.s.) by a concentration McMaster-method. Larval cultures were prepared on day 0, 12 and 28 p.s. and the larval yield determined. For C. oncophora, the EPG was significantly higher in vacuum packed samples after 28 days as compared to normal storage, regardless of temperature. However, O. dentatum EPG was significantly higher in samples kept at 5 degrees C as compared to 20 degrees C, irrespective of packing. For the horse strongyles, vacuum packed samples at 5 degrees C had a significantly higher EPG compared to the other treatments after 28 days. The highest larval yield of O. dentatum and horse strongyles were obtained from fresh faecal samples, however, if storage is necessary prior to setting up larval cultures O. dentatum should be kept at room temperature (aerobic or anaerobic). However, horse strongyle coprocultures should ideally be set up on the day of collection to ensure maximum yield. Eggs of C oncophora should be kept vacuum packed at room temperature for the highest larval yield.


Background: The Internal Transcribed Spacer 2 (ITS2) is a candidate diagnostic marker of the pathogenic cattle nematode Ostertagia ostertagi. The aims of this study were: (i) to document and quantify how the development of O. ostertagi eggs affects ITS2 copies under different storage conditions, and (ii) to suggest optimal storage conditions for faecal samples in a diagnostic pipeline that involves detection and semi-quantification by real-time semi-quantitative polymerase chain reaction (qPCR). Findings: Eggs of Ostertagia ostertagi were obtained from fresh faeces and stored at 4 degrees C or 25 degrees C under aerobic or anaerobic (vacuum packing) conditions. Development was monitored by microscopy for up to 336 h, and the ITS2 copies were determined by qPCR from a fixed number of parasites.
Under aerobic conditions at 25 degrees C, embryonation and a significant increase of ITS2 copies (P < 0.0001) were observed after 12 h. At 4 degrees C, embryonation occurred after 168 h with a trend towards increased ITS2 copies. Anaerobic conditions inhibited egg development at both temperatures and no significant increase in ITS2 copies was noticed (P = 0.90). ITS2 copies were analysed for each parasite stage: first-stage larvae (L1) exhibited significantly higher copy numbers (20,353 +/- 1,950) than unembryonated eggs (568 +/- 168; P < 0.0001) with lower coefficient of variation (33 vs 266 %). Conclusions: Aerobic storage of O. ostertagi eggs at 25 degrees C led to a significant increase in ITS2 copies after 12 h due to embryonation and subsequent hatching. In contrast, anaerobic storage (vacuum packing) at 25 degrees C completely inhibited egg development and any undesirable semi-quantification bias for up to 336 h. Hence, vacuum packing is an optimal storage strategy prior to molecular diagnostic analyses. Alternatively, aerobic storage at 4 degrees C for up to 72 h can be used. Due to high copy numbers and lower genetic variation, the L1 stage may be considered for diagnostics and further molecular research.


Detecting antibodies formed in serum in response to infection is the traditional function of serology. Diagnostic modalities have included complement fixation tests, agar gel immune-diffusion, radioimmunoassay, ELISA and immunofluorescence. More recent technology now allows for the direct detection of pathogens by PCR. This review details the options for diagnostic testing using specimen types other than serum, identifying the advantages and disadvantages of these options and providing evidence for more widespread use of these techniques and specimen types.


The survival of the nematode parasite free-living stages depends mainly on environmental factors (i.e. rainfall, temperature and relative humidity). To complete its life cycle outside the host the parasite eliminated as eggs in the fecal material, develop to three larval stages before is ingested by the host. Although the presence of infective third stage larvae (13) on pasture is of great importance for producers and researchers to determine the level of infectivity, the quantification of 13 on pasture is often neglected. Such condition can be determined by pasture larval count (PLC) from a target area. As anthelmintic resistance is widely overspread PLC technique supports population dynamic and epidemiological studies and can be used for measuring the success rate of livestock management strategies under different climatic conditions. The present review article brings together some of the field applications and difficulties to run and interpret this technique, encouraging its use as a diagnostic procedure.


Gastrointestinal nematode parasites cause major production losses to small ruminants. The most common way to diagnose or monitor the worm burdens in sheep and goats remains the quantitative parasitological examinations, i.e. the
faecal egg counts. However, the reliability of the results of such methods depends greatly on the conditions and duration of the storage of the faecal samples prior to examination. The aim of this research was to evaluate the reduction rate and the maximum storage period, without significant losses, of nematode egg counts and third-stage larvae development from sheep and goat faeces preserved at 4°C. Towards this end, a pooled faecal sample was formed by collecting faeces from naturally infected sheep and goats (separately). Faecal egg counts and coprocultures were performed on fresh faeces and on preserved ones every week and up to 119 days post sampling. It was concluded that the preservation at 4 degrees C. i.e. into a refrigerator of fresh faeces from sheep and goats for parasitological examinations poses danger of misdiagnosis, if not performed in a period not exceeding 3 weeks of time. The rate of reduction of the faecal nematode egg counts starts to be significant lower than the ones performed with fresh samples, for both sheep and goats, after the third week of storage. The percentage of the gastrointestinal nematode larvae developing to the infective third-stage alters significantly for the Haemonchus genus, soon after the first week of storage (p<0.05).

**GI Parasite Control – Cattle**


The mucus-dwelling parasite Ostertagia ostertagi is one of the most important gastrointestinal nematodes in cattle. Our group has previously demonstrated the protective capacity of a vaccine against this parasite based on a native activation-associated secreted protein ASP1 (nASP) in combination with the saponin adjuvant QuilA. The aim of the current study was to analyse the effect of both antigen and adjuvant on the cellular and humoral vaccine-induced immune responses by comparing the native ASP to a recombinant version expressed in Pichia pastoris (pASP) and replacing QuilA by Al(OH)(3). Immunization of cattle with the protective nASP+QuilA vaccine was associated with antigen-induced proliferation of natural killer (NK) cells combined with IFN-gamma secretion and the induction of a mixed IgG1/IgG2 antibody response. ASP-specific activation and proliferation of NK cells was also observed in mice following the same vaccination regime. Replacing QuilA by Al(OH)(3) or nASP by pASP significantly decreased the capacity of the vaccines to trigger both NK cell activation and antibody responses and failed to induce protection against a challenge infection. Reduction of the structurally anchoring disulphide bonds of the nASP completely abolished its ability to induce NK cell activation and antibody responses, highlighting the importance of protein conformation for the immunostimulatory activity.

Rehbein, S., et al. (2016). "Control of parasitic infection with ivermectin long-acting injection (IVOMECA (R) GOLD) and production benefit in first-season grazing cattle facing a high-level larval challenge in Germany." *Parasitology Research* 115(12): 4639-4648.

Gastrointestinal and pulmonary nematode infections are affecting the health and productivity of grazing cattle worldwide. To evaluate the effects of a single treatment with ivermectin long-acting injection (IVM LAI; IVOMECA (R) GOLD, Merial; 3.15 % ivermectin w/v) in first-grazing season cattle, two studies were conducted under continued stocking conditions for 84 or 100 days in Bavaria, Germany. Each study involved 68 naturally infected, approximately 4- to 6-month-old Brown Swiss bull
calves. Animals were blocked based on pretreatment body weights. Within each block of four animals, animals were randomly assigned to treatments: one to saline (control) and three to IVM LAI. Treatments were injected at 1 mL/50 kg body weight subcutaneously in front of the shoulder. Animals in both studies were managed as one herd each grazing together. Cattle were weighed and fecal samples were collected pretreatment and at intervals thereafter for determination of weight gain and treatment efficacy, respectively. Fecal examination including composite fecal culture indicated the presence of nematodes of the genera Cooperia (dominating), Haemonchus, Nematodirus, Ostertagia, Strongyloides, Trichostrongylus, Trichuris, and Dictyocaulus, and Moniezia cestodes in the cattle. Following treatment, IVM LAI-treated cattle did not shed any Dictyocaulus larvae for 84 days while controls continued to pass larvae. Compared to the controls, IVM LAI-treated cattle had significantly (p < 0.01) lower strongylid egg counts at each occasion. Percentage reductions were 94 % up to 70 days after treatment and were 83.9 and 58.9 % at 84 and 100 days. Over the 84- or 100-day study periods, IVM LAI-treated cattle gained significantly more weight than the controls: 22.7 and 12.4 kg, respectively. The two studies demonstrated a high efficacy of IVM LAI against gastrointestinal and pulmonary nematode infections under field conditions in Germany which was associated with significant benefit as to weight gain.


Predicting the effectiveness of parasite control strategies requires accounting for the responses of individual hosts and the epidemiology of parasite supra- and infra-populations. The first objective was to develop a stochastic model that predicted the parasitological interactions within a group of first season grazing calves challenged by Ostertagia ostertagi, by considering phenotypic variation amongst the calves and variation in parasite infra-population. Model behaviour was assessed using variations in parasite supra-population and calf stocking rate. The model showed the initial pasture infection level to have little impact on parasitological output traits, such as worm burdens and FEC, or overall performance of calves, whereas increasing stocking rate had a disproportionately large effect on both parasitological and performance traits. Model predictions were compared with published data taken from experiments on common control strategies, such as reducing stocking rates, the 'dose and move' strategy and strategic treatment with anthelmintic at specific times. Model predictions showed in most cases reasonable agreement with observations, supporting model robustness. The stochastic model developed is flexible, with the potential to predict the consequences of other nematode control strategies, such as targeted selective treatments on groups of grazing calves.


ObjectiveTo determine whether (i) the drenching practices of beef producers in the Central Tablelands of NSW utilising short-acting anthelmintics limit the post-weaning growth of heifers and (ii) a single weaning treatment of a long-acting anthelmintic improves post-weaning growth. MethodsCommencing at weaning, 20/100 heifers in each of five herds were injected four times at 90-day intervals with long-acting moxidectin to suppress gastrointestinal nematodes. In year 1 the balance was drenched using the owner's usual commercial practice with short-acting anthelmintics (5 herds) or left undrenched (1 herd). In year 2 the balance was...
drenched with long-acting moxidectin within 3 months of weaning (4 herds) or with short-acting drenches only (2 herds). At each visit live weight (LW) was recorded and a subsample tested for worm egg count (WEC) and plasma pepsinogen.

Results
In year 1 by 3 and 6 months' post weaning, suppressed heifers were significantly heavier than the undrenched and short-acting groups, and by 12 months were 40 and 28kg heavier, respectively. In year 2, the LW of the suppressed and long-acting heifers was not different, but by 3 and 6 months both groups were significantly heavier than the short-acting group and by 12 months were 22kg heavier.

Conclusion
The post-weaning growth of beef heifers treated with short-acting drenches as adopted by Tablelands beef producers was slower than heifers drenched suppressively or with a single long-acting drench at weaning.


Helminth infections of cattle affect productivity in all classes of stock, and are amongst the most important production-limiting diseases of grazing ruminants. Over the last decade, there has been a shift in focus in the diagnosis of these infections from merely detecting presence/absence of infection towards detecting its impact on production. This has been facilitated by studies observing consistent negative correlations between helminth diagnostic test results and measures of productivity. Veterinarians are increasingly challenged to consider the economic aspects of their work, and the use of these tests should now be integrated in economic evaluation frameworks for improved decision making. In this paper, we review recent insights in the farm-specific economic impact of helminth infections on dairy cattle farms as well as in farmer attitudes and behaviour regarding helminth control. Combining better economic impact assessments of helminth infections together with a deeper understanding of the non-economic factors that drive a farmer's animal health decisions should result in more effective control strategies and increased farmer satisfaction.


Plants containing condensed tannins (CT) may have potential to control gastrointestinal nematodes (GIN) of cattle. The aim was to investigate the anthelmintic activities of four flavan-3-ols, two galloyl derivatives and 14 purified CT fractions, and to define which structural features of CT determine the anti-parasitic effects against the main cattle nematodes. We used in vitro tests targeting L1 larvae (feeding inhibition assay) and adults (motility assay) of Ostertagia ostertagi and Cooperia oncophora. In the larval feeding inhibition assay, O. ostertagi L1 were significantly more susceptible to all CT fractions than C. oncophora L1. The mean degree of polymerization of CT (i.e. average size) was the most important structural parameter: large CT reduced larval feeding more than small CT. The flavan-3-ols of prodelphinidin (PD)-type tannins had a stronger negative influence on parasite activity than the stereochemistry, i.e. cis-vs trans-configurations, or the presence of a gallate group. In contrast, for C. oncophora high reductions in the motility of larvae and adult worms were strongly related with a higher percentage of PDs within the CT fractions while there was no effect of size. Overall, the size and the percentage of PDs within CT seemed to be the most important parameters that influence antiparasitic activity.

Background: Increasing anthelmintic-resistance in nematodes of ruminants emphasises the need for sustainable parasite control. Condensed tannin-containing legume forages such as sainfoin (Onobrychis viciifolia) have shown promising anthelmintic properties in small ruminants but this has never been explored in cattle. Therefore, our aim was to examine the efficacy of sainfoin against cattle nematodes in vivo. Methods: Fifteen Jersey male calves (2-4 month-old) were allocated into two groups and fed isoproteic and isoenergetic diets mainly composed of sainfoin pellets (Group SF; n = 9, three pens) or concentrate and grass-clover hay (Group CO; n = 6, two pens). After 16 days of adaptation, all animals were experimentally infected with 10,000 and 66,000 third-stage larvae of Ostertagia ostertagi and Cooperia oncophora, respectively. Egg excretion, blood parameters and bodyweights were recorded throughout the study. Worms were harvested by sieving for quantification and scanning electron microscopy (SEM) 42 days post-infection (dpi) when the calves were necropsied. Results: The number of O. ostertagi adults in the abomasum was reduced by 50 % in Group SF compared with Group CO (P < 0.05). This was further reflected in higher albumin (P < 0.1) and lower pepsinogen levels (P < 0.05) in Group SF at 21 dpi, and structural damage of the worm cuticle could be visualised by SEM. Yet, the nematode egg excretion in Group SF was not significantly different from that of the controls (P > 0.05). Likewise, no statistical difference in total worm burdens of C. oncophora was found between the groups. Weight gains were lower for Group SF (P < 0.05), which may reflect lower digestibility and phosphorus levels in the SF diet, despite similar feed intake at pen-level. Conclusions: Overall, the effect of sainfoin on abomasal nematodes corroborates results from studies with small ruminants and encourages further investigations of the use of this crop for control of cattle nematodes.


Two experiments studied the effects of dietary chicory against gastrointestinal nematodes in cattle. In Experiment (Exp.) 1, stabled calves were fed chicory silage (CHI1; n = 9) or ryegrass/clover hay (CTL1; n = 6) with balanced protein/energy intakes between groups. After 16 days, all calves received 10 000 Ostertagia ostertagi and 66 000 Cooperia oncophora third-stage larvae (L3) [day (D) 0 post-infection (p.i.)]. In Exp. 2, calves were assigned to pure chicory (CHI2; n=10) or ryegrass/clover (CTL2; n = 10) pastures. After 7 days, animals received 20 000 O. ostertagi L3/calf (D0 p.i.) and were moved regularly preventing pasture- borne infections. Due to poor regrowth of the chicory pasture, CHI2 was supplemented with chicory silage. At D40 p.i. (Exp. 1) and D35 p.i. (Exp. 2) calves were slaughtered for worm recovery. In Exp. 1, fecal egg counts (FEC) were similar between groups. However, O. ostertagi counts were significantly reduced in CHI1 by 60% (geometric mean; P < 0.01), whereas C. oncophora burdens were unaffected (P = 0.12). In Exp. 2, FEC were markedly lowered in CHI2 from D22 p.i onwards (P < 0.01). Ostertagia ostertagi adult burdens were significantly reduced in CHI2 by 66% (P < 0.001). Sesquiterpene lactones were identified only in chicory (fresh/silage). Chicory shows promise as an anti-Ostertagia feed for cattle and further studies should investigate its on-farm use.
Gastrointestinal nematodes (GIN) infection can impair milk production (MP) in dairy cows. To investigate whether MP would be optimized by spring targeted-selective anthelmintic treatment in grazing cows, we assessed (1) the effect on MP of an anthelmintic treatment applied 1.5 to 2 months after turn-out, and (2) herd and individual indicators associated with the post-treatment MP response. A randomized controlled clinical trial was conducted in 13 dairy farms (578 cows) in western France in spring 2012. In each herd, lactating cows of the treatment group received fenbendazole orally, control cows remained untreated. Daily cow MP was recorded from 2 weeks before until 15 weeks after treatment. Individual serum pepsinogen and anti-Ostertagia antibody levels (expressed as ODR), faecal egg count and bulk tank milk (BTM) Ostertagia ODR were measured at treatment time. Anthelmintic treatment applied during the previous housing period was recorded for each cow. In each herd, information regarding heifers' grazing and anthelmintic treatment history was collected to assess the Time of Effective Contact (TEC, in months) with GIN infective larvae before the first calving. The effect of treatment on weekly MP averages and its relationships with herd and individual indicators were studied using linear mixed models with two nested random effects (cow within herd).

Unexpectedly, spring treatment had a significant detrimental effect on MP (-0.92 kg/cow/day on average). This negative MP response was particularly marked in high producing cows, in cows not treated during the previous housing period or with high pepsinogen levels, and in cows from herds with a high TEC or a high BTM ODR. This post-treatment decrease in MP may be associated with immunoinflammatory mechanisms. Until further studies can assess whether this unexpected result can be generalized, nonpersistent treatment of immunized adult dairy cows against GIN should not be recommended in early grazing season.

**GI parasite control-sheep**


Research has shown that Toll-like receptor 4 (TLR4) is important in immune responses to some helminth parasites. In sheep, variation in the PAMP region of TLR4 may result in structurally and thus functionally different TLR4 molecules, and this may consequently lead to variation in the TLR4 response to parasite infections. This study involved three separate, but related sheep breeds (Merino, Polwarth and Corriedale sheep) and a total of 885 lambs from five New Zealand farms that underwent a mixed field-challenge from gastro-intestinal parasites. Faecal samples were collected at approximately 4 and 9 months of age and faecal egg counts (FECs) for Nematodirus spp. and Strongyle species determined, along with the total number of eggs per gram (EPG). Analysis of the five farms collectively revealed an association (P=0.023) between the presence of TLR4 variant *02 (mean 24 EPG) and the absence of the variant (mean 32 EPG) at 9 months of age. Conversely the presence of *03 had a significantly (P=0.047) higher mean Nematodirus spp. FEC (mean 42 EPG) compared to the absence (mean 28 EPG) at 9 months of age. More associations were revealed when the data were split according to the dominant faecal parasite species. With a predominantly Trichostrongylus spp. FEC group of lambs at 9 months of age, the presence of TLR4 variant *02 was found to have significantly (P=0.003) lower Nematodirus spp. FEC (mean 4 EPG), and also
significantly (P=0.033) lower total FEC (mean 312 EPG) when compared to sheep without the variant (mean 15 EPG and 449 EPG, respectively). The presence of TLR4 variant *03 and *04 were associated or tended to be associated (P=0.010 and P=0.088, respectively) with higher Nematodirus spp. FEC (mean 25 EPG and 22 EPG, respectively) when compared to lambs without the variant (mean 10 EPG and 11 EPG, respectively). These results suggest that TLR4 variation may be affecting the immune response to gastro-intestinal parasites in sheep, although principally to Nematodirus spp. infections and not Strongyle species infections.


Targeted selective treatment (TST) requires the ability to identify the animals for which anthelmintic treatment will result in the greatest benefit to the entire flock. Various phenotypic traits have previously been suggested as determinant criteria for TST; however, the weight gain benefit and impact on anthelmintic efficacy for each determinant criterion is expected to be dependent upon the level of nematode challenge and the timing of anthelmintic treatment. A mathematical model was used to simulate a population of 10,000 parasitologically naive Scottish Blackface lambs (with heritable variation in host-parasite interactions) grazing on medium-quality pasture (grazing density =30 lambs/ha, crude protein = 140 g/kg DM, metabolisable energy =10 MJ/kg DM) with an initial larval contamination of 1000, 3000 or 5000 Teladorsagia circumcincta L-3/kg DM. Anthelmintic drenches were administered to 0, 50 or 100% of the population on a single occasion. The day of anthelmintic treatment was independently modelled for every day within the 121 day simulation. Where TST scenarios were simulated (50% treated), lambs were either chosen by random selection or according to highest faecal egg count (FEC, eggs/g DM faeces), lowest live weight (LW, kg) or lowest growth rate (kg/day). Average lamb empty body weight (kg) and the resistance (R) allele frequency amongst the parasite population on pasture were recorded at slaughter (day 121) for each scenario. Average weight gain benefit and increase in R allele frequency for each determinant criterion, level of initial larval contamination and day of anthelmintic treatment were calculated by comparison to a non-treated population. Determinant criteria were evaluated according to average weight gain benefit divided by increase in R allele frequency to determine the benefit per R. Whilst positive phenotypic correlations were predicted between worm burden and FEC; using LW as the determinant criterion provided the greatest benefit per R for all levels of initial larval contamination and day of anthelmintic treatment. Hence, LW was identified as the best determinant criterion for use in a TST regime. This study supports the use of TST strategies as benefit per R predictions for all determinant criteria were greater than those predicted for the 100% treatment group, representing an increased long-term productive benefit resulting from the maintenance of anthelmintic efficacy. Whilst not included in this study, the model could be extended to consider other parasite species and host breed parameters, variation in climatic influences on larval availability and grass growth, repeated anthelmintic treatments and variable proportional flock treatments.

