Executive Summary
This is a summary of the literature, produced from peer and non-peer reviewed papers in parasitology published in 2015 (and early 2016), of relevance to Defra, APHA and the wider industry.

Highlights
Gastro-intestinal nematodes of grazing ruminants
- Work published that estimates losses in growth rate due to specific GI parasites or the host’s immune response
- Mapping and modelling major parasites in Europe, an output of GLOWORM project published.
- Ongoing work on Haemonchus contortus vaccine development using specific antigens (a whole worm vaccine is now used in Australia)
- Work on SCOPS principles in UK showed that SCOPS farms had, on average, a higher daily weight gain and finish weight than CONTROL farms when comparing observed means. For both ewes and lambs, significantly fewer treatments were carried out on the SCOPS farms.
- Use of multiple active anthelmintics together with best practice advice in New Zealand found that almost exclusive use of multiple actives did not result in further resistance development despite all farms exhibiting resistance to different classes at the outset. What is more, the measured increases in anthelmintic efficacy suggests that adoption of best practice management strategies may extend the useful life of anthelmintics even after resistance has been diagnosed.
- Work on Cooperia oncophora vaccination in cattle continues
- Studies into targeted selective treatment (TST) with anthelmintic of dairy and beef calves in Ireland was published
- Two studies (in New Zealand and Australia) confirmed the transfer of MLs in the milk of treated ewes to the sucking lamb which prevented susceptible larvae establishing. This could increase pressure for anthelmintic resistance development
- Paper published on sheep farm in the Netherlands with a Haemonchus contortus resistant to monepantel (highlighted in previous annual review)
- A study to evaluate the efficacy of injectable formulations of ivermectin and moxidectin bodyweight against naturally acquired gastro-intestinal nematodes in cattle in various countries in Europe was published. For moxidectin, resistance was confirmed on 3 farms in France, and on 1 farm in Germany and the UK. For ivermectin, resistance was confirmed on 3 farms in the UK, and on 1 farm in Germany and France. After treatment Cooperia spp. larvae were most frequently identified, though Ostertagia ostertagi was also found, in particular within the UK and Germany

Besnoitia
- Papers published included one that recommended that control strategies, in the absence of effective treatment and vaccination, are based primarily on medical surveillance after widespread serological screening of all animals in the herd aged over six months.
• Studies in four chronically affected animals using PCR on skin samples, suggested that samples from the ‘Rump’ region (Regio fermoris) showed high parasite DNA concentrations. They conclude that this skin location appears to be optimal for taking skin biopsies for detection or isolation of *B. besnoiti*.

**Cestodes**

• Studies in Argentina looked at the effect of vaccination of animal intermediate hosts (lambs) of Cystic Echinococcus (*E. granulosus* or hydatid disease) with the EG95 vaccine was a useful add to control.
• A review paper on *E. multilocularis* stated that the European endemic area of *Echinococcus multilocularis* in foxes is larger than previously anticipated, and there is new evidence that both fox populations and the prevalence of *E. multilocularis* have increased in many areas, indicating increased pressure for infection with *E. multilocularis* eggs in intermediate and accidental hosts, including humans.

**Fasciola hepatica**

• Histological changes associated with closantel susceptibility in fluke were described. Histopathological studies could be complementary to the efficacy control test for closantel resistance in fluke populations.
• A paper detailing a novel monoclonal antibody-based immunoassay to detect *F. hepatica* parasites in the snail *Galba cubensis* from day eight post infection. *No previous immunoassay had been used to detect helminth infected snails prior to this paper and the assay can be used to detect infection status in natural populations of lymnaeid snails. This type of test may be useful in prediction of infection for the periods of highest risk by sampling snails if it could also be applicable to our most common parasitised snail Galba truncatula.*
• The first case of closantel treatment failure in Sweden was reported. This study involved the topical formulation containing closantel and ivermectin. Based on faecal egg count closantel efficacy, at 21 days post-treatment, was 72% and 97% on two farms respectively. Whereas no faecal egg count reduction was observed on a further farm. They question the use of this topical means of treatment in terms of closantel treatment failure.

**Paramphistomes (rumen fluke)**

• Details of an outbreak of acute diarrhoea in cattle western France were published. This was due to immature paramphistomes.
• A study into the pathological changes in cattle naturally infected by *Calicophoron daubneyi* adult flukes was published. Affected papillae showed morphological changes denoted by very narrow stalks and expanded heads. Histologically, these changes were characterized by epithelial acanthosis-hyperkeratosis of the epithelium. Infiltration of inflammatory cells was often related to the epithelial changes, although it was also found in the duodenal mucosa and submucosa. Tissue damage and inflammatory reaction were more severe in the ruminal atrium, where the largest number of flukes and affected papillae were observed. In contrast, lesions in the ruminal dorsal sac were absent or mild.
• A paper confirmed *Galba truncatula* as an intermediate host snail for *Calicophoron daubneyi* in Great Britain was published, as well as evidence of alternative snail species hosting *F. hepatica*.
• A paper on bovine and ovine rumen fluke in Ireland was published. The authors conclude that although *C. daubneyi* is thought to share an intermediate host snail with *Fasciola hepatica*, the differences in prevalence between host species over time suggest that the epidemiology of *C. daubneyi* is distinct from that of *F. hepatica*. 
Gastro-intestinal parasites in sheep

While Texel lambs have increased resistance to infection with the gastrointestinal nematode *Teladorsagia circumcincta* compared to Suffolk lambs, the underlying resistance mechanisms are still unknown. The aim of this study was to compare parasitological, humoral and cellular responses of Texel and Suffolk lambs over time following a single experimental infection with *T. circumcincta*. Gastrointestinal nematode free (but not naive) lambs received a single oral dose of 3 x 10(4) infective *T. circumcincta* larvae. The variables examined included worm burden, mucosal and serum IgA, abomasal mast cells and eosinophils, haematological parameters and plasma pepsinogen. Texel lambs had significantly lower worm burden on day 14 and lower plasma pepsinogen concentration from day 14 onwards than Suffolks and their response in mucosal IgA to infection occurred earlier. The results from the study suggest that an earlier local IgA response in the Texel contributes to the resistant characteristics of the breed, while the increased level of plasma pepsinogen in the Suffolk lambs implies greater abomasal tissue damage arising from the nematode infection. (Ahmed et al., 2015)

The effects of, and interactions between chronic *Trichostrongylus vitrinus* infection and immune suppression with methylprednisolone were investigated for a period of 112 days in a grazed flock of 176 crossbred meat lambs. Worm egg count of non-immune-suppressed lambs increased rapidly from days 21 to 42 post-infection, and then steadily declined. Infection was associated with significantly decreased fat depth, eye muscle area and cold carcase weight, and increased circulating anti-*T. vitrinus* IgG and IgA. Immune suppression led to sustained increases in WEC, and significantly greater worm count, liver weight, fat depth and carcase dressing percentage, and significantly reduced IgG and IgA anti-*T. vitrinus* titres, lymphocyte counts, adrenal weight, eye muscle area and cold carcase weight. Both infection and immune suppression were associated with significant body weight reductions. Only 39% of reduced growth rate due to infection was attributable to the host immune response to *T. vitrinus*. (Blackburn et al., 2015)

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 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 (SUP) 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. (Dever et al., 2016)

*Haemonchus contortus* is a gastrointestinal nematode parasite of small ruminants, which feeds on blood and causes significant disease and production loss in sheep and goats, especially in warmer parts of the world. The life cycle includes free-living immature stages, which are subject to climatic influences on development, survival and availability, and this species therefore exhibits spatio-temporal heterogeneity in its infection pressure based on the prevailing climate. Models that better explain this heterogeneity could predict future epidemiological changes. The basic reproduction quotient (Q(0)) was used as a simple process-based model to predict climate-driven changes in the potential transmission of *H. contortus* across widely different geo-climatic zones, and showed good agreement with the observed frequency of this species in the gastrointestinal nematode fauna of sheep (r = 0.81, P < 0.01). Averaged monthly Q0 output was further used within a geographical information system (GIS) to produce preliminary haemonchosis risk maps for the United Kingdom (UK) over a four-year historical span and under future climate change scenarios. Prolonged transmission seasons throughout the UK are predicted, especially in the south although with restricted transmission in peak summer due to rainfall limitation. Additional predictive ability might be achieved if information such as host density and distribution, grazing pattern and edaphic conditions were included as risk layers in the GIS-based risk map. However, validation of such risk maps presents a significant challenge, with georeferenced observed data of sufficient spatial and temporal resolution rarely available and difficult to obtain. (Bolajoko et al., 2015)

To evaluate the effects of Diet and corticosteroid-induced immune suppression during infection by *Haemonchus contortus*, 28 lambs were allocated to one of four groups treated as follows: Group Basal Diet - Normal; Group Basal Diet - Immune-Suppressed; Group Supplemented Diet - Normal; and Group Supplemented Diet - Immune-Suppressed. The Basal Diet contained Cynodon dactylon (cv. coast cross) hay with 82 g crude protein (CP)/kg dry matter (DM), which was
Gastrointestinal nematode (GIN) infections are common in domestic sheep and impact directly and indirectly on the health of infected animals as well as on the associated economic production. In this study, we aim at summarizing the current knowledge on the influence of GIN infections on sheep production by conducting a systematic review. A subsequent meta-analysis of relevant studies was performed to provide an estimate of the effect of GIN infections on weight gain, wool production and milk yield. A literature search was performed on the CAB, Pubmed and Web of Science database for the period 1960-2012. Inclusion criteria were: 1) Measurement of at least one production parameter. 2) Comparison between groups of sheep with different nematode burdens. 3) Same conditions regarding all aspects except parasite burden between groups. 4) Quantitative measurements of one or more production traits. Altogether, 88 studies describing 218 trials were included in this review. The majority of studies (86%) reported that GIN infections had a negative effect on production but this was reported to be statistically significant in only 43% of the studies. Meta-analysis indicated that performances of sheep infected with nematodes was 85, 90 and 78% of the performance in uninfected individuals for weight gain, wool production and milk yield respectively. Our results suggest a possible reporting bias or small study effect for the estimation of the impact of GIN infections on weight gain. Finally, a general linear model provided an estimate for the decrease in weight gain in relation to the increase in faecal egg count of nematodes. This study underlines the importance of GIN infections for sheep production and highlights the need to improve parasite management in sheep, in particular in face of challenges such as anthelmintic resistance. (Mavrot et al., 2015)

Mapping and modelling helminth infections in cattle and sheep in Europe through advanced geospatial research was one of the main task of GLOWORM, a three year project (2012-2014) funded under the European Commission’s (EC) seventh framework programme (FP7). Liver flukes as Fasciola hepatica and gastrointestinal nematodes, such as Haemonchus contortus were chosen for the project since these parasites constitute a major cause of lost productivity in small and large ruminants. The output of the GLOWORM project delivered guidelines for standardized and harmonized cross-sectional surveys of helminth parasites in ruminants allowing the development of updated prevalence maps and multi-scale, spatial models for the European area. (Rinaldi et al., 2015)
Gastrointestinal nematodes are important parasites of livestock and wildlife worldwide, causing mortality and morbidity, regulating host populations and threatening food security through reduced productivity of ruminant livestock. A significant part of the life cycle of most GINs is completed outside of the host. GINs are therefore susceptible to changes in climate, and evidence of climate-driven changes in the phenology of GINs and the seasonal incidence of disease already exists. A modelling framework, GLOWORM-FL was developed to predict changes in the seasonal dynamics of the free-living stages of trichostrongylid GINs on pasture as a first step towards evaluating potential mitigation strategies. The general model framework was parameterised and validated for three GIN species that infect a range of ruminants worldwide: *Haemonchus contortus*, *Teladorsagia circumcincta* and *Ostertagia ostertagi*. The model builds significantly on previous models of GIN population dynamics by incorporating the behaviour of nematodes in response to climate variability, facilitated by recent advances in our understanding of the ecology of GINs. Simulations using historical and predicted future climatic data for a temperate region reveal the potential for an increase in annual infection pressure of *H. contortus* and *T. circumcincta* in small ruminants as increasing temperatures accelerate development and remove constraints on the development of *H. contortus* during the winter months. In contrast, a significant decrease in annual infection pressure is predicted for *O. ostertagi* in cattle due to accelerated development being offset by rapid mortality at higher temperatures. A similar trade-off is predicted during the summer months for *H. contortus* and *T. circumcincta* resulting in complex seasonal dynamics of the availability of infective stages on pasture. These changes could have significant impacts on the seasonal incidence and pathology of infection by GINs. GLOWORM-FL therefore provides an important tool to predict the seasonal risk of transmission of GINs and will aid in the design of climate-driven, risk-based GIN control strategies. (Rose et al., 2015b)

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 faecal 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 69.56% of the ewes classified as RR and 46.67% of the ewes classified as S 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. (Pereira et al., 2016)

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. (Rose et al., 2016)
Gastro-intestinal parasitism diagnosis and control

Sustainable and effective control of nematode and trematode infections of ruminants requires an understanding of the farm-specific epidemiology of the parasites. To this end, a variety of diagnostic and monitoring techniques can be employed by the veterinary practitioner, of which the most commonly used is the faecal worm egg count (FWEC). This article discusses other techniques which can add detail to the information gained from FWECs and detect parasites not amenable to egg flotation techniques. (Crilly and Sargison, 2015)

The investigation aimed to assess factors affecting the uptake of novel targeted selective treatment (TST) strategies by sheep farmers in Western Australia where the most common nematode species present were Teladorsagia circumcincta, Trichostrongylus spp. and Nematodirus spp. (“scour worms”). The study used a questionnaire format with questions concentrated on current worm control practices and farmers’ current understanding and adoption of putative TST strategies. Participants represented a range of environments (derived from four farming regions) and sheep management situations, and it is therefore likely that the results of this investigation will apply in other locations where scour worms predominate. Sixty-five percent of participants were aware of the TST concept and 25% had implemented it in some form. The awareness of the TST approach was greatest where sheep farmers were concerned about anthelmintic resistance, where tools such as worm egg counts and faecal worm egg count resistance tests were employed, and where professional advisers were consulted regarding worm control. Respondents that sought advice chiefly from rural merchandise retailers were considerably less (0.1-0.6 times) likely to be aware of these management tools or to be aware of TST approaches. The findings indicated that the adoption of TST strategies will require greater use of professional advisers for worm control advice by sheep farmers, and that advisers are conversant with TST concepts. (Cornelius et al., 2015)

Haemonchus contortus is a blood-sucking parasite causing the presence of faecal occult blood (FOB). The objective was to study three different FOB tests in order to have a new indicator of H. contortus infection in sheep that could be included in the genetic evaluation system as an alternative selection criterion to faecal worm egg count (FEC). A total of 29 Corriedale lambs were experimentally infected with 10,000 larvae of H. contortus. Stool samples were recorded for FEC and FOB tests (Hexagon, Hematest and Multistix), blood for packed cell volume (PCV), haemoglobin, white and red blood cell count (RBC), and FAMACHA for scoring anaemia. At the end of the experiment lambs were slaughtered to worm burden count. Field infection was achieved in 309 Merino lambs under natural parasite challenge. FEC data were normalized through logarithmic transformation (LnFEC). Pearson correlation was estimated to examine the relationship between all traits. The three tests were able to detect the presence of FOB at day 11. FEC, PCV and RBC decreased to sub-normal values from day 18. FAMACHA score 3 was considered to be indicative of anaemia. Most of the correlations were of high magnitude, with the exception of Multistix test that was moderately correlated with haematological parameters, LnFEC and FEC. In field infection, most samples were negative to FOB tests and the correlations were lower than those calculated under experimental infection. In conclusion, FOB tests were able to detect haemonchosis earlier than FEC under high experimental parasite challenge. However, they were not able to detect FOB under natural mixed parasite challenge. FAMACHA and PCV demonstrated to be good indicators of Haemonchus chlamydospores on its in vitro trapping ability against Haemonchus contortus L3 larvae. The treated batch (200 NP) contained 4 x 10(6) chlamydospores of the FTH0-8 strain, whereas the control batch (200 NP) was produced without spores. Both NP batches were exposed to four experimental storage conditions: (T1) shelves (indoors); (T2) refrigeration (48C); (T3) outdoors under a roof; and (T4) 100% outdoors. Each group comprised 48 NP with spores and 48 NP without spores (control). The ability of D. flagrans spores to trap H. contortus L3 larvae was evaluated for 8 weeks for each storage condition. For that purpose, six randomly selected NP with spores were compared to their respective control NP. Each NP was individually crushed. The crushed material (1 g) was placed on the surface of a 2% water agar plate with 200 H. contortus L3 larvae. Plates were sealed and were incubated at room temperature for 8 days. The whole
content of every plate was transferred to a Baermann apparatus to recover the remaining larvae. There was a clear larval reduction in the NP with spores, compared to the respective control NP in the four storage conditions (P < 0.05). The mean reductions (+/- SEM) of the storage conditions were 67 +/- 4.9 (T2), 77 +/- 6.1 (T1), 81.5 +/- 3.8 (T4) and 82.1 +/- 2.5 (T3). Larval reductions were similar at all times and were not affected by storage conditions or storage time (R < 2 < 0.2; P > 0.05). The long-term shelf-life of the chlamydospores in the NP suggests that this spore dosage technology is a viable option. (Fitz-Aranda et al., 2015)

A paper was published in which the author looks at production losses associated with nematode parasites, warns of the consequences of anthelmintic resistance and considers approaches for future control. (Sargison, 2015)

Haemonchus contortus parasite causes high mortality rates and drastic productivity reduction besides enhancing treatment costs. It has also been demonstrating great resistance against most of the drugs currently in use. Thus, the utilization of elements that can boost the host immune response to cope with the infection has been widely studied. Some researchers have shown that the use of zinc (Zn) and copper (Cu) as nutritional supplements can help controlling the parasites. The present study evaluated the immune response of lambs experimentally infected with H. contortus and treated with Zn and Cu. Twenty-one (21) lambs were divided into three groups: CG (non-infected control group), IG (infected group infection performed with 15,000 larvae of H. contortus/animal) and ITG (group infected and treated subcutaneously with 1.5 mg/kg of Zn and 0.45 mg/kg of Cu on days 10 and 45 post-infection (PI)). Blood samples were drawn on days 0, 14, 28, 42, 56 and 70 PI in order to determine the serum concentrations of interleukin 1 (IL-1), interleukin 4 (IL-4) and interleukin 6 (IL-6), interferon-gamma (IFN-gamma), tumor necrosis factor-alpha (TNF-alpha) and immunoglobulins (Igs) IgA, IgE, IgG and IgM. The parasitic infection was monitored by the counting of eggs per gram of feces (EPG) in the same intervals of blood sampling. At the end of the experiment, animals were euthanized and the parasite load was quantified. In addition, the concentrations of Zn and Cu in liver were assessed. A significant increase in EPG was observed only on day 14 PI for IG and ITG (P < 0.05). Between days 14 and 42 there was a significant increase (P < 0.001) of cytokine and immunoglobulin levels in IG and ITG, when compared with the animals of CG. After day 56, the IG animals showed serum level of interleukins and immunoglobulins lower than ITG animals (P < 0.001). Regarding the liver concentrations of Zn and Cu, the infection showed to lead a depletion of these minerals; therefore, the supplementation did not prevent this effect. The results of this study indicate that the administration of Zn and Cu was capable of enhancing the immune response in lambs experimentally infected with H. contortus. However, it did not reduce egg counts in the faeces or the number of adult parasites in the abomasum. (Schafer et al., 2015)

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 Katahdin 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. Faeces were collected on days 0 (day of COWP treatment), 7, and 14 for determination of faecal egg counts (FEC). Pooled (2014) or pooled treatment group faeces 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 faeces 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. (Burke et al., 2016)

A dynamic and innovative approach to managing the blood-consuming nematode Haemonchus contortus in goats is critical to crack dependence on veterinary anthelmints. 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/selected 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. (Kearney et al., 2016)

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. (Li et al., 2016)

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 prop paddocks each grazing hylactic treatment (SPT) and treatment upon observation of clinical signs (MT). Lambs were grazed on one of eight replicate 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 L1 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 PCRs. Worm-naïve 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 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. 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. (Melville et al., 2016)

Parasitic diseases are a major constraint to optimum livestock production and are the major cause of economic loss in UK sheep flocks, with farmers remaining dependant on anthelmintics for control. In the UK, research and evidence based, “best practice” guidelines for sustainable control of parasites in sheep (SCOPS) were first produced in 2004 and have been regularly updated since. This study was designed to evaluate the effect of these best practice guidelines for worm control on lamb production and infection levels, compared with more traditional management. Sixteen farms were selected based on a 2 cube factorial design with 3 factors known to affect worm epidemiology: control regimen; farm type; and climatic region. A formalised plan for worm control using 7 potential resistance-delaying practices was prepared for each of the 8 best practice (SCOPS) farms, in conjunction with the farms veterinarians. The 8 farms in the traditional management group (CONTROL farms) were selected based on ongoing evidence of them using
worm control strategies deemed to be “higher-risk”. A cohort of 40-50 study lambs at each farm was monitored from birth to finishing, allowing evaluation of lamb productivity, worm infection levels and for comparison of numbers of anthelmintic treatments. Birth and mid-season weights were used to calculate daily live-weight gain. Birth and finish dates were used to calculate time to finish and finish weights were also compared. Faecal egg counts, larval culture and species differentiation were undertaken throughout the year to assess the impact of the control strategies on worm burdens. There was no significant difference in results for any of the 3 production responses when comparing predicted means accounting for the differences in birth weight. In fact SCOPS farms had, on average, a higher daily weight gain and finish weight than CONTROL farms when comparing observed means. Statistical analysis of infection levels clearly showed no significant effect according to farm type \((p = 0.71)\) or treatment \((p = 0.81)\). In contrast the effect of region \((p = 0.08)\), although not significant, had a much larger effect size (standardised mean difference) with lower parasite burdens based on faecal egg counts on Northern farms compared to Southern farms. For both ewes and lambs, significantly fewer treatments were carried out on the SCOPS farms. (Learmount et al., 2015)

