



Animal &  
Plant Health  
Agency

# APHA Parasitology Group Annual Review of Literature and Horizon Scanning Report 2017

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## Contents

Summary .....	1
Parasites.....	1
<i>Nematodirus</i> spp.....	1
<i>Haemonchus contortus</i> .....	1
Anthelmintics .....	1
Anthelmintic resistance (AR).....	2
AR in small ruminants .....	2
AR in cattle.....	3
Parasite diagnostics.....	3
Cattle parasites.....	4
Prevalence .....	4
Parasite control .....	4
Economics.....	4
Parasites of birds .....	5
Poultry mites .....	5
<i>Cryptosporidium baileyi</i> in grouse .....	6
Wildlife and livestock parasite interactions.....	6
Cestodes.....	6
<i>Echinococcus</i> spp .....	6
<i>Taenia</i> spp .....	7
<i>Moniezia</i> spp .....	7
Other Parasite Zoonoses .....	7
Besnoitiosis.....	7
Apicomplexa .....	8
Ectoparasiticides.....	8



# Summary

## Parasites

### *Nematodirus* spp

A study in Northern Ireland revealed inefficient control strategies for *Nematodirus* spp. (not specified but likely *N. battus*) generally in lambs. Also that *Nematodirus battus* eggs – 43% were able to hatch without chilling (as described in GB), but 100% with chilling. This may represent an adaptation to climate change. (McMahon, Edgar et al. 2017)

### *Haemonchus contortus*

One of the most important parasites worldwide (but less so in UK) means that there is more research effort into this parasite than many others.

Papers were published on the use of the *H. contortus* vaccine (Babervax) in dairy goats (in Brazil) where mean efficacies of  $69.8 \pm 2.11\%$  and  $57.4 \pm 2.76\%$  for the Anglo Nubians and Saanens were obtained. (Matos, Nobre et al. 2017).

Use of the FAMACHA system to control *H. contortus* in South American camelids in the USA where *H. contortus* was the predominant parasite, indicated it was a useful tool, using similar parameters to those advised for sheep. (Storey, Williamson et al. 2017).

## Anthelmintics

The efficacy of eprinomectin pour-on (EPRINEX® Pour-on, Merial): against gastrointestinal and pulmonary nematodes and pharmacokinetics in sheep was published. (Hamel, Bosco et al. 2017).

A review of the effects and accumulation of anthelmintics in parasites was published. Oral administration improves drug efficacy against nematodes located in the gastrointestinal tract especially if parasites have a reduced susceptibility. There is large inter-animal variability in drug exposure and subsequent high variability in efficacy is observed after topical administration of anthelmintic compounds. (Lifschitz, Lanusse et al. 2017).

A study looking at the effects of a moxidectin pour-on in the dry period in dairy cattle in three European countries showed that the effect on milk yield after anthelmintic treatment over the whole subsequent lactation varied from no effect ( $-0.43$  kg/day;  $P = 0.35$ ) to an increase of milk yield with  $2.35$  kg/day ( $P = 0.01$ ), depending on the study region and parity of the cows. (Geurden, Bartram et al. 2017).

Studies continue to find new anthelmintic products. (Preston, Jiao et al. 2017).

## Anthelmintic resistance (AR)

There is considerable scientific evidence of a possible relation between the anthelmintic resistance to BZs with that to MLs. However, one study showed that while isotype 1  $\beta$ -tubulin gene SNPs (associated with BZ resistance) may have some involvement with ML resistance, the presence of these  $\beta$ -tubulin SNPs alone are not sufficient to develop ML resistance. (Santos, Vasconcelos et al. 2017).

### AR in small ruminants

Resistance to the anthelmintic drug monepantel has emerged in parasitic worms infecting sheep and goats. The drug targets nicotinic acetylcholine receptors belonging to the nematode-specific DEG-3 subfamily. Studies into a monepantel resistant isolate of *H. contortus* have shown that the highly resistant isolate has a number of mutations in the drug target Hco-mptl-1 gene that would most-likely result in a non-functional receptor, thus rendering the larvae insensitive to the drug. Also the presence of multiple separate mutations in the Hco-mptl-1 gene in this viable field-derived worm isolate may at least partly explain why resistance to monepantel has arisen rapidly in the field. (Bagnall, Ruffell et al. 2017).