Teladorsagiosis is a major production-limiting disease in ruminants in temperate regions throughout the world and one of the key interventions in the management of the disease is the prevention of pasture contamination with Teladorsagia circumcincta eggs by ewes during the periparturient relaxation in immunity which occurs in the period around lambing. Here, we describe the immunisation of twin-bearing ewes with a T. circumcincta recombinant subunit vaccine and the impact that vaccination has on their immune responses and shedding of parasite eggs during a continuous T. circumcincta challenge period spanning late gestation and lactation. In ewes which displayed a clear periparturient relaxation in immunity, vaccination resulted in a 45% reduction in mean cumulative faecal egg count (cFEC, p = 0.027) compared to control (immunised with adjuvant only) ewes. Recombinant antigen-specific IgG and IgA, which bound each of the vaccine antigens, were detected in the serum of vaccinated ewes following each immunisation and in colostrum taken from vaccinated ewes post-partum whereas low levels of antigen-specific IgG were detected in serum and colostrum from control ewes. Antigen-specific IgG and IgA levels in blood collected within 48 h of birth from lambs largely reflected those in the colostrum of their ewes.


Control of gastrointestinal nematodes (GIN) remains a critical issue due to the prevalence of anthelmintic resistance. The objective of the experiment was to determine the efficacy of copper oxide wire particles (COWP) from three commercial sources and a combination of COWP and albendazole to control GIN and/or Haemonchus contortus in lambs. Naturally infected Katadin lambs in early June 2014 and 2015 were randomly assigned to receive no COWP (CON; n=9 and 12) or 2g COWP in a gel capsule as Copasure (R) (COP; n=4 and 17; Animax Ltd.), copper oxide wire form (AUS; n=7 in 2014 only; Pharmplex), UltraCruz (TM) (ULT; n=8 and 15; Santa Cruz Animal Health (TM)), no COWP and albendazole (CON+alb; n=10 in 2015 only; 15 mg/kg BW; Valbazen (R); Zoetis Animal Health), or COWP + alb (n= 7 and 11; in 2014, lambs were administered alb on day 3). Lambs grazed grass pastures as a group and were supplemented with 227 g/lamb daily of a commercial grain mix (15% crude protein) and the same amount of alfalfa pellets. Feces were collected on days 0 (day of COWP treatment), 7, and 14 for determination of fecal egg counts (FEC). Pooled (2014) or pooled treatment group feces were cultured on days 0, 7, and 14 (2015 only) to determine GIN genera. Data were analyzed using repeated measures in a mixed model, and FEC were log transformed. The predominant GIN on day 0 was H. contortus (87%) in 2014, and there was a mixed population in 2015. The mean FEC was reduced by day 7 in AUS and ULT lambs (treatment x day, P=0.001), and all of the COWP products were similar. By day 14, the AUS FEC were lower than the CON and COP groups. When examining the combination of COWP and synthetic anthelmintic, the FEC of COWP+alb were reduced to nearly 0 eggs/g (back-transformed) and lower than the other groups (treatment x day, P=0.001). The percentage of H. contortus in cultured feces was reduced to a greater extent in the COWP than CON or CON+alb groups of lambs. In a mixed GIN population, the COWP products appeared to be similar in efficacy and using a combination of COWP + alb increased the efficacy not only against H. contortus, but all GIN genera present, offering options in the face of resistance to benzimidazoles.

Early immune events associated with reduced larval burden remain unclear in parasite-resistant breeds of sheep. Therefore, our objective was to determine breed differences in immune-related gene expression following infection with H. contortus. Gene expression in abomasal tissue and mucosa and in abomasal lymph nodes (ALN) was measured in 24 St. Croix (hair) lambs and 24 Dorset x (Finn-Rambouillet) (wool) lambs at 0 (uninfected), 3, 5 and 7 days after infection with 10 000 L3 H. contortus larvae. Expression of IL-4 in abomasal mucosa was detected on day 3 and increased to day 7 in hair lambs, but was not detectable in wool lambs. Genes that recruit neutrophils (CXCL1) and macrophages (MCP1) were upregulated in abomasal mucosa of hair lambs. Genes associated with alternative macrophage activation (ARG-1) and eosinophil activation (Gal-14) were also upregulated in the abomasal mucosa of hair lambs. Tissue remodeling genes (MMP13, PDGF) and tumour necrosis factor alpha (TNF-alpha) and MCP1 were upregulated in abomasal tissue of wool lambs; these lambs also had greater expression of forkhead box P3 in ALN. These data indicate a role for early IL-4 expression locally and demonstrate potential downregulation of immunity in wool sheep that could facilitate establishment of H. contortus.


Haemonchus contortus is an important gastrointestinal parasite on sheep farms in tropical regions. The resistance of the parasite against most anthelmintic drugs represents a great economic problem to sheep farming and is a major challenge that needs to be overcome. The searches for new anthelmintic agents that act on different stages of the parasite's life cycle are necessary for the development of new therapeutic options. The aim of this study was to evaluate the in vitro and in vivo anthelmintic activity of Thymus vulgaris essential oil against H. contortus and of its main component, the monoterpenic thymol. Despite the relative ineffectiveness of the oil in the in vivo test, which may be corrected in the future after technical improvements to increase the oil's bioavailability, the in vitro results validated the popular use of T. vulgaris oil as an anthelmintic agent, at least against H. contortus. In fact, both the essential oil and thymol, which accounts for 50.22% of the oil composition, were effective against the three main stages of H. contortus. The oil and thymol were able to inhibit egg hatching by 96.4-100%, larval development by 90.8-100%, and larval motility by 97-100%. Similar to the positive control (levamisole 20 mg/mL), the oil and thymol completely inhibited the motility of H. contortus adults within the first 8 h of the experiment. Since thymol reproduces the anthelmintic effects of the oil and because it is the main component of the oil, it is reasonable to assume that thymol is the most important compound responsible for the anthelmintic effect of T. vulgaris. These results are of ethnopharmacological importance and may contribute to the development of new drugs and even herbal medicines, increasing treatment options for the farm breeding.

Levamisole phosphate, chosen based on its 100% efficacy demonstrated by a previous fecal egg count reduction test (FECRT), was used as the exclusive anthelmintic treatment in the Embrapa Southeast Livestock sheep flock from 2009 to 2014 in a target selected treatment scheme. In the present study, the effectiveness of this nematode control scheme was evaluated after 5 years by FECRT, larval development test (LDT), and a molecular test to assess the development of levamisole resistance in Haemonchus contortus. Animals were submitted to treatments with albendazole, levamisole, closantel, ivermectin, moxidectin, and monepantel. Eggs per gram of feces (EPG) counts and fecal cultures were performed, and anthelmintic efficacy was calculated by the RESO 4.0 program. The helminths of the flock (GIN Embrapa2014) were compared to susceptible (McMaster) and resistant (Embrapa2010) H. contortus isolates in the LDT to estimate the LC50 and LC90 of levamisole and in a molecular test to evaluate the 63-bp indel in the acr8 gene associated with levamisole resistance. In the FECRT, parasites were susceptible to monepantel (99.6%) and closantel (98.3%), but resistant to moxidectin (93.8%), levamisole (70.4%), ivermectin (48.1%), and albendazole (0%). In the coproculture on D14, and the control group presented 80% H. contortus and 20% Trichostrongylus sp., while in the monepantel group L-1 were observed as well as Oesophagostomum sp. L-3. LDT and resistance factors provided good separation between susceptible and resistant parasites. The genotypic frequencies of the 63-bp insertion in the acr8 gene in H. contortus were 11.9, 6.7, and 0% in GIN Embrapa2014, Embrapa2010, and McMaster isolates, respectively. After 5 years of exclusive use, the nematodes developed resistance to levamisole, detected by FECRT and by increase in LC50 and LC90 for levamisole in the LDT. The 63-bp indel was not confirmed as a molecular marker of levamisole resistance in our isolates. The target selected treatment scheme was effective to control helminths in the sheep flock for 5 years, when levamisole's inefficacy was perceived because of no change in the clinical situation of treated animals. Through this scheme, it was possible to promote reversion towards susceptibility or increase of efficacy for other chemical classes. Thus, this is a valid recommendation to control worms and to delay the development of resistance, preserving other anthelmintic classes for future use.


Haemonchus contortus parasitism is a major disease of sheep, with these parasites frequently demonstrating multi-drug class anthelmintic resistance. Copper oxide wire particles (COWP) have shown potential as adjuncts or alternatives to anthelmintics in resistant flocks. The purpose of this study was to compare the efficacy of two different COWP treatment regimens or placebo in the control of H. contortus in weaned lambs within a flock historically shown to have multi-drug resistant H. contortus using the DrenchRite (R) assay. Data from 43 lambs within 3 treatment groups in a double blind study were included in the experiment. Treatments were administered as a total of 2 boluses, each given on separate occasions (day 0 and day 42), so that each lamb received either 2 placebos, a single dose of 2 g COWP followed by placebo, or two doses of 1 g COWP. Strongyle-type fecal egg counts (FEC) were performed at initial treatment (day 0), on day 10, at second treatment (day 42), on day 52, and at study end (day 84). At the start of the trial, mean standard deviation FEC were 1634.4 +/- 825.2, 2241.7 +/- 1496.8, and 2013.3 +/- 1194.2 epg for the 2 g, 1 g x 2, and control groups, respectively. At the end of the trial, FEC were 757.1 +/- 825.3, 483.4 +/- 557.2, and
Lambs given a 2 g single dose of COWP or a 1 g dose of COWP twice had reductions in strongyle-type FEC (p <= 0.01) from trial start to trial end, whereas lambs given placebo did not. Average daily gains did not differ significantly among groups. Although copper is potentially toxic to sheep, no signs of toxicity were observed during this trial, which was consistent with similar studies at this treatment dose. The study indicated that administering COWP to lambs at weaning reduced FEC.


Haemonchus contortus (H. contortus) is a haematophagous parasite which causes important economic losses in small ruminants. On the island of Gran Canaria, two sheep breeds coexist which differ in their susceptibility to the infection with H. contortus; the resistant Canaria Hair Breed (CHB) sheep and the susceptible Canaria Sheep (CS) breed. The major target of resistance mechanisms in CHB sheep are directed to the adult parasite stage, reducing the worm burden, and decreased length and fecundity of surviving worms. Mucosal IgA (mIgA) has been shown to be an important regulator of immunity in Haemonchus and Teladorsagia infections; through correlations with larval stages where such mechanisms as antibody dependent cell cytotoxicity and enzyme inhibition may mediate resistance. Here for the first time, we demonstrate a significant negative correlation between mIgA and adult worm length and fecundity only in the resistant CHB sheep. In contrast, and as reported in other sheep breeds, mIgA was only negatively correlated against the larval stage in the more susceptible CS breed. This study suggests mIgA may play a role in resistance to both larval and adult stages.


Gastrointestinal (GI) nematode infections are considered among one of the toughest challenges sheep farmers face worldwide. Control still is largely based on the use of anthelmintics, but anthelmintic resistance is becoming rampant. To facilitate implementation of alternative nematode control strategies and to reduce anthelmintic usage, the purpose of this study was twofold: (i) to gain insight in common practices, knowledge gaps and perceptions of farmers regarding nematode control, and (ii) to provide foci of attention for improving parasite control practices and transfer of knowledge within the sheep husbandry. An internet-based questionnaire was made available to all sheep farmers pertaining to the year 2013, resulting in 450 entered questionnaires for analysis. The two most important nematodes mentioned, were Haemonchus contortus and, to a lesser extent, Nematodirus battus. Of all respondents, 25.6% said they did not have any worm problems. Of these, almost a third did notice clinical signs that can be related to worm infections and about three quarters did use anthelmintics. Overall, clinical symptoms mentioned by farmers matched the worm species they identified as the cause of problems. Ewes and lambs were treated up to 6 times in 2013. On average, ewes were treated 1.53 and lambs 2.05 times. Farmers who treated their ewes more often, also treated their lambs more often (P < 0.001). Both ewes and lambs were frequently treated based on fixed moments such as around lambing, at weaning and before mating, rather than based on faecal egg counts. Treatments based on faecal egg counts were practiced, but on a minority of the farms (32.7%). The majority of the farms (75.6%) did not leave 2-5% of the sheep within a flock untreated. About 74% of farmers keep newly purchased animals quarantined for at
least 10 days, but some (13.4%) leave quarantined animals untreated nor check faecal egg counts. Of farmers who do treat their quarantined animals, just 12.6% check the efficacy of the treatment. Slightly over 40% of the respondents said they did not experience bottlenecks in parasite control. Yet, over half of these said having problems with worm infections, over half did see clinical signs related to worm infections and over three quarters used anthelmintics. Within the group of farmers experiencing difficulties in parasite control, the most often mentioned bottleneck concerned pasture management (75.8%). When asking farmers for solutions, 90% of all respondents indicated they are willing to adjust their pasture management. Farmers are also interested in other methods to reduce the risk of worm infections, such as possibilities to enhance the immune system of sheep in general (71%), to increase specific genetic resistance to worms and to apply anti-parasite forages, both about 40%. Results of this study gave the following potential foci of attention: (1) making complex scientific knowledge more accessible to farmers through simple tools and applicable in the daily farming process; (2) changing the mindset of farmers about their current worm control practices, i.e. breaking long-standing habits such as treating ewes and lambs at fixed moments rather than based on actual worm infection monitoring data; (3) demonstrating effective pasture rotation schemes on specific farms and using these in extension work; (4) making farmers more aware that checking anthelmintic efficacy is important; (5) improving quarantine procedures; (6) creating a wider array of applicable alternative control measures from which individual farmers can choose what fits them most; and finally, (7) improving mutual understanding among farmers, veterinary practitioners and parasitologists alike.


Effective control of gastrointestinal parasites is necessary in sheep production. The development of anthelmintics resistance is causing the available chemically based anthelmintics to become less effective. Biological control strategies present an alternative to this problem. In the current study, we tested the larvicidal effects of Bacillus thuringiensis var. israelensis Cry11Aa toxin against Haemonchus contortus larvae. Bacterial suspensions [2 x 10(8) colony-forming units (CFU) g(-1) of the feces] of B. thuringiensis var. israelensis and recombinant Escherichia coli expressing Cry11Aa toxin were added to naturally H. contortus egg-contaminated feces. The larvae were quantified, and significant reductions of 62 and 81% (P < 0001) were, respectively observed, compared with the control group. A 30 mL bacterial suspension (1 x 10(8) CFU mL(-1)) of B. thuringiensis var. israelensis and recombinant E. coli expressing Cry11Aa toxin were then orally administered to lambs naturally infected with H. contortus. Twelve hours after administration, feces were collected and submitted to coprocultures. Significant larvae reductions (P < 0001) of 79 and 90% were observed respectively compared with the control group. The results suggest that the Cry11Aa toxin of B. thuringiensis var. israelensis is a promising new class of biological anthelmintics for treating sheep against H. contortus.

Efficient control of gastrointestinal parasites is necessary in sheep breeding. However, the available chemically based anthelmintics are becoming less effective due to the development of parasite resistance. An alternative to this problem is biological control. In the present study, we tested the larvicidal effect of Bacillus circulans by administering a spore suspension (2 x 10^9 colony forming units/ml) orally to lambs naturally infected with Haemonchus contortus. The number of faecal larvae was quantified daily and a significant reduction (similar to 87%, P < 0.05) of larval development was observed after administration of B. circulans. Using a transformed B. circulans with green fluorescent protein, we were able to detect B. circulans in the faeces at 4 h post-administration and 72h after cessation of its administration. These results suggest the use of B. circulans as a promising biological alternative for parasite control.


Haemonchosis is often associated with late gestation and parturition in ewes in Canada. Due to widespread concerns about development of anthelmintic resistance (AR), targeted selective treatment (TST), where individual animals are treated with an anthelmintic rather than the entire flock, is a possible strategy to control clinical signs in recently lambed ewes while still maintaining parasite refugia. Performing fecal egg counts (FEC) on individual animals is often cost-prohibitive, so indicators that identify ewes with high FEC are essential for TST programs. The study objectives were to: a) evaluate the ability of four TST indicators to identify periparturient ewes with high Haemonchus sp. FEC and b) determine appropriate treatment thresholds for statistically-significant indicators. A field study was conducted during the 2013 and 2014 lambing seasons (February-May) on three client-owned farms in Ontario with documented AR and problems with haemonchosis in ewes. Ewes were examined within three days of lambing and selected for treatment with oral closantel (10 mg/kg body weight), a novel anthelmintic to Canada, if they met at least one of four criteria: a) the last grazing season was their first grazing season; b) body condition score <= 2; c) Faffa Malan Chart (FAMACHA (c)) score >= 3; and/or d) three or more nursing lambs. Fecal samples were collected per rectum on the treatment day from each of 20 randomly selected treated and untreated ewes on each farm. Haemonchus sp. percentages on each farm, as determined by coproculture, ranged from 53% to 92% of total fecal trichostrongyle-type egg counts. Mean Haemonchus sp. FECs were significantly higher in treated ewes (n = 136) than in untreated ewes (n = 103) on the day of treatment in both years (p = 0.001), suggesting the indicators were suitable for identifying animals with high Haemonchus sp. FEC. A linear mixed model was fit with logarithmic-transformed Haemonchus sp. FEC as the outcome variable, the four indicators and year as fixed effects, and farm as a random effect. FAMACHA (c) score was the sole indicator to remain significantly associated with FEC (p = 0.002). A receiver-operator curve determined that test sensitivity was maximized (92.4%) with FAMACHA (c) score >= 3 as the sole indicator. FAMACHA (c) score should therefore be included in TST programs to identify ewes requiring treatment at lambing due to Haemonchus sp.

Genetic markers for sheep resistance to gastrointestinal parasites have long been sought by the livestock industry as a way to select more resistant individuals and to help farmers reduce parasite transmission by identifying and removing high egg shedders from the flock. Polymorphisms related to the major histocompatibility complex and interferon (IFN)-gamma genes have been the most frequently reported markers associated with infection. Recently, a new picture is emerging from genome-wide studies, showing that not only immune mechanisms are important determinants of host resistance but that gastrointestinal mucus production and hemostasis pathways may also play a role.


With the aim of validating the FAMACHA (c) as a method for phenotypic selection of sheep resistant/resilient to gastrointestinal nematodes, 27 Suffolk ewes with known FAMACHA (c) score histories were experimentally infected with 25,000 larvae of Haemonchus contortus. From the day of infection (day 0) to 60 days post-infection, at intervals of 7-15 days, ewes were evaluated for packed cell volume (PCV) and fecal egg counts (FEC). A statistically significant increase (p < 0.05) in FEC occurred between day 0 and day 60. PCV values showed a decrease (p < 0.05) starting from day 21, compared to day 0. Based on the changes in FEC and PCV values from day 0 to day 60,15 ewes (55.56%) were classified as susceptible (S) and 12 ewes (44.44%) as resistant/resilient (RR). A comparison of the average FEC after infection between susceptible (4487.6 eggs per gram epg) and resistant/resilient (1317.9 epg) ewes showed a significant difference (p < 0.05) between the two groups. The difference in average PCV values after infection (24.8% and 30.3% for S and RR, respectively) was also significant (p < 0.05). Data from 980 previous evaluations of FAMACHA (c) scores from the 27 ewes showed that 58.33% of the ewes classified as RR and 46.67% of the S group had a history of only F1 and F2 scores. In the RR group, only one animal (8.33%) had an F4 score, occurring one time out of the 61 evaluations of this ewe. In contrast, 40.0% of S group ewes had F4 and/or F5 scores. During the period of FAMACHA (c) score history that was evaluated, 69.56% of the total number of anthelmintic treatments in the flock were administered to ewes from group S. Since ewes with F4 and/or F5 scores during the FAMACHA (c) score time period were classified as susceptible during the experimental infection (with the exception of one ewe), we conclude that the FAMACHA (c) score history is a useful tool for the selection of ewes that are resistant/resilient, as well as for the identification of susceptible animals that should be culled.