Maintaining production and economic viability in the face of resistance to multiple anthelmintic actives is a challenge for farmers in many countries. In this situation, most farmers in New Zealand rely on the use of combination products, containing multiple actives with similar spectra of activity, in order to maintain control. However, there are concerns that use of combinations, once resistance has already developed to the individual actives, could rapidly lead to complete failure of all actives. This study followed seven farms, previously diagnosed with resistance to at least two classes of anthelmintic, which were implementing a tailored programme of “best practice parasite management”. The aim was to ascertain whether the programmes, which included the almost exclusive use of combination anthelmintics, were able to prevent resistance from developing further. Strategies implemented on each farm varied, but had consistent underlying principles i.e. to avoid over-use of anthelmintics; to minimise parasite challenge to susceptible stock; to maintain refugia of susceptibility and to ensure that only effective anthelmintics were used. Annual faecal egg count reduction tests (FECRT) were undertaken in lambs on all farms to monitor anthelmintic efficacy over 5 years. The efficacy of albendazole, ivermectin and levamisole was calculated and the changes in efficacy against Teladorsagia circumcincta assessed. Overall, there was a significant improvement in the effectiveness of both levamisole and ivermectin against T. circumcincta, and a positive but non-significant trend in efficacy of albendazole, i.e. there was evidence for reversion towards susceptibility. Hence, the almost exclusive use of combination anthelmintics, integrated with other resistance management strategies, did not result in further resistance development despite all farms exhibiting resistance to multiple actives at the outset. What is more, the measured increases in anthelmintic efficacy suggests that adoption of best practice management strategies may extend the useful life of anthelmintics even after resistance has been diagnosed. (Leathwick et al., 2015a)

Recently, the epidemiology of nematode parasites has changed as they have adapted to climatic and management changes and as a consequence of the irresponsible use of anthelmintic drugs. This adaptability is conferred by large, polymorphic genomes and high biotic potential. A significant net effect of these factors has been the inevitable emergence of anthelmintic resistance. Consequently, suboptimal sheep productivity due to nematode parasites has become commonplace because blueprint control programmes have failed. The focus of veterinary nematode parasite control in intensively managed sheep flocks has had to move away 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. (Sargison, 2015b)

**Gastro-intestinal parasites in cattle**

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 3°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°C versus 17-22.6°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. (Knapp-Lawitzke et al., 2016)

Genetic hybridisation between parasitic nematode species has potentially important consequences. It could lead to the introgression of genes between species including those involved in pathogenicity, host specificity, transmission and drug resistance. It could also complicate diagnosis and control. However, there are few compelling examples of its occurrence in parasites in the field. *Haemonchus contortus* and *Haemonchus placei* are two closely related parasitic nematode species that predominantly infect small ruminants and cattle, respectively. They are capable of experimental hybridisation when adult worms of each species are transplanted into the same individual host. Given that co-infection occurs in both small ruminants and cattle, there is potential for hybridisation in the field. However, this has not been definitively demonstrated and its extent is unknown. We investigated the occurrence of co-infection and interspecies hybridisation in *H. contortus* and *H. placei* in field populations from small ruminants from Pakistan and southern India using a number of independent genetic markers. *Haemonchus contortus* and *H. placei* co-infections were common in Pakistan but not in southern India where *H. placei* appeared to be absent in small ruminant hosts. In the former region, a number of worms were identified that were heterozygous for fixed, species-specific rDNA internal transcribed spacer 2 (ITS-2) - single nucleotide polymorphisms. Genotyping of these ITS-2 heterozygotes with an additional four nuclear markers conclusively demonstrated them to be F1 interspecies hybrids. Mitochondrial NADH dehydrogenase subunit 4 haplotype analysis demonstrated that four of the hybrid worms had a *H. placei* maternal parent and one had a *H. contortus* maternal parent showing that hybridisation could occur in either direction. Interestingly, one of these hybrids contained an *H. contortus* isotype-1 beta-tubulin benzimidazole resistance allele, suggesting there is a potential for interspecies introgression of drug resistance loci. We believe this is the first definitive genetic evidence of hybridisation between *H. contortus* and *H. placei* in the field and represents the most comprehensive genetic evidence of F1 hybrids between any human or livestock parasitic nematode species to date. Further, it suggests that interspecies transmission of anthelmintic resistance mutations warrants further investigation. (Chaudhry et al., 2015a)

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 (G1); 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. (Favero et al., 2016)

Gastro-intestinal parasitism, diagnosis and control in cattle

The rationale for treating dairy cows for gastrointestinal parasites in order to enhance production rests on a firm scientific base. The evidence includes: observations in abattoir studies of the frequent presence of *Ostertagia ostertagi* in the abomasum and lesions often extensive of parasitised gastric glands in the abomasum of adult cattle; associations between antibody titres to *O. ostertagi* and abomasal pathology, plasma pepsinogen, nematode epidemiology and milk production; the demonstration that nematode parasitism can reduce the feed intake of dairy cows; and production responses to anthelmintic treatment. Treatment at calving appears to be optimal in defining the returns on the investment from worming cows. Quantitative diagnostic tests can help discriminate amongst herds or individual cows that are likely to respond to treatment, thus facilitating targeted selective treatment (TST) approaches. In addition TST allows the maintenance of large refugia of unexposed worms that helps mitigate the risk of selection for anthelmintic resistance. None of this implies an imperative to worm dairy cows, but an understanding of the under-pinning science is useful if advice is to be informed, balanced and without subjective bias. (Forbes, 2015)
A major constraint for the effective control and management of helminth parasites is the lack of rapid, high-throughput, routine diagnostic tests to assess the health status of individual animals and herds and to identify the parasite species responsible for these helminthoses. The capability of a multiplex platform for the simultaneous detection of three pasture associated parasite species was evaluated and compared to existing ELISAs. The recombinant antigens 14.2 kDa ES protein for Cooperia oncophora, major sperm protein for Dicyoacaulus viviparous and Cathepsin L1 for Fasciola hepatica were recombinantly expressed either in Escherichia coli or Pichia pastoris. Antigens were covalently coupled onto magnetic beads. Optimal concentrations for coupling were determined following the examination of serum samples collected from experimentally mono-infected animals, before and after their infection with the target species. Absence of cross-reactivity was further determined with sera from calves mono-infected with Haemonchus contortus, Ostertagia ostertagi and Trichostrongylus colubriniformis. Examination of negative serum samples was characterised by low median values. For this, samples of (Tuo et al., 2015) were collected from cattle serum samples. Examination of serum samples collected on different days post infection from different animals showed a high reproducibility of the assays. Serum samples were additionally examined with two already established ELISAs, an in-house ELISA using the recombinant MSP as an antigen and a DRG ELISA using Cathepsin L1 for liver fluke. The results between the assays were compared and kappa tests revealed an overall good agreement. A versatile bead-based assay using fluorescence detection (xMAP (R) technology) was developed to simultaneously detect antibodies against C. oncophora, D. viviparous and F. hepatica in cattle serum samples. This platform provides rapid, high-throughput results and is highly sensitive and specific in comparison to existing serological as well as coproscopical diagnostic techniques. (Karanikola et al., 2015)

Many modern-day studies require parasite samples essentially free of environmental contamination. Techniques used to prepare gastrointestinal nematode eggs from livestock are sufficient for biological studies but fall woefully short of generating pure preparations for downstream molecular, biochemical, and immunological studies. Consequently, a method to produce highly purified nematode parasite eggs free from faecal contamination is needed. The present study compared different procedures for egg isolation and attempted to improve the purity of Ostertagia ostertagi eggs isolated from cattle faeces. The Wisconsin method using saturated sucrose has been used widely to enrich eggs from faeces and is adequate for identification and counting. A more-recently developed method using the combination of salt and sucrose is robust and consistent and can be used to isolate eggs with much higher purity; however, residual faecal material is still present in the egg preparations, which could account for large levels of nonspecific DNA and RNA contamination in the final samples. While large numbers of eggs can be harvested from the medium of overnight, in vitro-cultured adult worms, this method is labour intensive, involves euthanasia of animals, and requires the purification of adult worms to high purity and subsequent culturing to acquire the eggs. In addition, a large proportion of the eggs initiate development and hatch during isolation. In the current method, Ostertagia eggs free from faecal contamination were secondarily purified using lymphocyte separation medium (LSM) from eggs previously enriched by the Wisconsin method. The egg recovery following the LSM step was 100%. The results indicate that this two-step method involving sucrose and LSM was simple, rapid, nonselective, and greatly improved the purity of Ostertagia eggs. This method will have broad application for isolating eggs of the superfamily of Trichostrongylidea, which includes the most-important nematode parasites infecting livestock. (Tuo et al., 2015)

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 faeces and blood of 86 calves were collected. Faecal egg counts (FEC) were determined using the modified McMaster technique with a sensitivity of 50 eggs per gram of faeces (EPG). Positive nematode FEC was processed for coproculture using pooled samples to identify Strongylidae infective larvae (L3). First-stage larvae (L1) of Dicyocaulus 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 (amylose and lipase); and minerals (phosphorus and calcium). The calves were divided into four groups according to their 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 two 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., Oesophagostrongum 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. 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. (De Cezaro et al., 2016)

Recently we reported the successful vaccination of calves against Cooperia oncophora with a double domain activation-associated secreted protein, purified from the excretory-secretory material of adult stage parasites. In an attempt to elucidate the immune mechanisms involved in protection, the humoral and cell-mediated immune responses following vaccination and infection were compared with non-vaccinated control animals. Antigen-specific IgG1, IgG2 and IgA levels were significantly increased in sera of vaccinated animals post vaccination, whereas no effect was observed for IgM. Antigen-specific intestinal IgG1 levels were significantly increased in the vaccinated animals, whereas no differences were observed for antigen-specific IgA, IgM and IgG2 levels. Upon re-stimulation in vitro with the vaccine antigen, a significant proliferation of both alpha beta- and gamma delta-T cells, and B cells, collected from mesenteric lymph nodes, was only observed in vaccinated animals. RNA-seq analysis of intestinal tissue yielded a list of 67 genes that were differentially expressed in vaccinated animals following challenge infection, amongst which were several cell adhesion molecules, lectins and glycosyl transferases. A correlation analysis between all immunological and parasitological parameters indicated that intestinal anti-double domain activation-associated secreted protein IgG1 levels correlated negatively with cumulative faecal egg counts and positively with the proportion of L4s and L5s. The proportion of immature stages was also positively correlated with the proliferation of alpha beta T cells. Worm length was negatively correlated with the transcript levels of several lectins and cell adhesion molecules. Overall, the results indicate that intramuscular administration of the vaccine resulted in an immune memory response particularly characterised by increased antigen-specific IgG1 levels in the intestinal mucosa. (Van Meulder et al., 2015)

With the increasing incidence of anthelmintic resistance worldwide, immunological control of worm infections through vaccination is often put forward as a rational and cost-effective alternative for anthelmintic drugs. In this study we report on the evaluation of a double-domain activation-associated secreted protein purified from the excretory-secretory material of the adult stage of the small intestinal parasite Cooperia oncophora as a vaccine antigen against this parasite. In a first experiment, calves were vaccinated three times i.m. with activation-associated secreted protein and Quil A adjuvant or with adjuvant alone, and subsequently challenged with a trickle infection of 25,000 infective larvae in total over 25 days. Vaccinated calves showed a significant reduction of 91% in their cumulative faecal egg counts and a significantly higher number of inhibited L4s present in their intestine compared with control animals. Furthermore, both female and male adult worms were significantly smaller in the vaccinated group than in the control group. In a second experiment, the vaccine antigen was further evaluated under field conditions. Calves were immunised as described above, followed by a natural challenge infection on pasture. Cooperia oncophora faecal egg counts in the vaccinated animals were reduced during the entire grazing period, resulting in a significant reduction in the cumulative faecal egg counts of 58.5%. Numbers of infective C. oncophora larvae were lower on plots grazed by vaccinated calves, with a reduction in mean pasture larval counts of 65% at housing. A significant reduction of 81.6% in total numbers of C. oncophora worms was shown in the vaccinated group compared with the control group. Taken together, the data highlight the protective capacity of the double-domain activation-associated secreted protein and the possibility of controlling C. oncophora infections through vaccination. (Vlaminck et al., 2015)

With increasing concerns of anthelmintic resistance in cattle nematode populations worldwide, there is a need to explore alternative approaches to nematode control. One alternative approach is the use of targeted selective treatments (TST) where only individual animals are treated instead of the entire group. This study reports the findings of a TST approach in dairy calves conducted over their first grazing season (FGS) to control both gastrointestinal nematode and lungworm challenge. Ninety-six calves with an initial mean (s.d.) age and live weight of 130 (28.3) days and 120 (23.6) kg, respectively, were randomised by breed, age and live weight to one of two treatments; Control (n = 24; x2) and TST (n = 24; x2). Control calves were treated three times at pasture with ivermectin by subcutaneous injection. Individual calves in the TST group were treated at pasture with ivermectin when one of the following thresholds was met: (1) positive for lungworm larvae using the modified Baermann technique or (2) positive or negative for lungworm larvae using the modified Baermann technique with plasma pepsinogen concentration (PP) > 2 international units of tyrosine/litre and faecal egg count (FEC) > 200 strongyle eggs per gram of faeces. Calves were rotationally grazed from July 3rd 2012 (day 0) to November 2nd 2012 (day 122) when calves were housed. Calves were weighed and sampled (blood and faecal) every three weeks. There was an effect of treatment and time on both FEC [treatment (P = 0.023), time (P < 0.001)] and PP [treatment (P = 0.002), time (P < 0.001)]. Both FEC and PP were higher in TST calves. There was a 50% reduction in anthelmintic use in TST calves compared to control calves. Clinical signs of lungworm infection, confirmed by the modified Baermann technique, were evident in TST calves on days 62 and 63 of the study. The average daily live weight gain for control and TST calves was 0.50 (0.02) kg day(-1) and 0.47 (0.03) kg day(-1), respectively (P = 0.41). Thus, performance in dairy calves can potentially be maintained with fewer anthelmintic treatments but farmers need to be vigilant of the challenge posed by lungworm. Any future approach into the use of TST in FGS calves must take into consideration the relative importance of lungworm as a pathogen. (O'Shaughnessy et al., 2015a)
With concerns over the development of anthelmintic resistance in cattle nematode populations, we must re-examine our approach to nematode control in cattle. Targeted selective treatments (TST), whereby individual animals are treated instead of entire groups, are being investigated as an alternative. The study objective was to determine if anthelmintic usage could be reduced using a TST-based approach to nematode control in spring-born suckler beef cattle over their first and second grazing seasons (SGS) without affecting performance. In the first grazing season (FGS), 99 calves with an initial mean (s.d.) calf age and live weight on day 0 (June 28th 2012) of 107 (23.1) days and 160 (32.5) kg, respectively, were used. The study commenced on day 0 when calves were randomised and allocated to one of two treatments; (1) standard treatment (control) and (2), TST. Control calves were treated subcutaneously with ivermectin on days 0, 41 and 82 in the FGS. All calves were treated with ivermectin on day 124 and housed on day 133. In the SGS, only heifer calves from the FGS were used and control heifers were treated with ivermectin on day 393. Animals were weighed, blood and faecal sampled every three weeks. The TST animals were treated with ivermectin if thresholds based on a combination of plasma pepsinogen concentrations, faecal egg count and/or the presence of Dictyocaulus viviparus larvae in faeces (FGS only) were reached. No TST calves reached the treatment threshold criteria in the FGS. The FGS average daily live weight gain (ADGs.e.m.) for control and TST group calves was 0.890.02 kg and 0.940.02 kg day⁻¹, respectively (P=0.17). In the SGS, all heifers were treated with ivermectin on day 431 due to clinical signs of respiratory disease. The ADG for control and TST heifers from turnout on day 321 to day 431 was 0.900.04 and 0.800.04 kg day⁻¹, respectively (P=0.03). Spring-born FGS suckler beef calves require minimal anthelmintic treatment to maintain performance. In contrast, clinical parasitic disease may develop in the SGS unless appropriate anthelmintic treatment is provided.(O’Shaughnessy et al., 2015b)

In this article the author discusses the impact of gastrointestinal infections in adult cattle and presents guidelines on how to minimize variable responses to treatment. Highlights include: GI nematode species in dairy cattle, life cycle of GI nematodes, immunity, risk of infection and impact on health, assessing the evidence, treatment during dry period, and anthelmintic options in dry period. (Pedersen, 2015)

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, lacating 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 immuno-inflammatory mechanisms. Until further studies can assess whether this unexpected result can be generalized, non-persistent treatment of immunized adult dairy cows against GIN should not be recommended in early grazing season. (Ravinet et al., 2016)

Anthelmintics and nutraceuticals

Haemonchus contortus is a nematode of livestock that can cause severe disease and mortality. Ivermectin, an anti-parasitic drug that targets glutamate-gated chloride channels, is widely used in humans, livestock, companion animals and agriculture. Although an association between genetic changes to beta-tubulin and exposure to ivermectin has been previously reported, direct binding between ivermectin and tubulin has not been demonstrated to date. Tubulin/microtubules are key targets for many anti-mitotic drugs used in anti-parasite and cancer therapies. We now report that ivermectin exposure increased the rate and extent of polymerisation of H. contortus recombinant alpha- and beta-tubulin, and protected the parasitic alpha- and beta-tubulins from limited trypsin proteolysis. Direct binding between ivermectin and the tubulin monomers exhibited low micromolar affinities, as determined using surface plasmon resonance. Subsequent equilibrium dialysis indicated that ivermectin and Taxol compete for binding to tubulin, supporting our molecular modelling that predicts ivermectin interacts with the Taxol binding pocket of both parasitic and mammalian tubulins. Collectively, our data indicate that ivermectin can bind to and stabilise microtubules (i.e. alter the tubulin polymerisation equilibrium) and this can then lead to mitotic arrest. This work extends the range of known pharmacological effects of ivermectin, and reveals its potential as an anti-mitotic agent. (Ashraf et al., 2015a)
A field study was undertaken on three Swiss sheep farms (A, B, C) to evaluate the efficacy of a long-acting moxidectin formulation (Cydectin (R) 2% LA) against gastrointestinal nematodes (GIN). Naturally infected ewes (all three farms) and their winter-borne lambs (farms A and B) were allocated to two groups (MOX, CON). At day 0 ewes of the MOX-groups were treated with 1 mg moxidectin s.c. CON- and MOX-groups were grazed on separate pastures and were sampled for faeces and blood at 28-day intervals. The survey showed that whole flock treatments of injectable 2% moxidectin were used to control sheep scab on 21% of farms. Injectable 2% moxidectin and oral moxidectin were used to control the periparturient rise in faecal nematode egg shedding by ewes on 13% and 55% of farms respectively. The effects of injectable 2% moxidectin treatment on faecal nematode egg shedding post-treatment in both the autumn and spring were investigated by faecal nematode egg counts at the time of treatment and at 2-weekly interval thereafter on eight and six farms in the autumn and spring, respectively. Faecal egg shedding recommenced at 8 weeks (autumn) and 4 weeks (spring) post-treatment. Counts increased to a peak and then declined. The mean (95% confidence interval) peak counts post-treatment were 2.8 (0.6, 5.1), 3.6 (1.7, 5.5) and 53.5 (25.1, 82.0) eggs per gram (EPG) for autumn-treated ewes, autumn-treated lambs and spring-treated ewes respectively. The spring treated sheep showed a statistically significantly earlier return to faecal egg shedding (p = 0.0125, p = 0.0342) compared to both other groups, statistically significantly higher peak in egg counts than the autumn treated sheep (p < 0.001) and a statistically significantly longer period of positive egg counts (p = 0.0148). There was no statistically significant difference in the timing of the peak FECs between autumn and spring (p = 0.211). The FECs of all groups of sheep treated with an injectable long-acting formulation of moxidectin became positive earlier than would be expected from the period of persistence given on the datasheet, but post-treatment FECs were very low compared to pre-treatment counts. (Crilly et al., 2015)

Two controlled studies were performed to assess the efficacy and pharmacokinetics of topical 0.5% w/v eprinomectin (EPRINEX (R) Pour-on, Merial) against nematode infections of goats. Each study included 16 male castrated goats, less than one year old, harbouring induced infections of adult gastrointestinal and pulmonary nematodes. Following blocking on pre-treatment bodyweight and random allocation to one of two groups, half the goats in each study were treated once with eprinomectin (1 mg/kg bodyweight, administered topically), and half remained untreated and served as controls. Plasma concentrations of eprinomectin were determined in blood samples collected prior to and at multiple time points up to necropsy. Efficacy was determined based on the nematode counts of the animals following necropsy 14 days after treatment. Efficacy of treatment was 100% against adult Haemonchus contortus, Nematodirus battus, Nematodirus spathiger, Oesophagostomum venulosum, Teladorsagia circumcincta and Dictyocaulus filaria was >94% against adult Cooperia curvica, Trichostrongylus axei and Trichostrongylus colubriformis, and was 89.4% against adult Strongyloides papillosus (p < 0.01). Basic pharmacokinetic parameters of eprinomectin (81a component) for the goats in the two studies were: AUC(last), 23.5 +/- 5.19/36.0 +/- 8.74 day*ng/mL; and C-max 3.65 +/- 1.12/5.25 +/- 1.39 ng/mL, respectively; individual maximum plasma concentrations were observed 1 or 2 days after treatment. Results of the studies consistently demonstrated a high therapeutic efficacy of topical eprinomectin at 1 mg/kg bodyweight against a broad range of gastrointestinal and pulmonary nematode parasites of goats. (Hamel et al., 2015)

Parasitic infections with gastrointestinal nematodes (GINs) still represent a worldwide major pathological threat associated with the outdoor production of various livestock species. Because of the widespread resistance to synthetic chemical anthelmintics, there is a strong impetus to explore novel approaches for a more integrated management of these infections. The use of nutraceuticals in the control of GINs is one of the alternatives which has been widely studied for 20 years. The objectives of this review are: (i) to define and illustrate the concept of 'nutraceutical' in the context of veterinary parasitology based on data obtained on the most studied models to control GINs in small ruminants, the tannin-containing legumes (Fabaceae); (ii) to illustrate how the 'nutraceutical concept' could be expanded
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 gastro-intestinal 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 pyreantan 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 M, 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 M derquantel alone and in combination with 0.3 μM M 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. (Abongwa et al., 2016)

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*. An 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. (Bartley et al., 2016)

The correct use of pharmacology-based information is critical to design successful strategies for the future of parasite control in livestock animals. Integrated pharmaco-parasitological research approaches have greatly contributed to optimize drug activity. In an attempt to manage drug resistance in helminths of ruminants, combinations of two or more anthelmintics are being used or promoted, based on the fact that individual worms may have a lower degree of resistance to a multiple component formulation, when each chemical has a different mode of action compared to that observed when a single compound is used. However, as emphasized in the current review, the occurrence of potential pharmacokinetic and/or pharmacodynamic interactions between drug components highlights the need for deeper and integrated research to identify the advantages or disadvantages associated with the use of combined drug preparations. This review article provides integrated pharmacokinetic/pharmacodynamic and clinical pharmacology information pertinent to preserve the traditional and modern active ingredients as practical tools for parasite control. Novel pharmacological data on derquantel and monepantel, as representatives of modern anthelmintics for use in livestock, is summarized here. The article also summarizes the pharmaco-parasitological knowledge considered critical to secure and/or extend the lifespan of the recently available novel molecules. (Lanusse et al., 2015)

Administration of long-acting anthelmintics to pregnant ewes prior to lambing is a common practice in New Zealand. Today, most of these products contain macrocyclic lactone (ML) actives, which because of their lipophilic nature, are detectable in the milk of treated animals and in the plasma of their suckling offspring. This study was conducted to confirm the transfer of ML actives to lambs in the ewe’s milk, and to assess whether this could result in selection for ML resistant nematodes in the lamb. Ninety, twin bearing Romney ewes were treated before lambing with a long-acting injectable formulation of moxidectin, a 100-day controlled release capsule (CRC) containing abamectin and albendazole, or remained untreated. After lambing, seven ewes from each treatment group were selected for uniformity
of lambing date and, along with their twin lambs, relocated indoors. At intervals, all ewes and lambs were bled, and samples of ewe’s milk were collected, for determination of drug concentrations. Commencing 4 weeks after birth all lambs were dosed weekly with 250 infective larvae (L3) of either an ML-susceptible or resistant isolate of Teladorsagia circumcincta. At 12 weeks of age all lambs were slaughtered and their abomasas recovered for worm counts. Moxidectin was detected in the plasma of moxidectin-treated ewes until about 50 days after treatment and in their lambs until about day 60. Abamectin was detected in the plasma of CRC-treated ewes until the last sample on day 80 and in the plasma of their lambs until about day 60. Both actives were detectable in milk of treated ewes until day 80 after treatment.