The rapid development of monepantel resistance in *H. contortus* populations was detailed in one study in an area where *H. contortus* was predominant. The use of a suppressive treatment regimen that included monepantel over a period of 3 months resulted in the emergence of a population of resistant *H. contortus*. In the Targeted Selective Treatment (TST) group, where only two lambs were treated there was a rapid and progressive reduction in the efficacy of monepantel, which at the end of the experiment was only 76%. A study on a commercial farm in Brazil also detected a monepantel resistant strain of *H. contortus* after suppressive use (more frequently than monthly) for less than one year. (de Albuquerque, Bassetto et al. 2017).

A study on commercial farms in New Zealand showed that anthelmintic resistance to both AB-LEV-OX and moxidectin was present in *Trichostrongylus* spp on a commercial sheep farm. Monepantel and DEQ-AB were both effective against *Trichostrongylus* spp. based on FECRT results. The authors question whether this combination (AB-LEV-OX) is suitable for use as a quarantine treatment in this country. (Hodgson and Mulvaney 2017).

Broad spectrum anthelmintic resistance was confirmed at a sheep farm in a *H. contortus* predominant area of Australia showing clinical signs of poor parasite control. Treatment efficacies ranging from 21.3% (monepantel) to 93.8% (derquantel/abamectin combination) against *H. contortus*. Furthermore, resistance to the multi-combination anthelmintic containing 4 active ingredients was evident (52.5%). This broad spectrum of resistance highlights the need for integration of alternative sustainable methods in parasite control in

order to slow development of resistance and increase the life time effectiveness of anthelmintics. (Lamb, Elliott et al. 2017).

## AR in cattle

A case study on a beef farm in the Netherlands suggested reduced efficacy of ivermectin against *O. ostertagi* by faecal egg count reduction test. (Holzhauer, Hegeman et al. 2017).

The maintenance of anthelmintic-susceptible parasite refugia to delay the onset of anthelmintic resistance is an accepted but difficult task to achieve. A trial was conducted in Argentina, to attempt to establish a new population of ivermectin-susceptible *Cooperia* sp. on a beef cattle farm with proven problems of ivermectin-resistant *Cooperia*. This involved deliberately infecting heifers with susceptible *Cooperia* spp. and allowing them to graze pastures known to be infected with resistant parasites, and monitoring them closely. This allowed the clinical efficacy of ivermectin to increase from an initial 73% to 99.4%, (FECRT) while the absolute efficacy increased from 54.1% to 87.5% (controlled efficacy trail) after just two animal production cycles. (Fiel, Steffan et al. 2017).

## Parasite diagnostics

A multiplexed-tandem PCR for the specific diagnosis of gastrointestinal nematode infections in sheep developed in Australia has been validated in Europe (Scotland and Belgium). The MT-PCR platform was shown to be an advanced method for the species/genus-specific diagnosis of gastrointestinal nematode infections in small ruminants. It can achieve a specific diagnosis from different types of sample templates, including larval culture and faecal samples. (Roeber, Morrison et al. 2017).

A Loop-mediated isothermal amplification (LAMP) method, a test that doesn't need expensive machines, has been published that detects *H contortus* in goat faecal samples, that was as sensitive as species specific PCR. (Yang, Qi et al. 2017).

Next-generation deep amplicon sequencing, or metabarcoding, based on deep-amplicon sequencing of internal transcribed spacer 2 (ITS-2) rDNA, was validated in its ability to quantitatively assess the species composition of cattle gastro-intestinal nematode (GIN) communities. Using coproculture derived L3s they showed in a screen of 50 Canadian beef herds revealed the majority of parasite communities were comprised of just two species; *Ostertagia ostertagi* and *Cooperia oncophora*. They also examined the impact of routine macrocyclic lactone pour-on treatments on GIN communities in the Canadian beef herds. Low treatment effectiveness was observed in many cases, and nemabiome sequencing revealed an overall increase in the proportion of *Cooperia* spp. relative to *O. ostertagi* post-treatment. They conclude that this work demonstrates the power of nemabiome metabarcoding to provide a detailed picture of GIN parasite community structure in large sample sets and illustrates its potential use in research, diagnostics and surveillance. (Avramenko, Redman et al. 2017).