The worldwide spread of resistance to anthelmintic (AH) drugs in gastrointestinal nematodes (GINS) imposes to explore alternative solutions. Amongst those, the possible use of tannin-containing nutraceuticals appears as a relevant option to replace (or decrease the frequency of) chemical-based treatments. Our objectives were to test the AH efficacy of sainfoin pellets against a multiresistant strain of Haemonchus contortus in experimentally infected lambs and to examine possible interaction between ivermectin (IVM) and condensed tannins (CT)-rich ressource. In vivo study was performed with twenty-four lambs were inoculated (Day 0) with
multiresistant H. contortus infective larvae. On D21 Post-Infection, the lambs were assigned to two dietary treatments (sainfoin vs lucerne control pellets). On D39, half of the animals per group received 0.25 ml/kg of an oral ivermectin treatment. On D47, animals were slaughtered to count worms. The consumption of sainfoin was associated with higher packed cell volume (PCV) values (P < 0.05) and reduced faecal egg counts (FECs) (P < 0.05). For the experimental feeding period, FECs were overall reduced by 50% in the sainfoin group. The diet did not have significant effect on the worm number but sainfoin significantly reduced female fertility. Decrease in plasma IVM concentrations was observed in the sainfoin-fed animals and was associated with a decrease of IVM efficiency when compared with the control group. Incubating tannin in vitro with ivermectin and rumen fluid showed a blocking of ivermectin by the tannins. This suggests that tannins lower the IVM intestinal absorption compromising thereby drug plasma bioavailability and efficacy. Tannin-containing nutraceuticals alter the biology of multiresistant nematodes, thus representing an option for their sustainable control. In vivo and in vitro interactions between nutraceuticals and chemicals impose caution when both tannin -rich diet and drug-based treatments are combined. Further studies are required to clarify the mechanisms that support such interactions.


The control of gastrointestinal nematodes (GIN) is mainly based on the use of drugs, grazing management, use of copper oxide wire particles and bioactive forages. Resistance to anthelmintic drugs in small ruminants is documented worldwide. Host genetic resistance to parasites, has been increasingly used as a complementary control strategy, along with the conventional intervention methods mentioned above. Genetic diversity in resistance to GIN has been well studied in experimental and commercial flocks in temperate climates and more developed economies. However, there are very few report outputs from the more extensive low-input/output smallholder systems in developing and emerging countries. Furthermore, results on quantitative trait loci (QTL) associated with nematode resistance from various studies have not always been consistent, mainly due to the different nematodes studied, different host breeds, ages, climates, natural infections versus artificial challenges, infection level at sampling periods, among others. The increasing use of genetic markers (Single Nucleotide Polymorphisms, SNPs) in GWAS or the use of whole genome sequence data and a plethora of analytic methods offer the potential to identify loci or regions associated nematode resistance. Genomic selection as a genome-wide level method overcomes the need to identify candidate genes. Benefits in genomic selection are now being realised in dairy cattle and sheep under commercial settings in the more advanced countries. However, despite the commercial benefits of using these tools, there are practical problems associated with incorporating the use of marker-assisted selection or genomic selection in low-input/output smallholder farming systems breeding schemes. Unlike anthelmintic resistance, there is no empirical evidence suggesting that nematodes will evolve rapidly in response to resistant hosts. The strategy of nematode control has evolved to a more practical manipulation of host-parasite equilibrium in grazing systems by implementation of various strategies, in which improvement of genetic resistance of small ruminant should be included. Therefore, selection for resistant hosts can be considered as one of the sustainable control strategy, although it will be most effective when used to complement other control
strategies such as grazing management and improving efficiency of anthelmintics currently.


The use of synthetic drugs against gastrointestinal nematodes of ruminants has led to a situation where resistance to anthelmintics is widespread, and there is an urgent need for alternative solutions for parasite control. One promising approach is to use polyphenol-rich bioactive plants in animal feeds as natural anthelmintics. In the present work, the in vitro activity of a series of 33 hydrolyzable tannins (HTs) and their hydrolysis product, gallic acid, against egg hatching and motility of L1 and L2 stage Haemonchus contortus larvae was studied. The effect of the selected compounds on egg and larval structure was further studied by scanning electron microscopy. The results indicated clear relationships between HT structure and anthelmintic activity. While HT size, overall flexibility, the types and numbers of functional groups, together with the linkage types between monomeric HTs affected the activity differently, the optimal structure was found with pentagalloylglucose.

GI parasite control-other species


Background: Tannin-rich plants have been examined as an alternative for controlling the gastrointestinal nematodes in ruminants. In vivo assays typically examine the anthelmintic activity in female fecundity and/or the adult worm burden, without considering other life-cycle stages or the impact on pasture contamination. The aim of the present study was to evaluate the anthelmintic activity of tanniniferous food from Bauhinia pulchella in goats and the potential impact on pasture contamination with the infective larval stage of gastrointestinal nematodes. Findings: Sixteen cross breed Boer goats that were naturally infected with gastrointestinal nematodes were fed tanniniferous concentrate from the leaves of B. pulchella and compared to a separate paddock of control animals without condensed tannin supplementation. A range of parasite characteristics were monitored throughout the 63 days of experimentation, including faecal egg count (FEC), egg hatching and relative numbers of hatched helminth larvae on herbage. Worm free tracer animals were used to assess the infective larval stage load of the contaminated pasture. The tanniniferous food did not reduce the combined FEC values, but egg hatching was significantly affected (p < 0.05). The pasture grazed by goats fed with tanniniferous food from B. pulchella showed reduced contamination through infective larval stages. Tracer goats maintained in paddocks grazed with animals fed with tanniniferous food had lower numbers of Trichostrongylus colubriformis than did those in the control group (86 % reduction). Conclusions: Condensed tannin from B. pulchella showed anthelmintic activity, affected egg viability and reduced pasture contamination, which led to the reduced infection of the animals by T. colubriformis.

A dynamic and innovative approach to managing the blood-consuming nematode Haemonchus contortus in goats is critical to crack dependence on veterinary anthelmintics. H. contortus management strategies have been the subject of intense research for decades, and must be selected to create a tailored, individualized program for goat farms. Through the selection and combination of strategies from the Toolbox, an effective management program for H. contortus can be designed according to the unique conditions of each particular farm. This Toolbox investigates strategies including vaccines, bioactive forages, pasture/grazing management, behavioural management, natural immunity, FAMACHA, Refugia and strategic drenching, mineral/vitamin supplementation, copper Oxide Wire Particles (COWPS), breeding and selection/selecting resistant and resilient individuals, biological control and anthelmintic drugs. Barbervax (R), the ground-breaking Haemonchus vaccine developed and currently commercially available on a pilot scale for sheep, is prime for trialling in goats and would be an invaluable inclusion to this Toolbox. The specialised behaviours of goats, specifically their preferences to browse a variety of plants and accompanying physiological adaptations to the consumption of secondary compounds contained in browse, have long been unappreciated and thus overlooked as a valuable, sustainable strategy for Haemonchus management. These strategies are discussed in this review as to their value for inclusion into the ‘Toolbox’ currently, and the future implications of ongoing research for goat producers. Combining and manipulating strategies such as browsing behaviour, pasture management, bioactive forages and identifying and treating individual animals for haemonchosis, in addition to continuous evaluation of strategy effectiveness, is conducted using a model farm scenario. Selecting strategies from the Toolbox, with regard to their current availability, feasibility, economical cost and potential ease of implementation depending on the systems of production and their complementary nature, is the future of managing H. contortus in farmed goats internationally and maintaining the remaining efficacy of veterinary anthelmintics.


This study aimed to investigate the effect of selection for low faecal egg count (FEC) in Scottish Cashmere goats in comparison to a control line of unselected goats grazing the same pasture. Goats from generations F2 through to F9 were monitored for FEC, bodyweight, peripheral eosinophilia and IgG, IgA and IgE response against Teladorsagia circumcincta from the end of their first grazing season, through winter housing (during which a single artificial challenge dose of 10,000 drug susceptible T. circumcincta was given) and the following full grazing season. The study demonstrated that selected line animals excreted a significantly lower number of parasite eggs (P < 0.01) in the majority of generations examined. Liveweight productivity was unaffected by selection. Although selected line animals had greater numbers of circulating eosinophils in many of the generations (four generations of males and six generations of females, P < 0.05), there was no direct link between eosinophilia and reduced FEC. Immunoglobulin levels showed no consistent difference between selected and control lines. IgG, IgA and IgE levels were not different between lines over the whole dataset (P > 0.05), although the selected line had significantly elevated or reduced levels (P < 0.05) for all three within individual generations. There were significant associations between increased IgG and reduced FEC under artificial infection conditions (P = 0.02). Increased IgA was also significantly associated with elevated FEC during the
second grazing season (P < 0.001). The study demonstrates that selection produced a line of goats with consistently reduced FEC compared with control animals, but did not identify a clear relationship between any of the immune markers measured and faecal egg output.


Forty goats, aged from 2 to 5 months were subjected to two different immunization protocols with a vaccine containing Haemonchus contortus gut membrane proteins H11/1-1-gal-GP to evaluate protection against H. contortus on pre-contaminated pastures. Goats were allocated to four groups of ten, three of them received their first vaccination before turnout. One group (V4) was then vaccinated at 4-week-intervals whereas another two groups (V6 and V6SEP) were vaccinated at 6-week-intervals. A control group (CTRL) remained unvaccinated. In May, after the second vaccination, all goats were turned out on pastures which had been previously contaminated with H. contortus eggs by seeder sheep for a period of six weeks. Goats of groups V4, V6 and CTRL were grazed together, whereas V6SEP was kept separately at an identical stocking rate. Clinical (PCV, FAMACHA, body weight), parasitological (faecal egg count, FEC) and serological (antibody titres) parameters were measured fortnightly. All goats were staibled in October, drenched with levamisole and two weeks later infected with 5000 L3 of H. contortus and slaughtered four weeks later for determination of abomasal worm burdens. Group mean FEC peaked 42-56 days after turnout. Significantly lower FEC were observed in V6SEP vs CTRL between D 28 and 70 (p < 0.01). Mean egg output of all groups decreased substantially and fluctuated at low levels until the end of the grazing period (D 154). Goats responded to vaccination with increasing antibody titres peaking after every booster. Mean worm burdens deriving from experimental infections were reduced by 89, 65 and 47% in groups V4, V6 and V6SEP, respectively, compared with the controls. The difference was significant for V4 (p < 0.01). Antibody titres measured 14 days before slaughter did not correlate statistically with the worm burdens. It was concluded that the vaccination protocol did not result in sufficient protection on pasture, as antibody titres were still low at the time the goats were exposed to larval contamination on pasture after turnout.

**General parasite control**


Gastrointestinal nematode (GIN) infection of ruminants represents a major health and welfare challenge for livestock producers worldwide. The emergence of anthelmintic resistance in important GIN species and the associated animal welfare concerns have stimulated interest in the development of alternative and more sustainable strategies aimed at the effective management of the impact of GINs. These integrative strategies include selective breeding using genetic/genomic tools, grazing management, biological control, nutritional supplementation, vaccination and targeted selective treatment. In this review, the logic of selecting for resistance
to GIN infection as opposed to resilience or tolerance is discussed. This is followed by a review of the potential application of immunogenomics to genetic selection for animals that have the capacity to withstand the impact of GIN infection. Advances in relevant genomic technologies are highlighted together with how these tools can be advanced to support the integration of immunogenomic information into ruminant breeding programmes.


The global increase in anthelmintic resistant nematodes of ruminants, together with consumer concerns about chemicals in food, necessitates the development of alternative methods of control for these pathogens. Subunit recombinant vaccines are ideally placed to fill this gap. Indeed, they are probably the only valid option for the long-term control of ruminant parasitic nematodes given the increasing ubiquity of multidrug resistance in a range of worm species across the world. The development of a subunit multicellular parasite vaccine to the point of practical application would be a ground-breaking step in the control of these important endemic infections of livestock. This review summarizes the current status of subunit vaccine development for a number of important gastrointestinal nematodes of cattle and sheep, with a focus on the limitations and problems encountered thus far, and suggestions as to how these hurdles might be overcome.


Helminth infections and nutrition can independently alter the composition and abundance of the gastrointestinal microbiota, however, their combined effect is poorly understood. Here, we used the T. retortaeformis-rabbit system to examine how the helminth infection and host restriction from coprophagy/read-to-absorb nutrients affected the duodenal microbiota, and how these changes related to the acquired immune response at the site of infection. A factorial experiment was performed where the bacterial community, its functionality and the immune response were examined in four treatments (Infect, Infect+Collar, Control+Collar and Control). Helminths reduced the diversity and abundance of the microbiota while the combination of parasites and coprophagic restriction led to a more diversified and abundant microbiota than infected cases, without significantly affecting the intensity of infection. Animals restricted from coprophagy and free from parasites exhibited the richest and most abundant bacterial community. By forcing the individuals to absorb nutrients from less digested food, the coprophagic restriction appears to have facilitated the diversity and proliferation of bacteria in the duodenum. Changes in the microbiota were more clearly associated with changes in the immune response for the infected than the nutrient restricted animals. The functional and metabolic characteristics of the duodenal microbiota were not significantly different between treatments. Overall, infection and diet affect the gut microbiota but their interactions and outcome can be complex. These findings can have important implications for the development of control measures to helminth infections where poor nutrition/malnutrition can also be a concern.
Among gastro-intestinal nematodes, the blood-sucking worms belonging to the subfamily of Haemonchinae are considered to be of pathogenic and economic great importance, particularly in small ruminants. Haemonchus contortus, primarily found in domestic ruminants and wild bovines (Mouflon, Chamois), is probably the most studied, but occurrence of Ashworthius sidemi has gradually increased over recent years, especially in Cervids and free roaming wild bovid as the European bison in eastern Europe, and some cases of co-infestation were recently observed on five Roe deer (Capreolus capreolus) and one Red deer (Cervus elaphus) in France. If the diagnosis is possible on the morphological features for adult worms for helminthologists, the identification on some stages (female, subadult, eggs and larvae) is difficult or impossible. Sequencing ND4 domain from the mitochondrial DNA of H. contortus and A. sidemi worms, we observed clearly two distinct clades, with an inter-specific divergence of 28.1%. Basing on this specific domain, a multiplex PCR-based method was developed: new primers were designed and used pooled in one mix PCR, producing amplicons of 454 bp for H. contortus and 330 bp for A. sidemi, allowing a trivial and an inexpensive taxonomic affiliation after migration. This multiplex PCR-based method was developed here to distinguish H. contortus and A. sidemi regardless their developmental stage, easy to use for highlighting co-infestation cases in both wild and domestic ruminants. It is a non-invasive approach appearing as a good diagnostic tool relevant to coprological cultures.


Thirty Haemonchus contortus male worms were collected from farmed red deer yearlings in order to determine whether routine administration of albendazole for a long-term period (17 years) could select anthelminthic resistance. PCR-RFLP method based on single-nucleotide polymorphism of codon 200 in isotype 1 -tubulin gene (Phe200Tyr) was applied. The results showed a significant frequency of either the resistant allele (85 %) or the homozygous resistant genotype (70 %). By chi-square test, Hardy-Weinberg equilibrium of the population was accepted (p = 0.334, power of test 0.01). True prevalence of the resistant genotype (RR) was estimated to be 46.5-87.2 % (confidence interval 95 %) calculated by Sterne's exact method. These results confirmed that long-term use of benzimidazoles could change the relative allele frequency of genes associated with drug resistance and may cause a large-scale spread of the resistant allele. To our knowledge, this study supported benzimidazole resistance in Haemonchus contortus in red deer for the first time.


South American camelids (SACs) are receiving increasing interest in Europe. One of the main health problems in SAC management worldwide is the control of endoparasites that may cause economic losses or even overt disease. To estimate the prevalence and distribution of endoparasites of SACs kept in Austria for future recommendations for parasite control measures, a nationwide survey was conducted. Faecal samples from 469 clinically healthy SACs in all federal states...
were examined. The most frequently detected endoparasites were GIS (82.8% of the samples), followed by Eimeria (77.2%). Although the comparison of southern, northern, western and eastern regions of Austria did not show statistically significant differences in distribution of parasites, E. macusaniensis clustered in the south and Nematodirus spp. and Capillaria spp. in the north, while D. dendriticum was much more prevalent in the south and west. Lung worms, tapeworms and the large liver fluke appear prevalence in ruminants in Austria. While coccidia of the genus Eimeria are strictly host-specific, nematodes of various genera as well as small liver flukes are probably cross-transmitted from ruminants and may cause considerable health problems and productivity losses.


North American bison (Bison bison) producers face many challenges, including the potential clinical and economics problems caused by trichostrongyle nematodes within their herds. Little is known about the prevalence, intensity, geographical distribution and clinical significance of these parasites in commercial bison herds, even from regions where bison production has become popular. This study involved a large herd of bison from eastern South Dakota that was experiencing clinical parasitism due to a temporary over-stocking problem. After documenting fecal egg counts (FECs) and trichostrongyle genera present among the 3 main age-categories (i.e. adults, yearlings, calves) of bison during this heavily infected grazing season, the effects of doramectin treatment on the different age groups was also evaluated. This is the first bison study using PCR to identify genera of trichostrongyles in fecal samples. Virtually all 103 bison fecal samples from all 3 age classes were shedding trichostrongyle eggs by the end of the season, and the mean FECs were 34 eggs/g (EPG) among the cows, 125 EPG in the yearlings, and 186 EGP among calves. Based upon this heavily-infected herd, there is evidence that the susceptibility of bison to trichostrongyles is more similar to beef cattle than to sheep. Other parasites such as Moniezia, Nematodirus, Trichuris, and coccidians were also identified in these samples. All but 3 of the 51 samples analyzed with PCR shown at least 1 trichostrongyle genera. Ostertagia was detected in 68.6% of the samples, Cooperia in 80.39%, Haemonchus in at least 73% and Trichostrongylus in 16% of the herd. Most commonly, bison were infected with combinations of Haemonchus/Ostertagia/Cooperia. After treatment with doramectin, the mean FECs dropped by 99.9% for all of the bison age classes.


Most small ruminant farms in tropical climates are plagued by Haemonchus contortus, a hematophagous, abomasal parasite. Heavy burdens of this parasite can cause anemia, hypoproteinemia, weight loss, and mortality in susceptible animals. Haemonchus contortus is becoming a major health concern in New World camelids as well, namely llamas (Llama glama) and alpacas (Vicugna pacos), yet little research has been conducted regarding its prevalence or pathology in these species. Herein, we present a retrospective review of llamas and alpacas that were admitted to The University of Georgia Veterinary Teaching Hospital and Athens Diagnostic Laboratory between the years 2002 and 2013. Antemortem fecal egg count (FEC) estimates performed on 30 alpacas were negatively correlated with
hematocrit, hemoglobin, and red blood cell count. Total protein was not significantly correlated with FEC. On postmortem examination, 55 of 198 camelids, including 2 from the aforementioned antemortem review, were infected with H. contortus, with llamas (42.6%) having a significantly higher infection rate than alpacas (22.2%). In 15.7% of the total cases, the parasite was the major cause of death. Common gross lesions included peritoneal, thoracic, and pericardial effusions, visceral pallor, subcutaneous edema, and serous atrophy of fat. Histologic lesions included centrilobular hepatic necrosis, hepatic atrophy, lymphoplasmacytic inflammation of the mucosa of the third gastric compartment (C3), extramedullary hematopoiesis in both the liver and spleen, and the presence of nematodes in C3. Our study emphasizes the importance of H. contortus diagnosis and herd monitoring in New World camelids, particularly llamas.

Pig parasites


There are major gaps in our knowledge of many molecular biological processes that take place during the development of parasitic nematodes, in spite of the fact that understanding such processes could lead to new ways of treating and controlling parasitic diseases via the disruption of one or more biological pathways in the parasites. Progress in genomics, transcriptomics, proteomics and bioinformatics now provides unique opportunities to investigate the molecular basis of key developmental processes in parasitic nematodes. The porcine nodule worm, Oesophagostomum dentatum, represents a large order (Strongylida) of socioeconomically important nematodes, and provides a useful platform for exploring molecular developmental processes, particularly given that this nematode can be grown and maintained in culture in vitro for periods longer than most other nematodes of this order. In this article, we focus on the moulting process (ecdysis) in nematodes; review recent advances in our understanding of molecular aspects of moulting in O. dentatum achieved by using integrated proteomic-bioinformatic tools and discuss key implications and future prospects for research in this area, also with respect to developing new anti-nematode interventions and biotechnological outcomes.

Avian parasites


The role that parasites play in regulating animal populations is debated, however recent research hints at their pervasiveness among free-living animal populations. Parasites exert both direct and indirect effects on host populations, and can act to regulate populations. The Ring-necked pheasant is an important game-bird species in the UK, and large numbers of birds are released annually. The impact of the ubiquitous tracheal nematode, Syngamus trachea on pheasant populations through effects on host condition was assessed on two pheasant estates in the south west of England. Pheasants infected with S. trachea demonstrated a significant reduction in host condition compared with uninfected controls, with as few as one pair of
worms per bird. Although there was no difference in worm burden between sexes, analysis of regression slopes revealed there was a significant difference between sexes in the magnitude of the effect of increasing worm burden on host condition, with detectable effects observed in hosts with one and three pairs of worms for males and females respectively. The observed reductions in host condition in birds with even sub-clinical infections could be the cause of poor reproductive success and survival of pheasants post-release.