Establishment of resistant L3 was not different between the treatment groups but treatment of ewes with moxidectin reduced establishment of susceptible L3 by 70%, confirming the potential of drug transfer in milk to screen for ML-resistance in the sucking lamb. (Leathwick et al., 2015b)

To compare the pharmacokinetics, distribution and efficacy (pharmacodynamic response) of intraruminal ivermectin (IVM) and moxidectin (MXD) administered at 0.2 and 0.4 mg/kg to naturally nematode-infected lambs, and to determine the ex vivo accumulation of these anthelmintics by Haemonchus contortus. Romney Marsh lambs, naturally infected with IVM-resistant H. contortus, were allocated to treatment groups based on faecal nematode egg counts. They received 0.2 or 0.4 mg/kg IVM or MXD (n=10 per group), or no treatment (Control; n=6), on Day 0. Samples from four animals from each treatment group, including abomasal parasites, were obtained on Day 1. Plasma samples were also collected from Day 0 to 14, and a faecal egg count reduction test (FECRT) and a controlled efficacy trial were carried out on Day 14. Concentrations of IVM and MXD in plasma, in abomasal and intestinal tissues and in H. contortus were evaluated by high-performance liquid chromatography. Additionally, the ex vivo drug accumulation of IVM and MXD by H. contortus was determined. Peak plasma concentrations and the area under the concentration vs. time curve for both IVM and MXD were higher for 0.4 than 0.2 mg/kg treatments (p<0.05), but there were no differences for other parameters. Concentrations of IVM and MXD in the gastrointestinal target tissues and in H. contortus were higher compared to those measured in plasma. Concentrations of both drugs in H. contortus were correlated with those observed in the abomasal content (r=0.86; p<0.0001). The exposure of H. contortus to IVM and MXD was related to the administered dose. Mean FECRT and efficacy for removal of adult H. contortus was 0% for IVM at 0.2 and 0.4 mg/kg. For MXD, FECRT were >95% for both treatments, and efficacy against H. contortus was 85.1% and 98.1% for 0.2 and 0.4 mg/kg, respectively. The ex vivo accumulation of IVM and MXD in H. contortus was directly related to the drug concentration present in the environment and was influenced by the duration of exposure. Administration of IVM and MXD at 0.4 compared with 0.2 mg/kg accounted for enhanced drug exposure in the target tissues, as well as higher drug concentrations present in within resistant nematodes. The current work is a further contribution to the evaluation of the relationship between drug efficacy and basic pharmacological issues in the presence of resistant parasite populations. (Lloberas et al., 2015)

P-glycoproteins (P-gps) play an important role in the sensitivity of nematodes to anthelmintic drugs. They have been implicated in a number of anthelmintic resistances, particularly for macrolcytic lactone drugs. Hence, inhibition of nematode P-gps has been suggested as a means of reversing some types of anthelmintic resistance. The present study aimed to investigate the ability of the most-recently developed group of P-gp inhibitors (the so-called ‘third generation’ of inhibitors) including tariquidar, zosuquidar and elacridar, to increase the sensitivity of Haemonchus contortus larvae to various anthelmintics (ivermectin, levamisole and thiabendazole) in vitro. We compared these compounds to some older P-gp inhibitors (e.g. verapamil and valsodar). Larval migration and development assays were used to measure the sensitivity of larvae to anthelmintic alone, or in combination with P-gp inhibitors. Significant increases in sensitivity to ivermectin were observed with zosuquidar and tariquidar in larval migration assays (synergism ratios up to 6-fold). Several of the inhibitors increased the sensitivity of both the drug-resistant and -susceptible isolates (e.g. tariquidar with ivermectin in migration assays, zosuquidar with ivermectin in larval development assays), while others had significant effects on the resistant isolate only (e.g. zosuquidar with ivermectin in migration assays, verapamil with ivermectin in development assays). This suggests that some of the inhibitors interact with P-gps representing intrinsic pathways present across nematode populations with quite different drug sensitivities, while other inhibitors interact with P-gps of significance only to resistant nematodes, and hence most likely representing an acquired resistance mechanism. The study highlights the potential of the third generation of P-gp inhibitors for increasing the sensitivity of nematodes to anthelmintics. (Raza et al., 2015)

**Anthelmintics-cattle**

The persistent activity of ivermectin long-acting injection (IVM LAI; IVOMECA (R) GOLD, Merial; 3.15 % ivermectin w/v) against nematode infections of cattle was evaluated under natural challenge conditions. Seventy nematode-free Brown Swiss calves were blocked by pre-treatment bodyweight and allocated randomly to seven groups of 10 animals each: saline (control) at 1 mL/50 kg bodyweight once on day 0 or IVM LAI at 1 mL/50 kg bodyweight (630 mcg IVM/kg)
on either days 0, 7, 14, 21, 28, or 35. After housing until day 35, calves were grazed as one herd on a naturally contaminated pasture for 42 days. Calves were then weighed and housed for 4 weeks before being necropsied for parasite counting. Treatment with IVM LAI prevented the establishment (> 90 %, p < 0.05) of Dictyocaulus viviparus (100 %), Bunostomum phlebotomum (100 %), Haemonchus contortus (98.6 %), Ostertagia ostertagi/lyrata (94.9 %), and Oesophagostomum radiatum (93.3 %) for at least 77 days; Ostertagia leptospicularis (99.1 %) for 63 days; Cooperia punctata (97.7 %), Trichostrongylus axei (96.5 %), and Ostertagia spp. inhibited larvae 4 (93.3 %) for 56 days; Cooperia oncophora/surnabada (96.9 %), Trichuris discolar (93.6 %), and Cooperia spp. inhibited larvae 4 (98.8 %); and Nematodirus spp. inhibited larvae 4 (97.1 %) for 42 days. Calves of groups treated with IVM LAI had significantly (p < 0.001) higher days 0 to 77 weight gains than the saline-treated controls (28.40-39.25 vs 2.60 kg); the weight gains of the IVM LAI-treated groups, however, were not different from one another (p > 0.3). This study demonstrated a very high efficacy of IVOMECA (R) GOLD in preventing the establishment of a wide range of bovine nematodes for extended periods of time which was associated with a significant benefit to productivity in terms of weight gain. (Rehbein et al., 2015)

Anthelmintic resistance- sheep

The main goals of the current work were: (a) to assess the ivermectin (IVM) systemic exposure and plasma disposition kinetics after its administration at the recommended dose, x5 and x10 doses to lambs, (b) to compare the clinical efficacy of the same IVM dosages in lambs infected with an IVM-resistant isolate of Haemonchus contortus, and (c) to assess the expression of the transporter protein P-glycoprotein (P-gp) in H. contortus recovered at 14 days after administration of the IVM dose regimens. There were two separated trials where IVM was administered either subcutaneously (SC, Experiment I) or intraruminally (IR, Experiment II). Each experiment involved twenty-four (24) lambs artificially infected with a highly resistant H. contortus isolate. Animals were allocated into 4 groups (n = 6) and treated with IVM at either 0.2 (IVMx1), 1 (IVMx5) or 2 mg/kg (IVMx10). Plasma samples were collected up to 12 days post-treatment and analysed by HPLC. An untreated-control Group was included to assess the comparative anthelmintic efficacy of the different treatments. The level of expression of Pgp in H. contortus specimens obtained from lambs both untreated and IR treated with the different NM doses was quantified by real time PCR. Parametric and non-parametric tests were used to compare the statistical significance of the results (P < 0.05). After the SC treatment, the IVM plasma area under the concentration-time curve (AUC(0-LLOQ)) increased from 41.9 (IVMSCx1) up to 221 (IVMSCx5) and 287 (IVMSCx10) ng.day/mL and after the IR treatment from 20.8 (IVMIRx1) up to 323 (IVMIRx10)ng.day/mL. Dose-adjusted AUC(0-LLOQ) and C-max were similar among doses, demonstrating dose proportionality for IVM after both SC and IR administration at the three different doses. The efficacies against resistant H. contortus after the SC treatment were 42% (IVMSC1), 75% (IVMSCx5) and 75% (IVMSCx10). However, the IR IVM treatment reached clinical efficacies ranging from 48% (IVMIRx1) up to 96% (IVMIRx5) and 98% (IVMIRx10). None of the IR NM treatments increased the expression of P-gp in adult H. contortus at 14 days post-treatment compared to samples collected from the untreated control group. An enhanced parasite exposure of the drug at the abomasum may explain the improved efficacy against this recalcitrant H. contortus isolate observed only after the IR administration at 5- and 10-fold the IVM therapeutic dosage. (Alvarez et al., 2015)

Haemonchus contortus is an abomasal nematode of ruminants that is widely present across the world. Its ability to cause death of infected animals and rapidly develop anthelmintic resistance makes it a dangerous pathogen. Ivermectin (IVM) and moxidectin (MOX) are macrocyclic lactones (MLs). They have been successfully used to treat parasitic nematodes over the last three decades. A genetic association between IVM selection and single nucleotide polymorphisms (SNPs) on the beta-tubulin isotype 1 gene was reported in H. contortus. These SNPs result in replacing phenylalanine (F, TTC) with tyrosine (Y, TAC) at position 167 or 200 on the beta-tubulin protein. Recently we reported a direct interaction of IVM with alpha- and beta-tubulin. It had been hypothesized that the SNPs (F167Y and F200Y) may change tubulin dynamics and directly affect IVM binding. The goal of the current study was to observe the effects of SNPs (F167Y and F200Y) on tubulin polymerization and IVM binding. It was also of interest to evaluate the differences between IVM and MOX on tubulin polymerization. We conclude that the SNPs cause no difference in the polymerization of wild and mutant tubulins. Furthermore, neither of the SNPs reduced IVM binding. Varying results were obtained in the degree of polymerization of parasitic and mammalian tubulin for IVM and MOX, i.e., the extent of polymerization was greater for IVM compared with MOX for H. contortus tubulin, and vice versa for mammalian tubulin. Molecular modeling showed that IVM and MOX docked into the taxane binding pocket of both mammalian and parasitic wild type and mutant tubulins. However the binding was stronger for mammalian tubulin as compared to parasitic tubulin. (Ashraf et al., 2015b)

Monepantel (MPTL) is one of two new anthelmintic compounds introduced onto the sheep market to control gastro-intestinal nematodes. Resistance to this compound is rare but has been reported. In order to preserve the efficacy of this and other anthelmintics, it is essential to understand both (a) the mechanisms involved in the selection of resistance and (b) how the parasites evolve to deal with these compounds. To address these questions three MPTL-resistant Teladorsagia circumcincta isolates (MTci2-11, MTci5-13 and MTci7-12) have been artificially selected in vivo from
phenotypically characterised parent isolates (MTci2, MTci5, MTci7 respectively). The selection process involved collecting and culturing eggs from surviving worms from sheep administered sub-optimal dosages of MPTL (Zolvix (R)) to provide infective larvae to infect further sheep until resistant isolates were generated (between 9 and 13 rounds of selection). A controlled efficacy test was conducted using the original parental isolates and the newly generated MPTL resistant isolates (n = 5 per group). Selected isolates were assessed both under anthelmintic stress (Zolvix (R), 2.5 mg/kg bodyweight; MTci-MPTL) and at rest (untreated, MTci-CON). A number of life-history traits were assessed, namely, worm establishment rates, time to patency, faecal egg output, body length of adults and eggs in utero. The estimated resistance status of the selected isolates was confirmed with 48%, 28% and 9% reductions in worm burden at 7 -days post Zolvix (R) administration for MTci2-11-MPTL, MTci5-13-MPTL and MTci7-12-MPTL, respectively, compared with untreated controls. One of the selected isolates MTci7-12-CON showed significantly greater total worm burden (p = 0.025), greater establishment rate (p = 0.033), decreased time to patency (p = 0.048), higher cumulative egg outputs (p = 0.002) compared with its parental derivative MTci7. The trial results suggest that anthelmintic selection in T. circumcincta, albeit under experimental conditions, can select for more prolific/fecund and quicker maturing populations. These data provide an insight into how parasites evolve in response to anthelmintic pressure. (Bartley et al., 2015)

In a sheep farm in the Netherlands with a suspected *Haemonchus contortus* resistant to monepantel (Zolvix (R), Novartis Animal Health), a faecal egg count reduction test was carried out in two groups of lambs, according to the method of the World Association for the Advancement of Veterinary Parasitology. Group 1 was the untreated control group, and group 2 was treated with monepantel at the manufacturer’s recommended dose rate. Efficacy of treatment with monepantel was 0%. Larval identification of pre- and post-treatment coprocultures revealed 100% *H. contortus* larvae. On this farm, after a perceived reduction in efficacy of ivermectin and doramectin, the sheep farmer started using monepantel in July 2012, and since then, monepantel was used as the sole anthelmintic. Breeding sheep were treated twice each year in 2013 and 2014, and lambs two times in 2012, four times in 2013, and three times in 2014, before monepantel resistance was suspected, and confirmed three weeks later. Although the frequency of monepantel treatments on this farm was relatively high with treatments on thirteen separate occasions in two years, possibly establishing favorable conditions for a competitive advantage for resistant *H. contortus*, it is remarkable that resistance to monepantel was established in such a very short period. This study confirms, to the best of our knowledge, the first case of *H. contortus* resistance to monepantel occurring in the field. (Vanden Brom et al., 2015)

Levamisole phosphate, chosen based on its 100% efficacy demonstrated by a previous faecal 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 faecal 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 LC 50 and LC 90 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 d McMaster isolates, respectively. After 5 years of exclusive use, the nematodes developed resistance to levamisole, detected by FECRT and by increase in LC 50 and LC 90 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. (Chagas et al., 2016)

A total of 160 sheep and 160 goats were necropsied to evaluate the degrees of susceptibility or resistance of different helminth species to 0.2 mg/kg ivermectin (subcutaneous route), 0.2 mg/kg moxidectin (subcutaneous route), 100 mg/kg trichlorfon (administered orally) and the combination of 5 mg/kg albendazole + 7.5 mg/kg levamisole + 0.2 mg/kg ivermectin (administered orally). To achieve this objective, eight experiments were performed, four with each animal species. In each experiment, naturally infected sheep or goats were divided into five groups with eight individuals each, as follows: T01, untreated control; T02, trichlorfon; T03, ivermectin; T04, moxidectin; and T05,
albendazole + levamizole + ivermectin, based on average counts of eggs per gram of faeces (EPG) before treatment (experimental dates -3, -2 and -1). Seven days post-treatment (DPT), all animals were euthanized and necropsied for the recovery of helminth burdens. Based on the obtained results, it is possible to conclude that the resistance of some helminth species parasing sheep and goats is different for the tested chemical groups. Ivermectin, at 0.2 mg/kg dosage, presented inferior anthelmintic efficacy against some of these parasites. Of these species, populations of *Haemonchus contortus*, followed by *Trichostrongylus colubriformis*, *Cooperia curticci* and *Oesophagostomum columbianum*, exhibited the greatest resistance to the aforementioned chemical compound, whereas *Trichostrongylus axei* displayed higher susceptibility to ivermectin. For moxidectin (0.2 mg/kg), 75% of all *H. contortus* populations were considered resistant to this drug, whereas all populations of *T. colubriformis*, *T. cowl*, *C. curticci*e and *O. columbianum* were susceptible. Trichlorfon and albendazole + levamizole + ivermectin were effective against the analyzed nematode populations, except against one strain of *H. contortus* and one strain of *T. colubriformis*. All three *Strongyloides papillosus* populations evaluated were susceptible to the tested formulations, except for moxidectin, as this compound presented low efficacy indices against all populations of this helminth species. (Bichuette et al., 2015)

An in vivo study in goats evaluated the effect of superimposing a single artificial infection with a benzimidazole (BZ)-susceptible *Haemonchus contortus* isolate upon established *H. contortus* populations of known BZ resistance by measuring the phenotypic BZ resistance of eggs collected from faeces before and after re-infection. Two *H. contortus* isolates, one benzimidazole resistant (BZR) and the other susceptible (BZS), were used to infect worm-free goats. Eight goats were initially infected with 2000 third-stage larvae (L3). In each case the inoculum contained a pre-determined proportion of the two isolates: 100% BZS (one goat), 75% BZS/25% BZR (two goats), 50% BZS/50% BZR (two goats), 25%BZS/75% BZR (two goats) and, finally, 100% BZR (one goat). The phenotypic BZ susceptibility of the *H. contortus* population formed in each goat after the first infection was determined on days 30 and 36 post-infection using an egg-hatch assay (EHA) that estimated the concentration of thiabendazole (TBZ) required for 95% inhibition of larval hatching (EC95) with a 95% confidence interval (95% CI). On day 49 post-infection, goats were re-infected with 2000 L3 of the BZS isolate alone. A second set of EHA bioassays was performed 28 days and 34 days after re-infection. The first infection protocol produced three populations classified as BZS (EC95 0.055–0.065 g TBZ/ml) while four were categorized as BZR (EC95 0.122–0.344 g TBZ/ml). The status of one other population could not be determined. After re-infection with BZS L3, the number of susceptible populations increased to six (EC95 0.043–0.074 g TBZ/ml) while the remaining two were deemed resistant (EC95 0.114–119 g TBZ/ml). Re-infection with BZS L3 thereby reduced the resistance status of most *H. contortus* populations. (Chan-Perez et al., 2015)

It is important to understand how anthelmintic drug resistance mutations arise and spread in order to determine appropriate mitigation strategies. We hypothesised that a molecular genetic study of *Haemonchus contortus* in southern India, a region where resistance may be less advanced than in western Europe and North America, might provide some important insights into the origin and spread of anthelmintic resistance. The F200Y (T (A) under barC) isotype -1 beta-tubulin benzimidazole resistance mutation is common in *H. contortus* throughout the world and the F167Y (T (A) under barC) and E198A (G (C) under barA) mutations, although less common, have been reported in a number of different countries. We have investigated the haplotypic diversity and phylogenetic relationship of isotype -1 beta-tubulin benzimidazole resistance alleles for 23 *H. contortus* populations from small ruminants across southern India. The F200Y (T (A) under barC) mutation was most common, being detected in 18/23 populations at frequencies between 9% and 84% and the E198A (G (C) under barA) mutation was also detected in 8/23 populations at frequencies between 8% and 18%. The F167Y (T (C) under barC) mutation was not detected in any of the 23 populations. Phylogenetic haplotype network analysis suggested that the F200Y (T (A) under barC) mutation has arisen multiple independent times in the region with at least three independent origins of resistance alleles across the populations surveyed. In contrast, the E198A (G (C) under barA) mutation was present on a single haplotype which, given the high level of haplotypic diversity of the susceptible alleles in the region, suggests this particular mutation has spread from a single origin, likely by anthropogenic animal movement. Population genetic analysis of 12 of the *H. contortus* populations, using a panel of eight microsatellite markers, revealed extremely low genetic differentiation between populations, consistent with the hypothesis of high gene flow among sites. Additionally, there was no significant genetic differentiation between *H. contortus* taken from sheep and goats which is consistent with *H. contortus* populations being freely shared between these two different hosts. Overall, we believe these results provide the first clear genetic evidence for the spread of an anthelmintic resistance mutation to multiple different locations from a single origin. (Chaudhry et al., 2015b)

Studies on microtubule inhibitors have shown that the loss of equilibrium between tubulin and microtubules can generate a multitude of histochemical changes in mitochondria. This disruption of balance is also considered the basis of benzimidazole anthelmintic (BZ) activity. Studies have shown that BZ does not bond to the tubulin of the BZ-resistant *Haemonchus contortus*, as opposed to sensitive ones. This affinity alteration can be easily recognised by changes in the optical density and can help in the classification of *H. contortus* mitochondria, into sensitive (dark) and resistant (clear, unmodified zones). In order to confirm this hypothesis, we started our study from albendazole (ABZ) resistant and sensible *H. contortus* individuals, collected from the intestinal tract of sheep, aiming towards the identification of
mitochondrial features, using the Electron Microscopy Transmission (EMT) technique. The EMT has confirmed that the structure of sensitive trichostronglian populations was affected rapidly, only four hours after ABZ treatments. The main changes that appeared in the intestinal mitochondria of sensitive helminths were: cristae thickening and decreasing in number and cellular membrane thickening. Twelve hours after anthelmintic administration, a total blocking of metabolic functionality was observed, and finally, these changes completely altered the optical density of the mitochondria. In ABZ resistant populations, the optical density has remained normal; and the cristae number, size or functionality of resistant nematode mitochondria has remained unchanged. (Cristina et al., 2015)