# Cattle parasites

## Prevalence

A survey of British dairy farms by bulk milk analysis showed that the apparent prevalence of antibodies against *F. hepatica* was 55% (95% CI: 48–62%), *N. caninum* 46% (95% CI: 38–54%), and *O. ostertagi* 95% (95% CI: 91–98%). (van der Voort, Van Meensel et al. 2017).

## Parasite control

A study into targeted selective treatment (TST) with anthelmintics, in which only individual animals that will most benefit are treated, rather than whole groups of at-risk cattle, on commercial farms in Scotland (two of whom were organic) showed that daily live weight gains was the most useful measure to take to determine need to treat in first year grazing animals. A pre-determined threshold weight gain of 0.75 kg/day was used. No individual animal received more than one treatment during the grazing season and all treatments were given in July or August; five animals were not treated at all because their growth rates consistently exceeded the threshold. Thus anthelmintic use was minimised. They conclude that implementation of TST at farm level requires regular (monthly) handling of the animals and the use of weigh scales or tape, but can be integrated into farm management practices. (Jackson, Ellis et al. 2017) You need to be alert for signs of lungworm however, or advise vaccination before turnout.

A simulation study was used to assess the best way to administer TST to control *O. ostertagi* in cattle for the best benefit per R (BPR), the ratio of average benefit in weight gain to change in frequency of resistance alleles R (relative to an untreated population). When treating a fixed percentage of the herd, treatments according to plasma pepsinogen or random selection were identified as the most beneficial (i.e. resulted in the greatest BPR) for all levels of initial pasture contamination and all stocking rates. Conversely when treatments were administered according to threshold values, Average Daily body weight Gain was most beneficial, and was identified as the best TST strategy (i.e. resulted in the greatest overall BPR) for all levels of initial pasture contamination and all stocking rates (Berk, Laurenson et al. 2017).

Another study looked at a targeted mid-season treatment of first year grazing calves that were weighed only three times in a season, with animals only treated mid-season if their daily live weight gain was considered too low. (Merlin, Ravinet et al. 2017).

## Economics

The economic assessment of *Ostertagia ostertagi* and *Fasciola hepatica* infections in dairy cattle herds in Germany using the online calculation programme Paracalc® was published.

In this study bulk tank milk (BTM) samples and additionally up to six serum samples collected from first season grazing calves were analysed, for antibodies to *O. ostertagi* and *F. hepatica* and plasma pepsinogens. Median costs per farm and year were estimated for gastrointestinal (GI) nematode infections (€721.38) and *F. hepatica* infection (€565.61). The study demonstrated that if all required information is provided, the Paracalc® tool can assist to identify production losses in dairy cattle herds due to helminth infections and to optimise farm economics. Decreases in milk yield in multiparous cows were the major reason for annual production losses due to GI nematodes (€13.33 per cow) and *F. hepatica* infections (€7.95 per cow). (Fanke, Charlier et al. 2017).

Grazing management (GM) interventions, such as reducing the grazing time or mowing pasture before grazing, have been proposed to limit the exposure to gastrointestinal (GI) nematode infections in grazed dairy cattle. The farm-level economic effects of these interventions on Flemish farms were investigated. The results suggest that the dairy farms modelled can improve their economic performance by postponing the turnout date, but that advancing the housing date or reducing daily grazing time mostly leads to a lower net economic farm performance. Overall, the GM interventions resulted in a higher technical efficiency and milk production but these benefits were offset by increased feed costs as a result of higher maintenance and cultivation costs, but varied with each farm. (van der Voort, Van Meensel et al. 2017).

## Parasites of birds

### Poultry mites

The poultry red mite, *Dermanyssus gallinae*, has been described for decades as a threat to the egg production industry, posing serious animal health and welfare concerns, and adversely affecting productivity. A review of the factors contributing to this growing threat and a discussion of their recent development in Europe was published. They conclude that effective and sustainable treatment approach to control poultry red mite infestation is urgently required, included integrated pest management. (Sigognault Flochlay, Thomas et al. 2017).