Surveillance


Meat inspection has the ultimate objective of declaring the meat and offal obtained from carcasses of slaughtered animals fit or unfit for human consumption. This safeguards the health of consumers by ensuring that the food coming from these establishments poses no risk to public health. Concomitantly, it contributes to animal disease surveillance. The Catalan Public Health Protection Agency (Generalitat de Catalunya) identified the need to provide its meat inspectors with a support structure to improve diagnostic capacity: the Slaughterhouse Support Network (SESC). The main goal of the SESC was to offer continuing education to meat inspectors to improve the diagnostic capacity for lesions observed in slaughterhouses. With this aim, a web-based application was designed that allowed meat inspectors to submit their inquiries, images of the lesions, and samples for laboratory analysis. This commentary reviews the cases from the first 6 years of SESC operation (2008-2013). The program not only provides continuing education to inspectors but also contributes to the collection of useful information on animal health and welfare. Therefore, SESC complements animal disease surveillance programs, such as those for tuberculosis, bovine cysticercosis, and porcine trichinellosis, and is a powerful tool for early detection of emerging animal diseases and zoonoses.


Introduction - Gastro-intestinal nematodes are important helminth parasites in all animal species. However, they must regarded particularly dangerous in domestic ruminant species, also in relation to consequent economic losses. Aim - The present study focused on providing current data, missing since several decades, on gastro-intestinal nematode parasitic infection, prevalence and epidemiology in adult cattle (dairy and brood cows) bred in Italy. Materials and methods - The survey was performed collecting 427 fecal samples from a bovine slaughterhouse in the province of Bologna (Italy). Samples, obtained from single animals processed, were analyzed by qualitative coprological examinations. From the same animals 100 abomasa were randomly selected and examined by necropsy technique to assess the presence of worm burdens. Results - Gastro-intestinal nematode eggs were detected in 31% of individual fecal samples examined. Evaluation of abomasa exhibited a prevalence of 13% of helminthes. Ostertagia, Trichostrongylus and Cooperia were the isolated genera. The fecal output of nematode eggs was significantly related with the livestock category and the stocking density. Discussion - The influence of livestock category on the occurrence of positive coprological
results can be attributed to the condition of animal husbandry: brood cows are often pasture raised. The correlation observed between positive samples and herd size, with intermediate class (50-99 animals) associated with higher prevalence, may be explained by a different effectiveness of hygiene management among classes of stocking density. Conclusions - The study results show that endoparasitic infection by nematodes is a problem that must be considered ubiquitous in Italy in adult cattle, with relatively high prevalence rate. Nevertheless, it seems to be still underestimated by technicians in the field.

**Anthelmintics**


In phase I, faecal egg count reduction tests (FECRT) were conducted on six commercial cattle farms to compare the performance of two pour-on and one oral combination anthelmintic. Groups of 12-15 calves were sampled for faecal nematode egg count (FEC) before treatment with either abamectin oral, levamisole oral, an abamectin + levamisole oral combination or one of two abamectin + levamisole combination pour-ons. Samples were collected again 14 days after treatment to calculate the percentage reduction in FEC. The proportions of infective stage larvae (13) in faecal cultures were used to apportion egg counts to, and calculate efficacy against, the main parasite genera. Abamectin oral was effective against Ostertagia except on one farm where resistance was indicated, but had reduced efficacy against Cooperia on four farms. Levamisole oral was effective against Cooperia on all farms, but had variable efficacy against Ostertagia. The abamectin + levamisole oral was effective against both species on all farms. The abamectin + levamisole pour-ons were effective on some farms but not on others. In particular, pour-on 2 failed to achieve 95% efficacy in 45% of evaluations, 4/6 against Cooperia and 1/5 against Ostertagia. On some farms the combination pour-ons were less effective than their constituent actives administered alone as orals. In phase II, 8 groups of 6 calves, grazing parasite-free pasture, were infected with putatively ML resistant isolates of Cooperia oncophora and Ostertagia ostertagi. Once infections were patent groups were treated with oral or pour-on formulations of abamectin alone, levamisole alone, abamectin + levamisole (two pour-ons) or remained untreated. Blood samples were collected for analysis and after 8 days all calves were euthanized and abomasa and intestines recovered for worm counts. All treatments were effective against O. ostertagi and all treatments containing levamisole were effective against C. oncophora. Animals treated with the oral combination had higher Cmax and AUC values for abamectin in plasma than animals treated orally with abamectin alone. In contrast, animals treated with the combination pour-ons tended to have lower plasma levels for abamectin than those treated with abamectin alone as a pour-on, with differences in the Cmax and AUC values approaching statistical significance (p-values <= 0.07). There were no differences detected in plasma concentrations of levamisole. The inconsistent and sometimes poor efficacy of the combination pour-ons on-farm is likely due to reduced levels of abamectin in the plasma and hence less active reaching the target worms in the gut.
Anthelmintic resistance is a major problem in parasitic nematodes of livestock worldwide. One means to counter resistance is to use synergists that specifically inhibit resistance mechanisms in order to restore the toxicity, and hence preserve the usefulness, of currently available anthelmintics. P-glycoproteins (P-gps) eliminate a wide variety of structurally unrelated xenobiotics from cells, and have been implicated in anthelmintic resistance. Crizotinib is a tyrosine kinase inhibitor under development as a cancer therapeutic. The compound also inhibits P-gps, and has been shown to reverse multidrug resistance in cancer cells. We were therefore interested in determining if the compound was able to increase the sensitivity of Haemonchus contortus larvae to ivermectin, as measured by in vitro larval development and migration assays with a drug-resistant and a-susceptible isolate. In migration assays, co-administration of crizotinib increased the toxicity of ivermectin to resistant larvae (up to 5.7-fold decrease in ivermectin IC50), and rendered the resistant larvae equally or more sensitive to ivermectin than the susceptible isolate. On the other hand, co-administration of crizotinib had no effect on ivermectin sensitivity in the susceptible isolate. In development assays, significant increases in the sensitivity of both the resistant (up to 1.9-fold) and susceptible (up to 1.6-fold) larvae to ivermectin were observed, although the magnitude of the observed synergism was less than seen in migration assays, and the resistant larvae retained significant levels of ivermectin resistance. By highlighting the ability of the P-gp inhibitor crizotinib to increase the sensitivity of H. contortus larvae to ivermectin, this study provides further evidence that P-gp inhibitors are potential tools for modulating the efficacy of anthelmintics. In addition, the differences in the outcomes of the two assays, with 'resistance-breaking' effects being much more marked in migration assays, suggest that some life-stage-specific aspects may exist in the interaction of ivermectin with P-gps in the two worm isolates.


There is a substantial need to develop new medicines against parasitic diseases via public-private partnerships. Based on high throughput phenotypic screens of largely protozoal pathogens and bacteria, the Medicines for Malaria Venture (MMV) has recently assembled an open-access 'Pathogen Box' containing 400 well-curated chemical compounds. In the present study, we tested these compounds for activity against parasitic stages of the nematode Haemonchus contortus (barber's pole worm). In an optimised, whole-organism screening assay, using exsheathed third-stage (xL3) and fourth-stage (L4) larvae, we measured the inhibition of larval motility, growth and development of H. contortus. We also studied the effect of the 'hit' compound on mitochondrial function by measuring oxygen consumption. Among the 400 Pathogen Box compounds, we identified one chemical, called tolfenpyrad (compound identification code: MMV688934) that reproducibly inhibits xL3 motility as well as L4 motility, growth and development, with IC50 values ranging between 0.02 and 3 μM. An assessment of mitochondrial function showed that xL3s treated with tolfenpyrad consumed significantly less oxygen than untreated xL3s, which was consistent with specific inhibition of complex I of the respiratory electron transport chain in arthropods. Given that tolfenpyrad was developed as a pesticide and has already been tested for absorption, distribution,
excretion, biotransformation, toxicity and metabolism, it shows considerable promise for hit-to-lead optimisation and/or repurposing for use against H. contortus and other parasitic nematodes. Future work should assess its activity against hookworms and other pathogens that cause neglected tropical diseases.


Albendazole (ABZ), widely used benzimidazole anthelmintic, administered to animals enters via excrements into environment and may impact non-target organisms. Moreover, exposure of lower development stages of helminths to anthelmintics may also encourage the development of drug-resistant strains of helminths. In present project, the kinetics of ABZ (10 mg kg(-1) p.o.) and its metabolite (ABZ.SO, ABZSO(2)) elimination in faeces from treated Texel lambs were studied using UHPLC/MS/MS with the aim to find out their concentrations achievable in the environment. Consequently, the effect of these compounds on lower development stages of Barber’s pole worm (Haemonchus contortus) and on germination of white mustard (Sinapis alba) seeds was evaluated. The results showed that ABZ concentrations in faeces excreted in 4-60 h after treatment were above the concentrations lethal for H. contortus eggs. Moreover, pre-incubation with sub-lethal doses of ABZ and ABZ.SO did not increase the resistance of H. contortus eggs and larvae to anthelmintics. On the other hand, concentrations of ABZ and ABZ.SO in faeces are so high that might have negative influence on non-target soil invertebrates. As neither ABZ nor its metabolites affect the germination of mustard seeds, phytoremediation could be considered as potential tool for detoxification of ABZ in the environment.


The present in vitro study was designed to test and compare anthelmintic activity, hepatotoxicity, and biotransformation of four selected aminoacetonitrile derivatives (AADs): monepantel (MOP, anthelmintic approved for the treatment), AAD-970, AAD-1154, and AAD-1336. Micro-agar larval development test, MTT test of cytotoxicity, and biotransformation study coupled with Ultra high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) technique were used for this purpose. Larvae of two Haemonchus contortus strains (drug susceptible and multi-drug resistant) and primary cultures of rat and ovine hepatocytes served as model systems. All AADs (including MOP) exhibited significant larvicidal effect in H. contortus susceptible as well as multi-resistant strains, much higher than those of reference anthelmintics thiabendazole and flubendazole. AAD-1154 provides the best results for most tested parameters among all AADs in this study. The cytotoxicity test showed that all AADs can be considered as nontoxic for hepatocytes. In the biotransformation study, Phase I and Phase II metabolites of AADs were identified and schemes of possible metabolic pathways in ovine hepatocytes were proposed. Biotransformation of MOP was much more extensive than biotransformation of other AADs. Based on obtained results, AAD-1154 and AAD-1336 can be considered as promising candidates for further in vivo testing.

Nematode parasites may be controlled with drugs, but their regular application has given rise to concerns about the development of resistance. Drug combinations may be more effective than single drugs and delay the onset of resistance. A combination of the nicotinic antagonist, derquantel, and the macrocyclic lactone, abamectin, has been found to have synergistic anthelmintic effects against gastrointestinal nematode parasites. We have observed in previous contraction and electrophysiological experiments that derquantel is a potent selective antagonist of nematode parasite muscle nicotinic receptors; and that abamectin is an inhibitor of the same nicotinic receptors. To explore these inhibitory effects further, we expressed muscle nicotinic receptors of the nodular worm, Oesophagostomum dentatum (Ode-UNC-29:Ode-UNC-63:Ode-UNC-38), in Xenopus oocytes under voltage-clamp and tested effects of abamectin on pyrantel and acetylcholine responses. The receptors were antagonized by 0.03 μM abamectin in a non-competitive manner (reduced R-max, no change in EC50). This antagonism increased when abamectin was increased to 0.1 μM. However, when we increased the concentration of abamectin further to 0.3 μM, 1 μM or 10 μM, we found that the antagonism decreased and was less than with 0.1 μM abamectin. The bi-phasic effects of abamectin suggest that abamectin acts at two allosteric sites: one high affinity negative allosteric (NAM) site causing antagonism, and another lower affinity positive allosteric (PAM) site causing a reduction in antagonism. We also tested the effects of 0.1 μM derquantel alone and in combination with 0.3 μM abamectin. We found that derquantel on these receptors, like abamectin, acted as a non-competitive antagonist, and that the combination of derquantel and abamectin produced greater inhibition. These observations confirm the antagonistic effects of abamectin on nematode nicotinic receptors in addition to GluCl effects, and illustrate more complex effects of macrocyclic lactones that may be exploited in combinations with other anthelmintics.


This study investigated the interaction of ATP binding cassette (ABC) transport proteins with ivermectin (IVM) and levamisole (LEV) in larvae of susceptible and resistant isolates of Haemonchus contortus in vitro by measuring transcription patterns following exposure to these anthelmintics. Furthermore, we studied the consequences of drug exposure by measuring the sensitivity of L-3 to subsequent exposure to higher drug concentrations using larval migration assays. The most highly transcribed transporter genes in both susceptible and resistant L3 were pgp-9.3, abcf-1, mrp-5, abcf-2, pgp-3, and pgp-10. The resistant isolate showed significantly higher transcription of pgp-1, pgp-9.1 and pgp-9.2 compared to the susceptible isolate. Five P-gp genes and the haf-6 gene showed significantly higher transcription (up to 12.6-fold) after 3 h exposure to IVM in the resistant isolate. Similarly, five P-gp genes, haf-6 and abcf-1 were transcribed at significantly higher levels (up to 10.3-fold) following 3 h exposure to LEV in this isolate. On the other hand, there were no significant changes in transcriptional patterns of all transporter genes in the susceptible isolate following 3 and 6 h exposure to IVM or LEV. In contrast to these isolate-specific transcription changes, both isolates showed an increase in R-123 efflux following exposure to the drugs, suggesting that the drugs stimulated activity of existing transporter proteins in both isolates. Exposure of resistant larvae to IVM or LEV resulted, in some instances, in an increase in the proportion of the population able to migrate at the highest IVM concentrations in...
The significant increase in transcription of some ABC transporter genes following 3 h exposure to both IVM and LEV in the resistant isolate only, suggests that an ability to rapidly upregulate protective pathways in response to drugs may be a component of the resistance displayed by this isolate.


A new oil suspension containing 0.10% ivermectin (IVM) and 15% praziquantel (PZQ) (Tivm+pzq) for intramuscular injection was developed for sheep, and its pharmacokinetics was investigated in sheep. The quality of the new product met the technical standards set by the Ministry of Agriculture of the People's Republic of China. In pharmacokinetics, the commercially available single-component products approved by the Chinese Ministry of Agriculture and widely used in the livestock industry in China were selected as reference products (R-ivm and R-pzq). The results showed that all of the IVM pharmacokinetic parameters of Tivm+pzq were similar to those of the reference. However, after administration of Tivm+pzq, mean residence time (MRT) and plasma elimination half-life (t(1/2z)) were 20.36 h and 11.65 h, which were 2.61 and 3.22 times longer than those of R-pzq (7.81 h and 3.62 h). In summary, the MRT and t(1/2z) of PZQ in Tivm+pzq were prolonged and IVM pharmacokinetic parameters were similar to commercial product, therefore the new injection may be an alternative choice for sheep to control parasites sensitive to IVM and PZQ.


A series of novel carbazoles were synthesized based on structural modifications to lead carbazole 1 (EC100 = 2.5 μM against Haemonchus contortus in vitro), which was revealed in a small molecule screening program as a potentially promising platform for the development of new anthelmintic drugs. Subsequently, analogues 19, 21, 41, 42 (EC100 = 1.25 μM, all), and 39 (EC100 = 0.625 μM) were demonstrated to exhibit enhanced in vitro anthelmintic activity over the lead structure, with compound 39 also being shown to be active in vivo against Heligmosomoides polygyrus.


Carvacrol is a compound isolated from some essential oils. It has been reported to possess anthelmintic activity. Acetylation of this monoterpene has been proposed as a potential way to reduce the toxicity and enhance the pharmacological effects of carvacrol. This study aimed to evaluate the effect of carvacryl acetate (CA) using in vitro and in vivo assays with gastrointestinal nematodes of small ruminants. The egg hatching test (EHT), larval development test (LDT) and adult worm motility (AWM) assessment were conducted to evaluate the effect of the acetylated product and pure carvacrol on Haemonchus contortus eggs, larvae and adults. The structural changes induced in adult H. contortus were assessed using scanning electron microscopy (SEM). CA and carvacrol acute toxicity was evaluated in mice. Finally, the efficacy of 250 mg/kg CA and 2.5 mg/kg monepantel (positive control) were evaluated in 30 sheep naturally infected with gastrointestinal nematodes by the fecal egg count reduction test (FECRT). In vitro tests were analyzed by analysis of variance (ANOVA) followed by comparison with Tukey's test. The efficacy was
calculated by the Boot Street program using the arithmetic average. The number of eggs in feces (epg) of the groups were transformed to log (x+1) and subjected to ANOVA to compare differences among the groups by Tukey’s test. The level of significance was P < 0.05. CA and carvacrol inhibited larval hatching by 89.3 and 97.7% at doses of 8.0 and 1.0 mg/ml, respectively. At the concentration of 2 mg/ml, CA and carvacrol inhibited 100% of larval development. At a concentration of 200 μg/ml, CA and carvacrol inhibited the motility of adult worms by 100% and 58.3% at 24h post-exposure, respectively. CA caused cuticle and vulvar flap wrinkling and bubbles to emerge from the tegument. Carvacrol caused more discreet effects on the cuticle and vulvar flap. The LID10 and LD50 of CA were 566.7 mg/kg and 1544.5 mg/kg, respectively. The LD10 and LD50 of carvacrol were 546.8 mg/kg and 919 mg/kg, respectively. CA and monepantel reduced the epg of sheep by 65.9 and 96.4%, respectively, at 16 days post-treatment. CA showed in vitro and in vivo anthelmintic activity and was less toxic than carvacrol.


In Ontario, Canada, widespread resistance to ivermectin and fenbendazole, the only readily available ovine anthelmintics, has been documented, primarily in Haemonchus sp. In other parts of the world, closantel has been used to control such infections; however, the drug was not currently licensed for use in Canada and the USA. A randomized controlled trial was conducted on six client-owned farms in Ontario in 2013 and 2014 to determine the efficacy of closantel (Flukiver (R) 5% Oral Suspension, Elanco Animal Health, 10 mg/kg bodyweight) against ivermectin- and fenbendazole-resistant Haemonchus sp. infections in periparturient ewes and grazing lambs. Three farms were randomly assigned to treat all ewes, and three farms were randomly assigned to selectively treat individual ewes at lambing, using predetermined criteria. Fecal samples were collected from a minimum of 15 randomly selected ewes and 13 lambs per group on each farm at the time of treatment and approximately 14 days later. Trichostrongyle-type fecal egg counts (FEC) were performed using a modified McMaster technique with a lower detection limit of 8.3 eggs per gram of feces (epg). Haemonchus-specific FECs were determined by multiplying FECs by the proportion of Haemonchus sp. identified from coproculture for each farm; Haemonchus-specific FEC reductions were calculated for each farm. Twenty grazing lambs had FECs conducted monthly, and when mean monthly FECs surpassed 200 epg, all lambs were randomly allocated to either closantel, positive control (ivermectin, fenbendazole, or levamisole) or negative control groups. Pre-treatment Haemonchus-specific mean FECs ranged from 27 to 3359 epg in ewes and 0-5698 epg in lambs. Efficacy of closantel against Haemonchus sp. ranged from 99% (95% CI: 97%-99%) to 100% in recently lambed ewes on all farms in both years (total n = 274 ewes), and from 99% (95% CI: 98%-99%) to 100% in grazing lambs in both years on all but one farm (total n=171 lambs). On the latter farm, a whole flock treated farm, closantel efficacy in grazing lambs was 84% (95%CI: 81%-88%) in the first year, but 100% in the second year. Levamisole was effective against overall GIN in lambs on only two farms. Ivermectin and fenbendazole resistance continued to be present, particularly in Haemonchus sp. Closantel had excellent efficacy against Haemonchus sp. over the two year study period, regardless of treatment group, and therefore should be considered one viable component of sustainable integrated parasite control programs for farms with documented anthelmintic resistance and problems with haemonchosis.
Anthelmintic resistance


Ivermectin and moxidectin are the most widely administered anthelmintic macrocyclic lactones (MLs) to treat human and animal nematode infections. Their widespread and frequent use has led to a high level of resistance to these drugs. Although they have the same mode of action, differences in terms of selection for drug resistance have been reported. Our objective was to study and compare changes occurring upon ivermectin or moxidectin selection in the model nematode Caenorhabditis elegans. C. elegans worms were submitted to stepwise exposure to increasing doses of moxidectin. The sensitivity of moxidectin-selected worms to MLs was determined in a larval development assay and compared with those of wild-type and ivermectin-selected strains. Selection with either ivermectin or moxidectin led to acquired tolerance to ivermectin, moxidectin, and eprinomectin. Importantly, moxidectin was the most potent ML in both ivermectin- and moxidectin-selected strains. Interestingly, this order of potency was also observed in a resistant Haemonchus contortus isolate. In addition, ivermectin- and moxidectin-selected strains displayed constitutive overexpression of several genes involved in xenobiotic metabolism and transport. Moreover, verapamil potentiated sensitivity to ivermectin and moxidectin, demonstrating that ABC transporters play a role in ML sensitivity in ML-selected C. elegans strains. Finally, ivermectin- and moxidectin-selected strains displayed a dye-filling-defective phenotype. Overall, this work demonstrated that selection with ivermectin or moxidectin led to cross-resistance to several MLs in nematodes and that the induction of detoxification systems and defects in the integrity of amphidial neurons are two mechanisms that appear to affect the responsiveness of worms to both ivermectin and moxidectin.