The aim for this experiment was to look for evidence of milk transfer of anthelmintic actives from ewes to their suckling lambs by reference to lambs’ faecal worm egg count (WEC). The hypothesis was that WEC will decline in lambs suckling ewes treated with anthelmintics known to be lipophilic. One group of lactating Border Leicester x Merino ewes were treated (TX) with a combination of short (2.5 mg/kg monepantel) and long-acting (1 mg/kg moxidectin long-acting injection and a sustained release of 4.62 g albendazole over 100 days) anthelmintics to remove gastrointestinal nematode (GIN) burden on day 0. The other group of lactating ewes (UTX) and all lambs (White Suffolk sires) were not treated. Ewes and lambs grazed as a single group and were exposed to GIN (predominately Haemonchus contortus) infection from pasture. Measurements were taken on days 0 and 7. WEC of lambs suckling UTX ewes increased from 6441 to 10,341 eggs per gram (epg) between days 0 and 7, while there was a 51% reduction in WEC for lambs suckling TX ewes. Packed cell volume (PCV) was significantly higher for lambs suckling TX ewes on day 7 compared to lambs suckling UTX ewes (28.5% vs. 24.9%, p = 0.039). These results suggest that lambs suckling ewes treated with lipophilic anthelmintics received a sub-therapeutic dose via milk which would increase selection within the GIN (H. contortus) population for anthelmintic resistance. (Dever and Kahn, 2015)

Two cases of nematode resistance to macrocyclic lactones were suspected and were investigated in two sheep flocks in the ‘Centre’ area of France. A nematode faecal egg count reduction test was performed in the two flocks. One case of resistance was confirmed with a mean reduction in the treated groups of 0% and 13% for ivermectin (CI 95%: -228/58) and moxidectin (CI 95%: -152/70) respectively compared to a non-treated group. A follow-up study was conducted where eighteen, naïve lambs were experimentally infected with larvae isolated from the resistant flock (5000 L3/lamb). Abomasal round worm burdens (Teladorsagia circumcincta) were reduced by 90% and 85% after ivermectin and moxidectin treatment, respectively. While for the intestinal round worm (Trichostrongylus colubriformis), the reduction values reached 100% and 99% for ivermectin and moxidectin respectively. This trial demonstrated the first double resistance of ovine round worms to macrocyclic lactone es in France. (Devo et al., 2015)

Haemonchus contortus is a veterinary nematode that infects small ruminants, causing serious decreases in animal production worldwide. Effective control through anthelmintic treatment has been compromised by the development of resistance to these drugs, including the macrocyclic lactones. The mechanisms of resistance in H. contortus have yet to be established but may involve efflux of the macrocyclic lactones by nematode ATP-binding-cassette transporters such as P-glycoproteins. Here we report the expression and functional activity of H. contortus P-glycoprotein 2 expressed in mammalian cells and characterise its interaction with the macrocyclic lactones, ivermectin, abamectin and moxidectin. The ability of H. contortus P-glycoprotein 2 to transport different fluorophore substrates was markedly inhibited by ivermectin and abamectin in a dose-dependent and saturable way. The profile of transport inhibition by moxidectin was markedly different. H. contortus P-glycoprotein 2 was expressed in the pharynx, the first portion of the worm’s intestine and perhaps in adjacent nervous tissue, suggesting a role for this gene in regulating the uptake of avermectins and in protecting nematode tissues from the effects of macrocyclic lactone anthelmintic drugs. H. contortus P-glycoprotein 2 may thus contribute to resistance to these drugs in H. contortus. (Godoy et al., 2015)

Anthelmintic resistance is a major problem for the control of parasitic nematodes of livestock and of growing concern for human parasite control. However, there is little understanding of how resistance arises and spreads or of the “genetic signature” of selection for this group of important pathogens. We have investigated these questions in the system for which anthelmintic resistance is most advanced; benznidazole resistance in the sheep parasites Haemonchus contortus and Teladorsagia circumcincta. Population genetic analysis with neutral microsatellite markers reveals that T. circumcincta has higher genetic diversity but lower genetic differentiation between farms than H. contortus in the UK. We propose that this is due to epidemiological differences between the two parasites resulting in greater seasonal bottlenecking of H. contortus. There is a remarkably high level of resistance haplotype diversity in both parasites compared with drug resistance studies in other eukaryotic systems. Our analysis suggests a minimum of four independent origins of resistance mutations on just seven farms for H. contortus, and even more for T. circumcincta. Both hard and soft selective sweeps have occurred with striking differences between individual farms. The sweeps are generally softer for T. circumcincta than H. contortus, consistent with its higher level of genetic diversity and consequent greater availability of new mutations. We propose a model in which multiple independent resistance mutations recurrently arise and spread by migration to explain the widespread occurrence of resistance in these parasites. Finally, in spite of the complex haplotypic diversity, we show that selection can be detected at the target locus using simple measures of genetic diversity and
There is some evidence that **resistance to levamisole and pyrantel in trichostrongylid nematodes** is due to changes in the composition of nicotinic acetylcholine receptors (nAChRs) which represent the drug target site. Altered expression patterns of genes coding for nAChR subunits, as well as the presence of truncated versions of several subunits, have been implicated in observed resistances. The studies have mostly compared target sites in worm isolates of very different genetic background, and hence the ability to associate the molecular changes with drug sensitivity alone have been clouded to some extent. The present study aimed to circumvent this issue by following target site gene expression pattern changes as resistance developed in *Haemonchus contortus* worms under laboratory selection pressure with levamisole. We applied drug selection pressure to early stage larvae in vitro over nine generations, and monitored changes in larval and adult drug sensitivities and target site gene expression patterns. High level resistance developed in larvae, with resistance factors of 94-fold and 1350-fold at the IC50 and IC95, respectively, in larval development assays after nine generations of selection. There was some cross resistance to bephenium (70-fold increase in IC95). The expression of all the putative subunit components of levamisole-sensitive nAChRs, as well as a number of ancillary protein genes, particularly Hco-unc-29.1 and ric-3, were significantly decreased (up to 5.5-fold) in the resistant larvae at generation nine compared to the starting population. However, adult worms did not show any resistance to levamisole, and showed an inverse pattern of gene expression changes, with many target site genes showing increased expression compared to the starting population. A comparison of the larval/adult drug sensitivity data with the known relationships for field-derived isolates indicated that the adults of our selected population should have been highly resistant to the drug if the larval/adult sensitivity relationships were in accordance with previous field isolates. Hence, our selected worms showed a life-stage drug sensitivity pattern quite different to that seen in the field. The present study has highlighted an association between drug target site changes and resistance to levamisole in *H. contortus* larvae. However, it has also highlighted the artificial nature of the larval selection method with levamisole, as the resistance phenotype and the associated molecular changes were only observed in the drug - pressured life stage. The study therefore reinforces the need for caution in extrapolating larval based laboratory selection outcomes to field resistances. (Satyavir et al., 2015)

Faecal egg count reduction test (FECRT) was conducted to determine the **efficacy of levamisole and ivermectin alone and in combination against gastrointestinal nematodes in sheep**. The experiment was conducted in two parts in animals with eggs (>150) per gram faeces. In first part, sheep (n=45) were divided into three groups (A-I, A-II and A-III) having 15 animals in each group. Groups A-I and A-II were treated with levamisole (7.5 mg/kg b.wt. subcutaneously) and ivermectin (0.2 mg/kg b.wt. subcutaneously), respectively. Group A-III served as untreated control. Faecal egg counts of each animal in each group were ascertained on 0 and 11th day post treatment (PT) by modified Mc Master technique. Percent FECR in groups A-I and A-II was 92.5 and 91.34%, respectively. Identification of infective larvae from both pre- and post treatment faecal culture revealed the predominance of *Haemonchus contortus*. The results indicated the partial resistance of levamisole and ivermectin against *H. contortus*. In the second part, 30 sheep were divided in two equal groups (B-I and B-II) of 15 sheep each. Group B-I was administered levamisole (@7.5 mg/kg b. wt. subcutaneously) + ivermectin (0.2 mg/kg b. wt. subcutaneously) and group B-II served as control. FECR was 99.37% on 11th day PT in group B-I. Coproculture from pre-treatment faecal samples revealed the predominance of *H. contortus* whereas no larvae were recovered from post treatment (PT) faecal culture in group B-I. Thus, the present study indicated that the combination of levamisole + ivermectin was more effective against resistant *H. contortus* than when these drugs were administered individually. (Satyavir et al., 2015)

There have been a number of **significant advances in recent years to the theory and practice of managing anthelmintic resistance in sheep in Australasia**. The general principles of resistance management are, firstly identification and mitigation of high-risk practices, secondly using effective anthelmintics, and thirdly maintaining a refuge of unselected parasites. The first of these principles has been updated recently with the findings from a series of farm-based trials in New Zealand, in which the economic benefits of both short- and long-acting anthelmintic treatments in ewes pre-lambing were found to be inconsistent and not always positive. There have also been significant changes to the second principle, particularly given the introduction of new active families onto the market. Evidence continues to favour the use of combination products to maximise efficacy and delay the onset of treatment failure. Many farmers have readily accepted the effectiveness of maintaining a refuge of unselected parasites; the challenge for researchers and advisers is now to improve adoption of properly designed and implemented resistance management programmes. A recently completed education programme in New Zealand has demonstrated that when this is achieved, then anthelmintic resistance can be controlled, and in many cases reduced in severity. (Sutherland, 2015)
Multiple drug resistance of nematodes against anthelmintics has become one of the most important economic problems in sheep production worldwide. The aim of this experiment was to evaluate the efficacy of monepantel (2.5 mg/kg) against gastrointestinal nematodes in faecal egg count reduction test (FECRT) and controlled efficacy test (CT) in naturally infected sheep. We used 30 sheep for the FECRT and 20 sheep for the CT, equally divided into control and treated groups. In the FECRT, the reduction was 98%. Larval identification of pre-treatment coprocultures revealed 100% Haemonchus spp. for both control and treated groups. Post-treatment culture of treated sheep was 100% Oesophagostomum spp., but only few larvae were recovered. In the control group, they were 99% Haemonchus spp and 1% Oesophagostomum spp. larvae. Based on the FECRT, Haemonchus spp. was considered susceptible to monepantel. The efficacy of monepantel in the CT against Haemonchus contortus and Trichostrongylus axei was 100% and against Cooperia curticei was 99.7%. For Trichostrongylus colubriformis, the efficacy was -21.5%. In both treated and untreated animals, Oesophagostomum columbianum was recovered from the large intestines. Based on FECRT and CT and in accordance with WAAVP standards, monepantel was ineffective against T. colubriformis and O. columbianum, but effective against H. contortus, T. axei and C. curticei in the studied flock. (Cintra et al., 2016)

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). (Raza et al., 2016)

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 the extent 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 (Traversa and von Samson-Himmelstjerna, 2016)

**Anthelmintic resistance-cattle**

The objective of the present study was to determine simultaneously the status of resistance against ivermectin (IVM) in gastrointestinal nematodes (GIN) and *Rhipicephalus microplus* (Canestrini, 1888) ticks in 12 cattle farms where IVM was used for the control of GIN in the Mexican tropics. Six farms had frequent use of IVM (≥ 4 times per year) and six farms had low frequency of IVM use (1-2 times per year). The faecal egg count reduction test and the larval immersion test were used to determine the resistant status of GIN and *R. microplus* against IVM, respectively. The results indicated that 100% of the surveyed farms had IVM-resistant GIN (reduction % from 0 to 67%). The genera involved were *Haemonchus, Cooperia, Ostertagia, Trichostrongylus*, and Oesophagostomum. Although the IVM was never used for the control of ticks, 50% of the surveyed farms presented GIN and *R. microplus* simultaneously resistant to IVM. Furthermore, two *R. microplus* populations showed high resistance ratio (RR) to IVM (farm TAT: RR50% = 7 and RR99% = 40.1; and farm SLS: RR50% = 2.4; RR99% = 11.0). A high frequency of IVM use (≥ 4 times per year) seemed to promote IVM resistance amongst *R. microplus* ticks compared with the farms with low frequency of IVM use (1-2 times per year; 66.6
This study reports the percentage of cattle farms with ivermectin (IVM) resistant gastrointestinal nematodes (GINs) in Veracruz, Mexico, and identifies the GIN genera involved in the resistances. It also describes management practices of anthelmintic (AH) use on the surveyed farms. Twenty-one farms were assessed by means of the faecal egg count reduction test using the McMaster technique. Only two farms had GIN populations susceptible to IVM (9.5%). The proportion of farms with IVM resistant GIN was 71.4% (15/21). Seven of these farms had less than 80% egg count reductions. Haemonchus and Cooperia were the genera most commonly found in the resistant populations, followed by Oesophagostomum. Inappropriate AH treatment practices were identified from the completed questionnaires. Further management practices such as selective treatment and quarantine treatments are proposed to further reduce the spread of IVM resistance between farms. (Alonso-Díaz et al., 2015)

The use of long-acting avermectins (AVMs) in cattle to treat infections with gastrointestinal nematodes was common in Brazil until its prohibition by state authorities. The prohibition; however, was rescinded in 2015, but a scientific discussion of the pros and cons of the use of these formulations is necessary. We evaluated the levels of resistance to 1.0 and 3.5% doramectin and to 3.15% ivermectin in cattle. The worms in animals treated with 3.5% doramectin were characterized by the suppression of oviposition and by a higher proportion of adult females carrying no eggs. Haemonchus placei, Cooperia punctata, C. pectinata, C. spatulata, and Oesophagostomum radiatum were resistant to the above compositions. The administration of long-acting AVM formulations did not result in a higher efficacy against these helminth populations. (Borges et al., 2015)

Anthelmintic resistant Ostertagia ostertagi, Trichostrongylus axei and Cooperia spp. on 20 commercial dairy farms in Victoria, Australia. (Bullen, 2015)

Anthelmintic resistance in nematodes of beef cattle is an emerging issue globally with implications for effective parasite control. The prevalence of resistance in beef cattle in the Mediterranean-style climatic zone of south-west Western Australia was assessed on 19 farms, using faecal egg count reduction tests. Pre-treatment faecal worm egg counts were compared with counts at 14 days after treatments with ivermectin (injectable), fenbendazole (oral), or levamisole (oral). A separately grazed group treated with topical ivermectin (pour-on) and sampled at 28 days was included as a comparison against injectable ivermectin. The results demonstrate that resistance is common, with failure of at least one anthelmintic (<95% reduction for each species, by arithmetic means) for either of the major species Cooperia oncophora or Ostertagia ostertagi on 17 of the 19 properties. Resistance to ivermectin (injectable) was demonstrated in C. oncophora in 59% of tests, but ivermectin was fully effective against O. ostertagi by this route. Conversely, O. ostertagi resistant to fenbendazole and levamisole were present on 50% and 67% of farms respectively, with both fully effective against C. oncophora. The finding of Haemonchus placei on several properties was unexpected but the egg counts were low and there is no suggestion of pathogenic effects. An indication of reduced efficacy of the pour-on ivermectin formulation compared to the injectable was apparent against both C. oncophora and O. ostertagi, and this may have implications for resistance development, given the widespread use of topical treatments reported in this region. This survey confirms that anthelmintic resistance in nematodes of beef cattle is common in Western Australia and the pattern of occurrence is in general agreement with surveys elsewhere in Australia and in other countries. (Cotter et al., 2015)

During the United States Department of Agriculture (USDA) National Animal Health Monitoring System’s (NAHMS) 2007-2008 beef study, producers from 24 states were offered the opportunity to evaluate their animals for internal parasites and for overall responses to treatment with anthelmintics. A lapse of 45 d was required between initial sampling and any previous treatments. Choice of anthelmintic (oral benzimidazoles, and both injectable and pour-on endectocides) was at the discretion of the producer so as not to alter the local control programs. Fresh faecal samples were collected from 20 animals, or from the entire group if less than 20, then randomly assigned to 1 of 3 participating laboratories for examination. Analyses consisted of double centrifugation flotation followed by enumeration of strongyle, Nematodirus, and Trichurus eggs (the presence of coccidian oocysts and tapeworm eggs was also noted). Where strongyle eggs per gram (epg) exceeded 30, aliquots from 2 to 6 animals were pooled for egg isolation and polymerase chain reaction (PCR) analysis for the presence of Ostertagia, Cooperia, Haemonchus, Oesophagostomum, and Trichostrongylus. Results from 72 producers (19 States) indicated that faecal egg count reductions were < 90% in 1/3 of the operations. All operations exhibiting less than a 90% reduction had used pour-on macrocyclic lactones as the anthelmintic treatment. While some of these less than expected reductions could have been the result of improper drug application, PCR analyses of the parasite populations surviving treatment, coupled with follow-up studies at a limited number of sites, indicated that less than expected reductions were most likely due to anthelmintic resistance in Cooperia spp. and possibly Haemonchus spp. (Gasbarre et al., 2015)
Anthelmintic resistance has been increasingly reported in cattle worldwide over the last decade, although reports from Europe are more limited. The objective of the present study was to evaluate the efficacy of injectable formulations of ivermectin and moxidectin at 0.2 mg per kg bodyweight against naturally acquired gastro-intestinal nematodes in cattle. A total of 753 animals on 40 farms were enrolled in Germany (12 farms), the UK (10 farms), Italy (10 farms), and France (8 farms). Animals were selected based on pre-treatment faecal egg counts and were allocated to one of the two treatment groups. Each treatment group consisted of between 7 and 10 animals. A post-treatment faecal egg count was performed 14 days (+/- 2 days) after treatment. The observed percentage reduction was calculated for each treatment group based on the arithmetic mean faecal egg count before and after treatment. The resistance status was evaluated based on the reduction in arithmetic mean faecal egg count and both the lower and upper 95% confidence limits. A decreased efficacy was observed in half or more of the farms in Germany, France and the UK. For moxidectin, resistance was confirmed on 3 farms in France, and on 1 farm in Germany and the UK. For ivermectin, resistance was confirmed on 3 farms in the UK, and on 1 farm in Germany and France. The remaining farms with decreased efficacy were classified as having an inconclusive resistance status based on the available data. After treatment Cooperia spp. larvae were most frequently identified, though Ostertagia ostertagi was also found, in particular within the UK and Germany. The present study reports lower than expected efficacy for ivermectin and moxidectin (based on the reduction in egg excretion after treatment) on European cattle farms, with confirmed anthelmintic resistance on 12.5% of the farms. (Geurden et al., 2015)

Resistance to benzimidazoles (BZs) is widespread in sheep nematodes and increasing in those of cattle. Several reasons including the predominant use of pour-on anthelmintics and lack of scales in field conditions lead to under-dosing of cattle and therefore to increased selection pressure. In an field experiment the frequency of BZ-resistance associated allele (TAC) in codon 200 in the beta-tubulin isotype 1 gene of Ostertagia ostertagi was monitored over one grazing season (approximately 30 weeks). Group 1, consisting of four calves, was experimentally infected with a pure O. ostertagi population displaying similar to 50% of the TAC allele. The subsequently following groups of calves (four groups of two calves each) acquired natural infections by grazing contaminated pastures. Each group was treated with increasing percentages of sub-therapeutic dosages of albendazole (35-65%). Larvae obtained from faecal cultures pre and post treatment were subjected to species/genus-specific PCR as well as pyrosequencing to determine allele frequencies. PCR revealed the presence of Ostertagia, Trichostrongylus, Haemonchus and Cooperia in pre-treatment samples and predominantly Ostertagia as well as some Trichostrongylus in post treatment samples. Faecal egg count reduction was always less than 90% 7-10 days post treatment. In naturally infected calves TAC allele frequencies were significantly increased (p < 0.05) after treatment and they also rapidly increased during the grazing season (pre: 15-63%; post: 55-89%). The more than 4-fold increase in resistant genotypes before treatment indicates how fast selection for BZ resistance can occur when sub-therapeutic dosages are combined with a high treatment frequency, even under moderated climatic conditions and in the presence of a refugium. (Knapp-Lawitzke et al., 2015)

The objective of this study was to evaluate the status of anthelmintic resistance (AR) in ruminants and horses in Spain. The efficacy of commonly used macrocyclic lactones (MLs) - ivermectin (IVM) and moxidectin (MOX) - was measured in sheep, cattle and horses. In addition, albendazole (ABZ) and levamisole (LEV) were evaluated in sheep and oxibendazole (OXI) and pyrantel (PYR) in horses. Efficacy was evaluated based on the difference between the arithmetic mean pre- and post-treatment faecal egg count (in cattle and horses), or compared to an untreated control group (in sheep). AR was present when the percentage reduction in egg count was < 95% and the lower 95% confidence interval (CI) was < 90%; if only one of these two criteria was met, the finding was recorded as suspected AR (SAR). In horses, AR - PYR and OXI was considered when the percentage reduction in egg count was <= 90% and the lower 95% CI was <= 80%. For each animal species, at least 10 study sites were selected. AR to at least one of the drugs was detected in all 10 sheep flocks; the main parasite identified after treatment was Teladorsagia circumcincta. Moreover, in 5 flocks multidrug resistance was identified, on 4 farms to drugs from different families, on one farm to both MOX and IVM and on another farm to all drugs tested. In cattle, the efficacy of both MOX and IVM was 100% on 4 and 3 farms, respectively, and therefore 60% of these farms were considered to have AR or SAR to both MLs. The most frequent parasite identified after treatment was Trichostrongylus spp., although Ostertagia ostertagi was also identified after treatment on one farm. In contrast to ruminants, the 4 drugs evaluated in horses were highly efficacious against strongyles, with efficacies for the MLs and OXI between 95 and 100% and between 94 and 100% for PYR, although 3 herds were SAR against PYR. In conclusion, AR to at least one of the commonly used drugs was identified on all sheep flocks investigated in the northwest of Spain. The occurrence of AR to MLs in cattle was higher than expected but consistent with what was observed in sheep. In horses, all currently used drugs were confirmed as effective against strongyles. (Martinez-Valladares et al., 2015)

To report anthelmintic resistance in gastrointestinal nematode parasites of cattle on commercial dairy farms in the Macalister Irrigation District of Gippsland, Victoria. 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
dictors may substantially contribute to lungworm infections in dairy cows. Veterinarians detected on 15/20 (70%) farms, fenbendazole on 16/20 (80%) farms and or antibodies can help farmers and veterinarians plan and implement act, whole worm. Bulk tank milk mintic resistance. 

and 3 production parameters (milk production, (Schunn et al., 2015) were selected based on their BTM test result of November 2013, obtained (Dank et al., 2015) and 109 BTM+ accumulated uptake of , these losses cannot be attributed to one of the two parasites. However, it is clear that y significant association with a positive BTM test were included in a logistic regression model, which confidence interval <90% when the mean FEC of the control group, differentiated by genus, was greater than 25 eggs/g. 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. 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. (Bullen et al., 2016)