Helminth parasites cause significant morbidity and mortality in endemic countries. Given the severity of symptoms that helminths may elicit in the host, intervention with prophylactic and therapeutic measures is imperative. Treatment with benzimidazoles is the most widely used means of combatting these parasites. However, widespread use of these drugs can select for drug-resistant parasite strains. In this review, we approach the problem of benzimidazole resistance in helminths in both humans and animals, focusing on the properties of the drug, the molecular mechanisms of drug resistance and how resistance is diagnosed.


Resistance to xenobiotics remains a pressing issue in parasite treatment and global agriculture. Multiple factors may affect the evolution of resistance, including interactions between life-history traits and the strength of selection imposed by different drug doses. We experimentally created replicate selection lines of free-living Caenorhabditis remanei exposed to Ivermectin at high and low doses to assess whether survivorship of lines selected in drug-treated environments increased, and if this varied with dose. Additionally, we maintained lines where mortality was imposed randomly to control for differences in density between drug treatments and to distinguish between the evolutionary consequences of drug-
treatment versus ecological processes due to changes in density-dependent feedback. After 10 generations, we exposed all of the selected lines to high-dose, low-dose and drug-free environments to evaluate evolutionary changes in survivorship as well as any costs to adaptation. Both adult and juvenile survival were measured to explore relationships between life-history stage, selection regime and survival. Intriguingly, both drug-selected and random-mortality lines showed an increase in survivorship when challenged with Ivermectin; the magnitude of this increase varied with the intensity of selection and life-history stage. Our results suggest that interactions between density-dependent processes and life history may mediate evolved changes in susceptibility to control measures.


Background Soil-transmitted helminths (STHs) are the most prevalent intestinal helminths of humans, and a major cause of morbidity in tropical and subtropical countries. The benzimidazole (BZ) drugs albendazole (ABZ) and mebendazole (MBZ) are used for treatment of human STH infections and this use is increasing dramatically with massive drug donations. Frequent and prolonged use of these drugs could lead to the emergence of anthelmintic resistance as has occurred in nematodes of livestock. Previous molecular assays for putative resistance mutations have been based mainly on PCR amplification and sequencing. However, these techniques are complicated and time consuming and not suitable for resource-constrained situations. A simple, rapid and sensitive genotyping method is required to monitor for possible developing resistance to BZ drugs. Methods To address this problem, single nucleotide polymorphism (SNP) detection assays were developed based on the Smart amplification method (SmartAmp2) to target codons 167, 198, and 200 in the beta-tubulin isotype 1 gene for the hookworm Necator americanus. Findings Diagnostic assays were developed and applied to analyze hookworm samples by both SmartAmp2 and conventional sequencing methods and the results showed high concordance. Additionally, fecal samples spiked with N. americanus larvae were assessed and the results showed that the Aac polymerase used has high tolerance to inhibitors in fecal samples. Conclusion The N. americanus SmartAmp2 SNP detection assay is a new genotyping tool that is rapid, sensitive, highly specific and efficient with the potential to be used as a field tool for monitoring SNPs associated with BZ resistance. However, further validation on large numbers of field samples is required.


Xenobiotic-metabolizing enzymes (XMEs) modulate the biological activity and behavior of many drugs, including anthelmintics. The effects of anthelmintics can often be abolished by XMEs when the drugs are metabolized to an inefficient compound. XMEs therefore play a significant role in anthelmintic efficacy. Moreover, differences in XMEs between helminths are reflected by differences in anthelmintic metabolism between target species. Taking advantage of the newly sequenced genomes of many helminth species, progress in this field has been remarkable. The present review collects up to date information regarding the most important XMEs (phase I and phase II biotransformation enzymes; efflux
transporters) in helminths. The participation of these XMEs in anthelmintic metabolism and their possible roles in drug resistance are evaluated.

**Anthelmintic resistance - cattle**


Six suspected cases of ivermectin resistance in Ostertagia spp. in cattle were investigated after routine anthelmintic efficacy testing on commercial farms. On four farms a comprehensive faecal egg count reduction test (FECRT) was undertaken using oral formulations of ivermectin (0.2 mg/kg), albendazole (10 mg/kg) and levamisole (7.5 mg/kg) while on two farms only ivermectin was tested. The proportions of Ostertagia spp. in the untreated control and post-treatment larval cultures were used to apportion egg counts to genera and determine efficacy against this genus. Isolates of Ostertagia spp. recovered from three of the farms were each used to infect 18 six month old calves. The efficacy of oral formulations of ivermectin and moxidectin, both at 0.2 mg/kg, was determined against each isolate by slaughter and worm count. The efficacy of ivermectin against Ostertagia spp., based on differentiated FECRT for each of the farms varied from 0% to 88%. The efficacy of ivermectin based on worm counts in the slaughter trial varied from 13% to 75% but moxidectin was >99% effective against all isolates. In addition, in the FECRT albendazole, at a dose rate of 10 mg/kg, failed to achieve 95% efficacy against Ostertagia spp. on two farms (82% and 85%). Levamisole consistently failed to achieve 95% efficacy against Ostertagia spp. which is consistent with its known lesser efficacy against this parasite. These results confirm the presence of macrocyclic lactone resistant *O. ostertagi* in cattle in New Zealand and the likely presence of dual resistance, to macrocyclic lactones and albendazole, in some isolates. Resistant populations of this highly pathogenic parasite are probably not uncommon in New Zealand and pose a significant threat to animal production and welfare in the future.


**Objective**

To report anthelmintic resistance in gastrointestinal nematode parasites of cattle on commercial dairy farms in the Macalister Irrigation District of Gippsland, Victoria. Methods Faecal egg count reduction tests (FECRTs) were used to assess anthelmintic resistance on 20 Macalister Irrigation District dairy farms between May 2013 and June 2014. All three currently available anthelmintic classes for cattle nematodes in Australia were tested. Faecal samples were collected 10-14 days post-treatment for individual faecal egg counts (FEC) and larval differentiation. The arithmetic mean FEC for each treatment group was compared with an untreated control post-treatment. Resistance was defined as <95% reduction in FEC, with a lower 95% confidence interval <90% when the mean FEC of the control group, differentiated by genus, was greater than 25 eggs/g. Results Anthelmintic resistance was present on all 20 dairy farms involved in this study. Resistance to doramectin in at least one species was detected on 15/20 (70%) farms, fenbendazole on 16/20 (80%) farms and levamisole on 5/20 (25%) farms. On three farms, resistance by *Ostertagia ostertagi* to all three anthelmintic classes was detected. Conclusion This is the first report of anthelmintic-resistant *O. ostertagi* on Australian dairy farms. Resistance to all three available anthelmintic classes is of concern, given the high
pathogenicity of this species. The study highlights the need for veterinarians and dairy farmers to be aware of the risks posed by anthelmintic resistance.


Gastrointestinal nematodes resistant to anthelmintics have been reported in several regions of Brazil, and they may be associated with economic losses for the cattle industry. This study aimed to evaluate the resistance status of gastrointestinal nematodes from naturally infected beef cattle to several commercially available anthelmintics, as well as to test the efficacy of combinations of anthelmintics against multi-resistant gastrointestinal nematodes. Ten farms located in Rio Grande do Sul state were selected by: farmers' consent; extensive raising system; availability of calves aged from 7 to 9 months naturally infected by gastrointestinal nematodes; absence of anthelmintic treatment for 60 days before the study; and presence of 70-100 calves or more of both genders with >= 200 eggs per gram of feces (EPG) (sensitivity of 50 EPG). These calves were distributed into 10 groups (of 7-10 animals) per farm and treated with ivermectin, doramectin, eprinomectin, fenbendazole, closantel, nitroxynil, disophenol, levamisole, albendazole, or moxidectin. Feces were collected 2 days before treatment and 14 days after treatment. Additional groups of 7-10 calves were used to test six different two-drug combinations at four of the studied farms. In general terms, fenbendazole was the most effective drug, followed by levamisole, disophenol, and moxidectin. However, parasite resistance to multiple drugs was found in all herds, especially in the genera Cooperia spp., Trichostrongylus spp., and Haemonchus spp.. Some of the two-drug combinations were effective against nematode populations identified as resistant to the same compounds when used as single drugs. The most effective combinations were moxidectin + levamisole, doramectin + fenbendazole, and levamisole + closantel. In this study, parasites resistant to the main commercially available anthelmintics were found in all herds, and some combinations of two active components belonging to different chemical groups were effective against multi-drug resistant gastrointestinal nematodes.

**Anthelmintic resistance - sheep**


This study determined the prevalence of anthelmintic resistance (AR) in parasitic nematodes on smallholder sheep farms in Lithuania from April to November 2014. Faecal samples were collected from two groups of 10-15 sheep treated with fenbendazole (FBZ) or ivermectin (IVM) on 18 sheep farms. Two samples were collected from each group: on day zero (T1) and 10-14 days after treatment. Faecal egg counts (eggs per gramme, EPG) were determined using a modified McMaster technique. Animals with <140 EPG on day zero were removed from the analysis. The prevalence of AR was estimated using the in vivo faecal egg count reduction test. AR to FBZ was detected on three of 15 farms where FBZ was used (20 %) and was suspected on one farm (6.7 %). AR to IVM was detected on two of 16 farms where IVM was used (12.5 %). The main species of resistant gastrointestinal nematodes (GINs) identified after treatment were Teladorsagia spp. and Trichostrongylus spp. A questionnaire surveying 71 sheep farmers estimated that
71.8% of sheep farmers used anthelmintics against GINs. IVM was the most frequently (68.6%) applied anthelmintic, and 62.7% of the respondents reported treating their animals twice a year. This study confirmed the presence of AR to GIN infections on sheep farms in Lithuania. Future studies should assess the prevalence of AR to GIN infection using in vitro methods.

Sales, N. and S. Love (2016). "Resistance of Haemonchus sp to monepantel and reduced efficacy of a derquantel/abamectin combination confirmed in sheep in NSW, Australia." *Veterinary Parasitology* **228**: 193-196.

Early in 2015, sheep in a summer rainfall area of NSW, Australia, displayed signs of haemonchosis despite treatment with monepantel. A faecal egg count reduction test (FECRT) was performed on yearlings with natural field infections using various anthelmintics. Only a four-way combination drench achieved a reduction in faecal egg count (FEC) greater than 95%. The combination contained abamectin, albendazole, levamisole and closantel. Treatments with a derquantel/abamectin combination, monepantel and moxidectin reduced FECs by 93, 31, and 30% respectively. Sheep treated with abamectin displayed an increase in FEC of 22%. Larval differentiation counts conducted 10 days post-treatment showed that 100% of survivors were Haemonchus sp. This result confirms for the first time monepantel resistant Haemonchus in sheep in NSW, and is amongst the first of the Australian cases in sheep not associated with goats. A second FECRT was performed using sheep from the moxidectin and abamectin treatment groups in the first FECRT. In this second FECRT, monepantel treatment reduced FECs by 51% and 29% in the sheep previously treated with moxidectin and abamectin respectively. This suggests monepantel, in combination with moxidectin, may give some control against severely abamectin resistant Haemonchus.


Despite strong economic opportunities and incentives for small ruminant production, their health and productivity are often severely affected by parasitic disease. To combat these effects, most farms administer anthelmintics to their animals at frequent intervals, and without consideration to principles of sustainable integrated parasite management (SIPM). This has led to growing problems caused by the development of drug-resistant populations of gastrointestinal nematodes (GIN) in much of the world, particularly in Haemonchus contortus. The objectives of this research were to characterize levels of anthelmintic resistance on small ruminant farms located in the mid-Atlantic US and to compare the faecal egg count reduction test (FECRT) and larval development assay (LDA) for detecting resistance. To achieve these objectives, the DrenchRite (R) LDA was used to evaluate resistance status to benzimidazoles, ivermectin, moxidectin, and levamisole on 20 goat and 14 sheep farms in the Mid-Atlantic US over a 2-year period. A FECRT was also conducted on 14 of the same farms and on 2 additional farms in which the LDA was not completed. For the LDA and coprocultures, fecal samples were collected rectally from a minimum of 10 individual animals, pooled, and express-mailed to the University of Georgia for analysis. For the FECRT, albendazole, ivermectin, moxidectin, and/or levamisole were tested on each farm. Animals were allocated randomly based on FAMACHA (c) scores to 2-5 treatment groups, which included an untreated control group. The number of treatment groups on a farm depended on the number of qualified animals present. Haemonchus contortus was the most

Background: There is some evidence that ATP binding cassette (ABC) transporters play a role in resistance to anthelmintics, particularly against macrocyclic lactones. Some anthelmintics, including ivermectin (IVM), have been shown to induce transcription of multiple ABC transporters in nematodes; however, the effects of monepantel (MPL) on transcription of these transporter genes has not been studied.

Methods: Larvae of two MPL-susceptible isolates of Haemonchus contortus were exposed to MPL at two concentrations (2.5 and 250 μg/ml) for periods of 3, 6 and 24 h. Transcription levels of sixteen ABC transporter genes were measured at the end of the incubation periods. The consequences of MPL exposure were examined by measuring rhodamine-123 efflux from the larvae, and their sensitivity to subsequent treatment with IVM or levamisole.

Results: Multiple ABC transporter genes showed significantly higher transcription in both worm isolates following exposure to MPL at 250 μg/ml for 3, 6 or 24 h, particularly the P-glycoprotein (P-gp) genes pgp-11, pgp-12 and pgp-14. Of these, only pgp-11 maintained the elevated levels 24 h after the end of the drug exposure period. In contrast, there was only a single instance of low-level upregulation as a result of exposure to MPL at 2.5 μg/ml. Larvae exposed to MPL at 250 μg/ml showed an increased efflux of rhodamine-123 and a proportion of the larval population showed an ability to subsequently tolerate higher concentrations of IVM in migration assays. There was no increased tolerance to IVM following pre-exposure to MPL at 2.5 μg/ml.

Conclusions: Exposure of H. contortus larvae to 250 μg/ml MPL results in increased transcription of multiple transporter genes and increased R-123 efflux. The subsequent ability of a proportion of the larvae to tolerate IVM suggests a protective role of ABC transporters across different chemical entities. However, these observations were only made at a concentration of MPL well above that experienced by parasitic life stages in vivo, and hence their significance remains unclear.


The in vitro ovicidal activity of the amino acetonitrile derivative, monepantel (MPTL) and its active metabolite monepantel sulfone (MPTL-SO2) were assessed against a number of commercially important nematode species of ruminants, namely Teladorsagia circumcincta, Haemonchus contortus and Trichostrongylus axei.
egg hatch test (EHT) was used to make the assessment of both drug sensitive and drug resistant isolates. Both MPTL and MPTL-SO2 showed moderate ovicidal activity in vitro against all of the species examined, although species specific differences as measured by inhibitory concentration were observed. Analysis of the drug sensitive isolates showed H. contortus to be the most sensitive to both MPTL and MPTL-SO2 (ED50 1.7 and 2.7 μg/ml respectively) followed by T. circumcincta (ED50 2.1 and 2.7 μg/ml respectively) followed by T. axei (ED50 68.7 and 60.1 μg/ml respectively). Overall the EHT results would suggest no "global" in vitro discriminatory dose for detection of MPTL resistance is likely to be achievable, using the egg hatch test, due to large inherent variability observed between species. The test identified a dose dependent increase in MPTL and MPTL-SO2 sensitivity in two MPTL resistant T. circumcincta isolates and therefore offers to be a promising tool for the phenotypic characterisation of MPTL sensitivity, allowing exploration into the mechanisms involved in selection and development of MPTL resistance.


Haemonchus contortus has shown a great ability to develop resistance to anthelmintic drugs. In many instances, resistance has appeared less than 10 years after the introduction of a new drug class. Field populations of this species now show resistance to all major anthelmintic drug classes, including benzimidazoles (BZs), imidazothiazoles and macrocyclic lactones. In addition, resistance to the recently introduced aminoacetonitrile derivative class (monepantel) has already been reported. The existence of field populations showing resistance to all three major drug classes, and the early appearance of resistance to monepantel, threatens the sustainability of sheep and goat production systems worldwide. This chapter reviews the history of the development of resistance to the various anthelmintics in H. contortus and examines the mechanisms utilized by this species to resist the effects of these drugs. Some of these mechanisms are well understood, particularly for BZ drugs, while our knowledge and understanding of others are increasing. Finally, we summarize methods available for the diagnosis of resistance. While such diagnosis currently relies largely on the faecal egg count reduction test, which suffers from issues of expense and sensitivity, we describe past and current efforts to utilize cheaper and less laborious phenotypic assays with free-living life stages, and then describe progress on the development of molecular assays to provide sensitive resistance-detection tests.


Sheep farmers in the UK rely on strategic anthelmintic use to treat and control gastrointestinal roundworms in their flocks. However, resistance to these drugs is now widespread and threatens the sustain ability of sheep production. The mechanisms underlying resistance to the most commonly used class, the macrocyclic lactones, are not known and sensitive diagnostic tools based on molecular markers are not currently available. This prohibits accurate surveillance of resistance or assessment of strategies aimed at controlling its spread. In this study, we examined four UK field populations of Haemonchus contortus, differing in macrocyclic lactone treatment history, for evidence of selection at 'candidate gene'
loki identified as determining macrocyclic lactone resistance in previously published research. Individual worms were genotyped at Hc-lgc-37, Hc-glc-5, Hc-avr-14 and Hc-dyf-7, and four microsatellite loci. High levels of polymorphism were identified at the first three candidate gene loci with remarkably little polymorphism at Hc-dyf-7. While some between-population comparisons of individual farms with and without long-term macrocyclic lactone use identified statistically significant differences in allele frequency and/or fixation index at the Hc-lgc-37, Hc-glc-5 or Hc-avr-14 loci, we found no consistent evidence of selection in other equivalent comparisons. While it is possible that different mechanisms are important in different populations or that resistance may be conferred by small changes at multiple loci, our findings suggest that these are unlikely to be major loci conferring macrocyclic lactone resistance on UK farms or suitable for diagnostic marker development. More powerful approaches, using genome-wide or whole genome sequencing, may be required to define macrocyclic lactone resistance loci in such genetically variable populations.


Resistance to benzimidazoles (BZs) in trichostrongyloid nematodes is a worldwide problem for livestock production, particularly regarding small ruminants. Sensitive and reliable methods are required to assess anthelmintic resistance status. Currently available methods for BZ resistance detection can be divided into three main groups, in vivo (e.g. faecal egg count reduction test), in vitro (e.g. egg hatch assay) and molecular tests. Three single nucleotide polymorphisms (SNPs) in the isotype-1 beta-tubulin gene of various nematode species correlate with BZ resistance. While PCR-based methods have been reported for the three most economically important nematodes of sheep, namely, Trichostrongylus, Haemonchus and Teladorsagia, pyrosequencing assays are so far only available for the latter two. Here, the design and evaluation of pyrosequencing assays for isotype-1 and isotype-2 beta-tubulin genes of Trichostrongylus colubriformis are described. PCR fragments carrying the susceptible and corresponding resistant genotype were combined in defined ratios to evaluate assay sensitivity and linearity. The correlation between the given and the measured allele frequencies of the respective SNPs (codons F167Y, E198A and F200Y) was very high. Pyrosequencing assays for Haemonchus, Teladorsagia and Trichostrongylus were subsequently used for a BZ resistance survey, carried out in the three European countries, namely Ireland, Italy and Switzerland. Larval cultures obtained from field survey samples in 2012 and 2013 were used for pyrosequencing. The test was applied when the target species represented at least 10% of the sample. Trichostrongylus and Teladorsagia were detected in all countries’ samples whereas Haemonchus was not detected in samples from Ireland. SNPs in isotype-1 associated with resistance were detected for all three species, with frequencies at codon F200Y far exceeding those at codons F167Y and E198A. Elevated SNP frequencies in isotype-2 of Trichostrongylus were only rarely detected. Farms with BZ resistance-associated SNP frequencies above 10% were most often found in Switzerland followed by Ireland and Italy.