Dictyocaulus viviparus

In November 2008, a total of 19,910 bulk tank milk (BTM) samples were obtained from dairy farms from all over Germany, corresponding to about 20% of all German dairy herds, and analysed for antibodies against the bovine lungworm Dictyocaulus viviparus by use of the recombinant major sperm protein (MSP). A total number of 3,397 (17.1%) BTM samples tested seropositive. The prevalences in individual German federal states varied between 0.0% and 31.2% positive herds. A geospatial map was shown to draw the distribution of seropositive and seronegative herds per postal code area. ELISA results were further analysed for associations with land-use and climate data. Bivariate statistical analysis was used to identify potential spatial risk factors for dictyocaulosis. Statistically significant positive associations were found between lungworm seropositive herds and the proportion of water bodies and grassed area per postal code area. Variables that showed a statistically significant association with a positive BTM test were included in a logistic regression model, which was further refined by controlled stepwise selection of variables. The Pseudo R2 value of 6% for the final model indicates that additional, unrecorded factors may substantially contribute to lungworm infections in dairy cows. Veterinarians should include lungworms in the differential diagnosis of respiratory disease in dairy cattle, particularly those at pasture. Monitoring of herds through BTM screening for antibodies can help farmers and veterinarians plan and implement appropriate control measures. (Schunn et al., 2015)

Infections with Dictyocaulus viviparus and Ostertagia ostertagi nematode parasites are of importance to bovine health and production in temperate areas across the world. Losses due to these parasites in dairy herds can be considerable due to decreased milk productivity and fertility. However, information on current epidemiological patterns in Irish dairy herds is limited. Bulk milk samples were collected from a total of 319 dairy farms across the Republic of Ireland. The D. viviparus samples were tested with an ELISA based on recombinant major sperm protein, while the O. ostertagi samples were tested with an ELISA based on crude saline extract, whole worm O. ostertagi antigen. Management data were collected from the farms using a questionnaire. Logistic regression was used to find significant associations between the presence of antibodies against D. viviparus and O. ostertagi and management factors. The overall prevalence of D. viviparus infection was 62.8%, while over 98% of herds had antibodies to O. ostertagi at the specified cut-off. Both D. viviparus and O. ostertagi antibodies were highest in November, which could be explained by the accumulated uptake of larvae through the grazing season. In herds of farmers that dosed their in-calf heifers with anthelmintics, the results more likely to be positive for antibodies against D. viviparus infection. This study highlights that both D. viviparus and O. ostertagi infections are widespread in dairy herds in Ireland throughout the grazing season. (Bloemhoff et al., 2015)

The objective of this study was to estimate the association between Dictyocaulus viviparus bulk tank milk (BTM) test results and milk production and milk composition parameters in adult Dutch dairy cattle herds. Bulk tank milk samples were collected in August and November 201, and ELISA tests were performed. Two hundred BTM positive (BTM+) and 200 BTM negative (BTM-) herds were selected based on their BTM test result of November 2013, obtained from a list of farms that participated in the Dutch GD Animal Health voluntary monitoring program for controlling nematode infections. The relationship between D. viviparus BTM status and 3 production parameters (milk production, milk fat %, and milk protein %) in summer (June to August 2013) and autumn (September and October 2013) was investigated using generalized linear mixed models. Production data were available for 126 BTM herds and 109 BTM+ herds. Results showed that a positive D. viviparus status was associated with decreased milk production (June: -1.01, July: -1.19, August: -1.68, September and October: -1.33 kg/cow per d). Milk fat percentage was 0.14% and 0.08% lower during summer and autumn, respectively, in BTM+ herds. No significant association was demonstrated between a positive BTM test result and milk protein percentage. Because a strong correlation was present between the BTM status for D. viviparus and that for Ostertagia ostertagi, these losses cannot be attributed to one of the two parasites. However, it is clear that these parasite infections have a considerable effect on production. (Dank et al., 2015)

The lungworm Dictyocaulus viviparus, causing parasitic bronchitis in cattle, induces a temporary protective immunity that prevents clinical disease. A radiation-attenuated larvae based vaccine is commercially available in a few European
countries, but has the disadvantages of a live vaccine. As a recombinant subunit vaccine would overcome these disadvantages, the parasite's muscle protein paramyosin (PMY) was tested as a recombinant vaccine antigen. *D. viviparous*-PMY was recombinantly expressed in *Escherichia coli* as a glutathione-S-transferase (GST)-fused protein. Emulsified in adjuvant Saponin Quil A, the protein was given intramuscularly into calves. Two independent recombinant PMY (rPMY) vaccination trials with negative control groups (first trial: adjuvant only; second trial: non-fused GST) as well as an additional positive control group in the second trial, using the Bovilis®Dictol live vaccine to verify vaccination results, were performed. To determine the vaccination success, shedding of larvae as well as worm burden and worm sizes were analyzed. Additionally, ELISA-based determination of development of immunoglobulins IgM, IgA, IgE, IgG as well as the subclasses IgG1 and IgG2 was performed. To analyze PMY localization in the bovine lungworm, immunohistochemical staining of adult worms was carried out. Immunohistochemical staining revealed that PMY is part of the bovine lungworm's pharyngeal and body wall muscles. Vaccination with rPMY resulted in 47% (geometric mean: 67%) and 57% (geometric mean: 71%) reduction of larvae shedding in the first and second vaccination trial, respectively. Worm burden was reduced by 54% (geometric mean: 86%) and 31% (geometric mean: 68%), respectively, and worms of rPMY-vaccinated cattle were significantly shorter in both trials. Furthermore, ELISAs showed a clear antibody response towards rPMY with exception of IgE for which titers could not be detected. After challenge infection, rPMY antibodies were only exceptionally elevated among study animals indicating PMY to be a hidden antigen. Even though vaccination with the attenuated live vaccine was with 94% (geometric mean: 95%) reduction in larvae shedding and 93% (geometric mean: 94%) reduction in worm burden superior to rPMY vaccination, results using the latter are promising and show the potential for further development of a recombinant PMY-based vaccine against the bovine lungworm. (Strube et al., 2015)

**Besnoitia sp**

Bovine besnoitiosis by *Besnoitia besnoiti* is a protozoal disease of cattle spreading in Europe. A vector-borne disease, its spread within a herd is closely linked to the presence of individuals with high potential for transmission (whose dermis is rich in cysts) and to the proliferation of vector insects: horseflies and stable flies, mostly. Recently, considerable progress has been made thanks to serological diagnostics and real-time PCR. Control strategies, in the absence of effective treatment and vaccination, are based primarily on medical surveillance after widespread serological screening of all animals in the herd aged over six months. (Alzieu and Jacquet, 2015)

Bovine besnoitiosis control remains a challenge because the disease continues to spread and control relies solely on accurate diagnosis coupled to management measures. However, recent studies have reported that routinely used ELISAs may raise a high number of false-positive results. Herein, cross-reactions between *Besnoitia besnoiti* antigens and anti-*Neospora caninum* and/or anti-*Sarcocystis* spp.-specific antibodies were studied in an in-house ELISA since *N. caninum* and *Sarcocystis* spp. are closely related parasites, and both infections are highly prevalent in cattle worldwide. The serum panel was composed of the following categories: sera from *B. besnoiti*-seronegative (n = 75) and -seropositive cattle (n = 66). *B. besnoiti*-based ELISA false-positive reactors (n = 96) together with *N. caninum* (n = 36) and *Sarcocystis* spp. (n = 42) -seropositive reference cattle sera. *B. besnoiti* tachyzoite based western blot (WB) results classified animals as seropositive or seronegative. Sera were analyzed for the detection of anti-*N. caninum* by WB and ELISA and anti-*Sarcocystis* spp.-specific antibodies by WB and IFAT. Those samples recognizing a *Sarcocystis* spp. 18-20 kDa antigenic region and *N. caninum* 17-18 kDa immunodominant antigen were considered to be *Sarcocystis* spp. and *N. caninum* seropositive, respectively. The category of *B. besnoiti* based-ELISA false-positive reactors showed the highest number of sera with specific anti-*Sarcocystis* spp. and anti-*N. caninum* antibodies (74%; 71/96), followed by the *N. caninum*-seropositive cattle category (52.8%; 19/36). In contrast, few *B. besnoiti*-seronegative and -seropositive cattle showed antibodies against *Sarcocystis* spp. and *N. caninum* (10.7%; 8/75 and 1.5%; 1/66), respectively. This study revealed that *B. besnoiti* false-positive ELISA results were associated not only with the presence of anti-*N. caninum* and anti-*Sarcocystis* spp. antibodies (chi 2: 78.36; p < 0.0001; OR: 34.6; CI: 14-88) but also with high antibody levels against them using ELISA and IFAT tests, respectively (p < 0.05; t-test). These results may explain why only some animals seropositive to *Sarcocystis* spp. and/or *N. caninum* are Besnoitia false-positive reactors. Therefore, sera meeting these requirements should be included in future validations of serological tests for bovine besnoitiosis. (Garcia-Lunar et al., 2015)

Bovine besnoitiosis is an emerging protozoan disease in cattle. Neither vaccines nor chemotherapeutic drugs are currently available for prevention and treatment of *Besnoitia besnoiti* infections. Therefore the implementation of appropriate disease management strategies is of utmost importance. The aim of this longitudinal study was to complement current knowledge on the chronology of disease progression. This was realized by correlating clinical findings in early stages of naturally acquired bovine besnoitiosis with results of real-time PCR of skin biopsies and of two western immunoblots and an immunofluorescent antibody test (IFAT). Animals for this study were obtained by i) closely monitoring a cow-calf operation with a high prevalence of bovine besnoitiosis for cases of acute disease, and by ii) conducting a 12-week cohabitation experiment on pasture with five healthy heifers, a healthy bull and five *B. besnoiti*
infected cows. A control group of six healthy heifers was kept at a minimal distance of 20m. Further, the spectrum of potential insect vectors was determined. Infected cattle were followed up to a maximum of 221 days after first detection of *B. besnoiti* antibodies. Two severely affected cows developed visible and palpable alterations of skin, a decrease in body condition despite good feed intake, and chronic bovine besnoitiosis-associated laminitis leading to non-healing sole ulcers. The cows also had high reciprocal IFAT titers and high loads of parasite DNA in skin samples. Two heifers developed a mild clinical course characterized by few parasitic cysts visible in the scleral conjunctivae and vestibula vaginae. Both heifers became infected during the time of high insect activity of the species *Musa domestica*, *Musca autumnalis*, *Haematobia irritans*, and *Stomoxys calcitrans*. When a third heifer became subclinically infected, low insect activity was recorded. None of the six control heifers contracted a *B. besnoiti* infection. In chronic besnoitiosis, the severe clinical course apparently corresponded with high reciprocal IFAT titers and high loads of parasite DNA in skin, whereas mild and subclinical cases displayed lower values. Bovine besnoitiosis-associated laminitis represents an important complication in severe chronic disease which severely impairs animal welfare. (Gollnick et al., 2015)

*Besnoitia besnoiti* is a protozoan parasite known to cause important economic losses in the cattle industry in Africa, Asia and the Mediterranean area. In the last years, (re-) emergence of the parasite has been reported in France, Germany, Hungary and Italy with in some cases, establishment of an endemic infection. In this article, the first case of besnoitiosis in Belgium in a Blonde d’Aquitaine bull imported from the south of France is described. Additionally, a brief overview of the epidemiology of the disease is provided. (Vanhoudt et al., 2015)

Bovine besnoitiosis, caused by *Besnoitia besnoiti*, is a re-emergent disease, which is linked to considerable economical losses in Europe. Although in Portugal bovine besnoitiosis is generally associated to the Alentejo, recently, seropositive farms were identified in the North and Centre and the first clinical cases were detected in Beira Interior. Since clinical signs can be confounded with other conditions, bovine besnoitiosis may be an under-diagnosed disease, particularly in non-endemic areas or in regions where it has been recently introduced. The aim of this study was to collect information from veterinary field practitioners (MV) in order to evaluate the general perception of bovine besnoitiosis. An online questionnaire was sent between January-March 2013 to all coordinators of the farmer producer’s organizations in mainland Portugal. Responses to the questionnaire showed that knowledge on the disease is geographically limited, accompanying the concentration of cases in Alentejo. MV with clinical experience (n = 14) were from the districts Santarem, Lisbon, Setubal, Evora, Beja and Portalegre while answers indicating unawareness of the disease (n = 66) came mainly from the north of Portugal. In 2013, the respondents detected cases in 22 farms in 14 counties in Alentejo. The culling of symptomatic animals, adopted by 71.4%, was the preferred control strategy. The majority of practitioners with clinical experience (71.4%) considered that bovine besnoitiosis has a medium to high economic importance. The present findings underline the need to disseminate information on the clinical picture and available laboratory resources among MV and livestock producers in order to promote early detection of the disease. (Waap et al., 2015)

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 oedema. 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 indiv 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 ferroris) 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*. (Schares et al., 2016)
**Echinococcus sp.**

*Echinococcus granulosus*, formerly regarded as a single species with a high genotypic and phenotypic diversity, is now recognised as an assemblage of cryptic species, which differ considerably in morphology, development, host specificity (including infectivity/pathogenicity for humans) and other aspects. This diversity is reflected in the mitochondrial and nuclear genomes and has led to the construction of phylogenetic trees and hypotheses on the origin and geographic dispersal of various taxa. Based on phenotypic characters and gene sequences, *E. granulosus (sensu lato)* has by now been subdivided into *E. granulosus sensu stricto* (including the formerly identified genotypic variants G1-3), *Echinococcus felidis* (the former 'lion strain'), *Echinococcus equinus* (the 'horse strain', genotype G4), *Echinococcus ortleppi* (the 'cattle strain', genotype G5) and *Echinococcus canadensis*. The latter species, as recognised here, shows the highest diversity and is composed of the 'camel strain', genotype G6, the 'pig strain', genotype G7, and two 'cervid strains', genotypes G8 and G10. There is debate whether the closely related G6 and G7 should be placed in a separate species, but more morphological and biological data are needed to support or reject this view. In this classification, the application of rules for zoological nomenclature led to the resurrection of old species names, which had before been synonymised with *E. granulosus*. This nomenclatural subdivision of the agents of cystic echinococcosis (CE) may appear inconvenient for practical applications, especially because molecular tools are needed for identification of the cyst stage, and because retrospective data on *E. granulosus* are now difficult to interpret without examination of voucher specimens. However, the increased awareness for the diversity of CE agents - now emphasised by species names rather than genotype numbers - has led to a large number of recent studies on this issue and a rapid increase of knowledge on geographical spread, host range and impact on human health of the various species. *E. granulosus s.s.*, often transmitted by sheep, is now clearly identified as the principal CE agent affecting humans. Contrary to previous assumptions, genotypes G6/7 of *E. canadensis* readily infect humans, although CE incidences are rather low where *E. canadensis* predominates.

Sub-Saharan Africa seems to be the region with the highest diversity of *Echinococcus*, and wild carnivores may play a more important role in the lifecycles of various species than previously assumed. Still, a number of issues remain unclear, e.g. possibly diverging parameters of diagnostic tests among the species, different responses to vaccines and, importantly, possibly required modifications of clinical management due to differences in pathogenicity. (Romig et al., 2015)

**Echinococcus granulosus**

Diagnosis and detection of *Echinococcus granulosus (sensu lato)* infection in animals is a prerequisite for epidemiological studies and surveillance of echinococcosis in endemic, re-emergent or emergent transmission zones. Advances in diagnostic approaches for definitive hosts and livestock, however, have not progressed equally over the last 20 years. Development of laboratory based diagnostics for canids using coproantigen ELISA and also coproPCR, have had a huge impact on epidemiological studies and more recently on surveillance during hydatid control programmes. In contrast, diagnosis of cystic echinococcosis (CE) in livestock still relies largely on conventional post-mortem inspection, despite a relatively low diagnostic sensitivity especially in early infections, as current serodiagnosticos do not provide a sufficiently specific and sensitive practical pre-mortem alternative. As a result, testing of dog faecal samples by coproantigen ELISA, often combined with mass ultrasound screening programmes for human CE, has been the preferred approach for monitoring and surveillance in resource-poor endemic areas and during control schemes. In this article we review the current options and approaches for diagnosis of *E. granulosus* infection in definitive and animal intermediate hosts (including applications in non-domesticated species) and make conclusions and recommendations for further improvements in diagnosis for use in epidemiological studies and surveillance schemes. (Craig et al., 2015)

**Echinococcus granulosus** is a cestode of zoonotic importance that infects a wide range of animals. The main definitive host of this parasite is the domestic dog, which most commonly becomes infected by ingestion of infected tissues from ruminant livestock. In some areas, however, wild carnivores have been reported to be infected with *E. granulosus* and to potentially have a role in the maintenance of infection. This study explores *E. granulosus* infection in free-ranging foxes in rural areas of the Coquimbo District. Faecal samples of live-trapped culpeo (*Lycalopex culpaeus*) and chilla (*L. griseus*) foxes were obtained in six of previously designed rural sites of the Coquimbo District in Chile between 2005 and 2006. Overall, *E. granulosus* coproantigen prevalence in chillas by a coproELISA test was 7% (2/28) and ranged from 0% to 20% in the different study sites. Only five culpeos were trapped and none was positive. The presence of *E. granulosus* in wild carnivores for the maintenance of this parasite in this District is discussed. (Acosta-Jamett et al., 2015)

The results of serological monitoring of larvae *Echinococcus granulosus* (Egl) and *Taenia hydatigena* (Thl) of sheep in farms of different zones of the Kabardino-Balkariya Republic carried out using the enzyme-linked immunosorbent assay (ELISA) and the comparability of the immunoassay data with the rate of animal infestation are presented. 225 blood serum samples collected from sheep were examined. As antigens for ELISA test served the excretory secretory products from *Echinococcus granulosus* (Egl) and *Taenia hydatigena* (Thl) protoscoleces, as conjugate - affinity purified, specific to
Cystic echinococcosis (CE) is an important zoonotic disease caused by the cestode parasite *Echinococcus granulosus*. It occurs in many parts of the world where pastoral activities predominate, including the Rio Negro province of Argentina. Although CE control activities have been undertaken in the western regions of Rio Negro for more than two decades, the disease continues to remain prevalent in both the human and livestock animal populations. **Vaccination of animal intermediate hosts of CE with the EG95 vaccine may provide a new opportunity to improve the effectiveness of CE control measures**, although data are lacking about field application of the vaccine. **Evaluate the impact of EG95 vaccination in sheep on the transmission of Echinococcus granulosus in a field environment.** Two trial sites were established in western Rio Negro province within indigenous communities. Vaccination of lambs born into one trial site was introduced and continued for 6 years. Prior to initiation of the trial, and at the end of the trial, the prevalence of CE in sheep was determined by necropsy. Weaned lambs received two injections of EG95 vaccine, approximately one month apart, and a single booster injection one year later. Vaccination was not implemented at the second trial site. A total of 2725 animals were vaccinated in the first year. Animals from this cohort as well as age-matched sheep from the control area were evaluated by necropsy. Introduction of the vaccine led to a statistically significant in the number and size of hydatid cysts in comparison to the situation prior to the introduction of the vaccine, or compared to CE prevalence in the control area where the vaccine was not applied. The prevalence of infection in the vaccinated area was also significantly reduced by 62% compared to the re-intervention level, being lower than the prevalence seen in the control area, although the difference from the control area after the intervention was not significant possibly due to limitations in the numbers of animals available for necropsy. Vaccination of sheep with the EG95 vaccine provides a valuable new tool which improves the effectiveness of CE control activities. Vaccination was effective even in a difficult, remote environment where only approximately half the lambs born into the communities were fully vaccinated. (Larrieu et al., 2015)

*Echinococcus granulosus* is a cestode parasite. The metacestode stage causes cystic echinococcosis (CE) mainly in the human liver and lung. **Current chemotherapy against CE is based on mebendazole and albendazole. However, benzimidazoles result in a low cure rate or are ineffective in many patients; therefore, novel compounds for the treatment of this disease have been studied.** Mefloquine was reported to be dramatically effective on cultured *Echinococcus multilocularis* metacestodes in *vitro*. And, nitazoxanide has a prominent protoscolicidal effect. However, these compounds have no impact on the growth of cysts harbored in mice. In this study, we investigated the *in vitro* and *in vivo* efficacy of mebendazole, mefloquine, and nitazoxanide against *E. granulosus* protoscolecites, germinal cells, and infected mice. The effect of mebendazole on protoscolecites and germinal cell was proved to be dose-dependent *in vitro*. And, a reduction of the cyst weight was also the found after oral application of mebendazole to infected mice. Mefloquine (5 and 10 mg/ml) caused death within 24 h of protoscolecites and germinal cells in vitro, whereas a lower concentration of 1 mg/ml was ineffective. In mice infected with *E. granulosus*, oral mefloquine (200 and 400 mg/kg twice weekly for 2 weeks) showed no reduction in parasite weight. Without affecting the viability of germinal cells and the growth of hydatid cysts, nitazoxanide only showed protoscolicidal effects in infected mice. In conclusion, mebendazole, mefloquine, and nitazoxanide showed various effects on *E. granulosus* under different conditions. These drugs could be useful to some extent in the treatment of CE. (Liu et al., 2015)

Cystic echinococcosis caused by *Echinococcus granulosus sensu lato* is one of the most important helminth zoonoses in the world; it affects both humans and livestock. The disease is endemic in Argentina and highly endemic in the province of Neuquen. Considerable genetic and phenotypic variation has been demonstrated in *E. granulosus*, and **ten different genotypes (G1-G10) have been identified using molecular tools.** *Echinococcus granulosus sensu lato* may be considered a species complex, comprised of *E. granulosus sensu stricto* (G1-G3), *E. equinus* (G4), *E. ortleppi* (G5) and *E. canadensis* (G6-G10). In endemic areas, the characterization of cystic echinococcosis molecular epidemiology is important in order to apply adequate control strategies. A cut-off value for larvae large hook total length to distinguish *E. granulosus sensu stricto* isolates from those produced by other species of the complex was defined for the first time. Overall, 1780 larval hooks of 36 isolates obtained from sheep (*n* = 11, G1), goats (*n* = 10, G6), cattle (*n* = 5, G6) and pigs (*n* = 10, G7) were analysed. Validation against molecular genotyping as gold standard was carried out using the receiver operating characteristic (ROC) curve analysis. The optimum cut-off value was defined as 26.5 mm. The proposed method showed high sensitivity (97.8%) and specificity (91.1%). Since in most endemic regions the molecular epidemiology of
Echinococcus multilocularis