Background: The existence of nematodes of veterinary importance such as Haemonchus contortus resistant to anthelmintic drugs, including the macrocyclic lactones, has become a major concern in animal health. Macrocyclic lactone resistance in H. contortus seems to be multigenic including the active efflux of these drugs by P-glycoproteins, members of the ABC transporter family, present in this parasite. The goals of the present work were to determine the activity of H. contortus P-glycoprotein 9.1 (Hco-PGP-9.1) and its interaction with the avermectins, ivermectin, abamectin, and also the milbemycin, moxidectin. Additionally, the localisation of Hco-PGP-9.1 was sought in adult worms. Methods: Hco-Pgp-9.1 was cloned and expressed in mammalian cells and its expression profile was determined at the transcriptional and protein level by qRT-PCR and Western-blot, respectively. The nematode transport activity was assessed using the tracer dye Rhodamine 123. A ligand competition assay between different macrocyclic lactones and Rhodamine 123 was used to establish whether or not there was interaction between Hco-PGP-9.1 and the avermectins (abamectin and ivermectin) or moxidectin. In addition, immunostaining was carried out to localise Hco-PGP-9.1 expression in the transgenic cells and in adult female parasites. Results: Hco-PGP-9.1 was expressed in the cell membrane of the transfected host cells and was able to extrude Rhodamine 123. Ivermectin and abamectin, but not moxidectin, had a pronounced inhibitory effect on the ability of Hco-PGP-9.1 to transport Rhodamine 123. Antibodies raised against Hco-PGP-9.1 epitopes localised to the uterus of adult female H. contortus. Conclusions: These results suggest a strong interaction of the avermectins with Hco-PGP-9.1. However, possibly due to its physicochemical properties, moxidectin had markedly less effect on Hco-PGP-9.1. Because of the greater interaction of the avermectins than moxidectin with this transporter, it is more likely to contribute to avermectin resistance than to moxidectin resistance in H. contortus. Possible over expression of Hco-PGP-9.1 in the female reproductive system in resistant worms could reduce paralysis of the uterus by macrocyclic lactones, allowing continued egg release in drug challenged resistant worms.


Gastrointestinal parasitic nematodes in sheep cause severe economic losses. Anthelmintics are the most commonly used drugs for prophylaxis and therapy against parasitic helminths. The problem of drug resistance has developed for all commercially available anthelmintics in several genera and classes of helminths. In vitro and in vivo tests are used to detect anthelmintic resistance. Two in vitro methods (larval migration inhibition test and micromotility test) for the detection of ivermectin (IVM) resistance were compared using IVM-resistant and IVM-susceptible isolates of Haemonchus contortus. The degree of resistance for each test was expressed as a resistance factor (RF). The micromotility test was more sensitive for quantitatively measuring the degree of resistance between susceptible and resistant isolates. The RFs for this test for IVM and eprinomectin ranged from 1.00 to 108.05 and from 3.87 to 32.32, respectively.


The epidemiology of nematode parasites has changed as they have adapted to climatic and management changes and as a consequence of the inappropriate use of anthelmintic drugs. This adaptability is conferred by large, polymorphic genothes.
and r-reproductive strategies. A significant net effect of these factors has been the emergence of anthelmintic resistance. Consequently, blueprint control programmes have failed and suboptimal sheep productivity due to nematode parasites has become commonplace. The focus of veterinary nematode control in intensively managed sheep flocks and goat herds must shift from attempts to eliminate parasite populations, towards the adoption of management and anthelmintic drug treatment strategies aimed at maintaining adequate standards of health in the face of a low level of challenge. Conventional parasitological methods are useful for the diagnosis of disease and for monitoring of nematode management over time, but they lack the sensitivity needed to mitigate effects of climate and management on population genetics of the parasites. The publication of a draft genome and transcriptome for the model nematode parasite, Haemonchus contortus, affords opportunities for post genomic research to identify sensitive molecular markers to evaluate resistance management strategies and potential candidates for novel control methods.

Raza, A., et al. (2016). "Larval development assays reveal the presence of sub-populations showing high- and low-level resistance in a monepantel (Zolvix (R))-resistant isolate of Haemonchus contortus." Veterinary Parasitology 220: 77-82.

Resistance to the amino-acetonitrile derivative monepantel has been reported in several species of gastrointestinal nematodes over recent years. We were interested in the use of in vitro assays with free-living worm life-stages to detect resistance to this drug. We therefore used larval development and larval migration assays to examine dose response relationships for the drug against two susceptible and one resistant isolate of Haemonchus contortus. The resistant isolate was established by laboratory propagation of the survivors of a field treatment with Zolvix (R) that had originally resulted in a drug efficacy of over 99%. Drug efficacy against this field derived laboratory-propagated resistant isolate in vivo was approximately 15%. The larval development assay proved able to discriminate between the susceptible and resistant isolates, with larvae of the resistant isolate showing an ability to develop at higher drug concentrations than the two susceptible isolates. The resistant isolate showed the presence of two distinct subpopulations, separated by a plateau in the dose-response curve. Sub-population 1 (approximately 40% of the total population) showed a low level of resistance with an IC50 increased, approximately 7-fold compared to the baseline susceptible isolate, while sub-population 2 (the remaining 60% of the total population) showed an IC50 increased over 1000-fold compared to the baseline susceptible isolate. This level of resistance is unusually high for any gastrointestinal nematode species in drug dose-response in vitro assays. In contrast, the migration assay could not discriminate between the three isolates, with migration not reduced to zero at any of the drug concentrations tested. This study demonstrates that a larval development assay is able to detect resistance to monepantel in H. contortus, and that resistance can exist in two distinct forms. This suggests that at least two separate monepantel resistance mechanisms are acting within the worm isolate studied here, with one or more mechanisms conferring a much higher level of resistance than the other(s).


Haemonchus contortus is one of the most important parasitic nematodes of small ruminants around the world, particularly in tropical and subtropical regions. The
control of haemonchosis relies mainly on anthelmintics, but the excessive and prolonged use of anthelmintics is causing serious drug resistance issues in many countries. As benzimidazole (BZ) anthelmintics have been broadly used in China, we hypothesized that resistance is widespread. Given the link between three known single nucleotide polymorphisms (SNPs, designated F167Y, E198A and F200Y) in the isotype-1 beta-tubulin gene and BZ resistance, our goal here was to explore the presence of these mutations in H. contortus from small ruminants (sheep and goats) from eight provinces in China using PCR-coupled sequencing. In addition, the genetic diversity and genetic relationship of isotype-1 beta-tubulin sequence haplotypes were also investigated. Among 192 H. contortus adult individuals representing the eight populations, we identified six distinct sequence types, five of which had SNP E198A (GCA) and/or F200Y (TAC). Sequence analysis showed that the frequencies of SNPs E198A and F200Y were 0-70% and 0-31%, respectively. SNP F167Y (TAC) was not detected in any population. In addition, high haplotype diversities (0.455-0.939) and nucleotide diversities (0.018-0.039) were calculated. A network analysis of the isotype-1 beta-tubulin gene sequences showed that SNPs E198A and F200Y occurred in multiple distinct groupings, suggesting multiple independent origins of these SNPs. The findings of this first study of SNPs in the isotype-1 beta-tubulin gene of H. contortus populations suggest that BZ resistance is prevalent in some regions of China, and that any control strategy might focus on monitoring BZ resistance in this country.


The intensive use of anthelmintics for treating and control gastrointestinal strongyles affecting sheep has caused a global spread of parasite populations that are resistant to one or more parasiticide classes. This phenomenon, which represents a severe problem in the southern hemisphere, is recently becoming a threat also in Europe. In fact, the occurrence and possible spread of sheep strongyle populations resistant to benzimidazoles and tetrahydropyrimidines-imidazothiazoles and, with a lesser extent, to macrocyclic lactones has been recorded for the most important nematodes parasitizing sheep, i.e., Teladorsagia circumcincta, Haemonchus contortus and Trichostrongylus spp. in various countries. Of higher concern are also the recent descriptions of strongyle populations that are capable to survive to more than one anthelmintic class. These findings indicate that control programs based only on the use of anthelmintics are outdated and that novel and sustainable approaches, relying on a combination of integrated measures, are warranted, especially for preserving the still-effective molecules. The aim of the present article is to review the state-of-the-art of drug resistance in sheep strongyles in Europe, with a focus on current scenarios and measures necessary to limit the further expansion of drug resistance in sheep flocks.


This study utilised computer simulation modelling (Risk Management Model for Nematodes) to investigate the impact of different parasite refugia scenarios on the development of anthelmintic resistance and worm control effectiveness. The simulations were conducted for adult ewe flocks in a Mediterranean climatic region over a 20 year time period. Factors explored in the simulation exercise were...
environment (different weather conditions), drug efficacy, the percentage of the flock left untreated, the timing of anthelmintic treatments, the initial worm egg count, and the number of drenches per annum. The model was run with variable proportions of the flock untreated (0, 10, 20, 30, 40 and 50%), with ewes selected at random so that reductions in the mean worm burden or egg count were proportional to the treated section of the flock. Treatments to ewes were given either in summer (December; low refugia potential, hence highly selective) or autumn (March; less selective due to a greater refugia potential), and the use of different anthelmintics was simulated to indicate the difference between active ingredients of different efficacy. Each model scenario was run for two environments, specifically a lower rainfall area (more selective) and a higher rainfall area (less selective) within a Mediterranean climatic zone, characterised by hot, dry summers and cool, wet winters. Univariate general linear models with least square difference post hoc tests were used to examine differences between means of factors. The results confirmed that leaving a proportion of sheep in a flock untreated was effective in delaying the development of anthelmintic resistance, with as low as 10% of a flock untreated sufficient to significantly delay resistance, although this strategy was associated with a small reduction in worm control. Administering anthelmintics in autumn rather than summer was also effective in delaying the development of anthelmintic resistance in the lower rainfall environment where all sheep were treated, although the effect of treatment timing on worm control effectiveness varied between the environments and the proportion of ewes left untreated. The use of anthelmintics with higher efficacy delayed the development of resistance, but the initial worm egg count or number of annual treatments had no effect on either the time to resistance development or worm control effectiveness. In conclusion, the modelling study suggests that leaving a small proportion of ewes untreated, or changing the time of treatment, can delay the onset of anthelmintic resistance in a highly selective environment.


Resistance to ivermectin and moxidectin was explored by a faecal egg count reduction test in two sheep flocks with suspected anthelmintic resistance. The FECRT confirmed one suspicion, with a mean percentage of reduction in egg excretion within the treated groups of 0% for ivermectin (CI 95%: -228 to 58) and 13% for moxidectin (CI 95%: -152 to 70). This was further explored by a controlled efficacy test. An experimental infection of 18 naive lambs was set up using infective larvae isolated from this flock (5000 L3/lamb). Compared to the control group, abomasal worm burdens (Teladorsagia circumcincta) were reduced by 90% [CI 95%: 81.5-94.8] and 85% [CI 95%: 72.4-92.2] after ivermectin (p < 0.05) and moxidectin (p < 0.05) treatment respectively. Again, compared to the control group, there was a reduction for intestinal strongyles (Trichostrongylus colubriformis) of 100% and 99% [CI 95%: 97.5-99.7] for ivermectin and moxidectin respectively. No difference was found between the efficacy of moxidectin and ivermectin. Pharmacokinetic values indicated that the strongyles were submitted to anthelmintic concentrations usually lethal to them. This trial demonstrated the first multiple resistance of ovine strongyles in France.

The widespread development of anthelmintic resistance is a major global issue affecting the effective control of parasite diseases in grazing livestock production. Sustainable control strategies that reduce dependence on antimicrobials have the potential to slow the further development of resistance but there is little data on the effect of control strategies on resistance development in the field. This report documents a study undertaken to measure the temporal effect of the UK sustainable control of parasites in sheep (SCOPS) guidelines on the development of anthelmintic resistance. Farms carrying out SCOPS or traditional worm control (TRADITIONAL) were tested for resistance to the benzimidazole and imidazothiazole anthelmintics in vitro using a discriminating dose (dd) larval development test (LDT) in year 1 and then 7 years later. In years 5 and 7, resistance was also measured using a dose-response LDT assay. There was a significant increase in Teladorsagia survivors at the benzimidazole dd assay between year 1 and year 7 for both treatment groups, but the increase in survivors was greater for the farms carrying out their traditional worm control compared to the SCOPS farms. There was also a significant difference between benzimidazole dd results generated across years for Trichostrongylus, but the year and treatment interaction was not significant. Only one of the farm Teladorsagia populations had survivors in the imidazothiazole dd assay in years 1 and 7 and none of the Trichostrongylus populations survived in year 1 compared to isolates from three of the farms in year 7. Dose-response data showed a significant effect for time for both the benzimidazole and imidazothiazole anthelmintics and the increase was again significantly higher for the Teladorsagia populations in the TRADITIONAL group compared to the SCOPS group. This data suggests an increased sensitivity both to detect and to measure changes in response to anthelmintics with the dose-response assay compared to the dd and this is important particularly when allele frequencies are low as might be the case when novel compounds are released to the market. Anthelmintic use across years 5-7 was significantly lower for the farms in the SCOPS group compared to the TRADITIONAL group and farmers in the SCOPS group had selected products from the benzimidazole group less often than farmers in the TRADITIONAL group. Both groups had made minimal use of the imidazothiazole anthelmintic classes and the majority of ewe treatments were selected from the macrocyclic lactone class. Further research is required to determine the effect of these anthelmintic choices on the development of resistance to the macrocyclic lactones.


Haemonchus contortus is the leading parasitic nematode species used to study anthelmintic drug resistance. A variety of candidate loci have been implicated as being associated with ivermectin resistance in this parasite but definitive evidence of their importance is still lacking. We have previously performed two independent serial backcross experiments to introgress ivermectin resistance loci from two H. contortus ivermectin-resistant strains - MHco4(WRS) and MHcol 0(CAVR) - into the genetic background of the ivermectin-susceptible genome reference strain MHco3(ISE). We have interrogated a number of candidate ivermectin resistance loci in the resulting backcross populations and assessed the evidence for their genetic linkage to an ivermectin resistance locus. These include the microsatellite marker Hcms8a20 and six candidate genes Hco-glc-5, Hco-avr-14, Hco-lgc-37 (previously designated Hco-hg-1), Hco-pgp-9 (previously designated Hco-pgp-1),...
Hco-pgp-2 and Hco-dyf-7. We have sampled the haplotype diversity of amplicon markers within, or adjacent to, each of these loci in the parental strains and fourth generation backcross populations to assess the evidence for haplotype introgression from the resistant parental strain into the genomic background of the susceptible parental strain in each backcross. The microsatellite Hcms8a20 locus showed strong evidence of such introgression in both independent backcrosses, suggesting it is linked to an important ivermectin resistance mutation in both the MHco4(WRS) and MHcol 0(CAVR) strains. In contrast, Hco-glc-5, Hco-avr-14, Hco-pgp-9 and Hco-dyf-7 showed no evidence of introgression in either backcross. Hco-lgc-37 and Hco-pgp-2 showed only weak evidence of introgression in the MHco3/4 backcross but not in the MHco3/10 backcross. Overall, these results suggest that microsatellite marker Hcms8a20, but not the other candidate genes tested, is linked to a major ivermectin resistance locus in the MHco4(WRS) and MHcol 0(CAVR) strains. This work also emphasises the need for genome-wide approaches to identify mutations responsible for the ivermectin resistance in this parasite.

**Dictyocaulus viviparus**


The objective of this study was to conduct a comprehensive field survey using a Dictyocaulus viviparus major sperm protein ELISA on bulk tank milk samples from Belgian dairy herds to gain insights in: (1) the sensitivity (Se) and specificity (Sp) of the test under field conditions; (2) the value of the test to predict a future clinical lungworm outbreak; (3) its associations with milk production parameters and (4) its associations with herd management factors. A total of 1248 herds were sampled, with samplings occurring in the middle ("August") and towards the end ("October") of the grazing season. A completed questionnaire on potential risk factors and potentially lungworm-induced clinical signs was obtained from 587 farms and milk production records could be obtained from 343 herds. The median (25th-75th percentile) D. viviparus antibody level (ODR) was 0.25 (0.19-0.31) in "August" and 0.24 (0.19-0.32) in "October". At a threshold of 0.41 ODR, the Se and Sp were estimated using mixture models at 50 and 99%, respectively. At the same threshold, the positive and negative predictive value of the ELISA applied in "August" on the occurrence of farmer-reported lungworm symptoms in the period August-November were 65% and 69%, respectively. D. viviparus antibody levels were significantly higher in the north vs. the south of the country, in large herds and in herds that did not mow pastures or that frequently purchased new animals. An increase in the ELISA result of "August" over the interquartile range was associated with a drop in the annual average milk yield, milk protein% and milk fat% of -0.50 kg cow(-1) day(-1), 0.02 and 0.02, respectively. The relationships between the ELISA results in "October" and milk production parameters were also negative, but lower and non- or only marginally significant. We conclude that the bulk tank milk ELISA has a low value to predict lungworm disease on an individual farm based on a fixed sampling date in the middle of the grazing season. On the other hand, the test has been potential to detect subclinical production impacts and study risk factors through epidemiological surveys.

The bovine lungworm, Dictyocaulus viviparus (order Strongylida), is an important parasite of livestock that causes substantial economic and production losses worldwide. Here we report the draft genome, variome, and developmental transcriptome of D. viviparus. The genome (161 Mb) is smaller than those of related bursate nematodes and encodes fewer proteins (14,171 total). In the first genome-wide assessment of genomic variation in any parasitic nematode, we found a high degree of sequence variability in proteins predicted to be involved host-parasite interactions. Next, we used extensive RNA sequence data to track gene transcription across the life cycle of D. viviparus, and identified genes that might be important in nematode development and parasitism. Finally, we predicted genes that could be vital in host-parasite interactions, genes that could serve as drug targets, and putative RNAi effectors with a view to developing functional genomic tools. This extensive, well-curated dataset should provide a basis for developing new anthelmintics, vaccines, and improved diagnostic tests and serve as a platform for future investigations of drug resistance and epidemiology of the bovine lungworm and related nematodes.


It describe the clinical case of a possible resistance to fenbendazole in a Jersey calf infested by Dictyocaulus viviparous described. In the municipality of Enterrrios (Antioquia, Colombia) a female Jersey breed of 6 months of age, presented serious sings of bronchopneumonia. which did not improve with the application of fenbendazole and enrofloxacin as maintenance therapy. At the necropsy, a large number of live parasites compatible with Dictyocaulus viviparous were observed, determining a verminous bronchitis. This nematode, which is routinely treated with fenbendazole in Colombian dairy farms, has been proved resistance when is trated with some benzimidazoles. This specific case shows the possible causes of the Dictyocaulus viviparous resistance in Jersey calf.

Cestodes


Praziquantel (PZQ) is the treatment of choice against various trematode and cestode infections. To study the pharmacokinetics of PZQ in patients infected with the liver fluke Opisthorchis viverrini, we developed and validated an enantioselective liquid chromatography coupled to tandem mass spectrometry method for the analysis of R- and S-PZQ and its R-trans-4-OH-PZQ metabolite in human plasma, blood and dried blood spots (DBS). The analytes were detected in the positive mode using selected reaction monitoring (R- and S-PZQ: m/z 312.2 -> 202.2; R-trans-4-OH-PZQ: m/z 328.0 -> 202.0). Prior to the chiral separation with a cellulose tris(3-chloro-4-methylphenylcarbamate) column, the analytes were purified from matrix contaminants and concentrated on a C-18 trapping column. The
The analytical range for each PZQ enantiomer was 0.01-2.5 μg/mL, and 0.1-25 μg/mL for the metabolite. The method met the requirements regarding precision (+/-15%, +/- 20% at the lower limit of quantification-LLOQ), intra- and inter-assay accuracy (85-115%, 80-120% at LLOQ), and linearity (R^2 >= 0.998). The analytes were stable in stock solutions as well as in plasma, blood and DBS. For DBS, the influences of hematocrit and blood spot size were considered as minor. Our validation results show that the method presented here is precise, accurate and selective, and can be used for pharmacokinetic studies. Moreover, the enantioselective separation was achieved with a run time of 11.5 min and a simple sample processing method.


Reliable and rapid molecular tools for the genetic identification and differentiation of Echinococcus species and/or genotypes are crucial for studying spatial and temporal transmission dynamics. Here, we describe a novel dual PCR targeting regions in the small (rrnS) and large (rrnL) subunits of mitochondrial ribosomal RNA (rRNA) genes, which enables (i) the specific identification of species and genotypes of Echinococcus (rrnS + L-PCR) and/or (ii) the identification of a range of taeniid cestodes, including different species of Echinococcus, Taenia and some others (17 species of diphylloidean helminths). This dual PCR approach was highly sensitive, with an analytical detection limit of 1 pg for genomic DNA of Echinococcus. Using concatenated sequence data derived from the two gene markers (1225 bp), we identified five unique and geographically informative single nucleotide polymorphisms (SNPs) that allowed genotypes (G1 and G3) of Echinococcus granulosus sensu stricto to be distinguished, and 25 SNPs that allowed differentiation within Echinococcus canadensis (G6/7/8/10). In conclusion, we propose that this dual PCR-based sequencing approach can be used for molecular epidemiological studies of Echinococcus and other taeniid cestodes.