Alveolar echinococcosis (AE), caused by the larval (metacestode) stage of Echinococcus multilocularis, is considered one of the most serious parasitic zoonoses in Central and Eastern Europe and is emerging also in large parts of Asia and in North America. The red fox represents the main definitive host of E. multilocularis in Europe, but the raccoon dog, the domestic dog and to a much lesser extent the domestic cat also represent potential definitive hosts. The natural intermediate hosts of E. multilocularis are mainly voles. The spectrum of accidental hosts is broad and includes many species of monkeys, pigs, dogs and humans which get infected by oral uptake of the viable eggs. Yet, human AE is a very rare disease in Europe; incidences have increased in recent years, while the infection is widely distributed in foxes with high prevalences reaching up to 70% in some areas. Generally, infected foxes represent a zoonotic risk, which may be particularly relevant in urban areas. Furthermore, there is concern that the risk for humans to acquire AE may rise due to the suspected geographical spread of the parasite as assessed by infections in its definitive hosts and the high prevalences in some regions. Monitoring and surveillance activities have therefore been initiated in a few European countries. Several diagnostic strategies have been developed and validated in recent years, applying classical worm detection by microscopy, but also immunological (ELISA for coproantigen detection) and molecular tests (coproDNA detection by PCR). However, there is an urgent need for defining minimal requirements and harmonised approaches for these activities to allow for a reliable assessment of the epidemiological situation in Europe and comparable results from different countries.(Conraths and Deplazes, 2015)

Alveolar echinococcosis (AE), a parasitic disease primarily of the liver caused by the larval stage of Echinococcus multilocularis, is highly endemic in Switzerland. In contrast to well-established management protocols in people, little is known with regard to optimal treatment strategies in dogs. The objective of this study was to describe the clinical signs and diagnostic procedures in dogs with AE and to evaluate outcome following medical treatment alone or surgery and medical treatment. Of 23 putative AE cases between 2004 and 2014, 20 were classified as confirmed (n=18) or probable (n=2) AE, based on abdominal ultrasound, serology, cytology, histology and/or PCR. Most dogs presented with abdominal distension in an advanced stage of disease. Dogs receiving specific treatment (radical or debulking surgery together with medical treatment, or medical treatment alone) survived longer than dogs left untreated, but no difference was found between treatment types. Survival at one year was associated with absence of free abdominal fluid, absence of abdominal distension and treatment of any type. However, dogs treated with debulking surgery all faced relapse. Findings of this study suggest that in AE-affected dogs for which a therapeutic approach is regarded appropriate by owners and veterinarians, radical surgical resection and medical treatment or, if total resection is not possible, medical treatment alone should be considered. However, studies on larger numbers of dogs are necessary before definitive treatment recommendations can be made.(Corsini et al., 2015)

Echinococcus multilocularis is the causative agent of alveolar echinococcosis, a serious and emerging zoonotic disease in many parts of the northern hemisphere. Humans but also primates and other accidental hosts can acquire the infection by the ingestion of eggs excreted by the carnivore definitive hosts, e.g. after hand contact with egg-contaminated
The range of *Echinococcus multilocularis* has extended over the past 4 years, effectively removing the 'buffer zone' of land between the channel coast and the western-most edge of its endemic area. This means that pet owners taking their dogs and cats across the channels should consider treating their dogs or cats at monthly intervals with a suitable cestocide containing praziquantel or equivalent if there is any chance of the pet having access to rodents. The role of cats as significant producers of eggs is markedly less than that of foxes or dogs. However, the role of the cat as a source of infection within the domestic environment is less well defined. Infection of humans has a lag time of between 5-15 years before clinical signs are seen, thus it will be some time before the scale of zoonotic infections is known from the areas where the infection has now spread to. Diagnosis in humans relies on serology, PCR, and magnetic resonance imaging (MRI) or computer tomography (CT) scans to detect characteristic cystic lesions normally in the liver. Cure is most likely to be achieved if resection of the entire lesion is possible. Long-term benzimidazole treatment is normally administered to ensure control. In addition to regular cestoidal treatment of dogs and cats, cleanliness measures including hand washing before eating and wearing gloves when gardening can help with preventing human infection. (Fisher, 2015)

Alveolar echinococcosis (AE) is a neglected 'malignant' parasitic disease. The European endemic area of *Echinococcus multilocularis* in foxes is larger than previously anticipated, and there is new evidence that both fox populations and the prevalence of *E. multilocularis* have increased in many areas, indicating increased pressure for infection with *E. multilocularis* eggs in intermediate and accidental hosts, including humans. **This may result in more human AE cases within the next decades.** Current numbers of both immunocompetent and immuno-compromised AE patients, and the anticipated future increase, call for scaling-up research to rapidly improve the development and implementation of prevention measures, early diagnosis, and curative treatment of human AE. (Gottstein et al., 2015a)

Epidemiological studies have demonstrated that the majority of human individuals exposed to infection with *Echinococcus spp.* eggs exhibit resistance to disease as shown by either seroconversion to parasite specific antigens, and/or the presence of 'dying out' or 'aborted' metacestodes, not including hereby those individuals who putatively got infected but did not seroconvert and who subsequently allowed no development of the pathogen. For those individuals where infection leads to disease, the developing parasite is partially controlled by host immunity. In infected humans, the type of immune response developed by the host accounts for the subsequent trichotomy concerning the parasite development: (i) seroconversion proving infection, but lack of any hepatic lesion indicating the failure of the parasite to establish and further develop within the liver; or resistance as shown by the presence of fully calcified lesions; (ii) controlled susceptibility as found in the "conventional" alveolar echinococcosis (AE) patients who experience clinical signs and symptoms approximately 5-15 years after infection, and (iii) uncontrolled hyperproliferation of the metacestode due to an impaired immune response (AIDS or other immunodeficiencies). Immunomodulation of host immunity toward anergy seems to be triggered by parasite metabolites. Beside immunomodulating IL-10, TGF beta-driven regulatory T cells have been shown to play a crucial role in the parasite-modulated progressive course of AE. A novel CD4+CD25+ Treg effector molecule FGL2 recently yielded new insight into the tolerance process in *Echinococcus multilocularis* infection. (Gottstein et al., 2015b)

The European Food Safety Authority (EFSA) was required to support the European Commission in preparing the review of Regulation (EU) No 1152/2011. In Europe, red fox ( *Vulpes vulpes* ) is the main definitive host of the *Echinococcus multilocularis* (EM) lifecycle. There is no evidence that any other carnivore species can maintain the lifecycle in the absence of red fox, and this makes it to most relevant target species for surveillance. Movement of infected definitive hosts is an important introduction pathway. The knowledge on the geographical distribution of the environmental factors for the persistence of the lifecycle is scarce. In areas where no suitable autochthonous wild canid hosts and no highly suitable intermediate hosts are present, e.g. Malta, establishment of the EM cycle is considered close to impossible. Such
countries do not need to carry out surveillance on domestic dogs to substantiate absence of EM in the relevant animal population. Reconsideration of some aspects of the current legislation regarding surveillance activities might be relevant; for example the identification of epidemiologically relevant units should be independent from political borders. Studies to improve the knowledge on epidemiological risk factors should be encouraged to enable risk-based sampling.

Echinococcus notification should always be done at species level in order to discriminate between the more severe alveolar echinococcosis and the cystic echinococcosis. Praziquantel is the substance of choice for the treatment of dogs. However, the treatment window should be reconsidered to reduce the risk of re-infection: a general rule is to treat as close as possible to entry into a non-infected country. There is a lack of standardization of the diagnostic methods between laboratories. The diagnostic sensitivity of the tests should be established in accordance to the World Organisation for Animal Health (OIE) standards for validation. For the time being, the diagnostic sensitivity can be set conservatively to 78%. (Health and Welfare, 2015)

The life cycle of the zoonotic cestode Echinococcus multilocularis depends on canids (mainly red foxes) as definitive hosts and on their specific predation on rodent species (intermediate hosts). Host densities and predation rates are key drivers for infection with parasite eggs. We demonstrate that they strongly depend on multifaceted human-wildlife interactions: vaccination against rabies, elimination of top predators, and changing attitude towards wildlife (feeding) contribute to high fox densities. The absence of large canids, low hunting pressure, and positive attitudes towards foxes modify their anti-predator response (‘landscape of fear’), promoting their tameness, which in turn facilitates the colonization of residential areas and modifies parasite transmission. Such human factors should be considered in the assessment of any intervention and prevention strategy. (Heggin et al., 2015)

The aim of the study was to estimate the current prevalence of E. multilocularis in selected populations of red foxes in Poland and to evaluate the changes in prevalence of this parasite by comparison with the results obtained in the same area during earlier surveillance. The investigations were performed in the area of four Polish provinces: 2 eastern/south-eastern (Lubelskie and Podkarpackie) and 2 south-western (Slaskie and Opolskie). Five hundred red foxes coming from the investigated areas were examined between 2013 and 2014 to estimate the current situation in selected provinces. Moreover, 550 red foxes from the same areas examined between 2007 and 2013 were used for comparison of differences in E. multilocularis prevalences in time. Intestines were examined with the use of the sedimentation and counting technique. Among 500 foxes examined in the current study, 118 were positive for E. multilocularis. There were differences in prevalence between individual provinces: Podkarpackie Province - 54.6%, Lubelskie Province - 18.9%, Slaskie Province - 11.7%, and Opolskie Province - 3.9%. Statistical analysis demonstrated that in most cases there were no differences in prevalence between the current results and the results from previous studies. Only in Opolskie Province was a statistically significant increase observed between 2010 and 2014. A stable degree of infection in the region with high prevalence of this parasite was demonstrated. However, a significant increase in the region with very low prevalence of E. multilocularis points out the necessity to monitor this infection during the coming years to control the progress of the disease. (Karamon et al., 2015)

Alveolar and cystic echinococcosis, caused by the metacestode larval stages of the tapeworms Echinococcus multilocularis and Echinococcus granulosus, respectively, are life-threatening diseases and very difficult to treat. The introduction of benzimidazole-based chemotherapy, which targets parasite beta-tubulin, has significantly improved the life-span and prognosis of echinococcosis patients. However, benzimidazoles show only parasitostatic activity, are as associated with serious adverse side effects and have to be administered for very long time periods, underlining the need for new drugs. Very recently, the nuclear genomes of E. multilocularis and E. granulosus have been characterised, revealing a plethora of data for gaining a deeper understanding of host-parasite interaction, parasite development and parasite evolution. Combined with extensive transcriptome analyses of Echinococcus life cycle stages these investigations also yielded novel clues for targeted drug design. Recent years also witnessed significant advancements in the molecular and cellular characterisation of the Echinococcus ‘germinative cell’ population, which forms a unique stem cell system that differs from stem cells of other organisms in the expression of several genes associated with the maintenance of pluripotency. As the only parasite cell type capable of undergoing mitosis, the germinative cells are central to all developmental transitions of Echinococcus within the host and to parasite expansion via asexual proliferation. In the present article, we will briefly introduce and discuss recent advances in Echinococcus genomics and stem cell research in the context of drug design and development. Interestingly, it turns out that benzimidazoles seem to have very limited effects on Echinococcus germinative cells, which could explain the high recurrence rates observed after chemotherapeutic treatment of echinococcosis patients. This clearly indicates that future efforts into the development of parasitocidal drugs should also target the parasite’s stem cell system. (Koziol and Brehm, 2015)

Echinococcus multilocularis is a widespread parasite with zoonotic potential. Carnivores are the definitive hosts and rodents the alternate hosts. There have been reports of single cases of infected European beavers (Castor fiber). Humans are accidental alternate hosts of the parasite so-called alveolar echinococcosis is one of the most dangerous zoonotic diseases and can lead to death if not treated. During a project to monitor the population of the European beaver in
The life cycle of the cestode *Echinococcus multilocularis* primarily involves canids and small mammals (rodents, lagomorphs) as definitive and intermediate hosts, respectively. Several surveys have identified marked temporal and geographical variations at different scales in the parasite’s prevalence in both types of hosts, suggesting variations in the biological and ecological factors that control transmission processes. The parasite transmission from intermediate to definitive hosts is determined by the predator prey relationship, which theoretically depends on prey population dynamics and the complex dietary response of predators to varying densities of prey species and other food items. Parasite eggs are transmitted to intermediate hosts via carnivore faeces, whose distribution in the environment is driven by the defeating behaviour of final hosts. We reviewed field-based studies that address issues related to the trophic ecology and behaviour of definitive hosts, interactions between definitive and intermediate hosts, and *E. multilocularis* transmission both in wild and domestic animals in rural and urban environments. Two density-dependent mechanisms control the transmission dynamics in definitive hosts: one is based on the variations in the availability of intermediate hosts, and the other is based on the variations in the density of the definitive host and its faeces. Non-linearity and the direct and delayed responses of definitive host contamination in relation to intermediate host population variations were recorded. The dietary response of the red fox was shown to be complex when abundant alternative resources were available (anthropogenic food, multiple intermediate host prey species). Micro-local hotspots of parasite transmission to intermediate hosts in a landscape, as well as areas of higher risk for human contamination in village and urban settings, may be explained by the definitive hosts’ activity patterns and defecation behaviour. (Raoul et al., 2015)

**Other cestodes**

Cysticercosis caused by the metacestode stage of *Taenia hydatigena* is endemic in Sardinia. Information on the genetic variation of this parasite is important for epidemiological studies and implementation of control programs. Using two mitochondrial genes, the cytochrome c oxidase subunit 1 (cox1) and the NADH dehydrogenase subunit 1 (ND1) we investigated the genetic variation and population structure of *Cysticercus tenuicollis* from Sardinian intermediate hosts and compared it to that from other hosts from various geographical regions. The parsimony cox1 network analysis indicated the existence of a common lineage for *T. hydatigena* and the overall diversity and neutrality indices indicated demographic expansion. Using the cox1 sequences, low pairwise fixation index (Fst) values were recorded for Sardinian, Iranian and Palestinian sheep *C. tenuicollis* which suggested the absence of genetic differentiation. Using the ND1 sequences, *Cteniuicollis* from Sardinian sheep appeared to be differentiated from those of goat and pig origin. In addition, goat *C. tenuicollis* were genetically different from adult *T. hydatigena* as indicated by the statistically significant Fst value. Our results are consistent with biochemical and morphological studies that suggest the existence of variants of *T. hydatigena*. (Boufana et al., 2015)

The efficacy of oxendazole (OFZ) on *Taenia hydatigena* metacestodes, also called *Cysticercus tenuicollis* (*C. tenuicollis*), was studied in 648 raising pigs. This study was performed in Tumbes Department in Peru, an endemic area for cysticercosis. Pigs were randomized in two groups: untreated group (n = 142) did not receive any treatment and treated group (n = 506) received OFZ treatment at a single dose of 30 mg/kg body weight. Six months after treatment, the pigs were necropsied. The prevalence of infection by *C. tenuicollis* among the pigs was 27.5% (39/142) and 2.0% (10/506) in untreated and treated groups, respectively. Untreated group was infested only with viable cysts, whereas treated group had no viable cysts. All the cysts found in treated group presented degeneration, with a thick membrane, and they contained milky fluid and fibrous tissue. A single dose of OFZ was effective against *C. tenuicollis*, thus providing an alternative drug for controlling this parasite in pigs. (Gomez-Puerta et al., 2015)
This report describes two cases of **acute outbreaks of cysticercosis in lambs and kids** caused by *Cysticercus tenuicollis* the larval (metacestode) stage of the tapeworm *Taenia hydatigena*. The acute form of cysticercosis is rare and only a few cases have been described in the literature in the United Kingdom, Greece and Turkey. In the first case presented in this report 40% of lambs aged 2-3 months died over a period of 3-4 weeks during the month of March 2013. In the second case a few months later, 30% of lambs and 20% of kids succumbed. In both episodes adult sheep and goats were not affected. Pathological investigation revealed larval cestodes migrating through the liver and lungs. Investigation of the incidents led to the possibility that the main source of infestation was in the first case food borne in the concentrated feed manufactured by a local dealer, and in the second case due to contaminated hay harvested by the farmer and fed to the animals. The article describes the outbreaks, macroscopic and microscopic pathology findings and epidemiological investigations. (Perl et al., 2015)

Bovine cysticercosis is a zoonosis caused by the cestode *Taenia saginata* and involves cattle as the intermediate host and humans as the final host. This disease is both a public health issue and an economic concern for farmers. Cattle are infected after grazing on infected pasture. Humans are infected by the consumption of raw or under-cooked meat. This study aimed to identify geographical areas where animals are infected by bovine cysticercosis so as to implement adequate control measures and to provide a risk-based meat inspection process for improving disease detection. Considering both the long period of cyst development in cattle muscle and the complexity of cattle movements, a spatial analysis of slaughtered cattle found to be harboring viable and degenerated cysts was a challenge. Detection of clusters of bovine cysticercosis cases was performed using a spatial scan statistic with a discrete Poisson model adjusted for a variable combining age and sex. The novelty of this approach was that it used an animal-herd level weighted analysis to take into account the uncertainty of the location where animals became infected. This study included 4,557,593 (91.3%) cattle slaughtered in 2010 in France in 181 slaughterhouses. The meat inspection process enabled the detection of 6431 cattle harbouring at least one bovine cysticercosis lesion and 603 harbouring at least one viable cyst. Three significant clusters for cattle with all types of cysts were detected through the spatial analysis in north-western and eastern France. One significant cluster was detected in eastern France for cattle with viable cysts only. The difference in location of the clusters detected, when considering only cattle harbouring viable cysts or cattle harbouring all types of cysts, proved the relevancy of this novel approach. We identified areas in France with a higher risk of bovine cysticercosis in which investigations could be performed to identify the risk factors that explained this spatial distribution. These risk factors could then be used to suggest control measures in these areas and to implement a reinforced meat inspection protocol so as to increase the efficiency of the current meat inspection process. (Dupuy et al., 2015)

The bovine taeniasis-cysticercosis complex can be defined as a set of pathological changes caused by the adult form of *Taenia saginata* in humans and their larval form in animals (*Cysticercus bovis*). Data on the occurrence of bovine cysticercosis comes from the records of veterinary inspection of meat in slaughterhouses under fiscalization, where some positive cases may go unnoticed, especially in moderate infections. So, it is relevant the use of serological tests that have greater sensitivity than the post-mortem routine exams. Studies have shown the possible application of the ELISA test as a tool for epidemiological studies of the parasitosis and in the identification of animals with cysts. The aim of this study is to determine the detection threshold of the indirect ELISA test, using experimentally and naturally infected animals in detecting cases of cysticercosis. The sensitivity of the test to naturally infected animals using a cut-off of 1 and 2 added with 2 standard-deviation (SD) was of 12% and 24.4%, respectively. However, when using the cut-off of 1 and 2 added with 3 SD, the test sensitivity dropped, represented by 14.4% and 1.99% of the test sensitivity. As for the samples from experimentally infected animals, using the cut-off of 1 and 2 added with 2 SD the sensitivity was 55.9% and 92.5%; and adding 3 SD the values found were 31.2% and 86%, respectively. The specificity of the test in all situations tested was 100%. It is important to take into account the right choice of control sera used in the ELISA test, whereas, according to its application, it is necessary to increase the sensitivity or specificity. (Guimaraes-Prióxeto et al., 2015)

Bovine cysticercosis is cosmopolitan in its distribution, occurring in developing as well as in industrialized countries. Control of this parasite requires a good diagnostic test to identify infected animals carrying viable cysticerci. The currently applied meat inspection method has low sensitivity. While ELISA protocols were developed for detection of circulating antigens or antibodies against *T. saginata* cysticerci in cattle. But, these assays have not been validated and applied in field conditions yet. Therefore, the aim of this study was to **develop and optimize a milk Ab-ELISA protocol for the diagnosis of T. saginata cysticerci in cattle**. As no reference milk samples were available, the protocol was developed and optimized using milk spiked with reference serum samples. Series of tests were performed to develop and optimize the test. Finally, the protocol was established using sheep anti bovine IgG1 as conjugate, *T. saginata* metacestode excretory and secretory (ES) as antigen and 2% casein as blocking buffer. The test detected all reference positive samples as positive and negative samples as negative spiked in skim milk and cow milk. In a next step, both serum and milk samples should be collected from infected and non-infected dairy cows to confirm the use of the test. The results of this study showed that the protocol is promising test for diagnosis of *T. saginata* in dairy cattle. (Hailu and Gabriel, 2015)
This study focused on estimating the economic losses resulting from cysticercosis at beef cattle farms that supply an export slaughterhouse located in the state of Sao Paulo, Brazil, and to identify the epidemiological risks factors involved in the disease to ascertain if these farms adopt Good Agricultural Practices (GAP). To this, we used data recorded in 2012 by Brazil’s Federal Inspection Service (SIP) on the daily occurrence of the disease, according to the farm from which the animals originated. In addition, the associated risk factors were determined based on a case-control study at 48 farms. Cysticercosis was detected in 2.26% (95% CI 2.2-2.33) of the 190,903 bovines supplied by 556 farms in the following four states: 2.92% (95% CI 2.83-3.03) in Sao Paulo, 1.81% (95% CI 1.71-1.93) in Minas Gerais, 0.71% (95% CI 0.6-0.82) in Goias and 1.11% (95% CI 0.79-1.57) in Mato Grosso do Sul, with significant differences in the epidemiological indices of these states. Cysticercosis was detected at 58.45% (95% CI 54.36-62.55) of the farms of this study, representing estimated economic losses of US$312,194.52 for the farmers. Lower prevalence of this disease were found at the farms qualified for exports to the European Union, indicating a statistically significant difference from those not qualified to export to Europe. The access of cattle to non-controlled water sources, as well as sport fishing activities near the farms, was identified as risk factors. Cysticercosis causes considerable losses in Brazil’s beef supply chain, with lower prevalence appearing only at farms qualified to export to the European Union. As for the access of cattle to non-controlled water sources, this is an indication that GAP are not implemented by some farms, demonstrating the violation of international agreements by the industry and the farms. (Marques Rossi et al., 2015)

The larval stage of Taenia multiceps, also known as coenurus, is the causative agent of coenurosis, which results in severe health problems in sheep, goats, cattle and other animals that negatively impact on animal husbandry. There is no reliable method to identify coenurus infected goats in the early period of infection. We identified a full-length cDNA that encodes acidic ribosomal protein P2 from the transcriptome of T. multiceps (TmP2). Following cloning, sequencing and structural analyses were performed using bioinformatics tools. Recombinant TmP2 (r TmP2) was prokaryotically expressed and then used to test immunoreactivity and immunogenicity in immunoblotting assays. The native pr otins in adult stage and coenurus were located via immunofluorescence assays, while the potential of r TmP2 for indirect ELISA-based serodiagnosis was assessed using native goat sera. In addition, 20 goats were randomly divided into a drug treatment group and a control group. Each goat was orally given mature, viable T. multiceps eggs. The drug treatment group was given 10% praziquantel by intramuscular injection 45 days post-infection (p.i.), and all goats were screened for anti-TmP2 antibodies with the indirect ELISA method established here, once a week for 17 weeks p.i. The open reading frame (366 bp) of the target gene encodes a 12.62 kDa protein, which showed high homology to that from Taenia solium (93% identity) and lacked a signal peptide. Immunofluorescence staining showed that TmP2 was highly localized to the parenchymatous zone of both the adult parasite and the coenurus; besides, it was widely distributed in cystic wall of coenurus. Building on good immunogenic properties, r TmP2 antibody was detectable in the sera of naturally infected goats and sheep. In goats experimentally infected with T. multiceps, anti-TmP2 antibody was detectable in the control group from 3 to 10 weeks and 15 to 17 weeks p.i. In the drug-treated group, the anti-TmP2 antibody dropped below the cut-off value about 2 weeks after treatment with praziquantel and remained below this critical value until the end of the experiment. The indirect ELISA method developed in this study has the potential for detection of T. multiceps infections in hosts. (Huang et al., 2015)

Many interventions against Taenia solium are evaluated by assessing changes in the prevalence of porcine cysticercosis ascertained by carcass dissection. Financial and logistical difficulties often prohibit dissection of entire pig carcasses. We assessed 209 pigs from rural areas of Cameroon and Peru for the presence of T. solium cysticerci and determined the distribution of parasites within the infected animals. Considering the presence of cysts in the tongue, masticatory muscles and heart, 31 of the 38 (81%) naturally infected animals were identified as having cysts. Dissection of only the tongue, masticatory muscles and heart provides a relatively sensitive and highly specific method for diagnosis of porcine cysticercosis. (Lightowler et al., 2015)

Taenia multiceps is a globally distributed zoonotic cestode, which uses canids as definitive and the ungulates, especially sheep and goats as intermediate hosts. The resulting cerebral and non-cerebral cysts caused by the larval forms of this cestode are commonly known as coenurus. The location of cyst(s) outside the central nervous system, particularly in goats, suggests that they may be a different strain or genetic variants of T. multiceps or may reflect a different host response to the parasite in the goats. Therefore, conflicting views exist on their specification and based on typological grounds, T. multiceps and Taenia gaigeri have been recognized separately. In the present study, central nervous system, muscles, heart, lungs, kidneys and other visceral organs of the experimentally infected sheep and goats, challenged with T. multiceps eggs originated from the cerebral cysts, were evaluated by histopathological and molecular methods. Although acute and chronic forms of cerebral coenurus were developed in the experimentally infected sheep, non-cerebral chronic coenurus was observed in the experimentally infected goats. In addition, sequence analysis using the cytochrome c oxidase subunit I (cox1) and NADH dehydrogenase I (nad1) genes of mitochondrial DNA, showed that all cerebral and non-cerebral samples obtained from experimental infection were 100% identical to each other and their original source. Based on molecular assessment, pathogenesis and morphological criteria the larval
forms of *T. multiceps* were found originated from the same species. Therefore *T. gaigeri* may not be a distinct species from *T. multiceps* and *T. multiceps* is a single valid species within the genus *Taenia.*(Oryan et al., 2015)

This report describes two cases of **acute outbreaks of cysticercosis in lambs and kids** caused by *Cysticercus tenuicollis* the larval (metacestode) stage of the tapeworm *Taenia hydatigena.* The acute form of cysticercosis is rare and only a few cases have been described in the literature in the United Kingdom, Greece and Turkey. In the first case presented in this report 40% of lambs aged 2-3 months died over a period of 3-4 weeks during the month of March 2013. In the second case a few months later, 30% of lambs and 20% of kids succumbed. In both episodes adult sheep and goats were not affected. Pathological investigation revealed larval cestodes migrating through the liver and lungs. Investigation of the incidents led to the possibility that the main source of infestation was in the first case food borne in the concentrated feed manufactured by a local dealer, and in the second case due to contaminated hay harvested by the farmer and fed to the animals. The article describes the outbreaks, macroscopic and microscopic pathology findings and epidemiological investigations.(Perl et al., 2015)

Bovine cysticercosis in Europe has been known for centuries but the data showing the occurrence of this zoonosis are scarce. The aim of this paper is to **review and present the current knowledge on bovine cysticercosis in Europe.** We conducted a systematic review of studies published between 1990 and November 2014. Qualitative and quantitative data on prevalence, risk factors, burden and interventions were extracted and analysed. Reports on prevalence were available for 23 European countries, mostly from western and central Europe; for a few of these only data before 1990 were available. Prevalence based on meat inspection was generally low (below 6.2% in 95% of the records) and varied between and within countries. Serology and detailed meat inspection provided a higher prevalence range (0.41-14%). Only few studies analysing risk factors were identified. Reported factors related to access to pastures and risky waters, dairy production and uncontrolled human defecation in the proximity of the farm among others. Only one estimate of the economic impact of the disease could be identified. Recommended interventions were focused on increasing diagnostic tests sensitivity or the application of risk based surveillance strategies. There is a lack of complete and updated data on most countries, especially in eastern Europe. Further risk factor studies might be needed together with estimates on the burden of the disease in all European countries. Risk-based interventions are being encouraged but current data are limited to guide this approach.(Laranjo-Gonzalez et al., 2016)

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.(Scala et al., 2016)

Cestodes (tapeworms), as a group of parasites, have gained more prominence in recent years due primarily to improved abattoir feedback to fat stock producers on rejections. The zoonotic potential of some of this group adds to their importance. The aim of this article is to enable practising veterinary surgeons to easily answer their clients’ queries about these infections and **advise on effective control plans.**(Mitchell, 2015)

Many interventions against *Taenia solium* are evaluated by assessing changes in the prevalence of porcine cysticercosis ascertained by carcass dissection. Financial and logistical difficulties often prohibit dissection of entire pig carcasses. We assessed 209 pigs from rural areas of Cameroon and Peru for the presence of *T. solium* cysticerci and determined the **distribution of parasites within the musculature of infected animals.** Considering the presence of cysts in the tongue, masticatory muscles and heart, 31 of the 38 (81%) naturally infected animals were identified as having cysts. Dissection of only the tongue, masticatory muscles and heart provides a relatively sensitive and highly specific method for diagnosis of porcine cysticercosis.(Lightowler et al., 2015)

**Other zoonotic parasites**

**Paragonimiasis,** caused by the lung fluke *Paragonimus,* is a major food-borne helminthic disease. Differential diagnosis of paragonimiasis from tuberculosis and other infectious granulomas in the lung is a prerequisite to proper management of patients. Cysteine proteases of *Paragonimus westermani* (PwCPs) invoke specific antibody responses against patient sera, while antibody capturing activity of different PwCPs has not been comparatively analyzed. In this study, we assessed the expessional regulation of 11 species of different PwCPs (PwCP1-11). We expressed recombinant PwCPs and assessed diagnostic reliability employing sera from patients with P. westermani (*n = 138*), other trematodias (n = 80), cestodias (n = 60) and pulmonary tuberculosis (n = 20), and those of normal controls (n = 20). PwCPs formed a monophyletic clade into cathepsin F and showed differential expression patterns along with developmental stages of worm. Bacterially expressed recombinant PwCPs (rpPwCPs) exhibited variable sensitivity of 38.4-84.5% and specificity of 87.2-100% in
diagnosing homologous infection. rPwCPs recognized specific antibodies of experimental cat sera as early as 3 or 6 weeks after infection. Patient sera of fascioliasis, Schistosomiasis japonicum and clonorchiasis demonstrated weak cross-reactions. Our results demonstrate that diverse PwCPs of the cathepsin F family participate in inducing specific antibody responses. Most *P. westermani* cathepsin F, except for PwCP2 (AAF21461), which showed negligible antibody responses, might be applicable for paragonimiasis serodiagnosis. (Ahn et al., 2015)

Many waterborne helminthes are opportunistic parasites that can travel directly from animals to man and may contain forms capable of penetrating the skin. Among these, Sparganum is the pseudophyllidean tapeworm that belongs to the genus Spirometra, which is responsible for parasitic sparganosis; it is rarely detected in Europe and is caused by the plerocercoid infective larva. Thus far, only six cases of cutaneous and ocular sparganosis have been reported in Europe; two and four cases have occurred in France and Italy, respectively. Herein, we describe a new case of sparganosis in Italy that affected a male diver who presented to the Bambino Gesù Children’s Hospital of Rome. The patient’s skin biopsy was submitted to the Parasitology department who, in consultation with Pathology, concluded that the morphologic and microscopic findings were those of *Sparganum* spp. larvae. The patient recovered following a single dose of 600 mg praziquantel. (Bracaglia et al., 2015)

The aim of present study was to identify *Trichostrongylus* spp. among human population using excreted egg specimens, by the molecular method, in Mazandaran Province, northern Iran. Overall, 33 positive faecal specimens were randomly sampled and examined. PCR amplification of ITS2-rDNA region was performed on the isolated egg and then a restriction fragment length polymorphism (RFLP) profile was considered to discriminate of *Trichostrongylus* spp. A total of 33 positive faecal specimens, 29(78.9%), 4(12.1%) were found *Trichostrongylus colubriformis* and *T. axei* respectively. Our data appear the molecular evidence of both human *Trichostrongylus colubriformis* and *Trichostrongylus axei* infections in North of Iran. *Trichostrongylus colubriformis* was the probable most common zoonotic species causing human trichostrongylosis infection in the area (Gholami et al., 2015)

*Hymenolepis nana* is the most common tapeworm in humans; prevalence rates of 0.1%-58% have been reported. The aim of this study was to determine the prevalence in a health area of Southern Spain and identify the demographic variables potentially associated with increased rates of hymenolepiasis in this area. A retrospective study was performed with patients, who had *H. nana* eggs in faecal samples during January 2000 to December 2013. Parasitological diagnosis relied on microscopic detection in concentrated stool samples. During the study period, 73,660 stool samples were analyzed. *H. nana* eggs were observed in 158 patients (31 female) with a mean age of 18.9 years. The prevalence during the study period was 0.21% and 61% of the infected patients had more than one intestinal parasite. In conclusion, the prevalence of parasitism by *H. nana* in our population was higher than the national average and higher in adults than in children due to the characteristics of our population. (Isabel Cabeza et al., 2015)

Urban brown rats (*Rattus norvegicus*) carry microbial human pathogens but their role as reservoir hosts for helminths of public health importance is less well known. In this study, 42 brown rats trapped on Merseyside were subject to thorough combined helminthological and pathohistological post-mortem examination. Eggs of the *rodent-borne zoonotic nematode Calodium hepaticum* were initially detected in histological sections of the livers of 9.5% of rats, but overall diagnostic sensitivity increased to 16.6% when entire liver tissue was disrupted and the resulting filtrates were examined for released eggs. In their rat host, mainly trapped inside the dockland, infections with *Hepaticum* were associated with a chronic multifocal pyogranulomatous hepatitis with intralesional eggs and peripheral fibrosis. Mean intensity of hepatic *Hepaticum* egg infections was 1041 eggs. This is the first report of *Hepaticum* in an urban brown rat population in the UK and provides original data for liver egg burdens in this abundant commensal rodent. The zoonotic cestode *Rodentolepis nana* had a prevalence of infection of 14.3%. Rodent-specific, non-zoonotic helminths found were the spiruroid *Mastophorus muris* (16.0%) in the stomach, the trichuroid *Trichosomoides crassicauda* in the urinary bladder (31.0%); the ascarid *Heterakis spumosa* was the commonest helminth of the large intestine (76.2%). Many millions of brown rats inhabit cities and rural areas of the UK, and the infective stages of the zoonotic worm species, particularly *Hepaticum*, are likely to be widely distributed in the environment presenting a threat to public health. (McGarry et al., 2015)

**Misc farmed species**

There are distinctive specifications for veterinary medical care of South American camelids (SACs), namely, llamas, alpacas, vicunas and guanacos. Camelids are classified as food-producing animals, but as veterinary/medicinal products are often only licensed for domestic food-producing species such as horses, goats, sheep and cattle, treatment of SACs generally requires off-label use of drugs. Endoparasitism is a major health concern in camelids and can result in severe clinical diseases and economic losses. There is still a lack of work on the pharmacokinetics, safety and efficacy for most antiparasitic drugs used in SACs. Even when choosing an appropriate route of administration, several aspects must be
considered such as the fact that pour-on formulations are largely ineffective in camelids due to the unique features of llama and alpaca skin and hair that result in extremely low drug bioavailability. This review focuses on the main endoparasites of the digestive tract in llamas and alpacas in Europe and pharmacotherapeutic options based on current knowledge. (Franz et al., 2015)

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 llama*) 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 faecal 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 effusion, 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. (Edwards et al., 2016)

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. maccaniensis* 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. Lungworms, 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. (Lambacher et al., 2016)

*Nematodirus lamae* identified in an alpaca in the UK (Mitchell et al., 2016)

**Parasites of new world camelids** (Taylor, 2016)

Over a 13 month period, faecal and feather samples were collected from 85 ostriches and rheas from various geographical locations in England. Faecal samples were analysed using sedimentation and floatation methods for the presence of endoparasites. Faecal results showed eggs/oocysts from four genera of parasites in the screened ostrich samples. There was a high prevalence of *Ascaridia* spp. and *Trichostrongylus* spp., followed by *Eimeria* spp. and *Heterakis* spp. which are unusual and have only been documented in a small number of studies. However, the infection may support the theory of cross species contamination. Greater rheas were infected with 5 genera of parasites with an abundance of *Capillaria* spp. Other species included *Eimeria* spp., *Ascaridia* spp., *Trichostrongylus* spp., and *Deleurocephalus* spp. Feather sample results recorded a 100% infection rate on the ostrich samples identifying just one louse species, i.e. *Struthiolipeurus struthonis* (Tacey and Halan, 2015)

**Wildlife**

This special issue on wildlife parasitology features 3 opinion articles which discuss the following: human-wildlife interaction and zoonotic transmission of *Echinococcus multilocularis*; wildlife parasites in a One Health world; and, climate change and Arctic parasites. The issue also includes 4 review articles on the following topics: parallels, interactions and control of parasites and biological invasions; the role of antiparasitic treatment experiments in as sessing the impact of parasites on wildlife; an ecosystem approach to understanding and managing within-host parasite community dynamics; and, humans and their effect on wildlife nematodes. (Special Issue: Wildlife parasitology ecosystems. (2015b))

This special section presents papers from the International Congress on Parasites of Wildlife, held in Kruger National Park, South Africa held 15-18 September 2014. Some of the papers presented summarised the history and development of
research on wildlife parasites in southern Africa, the evolutionary ecology of parasite diversity and the role of wildlife in the transmission of parasitic zoonoses in peri-urban areas. The research papers included in this special issue further illustrated the diversity of current work in the field of wildlife parasitology, with specific reference to the parasites studied as well as their hosts. The selected papers range from leeches and haemoparasites in frogs, nematodes in eels to competition between ectoparasites (ticks and mites) parasitizing eastern rock sengis. Further papers, also included in this special issue, focused on the very important role that wildlife plays as a reservoir of parasitic zoonoses and zoonotic infections. The congress also highlighted the importance of diagnostics and epidemiology as illustrated with the case study on Theileria. One session was devoted to parasites as sentinels of the environment. Finally, the role of parasites in foodwebs and their importance in trophic linkages was emphasized. The collection of papers in this special issue represents the current trends in research on parasites of wildlife and also highlights the need for continued research into this extremely important topic. The geographical regions covered in this special issue (South Africa, Namibia, Caribbean, Germany, Australia, to name a few) also attest to the truly international nature of the congress. (International Congress on Parasites of Wildlife, Kruger National Park, South Africa, 15-18 September 2014) (2015a)

Pinnipeds are frequently infected by the lungworms *Otostongylus circumlitus* and *Parafilaroides gymnurus* (Metastrongyloidea). Infections are frequently associated with secondary bacterial bronchopneumonia and are often lethal. To date, a reliable lungworm diagnosis in individual seals is only possible during necropsy as examination of faeces collected from resting places does not allow assignment to individuals. Therefore, a diagnostic tool for lungworm detection in living seals is desirable for monitoring health of seals in the wild and in captivity. Previously, an ELISA based on recombinant bovine lungworm major sperm protein (MSP) as diagnostic antigen was developed for lungworm diagnosis in cattle. In the present study, this test was adapted for detection of antibodies against lungworms in harbour seals (*Phoca vitulina*) and grey seals (*Halichoerus grypus*). Furthermore, sera of northern elephant seals (*Mirounga angustirostris*) were tested to evaluate whether the harbour/grey seal ELISA is suitable for this seal species as well. For ELISA evaluation, lungworm-positive and -negative sera of harbour and grey seals were analysed using horseradish peroxidase (HRP)-conjugated Protein A as secondary antibody. Optical density was measured and a receiver operating characteristic (ROC) analysis was performed to determine a cut-off value. Potential cross-reactions were examined by testing serum of seals positive for gastrointestinal and heart nematodes, but negative for lungworm infections. In addition, sera of northern elephant seals were analysed. Harbour and grey seal serum samples showed significant differences in optical density (OD) between serum of infected and uninfected animals resulting in a cut-off value of 0.422 OD with a specificity of 100 % (95 % CI: 87.23-100 %) and a sensitivity of 97.83 % (95 % CI: 88.47-99.94 %). Cross-reactions with heart or gastrointestinal nematodes were not observed. Analysis of northern elephant seal samples resulted in detection of antibodies in animals positive for lungworm larvae at faecal examination. The ELISA presented is a valuable method for detection of lungworm infections in live harbour and grey seals, providing a monitoring tool to reveal epidemiological dynamics of lungworm infections during health surveillance in free-ranging seals. Furthermore, ELISA results may aid institutions with harbour and grey seals under human care on decisions regarding anthelmintic treatment of individual animals. (Ulrich et al., 2015)

**Ectoparasites**

The present study aimed to determine the susceptibility of 17 *Rhizophalus (Boophilus) microplus* populations, originating in the Southeast and Southern regions of Brazil, to different ivermectin concentrations (200, 500 and 630 mu g/kg), administered through subcutaneous or topical (pour-on) routes. *R. (B.) microplus* populations from the states of Minas Gerais (seven populations), Sao Paulo (seven populations) and Parana (three populations) were chosen for the tests. The selected cattle were allocated to treatment groups on day 0, and block formation was based on the arithmetic mean of female ticks (4.5-8.0 mm long) counted on three consecutive days (-3, -2 and -1). To evaluate the therapeutic and residual efficacies of these formulations, tick counts (females ranging from 4.5 to 8.0 mm long) were performed on days 3, 7 and 14 post-treatment, and continued on a weekly basis thereafter until the end of each experiment. The results obtained throughout this study, utilizing field efficacy studies, allowed us to conclude that the resistance of *R. (B.) microplus* against 200 and 500 mu g/kg ivermectin is widely disseminated because all tick populations that had contact with these specific concentrations were diagnosed as resistant. However, it is possible to infer that *R. (B.) microplus* resistance against 630 mu g/kg ivermectin was also widespread, diagnosed at six of ten analyzed properties. Resistance of these ectoparasites to 630 mu g/kg ivermectin is most likely emerging in three other populations of *R. (B.) microplus*. Strategies of resistance management need to be quickly determined to keep the selection pressure at a minimum level in Brazil. (Cruz et al., 2015)
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Fasciola hepatica

The liver fluke Fasciola hepatica causes considerable damage to the health, welfare and productivity of ruminants in temperate areas, and its control is challenged by anthelmintic resistance. Targeted selective treatment (TST) is an increasingly established strategy for preserving anthelmintic efficacy in grazing livestock, yet no practical indicators are available to target individuals for treatment against fluke infection. This paper evaluates the FAMACHA(C) system, a colour chart for the non-invasive detection of anaemia in small ruminants, for this purpose. FAMACHA(C) scores were collected from 288 sheep prior to slaughter during the winter period, when fluke infections were largely mature, and condemned livers were recovered and adult flukes extracted. Average FAMACHA(C) score was significantly higher (= paler conjunctivae) in animals whose livers were condemned (3.6, n = 62) than in those whose livers were not condemned (2.1). The number of adult flukes recovered ranged from 2 to 485, and was positively correlated with FAMACHA(C) score (r(2) = 0.54, p < 0.001). Packed cell volume was correlated negatively with both FAMACHA(C) score (n = 240, r = 0.23, p <0.001) and fluke number (r= 0.24, p <0.001). Nematode faecal egg count (FEC) did not correlate with FAMACHA(C) score, and selective treatment of individual sheep with FAMACHA(C) scores above 2 or 3 would have preserved between 27 and 100% of nematodes in refugia on the basis of FEC, depending on group and the threshold used for treatment. FAMACHA(C) holds promise as a tool for selective treatment of sheep against adult F. hepatica, in support of refugia-based control of fluke and nematode infections, and further field evaluation is warranted. (Olah et al., 2015)

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 closantel resistance in fluke populations. (Scarcella et al., 2016)

Flukicides are commonly administered at housing to cattle that have grazed fluke-infected pastures or that have been purchased from endemic areas. The choice of product is determined by numerous factors, one of which is the stages of Fasciola hepatica that are killed. Flukicides can be categorised into three main groups: (A) those that kill all juvenile stages and adults; (B) those that kill juveniles from six to eight weeks of age and adults and (C) those that kill adults only. This study was conducted on a commercial beef farm in Scotland and was designed to compare the efficacy of flukicides from each of these three classes in terms of their effects on faecal egg output, coproantigen and liveweight gain. The majority of animals in the untreated control group were positive for coproantigen, fluke eggs or both throughout the study duration of 16 weeks. Egg reappearance interval following housing treatment was eight weeks for clorsulon and 13 weeks for nitroxynil, though patent infections in both groups developed in only a small minority of animals; no fluke eggs were recovered from cattle treated with triclabendazole. Coproantigen was detected four weeks before the reappearance of fluke eggs in the dung. Animals treated with flukicides had significantly fewer faecal samples positive for eggs (P<0.006) and coproantigen (P<0.05) following treatment compared with the controls. Despite differences in the efficacy profiles among the flukicide-treated groups, there were no significant differences (P>0.05) in growth rates among any of the four treatment groups. There was, however, a significant negative association (P<0.001) between fluke positivity at housing and subsequent growth performance, irrespective of treatment group. (Forbes et al., 2015)


**FASCIOLA HEPATICA**

**INTRODUCTION**

This review is divided into five major areas of research of the liver fluke Fasciola hepatica. Research papers in the area of molecular research are the most numerous because of the increasing use of new techniques to target different molecules and metabolic pathways of the parasite. This is encouraging and will inform all other aspects of research in time including diagnostics, vaccinology and therapeutics.