Cestodes or tapeworms belong to a diverse group of helminths. The adult Taenia saginata and Taenia solium tapeworm can infest the human gut and the larval stage of Echinococcus spp. and T. solium can infect tissues of the human body, causing serious disease. Molecular diagnostics can be performed on proglottids, eggs and on cyst fluids taken by biopsy. Detection of cestodes when a helminthic infection is suspected is of vital importance and species determination is required for appropriate patient care. For routine diagnostics a single test that is able to detect and type a range of cestodes is preferable. We sought to improve our diagnostic procedure that used to rely on PCR and subsequent sequencing of the Cox1 and Nad1 genes. We have compared these PCRs with novel PCRs on the 12S rRNA and Nad5 gene and established the sensitivity and specificity. A single PCR on the 125 gene proved to be very suitable for detection and specification of Taenia sp. and Echinococcus sp. Both targets harbour enough polymorphic sites to determine the various Echinococcus species. The 12S PCR was most sensitive of all tested.
Taenia saginata


The monoclonal antibody-based circulating antigen detecting ELISA (B158/1360 Ag-ELISA) has been used elaborately in several studies for the diagnosis of human, bovine and porcine cysticercosis. Interpretation of test results requires a good knowledge of the test characteristics, including the repeatability and the effect of the borders of the ELISA plates. Repeatability was tested for 4 antigen-negative and 5 antigen-positive reference bovine serum samples by calculating the Percentage Coefficient of Variation (%CV) within and between plates, within and between runs, overall, for two batches of monoclonal antibodies and by 2 laboratory technicians. All CV values obtained were below 20% (except one: 24.45%), which indicates a good repeatability and a negligible technician error. The value of 24.45% for indicating the variability between batches of monoclonal antibodies for one positive sample is still acceptable for repeatability measures. Border effects were determined by calculating the %CV values between the inner and outer wells of one plate for 2 positive serum samples. Variability is a little more present in the outer wells but this effect is very small and no significant border effect was found.


Cysticercosis is an infection of muscles in cattle and buffaloes, which occurs due to the larval stage of Taenia saginata. Cysticercosis has worldwide distribution but is more common in developing countries due to poor hygienic measures. Hematological and biochemical tests may be used as alternate for the diagnosis of cysticercosis within the local setting. The present study was aimed to investigate hematological and serum biochemical changes in cysticercosis infested cattle and buffaloes. For this purpose, 60 cysticercosis seropositive animals (n=30 buffaloes; n=30 cattle) and 60 cysticercosis seronegative animals (n=30 buffaloes, n=30 cattle) based on ELISA were used. These animals were selected from private abattoir from November 2012 to October 2013. Blood and serum sample were collected from these animals and were tested for hemogram and serum biochemical profile using automated hematology and biochemistry analyzers, respectively. The results showed a significant (P<0.05) decrease in hemoglobin and platelet count while other hematological parameters like RBCs, WBCs, MCV, MCH and MCHC remained non-significant (P>0.05). Among serum biochemistry characters, minerals and glucose did not differ significantly (P>0.05). Significant decrease (P<0.05) was observed for total serum protein, cholesterol, and aspartate transaminase enzyme in infested animals. It was concluded that cysticercosis has deleterious effects on hemogram and serum biochemical profile of cattle and buffaloes.


Background Bovine Taenia saginata cysticercus infections (also called bovine cysticercosis or beef measles) is usually diagnosed in cattle only during post-mortem meat inspection. The aim of this study was to investigate the identification rates of these infections in and to identify predictors/determinants of variations in the identification rates in abattoirs in Gauteng province, South Africa. Methods
Retrospective data for over 1.4 million cattle carcasses inspected in 26 abattoirs between January 2010 and December 2013 were used for the study. The identification rates (proportion of bovine Taenia saginata cysticercus positive carcasses) were computed and generalized estimating equations used to identify predictors/determinants of identification rates. Results The overall identification rate was 0.70% (95% CI: 0.45, 0.95). Significantly (p< 0.05) lower rates were reported during summer (0.55%) than other seasons. Some geographic areas reported significantly (p<0.05) higher rates than others. The identification rates in high throughput abattoirs was significantly (p<0.05) higher (RR: 9.4; 95% CI: 4.7-19.1) than in low throughput abattoirs. Similarly, the identification rates among animals from feedlots were significantly (p<0.05) higher (RR: 1.6; 95% CI: 1.7-3.5) than those from non-feedlot sources. No significant (p>0.05) association was identified between identification rates and either the number of meat inspectors per abattoir or the provider of inspection services. Conclusion Although no significant association was found between identification rates and provider of inspection services, follow-up studies will need to be done to specifically investigate the potential conflict of interest arising from the fact that abattoir owners hire meat inspection services directly. Capture of abattoir surveillance data needs to include farm address and for each case to be reported separately. Finally, information on the type of identified cysts (alive or calcified) need to be collected to help better estimate risk to consumers. This study provides useful baseline data to guide future studies, surveillance and control efforts.


Bovine cysticercosis is a zoonotic infection widely spread throughout Brazil, creating a burden on hygiene maintenance and the economy. Diagnosis of cysticercosis usually relies on post mortem inspection of carcasses in slaughterhouses. This detection method provides only low sensitivity. Recent advancements have improved the performance of serologic tests, such as ELISA, providing greater sensitivity and specificity. The objective of the current study was to identify and evaluate a synthetic peptide derived from the Taenia saginata 18 kDa oncospheric surface protein for the diagnosis of bovine cysticercosis in ELISA. Test performance of the identified peptide was compared to an ELISA based on a heterologous crude Taenia crassiceps antigen (Tcra), widely used for the serodiagnosis of bovine cysticercosis. Based on the primary sequence of an in silico structural model of the 18 kDa protein, an epitope region designated EP1 was selected (46-WDTKDMAGYGVKKIEV-61). The peptide derived from this region yielded 91.6% (CI = 80-96%) sensitivity and 90% (CI = 82-95%) specificity when used in an ELISA, whereas the crude antigen yielded 70% (CI = 56-89%) sensitivity and 82% (CI = 73-89%) specificity. Thus, we conclude that EP1 has higher diagnostic potential for detecting bovine cysticercosis than the crude antigen Tcra.


Bovine cysticercosis is a zoonotic parasitic infection caused by the larval stage (Cysticercus bovis) of Taenia saginata. This study aimed to identify prevention and control measures for bovine cysticercosis indicated by experts in the fields of public and animal health. The study was conducted through three rounds of the Delphi method and had the participation of 44 experts from 13 Brazilian states in 30
educational institutions, research institutions, and sanitary inspection services. The first round comprised an open question, the answers to which formed the basis for structuring the second and third questionnaire. To reach a consensus in the second and third rounds, experts were asked to express their agreement or disagreement of each proposition on the questionnaire using a five-point Likert scale. A descriptive statistical analysis was performed at the end of each round. Twenty-three specialists (52.27%) participated in all three rounds. The first round resulted in 28 proposals categorized into six areas: health education, methods of diagnosis and treatment, sanitation measures, epidemiological studies, legislation and sanitary supervision, and intersectorality. In the second round, the experts reached a consensus on 16 propositions (57.14%). This percentage increased by 3.54% during the third round. At the end of the third round, the experts had reached a consensus on 17 of the 28 initial propositions (60.71%). The highest percentage of agreement (29.4%) was observed in the category of health education. This study allowed us to identify 17 recommendations pertaining to the prevention and control of bovine cysticercosis. These measures are not mutually exclusive, and require an integrated approach to the establishment of intervention actions at various points in the life cycle of the parasite.


Bovine cysticercosis is caused by Taenia saginata cysticercus, the larval stage of the human tapeworm Taenia saginata. Recent European initiatives have highlighted the poor sensitivity of current surveillance for this parasite in cattle at slaughter; calling for more targeted, risk based and cost effective methods of T. saginata cysticercus detection. The aim of this study was to provide evidence that could inform such improved meat inspection activities in the United Kingdom (UK). The study included three components: (i) a farm-level case control study; (ii) the characterization of the network of movements of T. saginata cysticercus infected and non-infected animals, and an assessment of the strength of association between having passed through a farm that had previously originated an infected animal and the risk of infection; (iii) the assessment of the relationship between bovine age and gender and risk of infection. Abattoir records and cattle movement history data were used to identify farms of likely acquisition of infection (case farms) and a suitable control group. A questionnaire was used to gather farm-level characteristics and logistic regression was carried out to identify farm-level risk factors for the production of cattle found to be infected at slaughter. The case-control study provided evidence that farms situated close to a permanent potential source of human faecal contamination, and farms which used manure from animals other than cattle, were at higher risk of producing cattle later found to be infected with T. saginata cysticercus at slaughter. No other farm characteristics were identified as a risk factor for this. Analysis of the networks of animal movements showed that some individual farms played a key role as a source of T. saginata cysticercus infection; it was estimated that cattle with a history of being on a farm which previously appeared in the movement history of an infected animal were 4.27 times (P < 0.001; 95% CI: 3.3-5.52) more likely to be diagnosed with T. saginata cysticercus infection at meat inspection. Male cattle aged 20 months or younger at the time of slaughter were found at lower risk of T. saginata cysticercus infection by comparison to other sex or age groups of cattle. These results, in combination with the consultation of experts and stakeholders, led to the conclusion that abattoir-based surveillance in low T. saginata cysticercus prevalence settings, such as
Great Britain, could be made more targeted by stratifying cattle based on their individual movement history, sex and age characteristics.

**Taenia hydatigena**


An acute outbreak of Taenia hydatigena cysticercosis, causing mortality in 5 of 21 (23.8%) female lambs, is reported. Gross post-mortem examinations and histology showed Cysticercus tenuicollis as the cause of death. Biochemical parameters in infected lambs confirmed severe hepatitis. Praziquantel, given once at 15mg/kg body weight (bw), was administered and a dramatic improvement in the clinical condition and biochemical parameters was observed up to 30 days following treatment.

**Taenia multiceps/ C cerebralis**


This study was designed to genetically characterise the larval stage (coenurus) of Taenia multiceps from ruminants in Greece, utilising DNA regions within the cytochrome c oxidase subunit 1 (partial cox1) and NADH dehydrogenase 1 (pnad1) mitochondrial (mt) genes, respectively. A molecular-phylogenetic approach was used to analyse the pccox1 and pnad1 amplicons derived from genomic DNA samples from individual cysts (n =105) from cattle (n=3), goats (n=5) and sheep (n=97). Results revealed five and six distinct electrophoretic profiles for pccox1 and pnad1, respectively, using single-strand conformation polymorphism. Direct sequencing of selected amplicons representing each of these profiles defined five haplotypes each for pccox1 and pnad1, among all 105 isolates. Phylogenetic analysis of individual sequence data for each locus, including a range of well-defined reference sequences, inferred that all isolates of T. multiceps cysts from ruminants in Greece clustered with previously published sequences from different continents. The present study provides a foundation for future large-scale studies on the epidemiology of T. multiceps in ruminants as well as dogs in Greece.


This survey describes the evolution in vivo of Coenurus cerebralis in small ruminants. At presentation, neurological signs and cerebrospinal fluid (CSF) features were suggestive of multifocal or diffuse inflammatory reaction. Magnetic resonance imaging (MRI) captured the transition between the invasive and quiescent phase of the infection, revealing the concurrent presence of meningitis and small cysts. During the quiescent phase, in all animals, neurological symptoms disappeared, and cerebrospinal fluid was unremarkable while cysts grew progressively. Subsequently, the onset of neurological symptoms coincided with MRI signs of diffuse or localized increase of intracranial pressure, as confirmed by direct intracranial pressure measuring. All the animals had an excellent post-surgical recovery. This is the first report describing the evolution of coenurosis in
Sequential imaging allowed describing interesting such as the death of some coenuri and different parasite growth rate in the same host.

**Echinococcus spp.**


Echinococcosis is a zoonotic infection caused by cestode species of the genus Echinococcus; in addition, this zoonosis has long been neglected as a parasitic disease and has limited treatment options. Clinical drugs such as benzimidazole derivatives have limited treatment efficacy. The current study evaluated a novel drug, osthole, with low toxicity and high activity against Echinococcus in vitro and in vivo. The results in vitro indicated that the viability of Echinococcus granulosus protoscoleces in the group treated with osthole (120 mu M) decreased by 100% within 3 days. In vivo experiments were conducted using parasite-infected mice. For this purpose, three groups of infected mice were treated daily for 6 weeks with albendazole (ABZ, 100 mg/kg, positive control group), osthole (100 mg/kg, experimental group), or honey/PBS (100 mg/kg, negative control group), respectively. The osthole- and ABZ-treated groups presented a significant reduction in wet weight of metacestodes, increase in the level of interleukin (IL)-4 and the percentage of eosinophils compared with the control group. Osthole exhibited a high activity against echinococcosis in vivo. In addition, the toxicity of osthole was evaluated via an in vitro 3-(4,5)-dimethylthiazol-2(5)-yl)-3,5-di-phenyltetrazoliumromide (MTT) assay, as well as via morphological observation and calculation of liver and kidney function indexes in vivo. No obvious toxic effects of osthole were observed in our study. Therefore, this novel drug may be a promising alternative to benzimidazole in anti-echinococcosis chemotherapy.


Currently, benzimidazoles are used as chemotherapeutic agents and as a complement to surgery and PAIR in the treatment of cystic echinococcosis (CE). They are generally applied at high doses causing side effects and, 50% of cases do not respond favorably to such chemotherapy. The use of essential oils obtained by distillation from aromatic plants would be an effective alternative or complementary to the synthetic compounds, because would not bring the appearance of side effects. Carvacrol and his isomer thymol are the main phenolic components from essential oils of Origanum vulgare (oregano) and Thymus vulgaris (thyme). The aim of the present work was to evaluate the in vitro and in vivo efficacy of carvacrol against Echinococcus granulosus metacestodes. For the in vitro assay, protoscoleces and cysts of E. granulosus were incubated with carvacrol at the following final concentrations: 10, 5 and 1 mu g/ml of carvacrol. The maximum protoscolicidal effect was found with 10 mu g/ml of carvacrol. Results of viability tests were consistent with the structural and ultrastructural damage observed in protoscoleces. Ultra structural studies revealed that the germinal layer of cysts treated with carvacrol lost the multicellular structure feature. In the clinical efficacy study, a reduction in cyst weight was observed after the administration of 40 mg/kg of carvacrol during 20 days in mice with cysts developed during 4 months, compared to that of those collected from control mice. Given that the in vivo effect of carvacrol was comparable with the treatment of reference with ABZ and the fact...
that is a safe compound, we postulated that carvacrol may be an alternative option for treatment of human CE.

**Echinococcus granulosus s.l. cystic echinococcosis in humans**


Echinococcosis is one of the 17 neglected tropical diseases (NTDs) recognized by the World Health Organization. The two major species of medical importance are *Echinococcus granulosus* and *Echinococcus multilocularis*. *E. granulosus* affects over 1 million people and is responsible for over $3 billion in expenses every year. In this minireview, we discuss aspects of the epidemiology, clinical manifestations, and diagnosis of cystic echinococcosis or cystic hydatid disease caused by *E. granulosus*.

**E. granulosus in animals**


Study on the incidence of hydatidosis in food animals meant for human consumption such as buffaloes was done at the time of slaughter by inspecting the carcasses and viscera for the presence of hydatid cysts with particular reference to lungs, liver, spleen etc., Based on the observation, the incidence of hydatid cysts in buffaloes examined was found to be 11.11 percent. With regard to the organ wise involvement, the presence of hydatid cysts was more in lungs, followed by liver and the fertility rate of hydatid cysts was high in lungs.


An oil-based formulation of the EG95 vaccine to protect grazing animals against infection with *Echinococcus granulosus* was formulated in Argentina. The efficacy of the vaccine was monitored by serology in sheep and llama (Lama glama) and was compared to the serology in sheep previously published using a QuilA-adjuvanted vaccine. Long-term efficacy was also tested in sheep by challenging with *E. granulosus* eggs of the G1 strain 4 years after the beginning of the trial. The serological results for both sheep and llama were similar to those described previously, except that there was a more rapid response after the first vaccination. A third vaccination given after 1 year resulted in a transient boost in serology that lasted for about 12 months, which was similar to results previously described. Sheep challenged after 4 years with three vaccinations presented 842% reduction of live cysts counts compared with control group, and after a fourth vaccination prior to challenge, this reduction was 947%. The oil-based vaccine appeared to be bio-equivalent to the QuilA vaccine.

The Echinococcus granulosus tapeworm is responsible for cystic echinococcosis (CE), a zoonotic disease with worldwide distribution. The life cycle of the parasite is mainly domestic and takes place between dogs and livestock species. A slaughterhouse survey was conducted in 2012 in the Republic of Moldova in order to estimate the prevalence of CE. A total of 1525 cattle, 5580 sheep and 12,700 pigs were surveyed. No CE infection was observed in pigs, while prevalence was estimated at 59.3% in cattle and 61.9% in sheep. Infection was significantly higher in animals raised in private households than in those from collective farms. The frequency of infection increased with age in both species. In cattle and in sheep, infection of both the liver and lungs was the most common, while infection in the lungs only was much less frequent. Farm type appears to be an important factor in CE infection in Moldova, due to the extensive farming and the home-slaughtering undertaken in the majority private sector, despite a high prevalence of CE also recorded in the public sector. The low fertility of cysts in cattle (1.1%) compared to sheep (47.6%) confirmed the maintenance of E. granulosus s.s. in a dog-sheep life cycle which excludes cattle. Further studies are needed to obtain a complete overview of the parasite’s epidemiology in its intermediate and definitive hosts, in order to implement control and preventive measures, with specific attention given to farms in the private sector.


We have investigated the morphological and structural aspects of hydatid cysts recovered from sheep raised in southern Sardinia during two surveys conducted over the 20 years since the last control attempt at the end of the 1980s. In the first study carried out during 1995-1997, a few years after the last control attempt, a total of 10,334 cysts were examined, recovered from 1029 infected sheep out of a total of 1375 inspected (prevalence 74.84%). In the second survey, conducted ten years later during the period 2005-2010 in the absence of specific control measures, a total of 6249 cysts were recovered from 916 parasitized sheep out of the 1414 examined (prevalence 64.78%). Cysts were grouped into 5 different types: "Unilocular", "Multisepted", "Calcified", "Caseous", "Hyperlaminated". Unilocular: fertile, consist of a single fluid-filled cavity; multisepted: generally sterile, fluid filled, with cavity divided into spheroidal chambers (3-dozen); calcified: sterile, small, with internal chambers almost virtual due to the thickening of internal septa; caseous: sterile, the cavity filled with a thick yellowish matrix; hyperlaminated: sterile, with a virtual cavity filled with extensively folded and overlapping sheets of hyperproduced laminated tissue. A thorough knowledge and the precise characterization of each type of lesion, making it possible to establish detailed parameters for cyst classification, appears very useful for the harmonization of data collection. From a surveillance and control perspective it is proposed that, in addition to other relevant epidemiological information, data from meat inspection be further improved, including morpho-functional indications about cyst type.

**Echinococcus multilocularis**


Background: In Europe, the life-cycle of Echinococcus multilocularis is predominantly sylvatic, involving red foxes (Vulpes vulpes) as the main definitive hosts and rodents such as muskrats and arvicolid as intermediate hosts. The
parasite is the etiological agent of human alveolar echinococcosis, a malignant zoonotic disease caused by the accidental ingestion of eggs shed by definitive hosts in their faeces. The aims of this study were to investigate the prevalence of E. multilocularis in red foxes and raccoon dogs (Nyctereutes procyonoides) and to study the environmental factors favouring the perpetuation of the parasite in Latvia. Methods: A total of 538 red foxes and 407 raccoon dogs were collected across Latvia from 2010 to 2015. The sedimentation and counting technique was used for collecting E. multilocularis adult worms from fox and raccoon dog intestines. The morphological identification of the parasite was confirmed by molecular analysis. Results: The prevalence of E. multilocularis was significantly higher in foxes (17.1%; intensity of infection 1-7,050 worms) (P < 0.001) than in raccoon dogs (6.1%; intensity of infection 5-815 worms). In foxes, a significant positive correlation (r(10) = 0.7952, P = 0.001) was found between parasite prevalence and the intensity of infection. A positive relationship (R-s = 0.900, n = 5, P = 0.037) between parasite prevalence and precipitation was also observed. In raccoon dogs, a significant negative relationship (F(1,F-8) = 9.412, P = 0.015) between animal density and parasite prevalence, and a significant positive relationship (F(1,F-8) = 7.869, P = 0.023) between parasite prevalence and agricultural land cover, were detected. Conclusions: The results of this study confirm the red fox as the most important definitive host of E. multilocularis and, consequently, as the main target for control programmes in the Baltic countries. Raccoon dogs seem to play a secondary role in the life-cycle of E. multilocularis within the investigated European region.