**Epidemiology**

There were a large number of papers on this topic, employing various diagnostic tests for fascioliosis, and the use of geographic information system (GIS). Not surprisingly many drew the same conclusions about an increased prevalence of disease in years where there was a high rainfall in specific months, as well as factors associated with grazing management.
Aleixo wrote a review paper and observed that PCR and ELISA tests as well as GIS were being developed and the objective was early diagnosis of infection, and the prevalence of infection.

Howell studied the epidemiology of *F. hepatica* exposure in high yielding dairy herds. High rainfall, grazing wet and muddy pasture, the presence of beef cattle on farm, access to streams or ponds, and smaller herd size, were associated with an increased risk of exposure. Howell also observed that there was a significant negative association between infection and milk yield at the herd level. There was no association between F hepatica exposure and any of the other production disease or fertility variables.

Selemetas studied climatic and environmental predictors of liver fluke exposure risk in Ireland using spatial cluster analysis. Rainfall, total wet days, rain days, and soil type were significant environmental variables explaining the difference between infection clusters. A risk map of exposure was constructed with higher probability of exposure in western and north-western regions.

The same author produced a second paper concerning risk mapping of infection in dairy herds in Ireland using the bulk tank milk ELISA. The most important predictors in descending order of importance were: average of annual total number of rain days for the period 1981 to 2010, total rainfall during September, winter and autumn of 2012, average of annual total number of wet days for the period 1981 to 2010 and the annual mean temperature of 2012. 2012 was the year when a significant increase of infection occurred in the UK and Ireland in both cattle and sheep.

A further investigation by the same author was conducted on 369 dairy farms where a single bulk tank milk sample was taken for fluke antibody detection. Analysis showed that cows on 78% of the farmers had been exposed to liver fluke. Exposure was greater where there was a higher total number of dairy cows and the size of the total grassland area which they grazed. Other parameters which indicated higher exposure included grazing dry cows with replacement cows, whether or not grassland was mowed for conservation, the type of drinking water provided, spreading of manure on grassland, and grazing season length. The grazing season length on farms with good drainage was 11 days longer than for those with moderate drainage. Albendazole was the most commonly used flukicide. The research also stressed the importance of appropriate advice on farm management practices and flukicide usage to control the liver fluke on dairy farms.

Selemetas also undertook cluster analysis of fasciolosis in dairy cows in Munster. He concluded that high-risk clusters were present in the northern and western regions in Munster compared to the southern and eastern regions. The most significant variables that could reflect the difference between high and low risk clusters were the total number of wet days and rain days, rainfall, the normalised difference vegetation index (NDVI), temperature and soil type. There was a bigger proportion of well-drained soils among the low risk clusters, whereas poorly drained soils were more common among high-risk clusters.

Similar observations were made by Bloemhoff also studying infection on pasture-based dairy herds in Ireland using a bulk tank milk ELISA. BTM antibody levels increased as the grazing season progressed reaching a peak in January. There was a significant association between F hepatica infection and age at first calving. The conclusion was that there were a large proportion of dairy herds in Ireland infected with *F. hepatica* and this information would be useful for control practices.
Bosco described an outbreak of acute fasciolosis in sheep in the Mediterranean as a consequence of climate change. The outbreak occurred in southern Italy between May and June 2014 and was investigated by post mortem examination and fluke egg detection. High parasitic burdens in the liver were detected in affected animals - between 124 and 426 fluke parasites. Climatic data indicated that there was a significant association between temperature, increased rainfall and increase in the number of rainy days in 2014 leading up to this outbreak compared to previous years.

Caminade reviewed recent and future risks of fasciolosis due to climate change. A climate driven disease model was used in order to assess the impact of recent and potential future climate changes on the incidence of fasciolosis in Europe. The simulations showed that recent weather trends are likely to continue in the future, with the estimated pattern of climate change from northern Europe possibly extending the season suitability for development of the parasite by up to four months. For southern Europe, the burden is likely to be lower but the model projected climate change will increase the risk during the winter months. F. hepatica would therefore present a serious risk to the health welfare and productivity of all ruminants livestock in Europe. Better control programs at farm and regional level were advocated, but there was also a risk of increased anthelmintic resistance.

Ducheyne described a spatial distribution model for the distribution of F. hepatica in dairy cattle in Europe. Bulk tank milk samples were taken in several countries for examination by ELISA. The model identified rainfall and temperature as the most important factors for probability of exposure. Areas of high and low exposure were identified using this model. The model could be used to map fasciolosis in dairy cattle in Europe.

Novobilsky investigated the distribution of F. hepatica in Swedish dairy cattle and association with pasture and management factors. The conclusion was that the parasite was common in Swedish dairy herds, and the month of heifer turnout and grazing period length were the most influential factors observed. There was no significant differences in seropositivity between organic and conventional herds, or due to pasture management routines. A small number of dairy herds were found to be positive by bulk tank ELISA despite there being no evidence of infection from cattle slaughtered on these premises.

Rinaldi conducted a standardised cross-sectional survey in three pilot areas in Ireland, Switzerland and Italy as part of the EU funded GLOWORM project. There was great variation in prevalence rates between countries, with a high value in Ireland of 61% compared to Italy 7.9% and Switzerland 4%. Latent factor analysis highlighted the importance of year-to-year variation of mean temperature, rainfall and seasonality within a country, while long-term trends of temperature and rainfall dominated between countries with respect to prevalence of infection.

Rondelaud investigated the susceptibility of the snail Lymnaea glabra to experimental infection with F. hepatica over successive generations. The results confirmed that the prevalence and intensity of infection progressively increased over six generations. This did not however match the level of infection of the usual intermediate snail host Galba truncatula. The results however explain why there were cases of fasciolosis on cattle farms where there are no Galba truncatula snails or paramphistome infection.

Vasquez-Prieto investigated temporal genetic variation of F. hepatica in sheep in north-west Spain. There was low genetic differentiation between two temporal samples taken in 2006 and 2008,
collected from nine sheep farms that share the same pasture for at least two years. There were however strong heterozygote deficits, and a high degree of genetic structure at the infra population level. This was unexpected and they explained the findings by the fact that the parasite can survive for many years in the definitive host. The high genetic divergence observed between infra populations was likely to be a consequence of strong genetic drift associated with the complexity of the life-cycle.

References


Selemetas, N. The effects of farm management practices on liver fluke prevalence and the current internal parasite control measures employed on Irish dairy farms. Veterinary Parasitology 207, 228-240.


Molecular Research

With the advances in technology associated with molecular analysis, there are a number of important papers in this area.

Alvarez-Rojas identified key genes involved in the sheep host’s liver associated with metabolism, fibrosis and repair and the immune response against F. hepatica. They claimed that this was the first detailed investigation of transcriptomic responses in the liver tissue of the host to F. hepatica infection. It defined the involvement of specific genes and an apparent overlapping function of many genes involved in this process.

Zintl used experimental infections to investigate whether fragments of mitochondrial DNA could be used as markers to determine the parasite’s ability to complete its tissue migration, and establish infection in the liver of the final host. The research indicated that parasites with heterozygote microsatellite alleles have a selective advantage over homozygote parasites during their migration in the final host.

Alba used a novel monoclonal antibody-based immunoassay to detect F. hepatica parasites in the snail Galba cubensis from day eight postinfection. No previous immunoassay had been used to detect helminth infected snails prior to this paper and the assay can be used to detect infection status in natural populations of lymnaeid snails.

I consider that this type of test is vital for prediction of infection for the periods of highest risk by sampling snails and seeing if they are infected. In this case the snail is Galba cubensis but it should also be applicable to our most common parasitised snail Galba truncatula.

Cancella investigated mucins as important mediators for the parasite’s establishment within its host. These mucins are produced by the parasite, and have a key role in immune evasion. Mucin cDNA was sequenced and they found that these mucins were upregulated in the mammalian-invasive newly existed juvenile stage of the parasite compared to the adult stage. The significance of identifying these genes and the upregulation was discussed.

Cwiklinski characterised a panel of microsatellite markers and discussed their application to study populations of F. hepatica. This multiplex panel of microsatellite markers could be used for several life-cycle stages. 2448 potential microsatellites were identified in the genome sequence of F. hepatica.

A second paper by this research worker described gene duplication and polymorphisms that revealed adaptation to the host’s environment and parasite’s capacity for rapid evolution. Gene analysis described here provided a new insight into the evolution of the parasite and its adaption
to the host environmental and to external selection pressures. This analysis also provided a platform for research into novel drugs and vaccines.

Cwiklinski also looked at extracellular vesicles, released by the parasite during its invasive stage, as an important role in its pathogenesis. Small exosome vesicles originate in the parasite's tegument and carry immunomodulatory molecules that could be delivered into host cells.

Hacariz also undertook transcriptomic profiling to detect virulence and immune modulation related genes in *F. hepatica*. They concluded that this was important to comprehend evasion mechanisms of the parasite and develop more effective strategies against fasciolosis. *In silico* analysis was an important aspect of this study.

Robles-Perez used a PCR test to examine the genetic variability of *F. hepatica* populations from different geographical locations. This was done in the north of Spain and a dendrogram was produced showing the different sub species of the parasite for this region. *F. hepatica* strains from England and Ireland and Mexico were also compared.

Rodriguez studied glycan conjugates and their possible role in the evasion of the immune response. Gycan conjugates are expressed on the surface of helminth parasites. Glycan rich secretions released from the surface are important in pathogenesis and parasite survival. The glycans could have a role in modifying TH-2 immune response that favoured parasite survival in the host. The results indicated that glycans of *F. hepatica* promote the production of IL-4 and IL-2 and suppress IFN-gamma production. These molecules modulated the hosts immunity and could be used in the design of vaccines against fasciolosis.

Skarcella used the random amplified polymorphic DNA PCR to characterise different strains of *F. hepatica*. Strains from different parts of Europe and America were looked at, as well as those that were susceptible or resistant to triclabendazole. The existence of genetically different populations could allow one or more populations of *F. hepatica* to be able to survive and create resistance or adaptability to selective pressure.

**References**


**Diagnostics**

There were not many relevant papers concerning pure diagnostic tests, those are given below.

Carnevale and others described the use of the PCR test to detect F. hepatica DNA in sheep faeces. The PCR test showed a detection limit of 20 pg of F hepatica DNA. No cross reaction was observed with samples containing coccidial oocysts or roundworm eggs. Carnevale claimed that the technique was effective for specific detection of F hepatica infections in sheep.

Kajugu investigated the specificity of the coproantigen ELISA for the diagnosis of fasciolosis in cattle and sheep faeces samples, where there was intercurrent infection with roundworms, coccidial oocysts, and rumen fluke. No false positives were found with any of these additional infections, but the sedimentation faecal egg detection test was more sensitive than the coproantigen test when there were less than 10 eggs per gram present in faeces. He concluded that diagnosis of fasciolosis should be based on both faecal egg count and coproantigen ELISA results, to ensure optimum sensitivity for prepatent and low-level infections.

Karanikola described the development of a multiplex fluorescent immunology test for the simultaneous detection of antibodies against Cooperia, Dictyocaulus and F hepatica antibodies in serum. The conclusion was that this platform provides a rapid high throughput of results, and is highly sensitive and specific in comparison to existing serological and coproscopical diagnostic techniques.
References


Human Infection

Most papers pertaining to human infection dealt with individual cases and the diagnostic methods employed. The diagnostic methods ranged from x-ray examination of the liver in infected patients to serological and PCR tests. The reports were from countries where human fasciolosis is recognised, either historically, or more recently because of the number of cases. These countries include Vietnam, Pakistan and Iran. An informative review article is detailed below, relating to fasciolosis in Iran.

The review by Ashrafi concluded that despite the high infection rates of livestock in southern areas of the country, human disease was more prevalent in northern provinces. The report concluded that human fasciolosis is an emerging public health issue in Iran and new foci of infection were being encountered and studied.

Reference


Therapeutics

Alvarez-Mercado examined the effect of 15 tropical plants extracts on excysted F. hepatica. The conclusion was that five tropical plant extracts had promising anthelmintic effects but further toxicity and in vivo biological evaluation was necessary in ruminant models to determine the anthelmintic potential of these extracts.

In an important study in the Australian state of Victoria, Elliott investigated drug efficacy against F. hepatica in this major dairy production state. No fluke prevalence data had been produced since the 1970’s in Victoria, which was prior to the introduction of triclabendazole. TCBZ resistance was now widespread. Using bulk tank ELISA data he showed that animals on six of the 15 farms were infected with F. hepatica and the herd prevalence of infected herds ranged from 47 to 100%. This exceeded the value at which production loss is said to occur, which is 25%. The intensity of fluke infection varied considerably within and between herds. They stated that Clorsulon and Oxyclozanide could be used to remove the adult stages of the fluke on TCBZ resistant fluke farms.
Conclusion - fasciolosis was a significant disease in Victoria and likely to cause production loss in the dairy industry in this irrigated dairy areas.

Forbes investigated the efficacy of treating cattle at housing, and the influence of differences in flukicidal activity against juvenile parasites. Flukicides are commonly administered at housing to cattle that have grazed fluke infested pasture, or that had been purchased from endemic areas. This study was conducted on a commercial beef farm and was designed to compare the efficacy of flukicides from each of the three classes in terms of effect on faecal egg output and live weight gain. Animals treated with flukicides had significantly fewer faecal samples positive for fluke eggs. Despite differences in the efficacy profiles of the flukicide treated groups, there were no significant differences in growth rates among any of the four treatment groups. There was however a significant association between fluke positivity at housing and subsequent growth performance irrespective of treatment group.

Hannah is one of the few research workers to examine this parasite histologically. In one paper he looked at the reproductive organs and differential effects of TCBZ treatment on these organs. He concluded that histological techniques conveniently screened samples of flukes in field trials designed to validate instances of drug resistance. Histology could also be used to test the efficacy of new products against drug resistant and drug susceptible fluke isolates. This account also provided reference criteria for drug induced histopathological changes in fluke reproductive structures, examination which may supplement and augment conventional diagnostic tests, such as coproantigen detection, and aid interpretation of electron microscopy findings.

In another study of upland flocks in Northern Ireland, he used different diagnostic tests to show that, in all flocks with high fluke burdens, TCBZ was ineffective in treating chronic fasciolosis. The results were also supported by the coproantigen ELISA. Histology of the reproductive organs of fluke from TCBZ treated sheep in flocks where there was suspected resistance showed these organs to be normal when compared with untreated flocks. This aided the diagnosis of TCBZ resistance in these flocks. There was a higher incidence of TCBZ resistance in intensively managed sheep production areas. The survey also emphasised the need for pre-emptive flukicide treatment against chronic fasciolosis using flukicides effective against egg producing adult fluke to minimise pasture contamination for next season’s lamb crop.

Knubben-Schweitzer and others advocated a control strategy based on the location of the intermediate snail host habitats on farm. They observed that even on individual farms there was epidemiological variation with risk of infection. They recommended that the source of infection be found by examination of infection in different epidemiological groups of the definitive host’s and of pastures on farm. Four factors were identified that promote transmission of bovine fasciolosis. 1. Snail habitats on pastures used for young stock prior to first calving or dry cows only. 2. Snail habitats on pastures used for dairy cows. 3. Snail habitats present on single pastures used for dairy cows. 4. Snail habitats present on hay fields. For each of these epidemiological situations an individual control strategy was advised.

Novobilsski reported the first case of closantel treatment failure in Sweden. This study involved the topical formulation containing closantel and ivermectin. Based on faecal egg count closantel efficacy, at 21 days post-treatment, was 72% and 97% on two farms respectively. Whereas no faecal egg count reduction was observed on a further farm. They question the use of this topical means of treatment in terms of closantel treatment failure.
Olah advocated FAMACHA as a potential tool for targeted selective treatment of chronic fasciolosis in sheep. Average FAMACHA scores were significantly higher in animals whose livers were condemned compared with those whose livers were not condemned. FAMACHA uses a colour chart as a non-invasive detection of anaemia in small ruminants for fluke and Haemonchus infection. The number of adult fluke is recovered in chronically infected animals range from 2 to 485 and were positively correlated with the FAMACHA score. Packed cell volume correlated negatively with the FAMACHA score.

O’Neill and others conducted a comparative study on the impact of two artemisinin derivatives on the female reproductive system of F. hepatica. Overall the results showed that artemisinin treatment had a severe impact on egg production by TCBZ resistant fluke, an effect that was mediated by destruction of the vitelline cells.

Robles-Perez undertook screening of anthelmintic resistance to TCBZ isolated from sheep by an egg hatch assay. He concluded that there was an ovicidal effect of TCBZ in F. hepatica eggs harvested from bile. The eggs were incubated in a commercial formulation of TCBZ diluted in DMSO. However in eggs recovered from faeces the results were not conclusive. The cleaning of eggs recovered from faeces is an important issue that should be reviewed and standardised before comparing results between susceptible and resistant isolates in this kind of egg hatch assay.

References


**Paramphistome infection.**

There were many papers produced worldwide on paramphistomosis, most were studies in specific countries, where, it was claimed, paramphistomes were more pathogenic. These countries were usually in the tropics and not relevant here. Relevant papers are given below.

Alba used a monoclonal antibody-based immunoenzymatic assay for epidemiological surveillance of the vector snails of *Fasciola hepatica*. No cross-reactivity was detected in lymnaeids infected with *Trichobilharzia* sp. or *Cotylophoron* sp. The ELISA enabled detection of the infection from day 8 p.i. in *G. cubensis* while in *P. columella* it was noted as early as day 4. They claimed that no previous immunoassays had been reported to detect helminth-infected snails and the sandwich ELISA method was applicable for infection status, of fluke and paramphistomes, in natural populations of lymnaeid snails.

Argente described an outbreak of acute diarrhoea in cattle western France. This was in cows due to immature paramphistomes. The clinical aspects, animal pathology, mortality, diagnosis and treatment of larval paramphistomosis were highlighted.

Choudharay reviewed the molecular characterization of digenean parasites using molecular markers with special reference to ITS region. According to this paper, the ITS or rDNA region of eukaryotes has an immense potential to resolve the evolutionary and phylogeny problems using molecular markers. The ITS region is more reliable and precise marker for demarcation and identification of species in combination with other DNA markers. Major studies were involved around the parasites of families *Fascioliidae* *Schistosomatidae*, *Opisthorchidae*, *Paragonimidae* and *Paramphistomidae*, *Clinostomidae*, *Diplostomidae*, *Haploporidae*, among others infecting humans, farm animals, birds, fishes, reptiles and amphibians on the clinical basis. In future, molecular and bioinformatics aspects based on genetic variations will lead to exploration of the untouched areas of trematode research.

The same author went on to describe transcriptome analysis of the adult rumen fluke *Paramphistomum cervi* following next generation sequencing. The conclusion was that the reference adult *P. cervi* transcriptome will serve as a basis for future work on the biology of this important parasite. Using the widely investigated trematode virulence factor and vaccine candidate Cathepsin L as an example, the epitope GPISIAINA was found to be conserved in *P. cervi* isolated from three different host species supporting its candidacy for vaccine development and illustrating the utility of the adult *P.cervi* transcriptome.

Chryssafidis, reported that standardisation of egg-viability assays for *Fasciola hepatica* and *Calicophoron daubneyi* could be a tool for evaluating new technologies of parasite control. Eggs were recovered from adult parasites collected in a commercial abattoir, from naturally infected cattle. A protocol for in vitro development of *F. hepatica* eggs was optimised based on previously published methods, with variations in duration and temperature of incubation. A new protocol for measurement of rumen fluke egg development in vitro was designed, based on testing different temperatures and periods of incubation, with or without light exposure. The protocols described could be
used for comparing experimental groups when new technologies for parasite control were tested. In addition, the methods described for C. daubneyi present new information on the biology of this parasite.

Fuertes produced a paper on an immunohistochemical study and mRNA cytokine profile of the local immune response in cattle naturally infected with Calicophoron daubneyi. The study suggested a predominant Th1 polarized local immune response with the probable involvement of Th regulatory cells in cattle to C. daubneyi natural infection.

A second paper by the same author described pathological changes in cattle naturally infected by Calicophoron daubneyi adult flukes. Local host response and parasite distribution were studied in the forestomachs, abomasum, duodenum and regional lymph nodes of cattle suffering from bovine paramphistomosis. The parasites were found attached, by the ventral sucker, to small conical papillae of the rumen and reticulum. Affected papillae, showed morphological changes denoted by very narrow stalks and expanded heads. Histologically, these changes were characterized by epithelial acanthosis-hyperkeratosis of the epithelium. Infiltration of inflammatory cells was often related with the epithelial changes, although it was also found in the duodenal mucosa and submucosa. Tissue damage and inflammatory reaction were more severe in the ruminal atrium, where the largest number of flukes and affected papillae were observed. In contrast, lesions in the ruminal dorsal sac were absent or mild.

Jones confirmed Galba truncatula as an intermediate host snail for Calicophoron daubneyi in Great Britain, as well as evidence of alternative snail species hosting F. hepatica. The study claimed that interactions between both species at intermediate host level could potentially occur. Radix balthica and P. antipodarum were found positive for F. hepatica but not C. daubneyi. This could indicate a role for alternative snail species other than G. truncatula in infecting pastures with F. hepatica in GB.

Malrait investigated the pathogenic importance, diagnosis and treatment of the rumen fluke (Calicophoron daubneyi) in cattle. In a case-control field survey to investigate the association between rumen fluke infection and herd-level problems with diarrhoea, no association was found. The use of closantel to treat rumen fluke infection in three herds showed no significant reduction in egg output post-treatment. Because this result was in contrast to a previous study using an oral dose of closantel, they concluded that more research was required into the effect of administration route on the efficacy of closantel on rumen fluke.

Toolan produced a paper on bovine and ovine rumen fluke in Ireland, and the risk factors and species identity based on passive veterinary surveillance and abattoir findings. At slaughter, 52% of 518 cattle from 101 herds were positive for rumen fluke, compared to 14% of 158 sheep. Prevalence in cattle was higher than reported in most studies from mainland Europe and varied by animal category, age, sex, abattoir visit and location (county) of farm from which the animal was submitted for slaughter, but in multivariate analysis, only sampling month and county were significantly associated with detection of rumen fluke. The identity of rumen fluke in cattle and sheep was confirmed as Calicophoron daubneyi. Although C. daubneyi is thought to share an intermediate host snail with Fasciola hepatica, the differences in prevalence between host species and over time suggest that the epidemiology of C. daubneyi is distinct from that of F. hepatica.
References