Two single-site, laboratory, negatively controlled, masked, randomised dose confirmation studies were performed: one in dogs, the other in cats. After a period of acclimatisation, both the dogs and cats were orally infected with Echinococcus multilocularis protoscoleces. In the dog study, 10 dogs received a single dose of MilproA (R) tablets at a minimum dose of 0.5 mg/kg milbemycin oxime and 5 mg/kg praziquantel 18 days post-infection and 10 dogs received no treatment. In the cat study, 10 cats received a single dose of MilproA (R) tablets at a minimum dose of 2 mg/kg milbemycin oxime and 5 mg/kg praziquantel 7 days post-infection, 10 cats received a single dose of the treatment 18 days post-infection and 10 cats remained untreated. In both studies, intestinal worm counts were performed 23 days post-infection at necropsy. No worms were retrieved from any of the 30 treated animals. Nine of 10 control dogs had multiple worms (geometric mean 91, arithmetic mean 304) and all 10 control cats had multiple worms (geometric mean 216, arithmetic mean 481). The difference in worm counts between all three treated groups and their controls was highly significant (ANOVA p values of log transformed data < 0.0001). Efficacy of 100 % was demonstrated for the elimination of adult E. multilocularis in dogs and cats as well as for elimination of immature E. multilocularis in cats as evidenced by the effectiveness of treatment 7 days post-infection. The treatments were well accepted and tolerated, and there were no adverse drug reactions observed.

A new method, based on a magnetic capture based DNA extraction followed by qPCR, was developed for the detection of the zoonotic parasite Echinococcus multilocularis in definitive hosts. Latent class analysis was used to compare this new method with the currently used phenol-chloroform DNA extraction followed by single tube nested PCR. In total, 60 red foxes and coyotes from three different locations were tested with both molecular methods and the sedimentation and counting technique (SCT) or intestinal scraping technique (IST). Though based on a limited number of samples, it could be established that the magnetic capture based DNA extraction followed by qPCR showed similar sensitivity and specificity as the currently used phenol-chloroform DNA extraction followed by single tube nested PCR. All methods have a high specificity as shown by Bayesian latent class analysis. Both molecular assays have higher sensitivities than the combined SCT and IST, though the uncertainties in sensitivity estimates were wide for all assays tested. The magnetic capture based DNA extraction followed by qPCR has the advantage of not requiring hazardous chemicals like the phenol-chloroform DNA extraction followed by single tube nested PCR. This supports the replacement of the phenol-chloroform DNA extraction followed by single tube nested PCR by the magnetic capture based DNA extraction followed by qPCR for molecular detection of E. multilocularis in definitive hosts.


Alveolar echinococcosis is considered to be the most serious zoonosis in the Northern Hemisphere's cold or temperate regions. In Europe, the parasite has a sylvatic life cycle based on predator-prey interactions, mainly between red foxes and small rodents. Echinococcus multilocularis has been observed to have spread across Europe over the last three decades. In France, a westward spread of the parasite's known endemic areas has been described. In this study, a retrospective analysis of fox feces by real-time PCR was carried out in four departements not previously investigated and considered free along with two endemic control departments. The fox feces collected from 2000 to 2004 were analyzed by real-time PCR. Fecal prevalence in the two endemic departments of Doubs and Cte d’Or were estimated at 12 % [6.4-20.0 %] and 4.2 % [1.1-10.3 %], respectively. No positive samples were identified among the 72 feces collected in Drme or the 112 from Allier, which is consistent with the very low expected prevalence should the parasite be present. Three positive samples were recovered in the Seine-Maritime and Hautes-Alpes departements, resulting in a prevalence of 3.5 % [0.7-10.0 %] and 2.5 % [0.5-7.1 %], respectively. From now on, Hautes-Alpes constitutes the new southern border of the endemic areas in France and confirms the southward expansion previously highlighted. Real-time copro-PCR proved useful in identifying new endemic areas even with low prevalence. Due to the spread of E. multilocularis in France and associated zoonotic risk, it is necessary to expand surveillance in order to fully define all the country's endemic areas. On a continental scale, the development and harmonization of surveillance programs are now needed in order to obtain a global overview of the presence of E. multilocularis and to tailor potential countermeasures.

The fox tapeworm *Echinococcus multilocularis* is the causative agent of alveolar echinococcosis, a severe zoonotic disease that may be fatal if untreated. A broad spectrum of mammalian species may be accidentally infected even in captivity. In April 2011, liver lesions due to *E. multilocularis* were observed during the necropsy of a captive born nutria (*Myocastor coypus*) in a French wildlife park, leading to initiation of a study to survey the parasite's presence in the park. A comparable environmental contamination with fox's feces infected by *E. multilocularis* was reported inside (17.8%) and outside (20.6%) the park. *E. multilocularis* worms were found in the intestines of three of the five roaming foxes shot in the park. Coprological analyses of potential definitive hosts in captivity (fox, lynx, wildcat, genet, wolf, bear and raccoon) revealed infection in one Eurasian wolf. Voles trapped inside the park also had a high prevalence of 5.3%. After diagnosis of alveolar echinococcosis in a Lemur catta during necropsy, four other cases in *L. catta* were detected by a combination of ultrasound and serology. These animals were treated twice daily with albendazole. The systematic massive metacestode development and numerous protoscoleces in *L. catta* confirmed their particular sensitivity to *E. multilocularis* infection. The autochthonous origin of the infection in all the captive animals infected was genetically confirmed by EmsB microsatellite analysis. Preventive measures were implemented to avoid the presence of roaming foxes, contact with potential definitive hosts and contaminated food sources for potential intermediate hosts.


Experimental *Echinococcus multilocularis* infection and deworming was repeated three or five times in nine dogs at various re-infection schedules. The mean number of worms decreased more than 91% in dogs with repeated infection, compared to first infection controls (n = 6). The copro-antigen assay and the egg count in the faeces suggested that the worm burden gradually decreased each time the dogs were re-infected. To examine whether such worm exclusion was a non-specific response, five dogs were sequentially infected with the parasite four times and subsequently fed freely for 6 months. Even after the 6-month interval, the five dogs that were infected five times with the parasite were still able largely to exclude the adult worms. The results suggested that the ability of worm exclusion in dogs that developed a resistance did not become rapidly extinct. Observation of the condition of faeces and the excretion of hooks in the faeces of repeatedly infected dogs revealed that the exclusion of worms started at the first week after the re-infection, and it continued during the patent period. Serum antibodies specific to the parasite antigen increased gradually until the third infection and significantly decreased during the 6-month interval. There was little enhancement of serum antibodies after the fifth infection in most dogs, although no clear correlation was observed between the antibody response and the worm burden. These findings suggested the possibility of developing a vaccine.

**Besnoitia besnoiti**

Bovine besnoitiosis, caused by the apicomplexan protozoan parasite Besnoitia besnoiti, was diagnosed in an Irish dairy herd. This is the first diagnosis of besnoitiosis in Ireland or the UK and the most northerly European outbreak yet described. The diagnosis occurred following a farm investigation in June 2015 into an unusual dermatological problem that had been ongoing since 2010. On an annual basis, 1-2 per cent of cows in the herd exhibited clinical signs, including skin thickening, alopecia, weight loss and poor performance. Others displayed pyrexia, limb oedema, respiratory distress and reduced milk yield. Histopathological examination of skin revealed granulomatous and eosinophilic dermatitis, with characteristic intradermal protozoal cysts, consistent with cutaneous besnoitiosis. Follow-up serological testing and clinical examination of cattle (n=228) on the farm found that 68 per cent (144/212) were seropositive for B. besnoiti. In addition, 51 per cent (117/228) had characteristic scleral conjunctival cysts and 68 per cent (134/198) had vulval cysts. Postmortem examination of a severely affected animal revealed typical gross and histopathological lesions of B. besnoiti infection. These results confirmed endemic infection with B. besnoiti. The identification of this exotic disease highlights the importance of veterinary surveillance at both local and national level, particularly in relation to emerging diseases.

Besnoitia besnoiti is an obligate intracellular and emerging coccidian parasite of cattle that mainly infects host endothelial cells during acute infection. We here analyzed early innate immune reactions of B. besnoiti-infected primary bovine umbilical vein endothelial cells (BUVEC). B. besnoiti infections significantly activated BUVEC since the gene transcripts of several adhesion molecules (P-selectin, intercellular adhesion molecule 1 (ICAM-1)), chemokines (CXCL1, CXCL8, CCL5), and of COX-2 were significantly upregulated during in vitro infection. Overall, the highest upregulation of most transcripts was observed at 24 or 48 h post infection (p.i.). Enhanced adhesion molecule expression in infected host cells was confirmed by PMN adhesion assays being performed under physiological flow conditions revealing a significantly increased PMN adhesion on B. besnoiti-infected BUVEC layers at 24 h p.i. Furthermore, we were able to illustrate neutrophil extracellular traps (NETs) being released by PMN under physiological flow conditions after adhesion to B. besnoiti-infected BUVEC layers. The present study shows that B. besnoiti infections of primary BUVEC induce a cascade of pro-inflammatory reactions and triggers early innate immune responses.

Background: Bovine besnoitiosis, caused by the protozoan Besnoitia besnoiti, reduces productivity and fertility of affected herds. Besnoitiosis continues to expand in Europe and no effective control tools are currently available. Experimental models are urgently needed. Herein, we describe for the first time the kinetics of standardised in vitro models for the B. besnoiti lytic cycle. This will aid to study the pathogenesis of the disease, in the screening for vaccine targets and drugs potentially useful for the treatment of besnoitiosis. Methods: We compared invasion
and proliferation of one B. tarandi (from Finland) and seven B. besnoiti isolates (Bb-Spain1, Bb-Spain2, Bb-Israel, Bb-Evora03, Bb-Ger1, Bb-France, Bb-Italy2) in MARC-145 cell culture. Host cell invasion was studied at 4, 6, 8 and 24 h post infection (hpi), and proliferation characteristics were compared at 24, 48, 72, 96, 120, and 144 hpi. Results: In Besnoitia spp., the key parameters that determine the sequential adhesion-invasion, proliferation and egress steps are clearly distinct from those in the related apicomplexans Toxoplasma gondii and Neospora caninum. Besnoitia spp. host cell invasion is a rather slow process, since only 50 % of parasites were found intracellular after 3-6 h of exposure to host cells, and invasion still took place after 24 h. Invasion efficacy was significantly higher for Bb-France, Bb-Evora03 and Bb-Israel. In addition, the time span for endodyogeny to take place was as long as 18-35 h. Bb-Israel and B. tarandi isolates were most prolific, as determined by the tachyzoite yield at 72 hpi. The total tachyzoite yield could not be predicted neither by invasion-related parameters (velocity and half time invasion) nor by proliferation parameters (lag phase and doubling time (dT)). The lytic cycle of Besnoitia was asynchronous as evidenced by the presence of three different plaque-forming tachyzoite categories (lysis plaques, large and small parasitophorous vacuoles). Conclusions: This study provides first insights into the lytic cycle of B. besnoiti isolates and a standardised in vitro model that allows screening of drug candidates for the treatment of besnoitiosis.


Bovine besnoitiosis is an emerging disease in Europe, presenting quick spread toward central and southern Spain. Characterization of an outbreak in a free-ranging Limousin and Avilea beef cattle herd from southwestern Spain territories is attempted. Serological survey in the herd revealed increase of number of infected animals, from 34.3 % on first diagnoses/exams on December 2013 to 42.5 % in the second on April 2014. Blood analysis and serum biochemistry showed important alterations like leukocytosis (+33.2 % of mean value), with lymphocytosis (+205.3 %) and increase of LDH (+25.1 %), associated with tissue damage. Clinical cases were only observed in Limousin animals. Along with typical lesions of acute and chronic besnoitiosis, inflammatory and degenerative processes and parasitic cysts were present in the corpus cavernosum and the corpus spongiosum of penis. By using polymerase chain reaction (PCR) sequencing of 18S rDNA, Besnoitia besnoiti was confirmed as causative agent; microsatellite sequence analyses showed the homology of isolates with previously studied strains.


Bovine besnoitiosis is a reemerging disease in Europe. The clinically Besnoitia besnoiti infection in bulls is characterized by fever, nasal discharge, and orchitis in the acute phase and by scleroderma in the chronic phase. However, in many bulls, B besnoiti infection remains at a subclinical stage. Bull infertility is an economically relevant consequence of besnoitiosis infection. It is not clear, however, if semen quality returns to normal levels when infected animals have clinically recovered. The aim of this study was to examine the relationship between chronic besnoitiosis and bull sexual function in a region of eastern France, where the disease is reemerging, by comparing semen quality and genital lesions in 11 uninfected, 17 subclinically infected, and 12 clinically infected bulls. The presence of anti-B besnoiti antibodies was detected by Western blot test. Semen was collected by
electroejaculation. Bulls clinically infected with B besnoiti showed significantly more genital tract alterations than uninfected or subclinically infected bulls. No relationship was evidenced between besnoitiosis infectious status and semen quality, whereas a significant relationship was noted between genital lesions and semen score. This means that in the absence of moderate to severe genital lesions, chronic bovine besnoitiosis is unlikely to alter semen quality. However, as the presence of infected animals could lead to spread of the disease, culling or separation of clinically infected bulls from the remaining healthy animals is strongly recommended.


Bovine besnoitiosis is caused by Besnoitia besnoiti, an apicomplexan parasite closely related to Toxoplasma gondii and Neospora caninum. In the acute stage of besnoitiosis, cattle suffer from pyrexia, swollen lymph nodes, anorexia and subcutaneous edema. In the chronic stage, tissue cysts are formed in a variety of tissues including the skin. Knowledge about the distribution of tissue cysts of different parts of the skin of infected animals is scarce. Four chronically infected cattle were euthanized and skin samples were taken from a total of 77 standardized cutaneous locations per animal. Portions of the dermis were taken, from which DNA was extracted and examined by real-time PCR. Cycle of transition (Ct) values reflecting the amount of parasite DNA in the samples were determined. For statistical analysis, samples were attributed to 11 larger skin regions ('OuterHindlegDistal', 'Rump, ForelegMiddle', 'NoseFrontEars', 'CheekEye', 'SideLowerPart', 'ForelegDistal', 'SideUpperPart,'LegsInner', 'VentralHeadNeck', 'DorsalNeckWithersBackTail'). While all samples revealed a positive result in three female cattle, only 63.6% (49/77) of the samples of a bull showed positive results. For statistical analysis, a Ct value of 45 was assumed for samples with a negative result. The dams showed median Ct values of 16.1, 17.5 and 19.4, while in skin samples of the bull a median Ct value of 37.6 was observed. To determine the differences in DNA concentrations between different locations of the skin of the animals, a relative Ct (relCt) was determined by subtracting for each animal indv the MedianCt(indv) from each sample Ct. Analyses of the relCt values showed that the highest relative parasite DNA concentrations were observed in the categories 'OuterHindlegDistal', 'Rump', 'ForelegMiddle' and 'NoseFrontEars'. The relCt values in these categories differed statistically significantly from those determined for the categories 'VentralHeadNeck' and 'DorsalNeckWithersBackTail'. The analysis showed clear differences in the distribution and the detectability of parasite DNA in the skin of cattle infected with B. besnoiti. In all four animals, samples from the 'Rump' region (Regio fermoris) showed high parasite DNA concentrations. Because this region is also easily accessible for veterinarians, this skin location appears to be optimal for taking skin biopsies for detection or isolation of B. besnoiti.

Eimeria sp.


Recent research has suggested that plants containing condensed tannins may offer a promising alternative approach for the control of coccidiosis in lambs and goat
The present study aimed to examine the potential effect of condensed tannins in sainfoin (Onobrychis viciifolia) and carob pods (Ceratonia siliqua) incorporated in sheep rations against lamb coccidiosis. The above tannin-rich sources were studied in three independent feeding trials in which the animals (naturally infected by Eimeria spp. ewes and their lambs) were allocated (i) in the control group and received a tannin-free diet (lucerne hay), or (ii) in the treatment groups and received a tannin-rich diet based on sainfoin hay (in trials 1 and 2), or in carob pod meal and a combination of carob pod meal and sainfoin hay (in trial 3). In total, 95 newborn lambs (and their 73 ewes) were enrolled in all trials which started a month before lambing and ended 8-10 weeks after lambs were born (at weaning). The course of coccidial infection was monitored in lambs by faecal oocyst counts and consistencies which were recorded at weekly intervals. Moreover, lambs total weight gain was evaluated at the end of each trial. During all trials, 100 % of the animals got naturally infected by Eimeria species and the infection burden was higher in trials 2 and 3 compared to trial 1 but in all cases, severe signs of diarrhoea were not observed. Tannin-rich diets were well accepted by the animals not affecting their feed intake and body weight gain when compared to the controls. The results suggest that incorporation of both tannin-rich resources (especially sainfoin) in sheep rations can reduce Eimeria oocyst excretion rates by the lambs, which can decrease subsequently the contamination of the farm environment with the parasite. However, the high variability noted on the results is not allowing us to draw any definite conclusions at least until the potential of those plants is further investigated.

**Fasciola hepatica**


Fasciolosis, a parasitic disease caused by the trematode Fasciola hepatica underreported is expanding both in human and animal population, throughout the world. The constant use of synthetic drugs to treat this condition has led to the natural selection of resistant strains of the parasite. Hence, there is a growing focus on the potential anti-helminthic properties of medicinal plants and phytopharmaceuticals. The current study assessed the potential anti-fasciolicide action of Momordica charantia leaf extracts and fractions on the eggs of F. hepatica parasites. The lyophilized crude extract (CE) of M. charantia leaves and its sub-fractions, obtained from liquid-liquid partitioning with organic solvents, were analysed by High Performance Liquid Chromatography (HPLC), suspended in 1% DMSO and used in in vitro tests. Quadruplicates of 50 F. hepatica eggs were incubated at 23 degrees C with M. charantia leaf CE in different concentrations. After 12 days no larvae were formed in eggs incubated with CE concentrations above 12.5 mg/mL. Eggs incubated with CE sub-fractions at concentrations of 1000, 100, 10, 1, 0.1, 0.01 mu g/mL affected embryonic development, with n-butanol presenting the strongest inhibition of miracidia formation. In contrast, on the 12th day, 90% of the miracidia hatched in the control experiments using 0.03% DMSO whereas embryo genesis was completely abolished with any concentration of albendazole sulphoxide ABZ(SO). Chemical analysis of the CE and sub-fractions revealed a prominent presence of flavonoids. HPLC-MS confirmed Quercetin to be one of the main flavonoids present in the CE and the n-butanol subfraction. This is the first study to analyse the potential anti-fasciolicide action of M. charantia leaf CE and subfractions.

Lambs infected with the Cullompton isolate of Fasciola hepatica were treated orally or subcutaneously with 10 mg/kg of closantel at 16 weeks post-infection. Adult flukes were recovered from the liver of individual animals at 12 h, 24 h, or 36 h post-treatment. The flukes were processed for histological analysis. In general, degenerative changes in the reproductive and somatic tissues were progressive, and were most marked in flukes exposed to closantel in vivo for 36 h. However, flukes from a 12 h subcutaneously-treated lamb showed marked deterioration of the testis, possibly because a portion of the dose has been delivered intravenously. Fewer intact eggs were seen in the uterus of flukes exposed to closantel for longer times (whether administered subcutaneously or orally to the host). The most conspicuous closantel-induced effect in flukes from treated hosts was progressive damage to the tegumental syncytium. While the flukes from 24 h-treated hosts showed relatively minor damage to limited areas of the syncytium, towards the posterior end, the flukes from 36 h-treated hosts (and flukes from the lamb that putatively received intravenous dosage) had lost large areas of the surface syncytium from the posterior end and dorsal surface, although the syncytium over the anterior end and the anterior ventral surface was largely spared. In areas where the syncytium had sloughed, the underlying structures such as the vitelline follicles, gut profiles and testis profiles, showed marked degeneration and breakdown. Other changes included cell depletion and early stage apoptosis in the testis, ovary and vitelline follicles. This study establishes a model for histological changes in closantel-sensitive F. hepatica exposed to closantel in vivo. Histopathological studies could be complementary to the efficacy controlled test for for closantel resistance in fluke populations.


Background: Coprological examinations are commonly used for diagnosis of fasciolosis. However, these methods are not useful during the acute phase of the infection and also show poor sensitivity during its chronic phase. In this study we compared the immunoreactivity of the native and recombinant forms of Fasciola hepatica excretory/secretory antigens and determined the most appropriate one for development of F. hepatica-specific immunoassays. Methods: The coding sequences of previously-determined immunogenic proteins including cathepsin L1 (CL1), fatty acid binding protein (FABP) and glutathione-S-transferase (GST) were cloned and expressed in E. coli BL-21 cells. Native forms of FABP and GST were also purified. We evaluated the immunoreactivity of the native and recombinant proteins by ELISA using sera from 40 healthy individuals, 15 fasciolosis patients, and 57 patients with other infectious diseases. Results: All of the studied proteins showed high sensitivity and specificity for F. hepatica serodiagnosis. However, CL1 was more sensitive and specific (100%) than the others for the detection of F. hepatica-specific antibodies. Notably, both FABP and GST showed significant cross-reactivity with hydatidosis patients' sera while CL1 did not. Conclusions: Cathepsin L1 has acceptable sensitivity and specificity for serodiagnosis of F. hepatica and its application could be advantageous in immunoassay development.