The use of product 'fluralaner', a novel systemic ectoparasiticide against *Dermanyssus gallinae* and its effect on mite vitality and reproduction after oral administration to laying hens was studied. Fluralaner administered orally to hens twice, 7 days apart, provides efficacy against experimental poultry red mite infestation for at least 2 weeks. (Brauneis, Zoller et al. 2017).

Northern fowl mites (*Ornithonyssus sylviarum*) are obligate hematophagous ectoparasites. Though predominantly a nest-parasite of wild birds, this mite can be a particular problem in laying hens. Economic damage is incurred by direct blood feeding and activation of the host's immune responses. This in turn causes decreased egg production and feed

conversion efficiency, and severe infestations can cause anemia or death to birds. A review of this parasite was published. (Murillo and Mullens 2017).

An *in-vitro* study into the effects of contact with various chemicals showed that Northern fowl mites were highly sensitive to fluralaner after contact exposure. They were moderately sensitive to phoxim and propoxur, and less sensitive to spinosad. Furthermore, the tested mite population appeared to be resistant to the pyrethroids, permethrin and deltamethrin, despite not being exposed to acaricides for at least 10 years. (Mullens, Murillo et al. 2017).

## **Cryptosporidium baileyi in grouse**

Infection by *Cryptosporidium baileyi* causes respiratory cryptosporidiosis in red grouse *Lagopus lagopus scotica*. First diagnosed in 2010, it has since been detected across half of moors managed for grouse shooting in northern England. A study looking at the potential sources of infection, in particular contaminated grouse faeces within communal trays visited by grouse containing grit coated with flubendazole, provided to control *Trichostrongylus tenuis*. The study suggested that these trays were a potential source of infection and that the design had to be addressed. (Baines, Giles et al. 2017).

## **Wildlife and livestock parasite interactions**

A study in one area of Hungary showed that the prevalence of benzimidazole resistance in *H. contortus* was 17.1% and 68.6% in the wild roe deer and sheep, respectively. Another study suggested that overlapping habitats of sheep flocks and roe deer could contribute to the occurrence and spread of resistant alleles. (Csivincsik, Nagy et al. 2017).

Red deer can be infected with some gastrointestinal nematodes (GIN) of cattle but it is unknown to what extent. An indoor study was conducted to determine the establishment rate of cattle GIN in young deer. It showed that some cattle-origin GIN can establish in red deer. In particular, the establishment of *H. contortus* and *T. axei* could allow sufficient burdens to build up to be clinically significant. Importantly, almost no cattle *Ostertagia* species or small intestinal species established in the deer. (Nagy, Csivincsik et al. 2017).

## **Cestodes**

### **Echinococcus spp**

An article was published which provides an overview on the general history of echinococcosis. (Eckert and Thompson 2017).

In Europe the principal definitive host for *Echinococcus multilocularis*, causing alveolar echinococcosis in humans, is the red fox (*Vulpes vulpes*). Obtaining reliable estimates of the prevalence of *E. multilocularis* and relevant risk factors for infection in foxes can be

difficult if diagnostic tests with unknown test accuracies are used. Latent-class analysis was used to obtain estimates of diagnostic test sensitivities and specificities in the absence of a perfect gold standard. Four different diagnostic tests including necropsy followed by sedimentation and counting technique (SCT), an egg-PCR, a monoclonal and a polyclonal copro-antigen ELISA. SCT, assuming a specificity of 100%, performed best among the four tests. (Otero-Abad, Armua-Fernandez et al. 2017).

## **Taenia spp**

Human taeniosis may be caused by *T. solium*, *Taenia saginata* and *Taenia asiatica* tapeworms. A review of diagnostic methods used in humans in Europe was published. (Gómez-Morales, Gárate et al. 2017).

## **Moniezia spp**

A questionnaire to obtain information on tapeworm control practices was sent to 252 sheep farmers in Northern Ireland (NI) in 2012. The majority of respondents (61.8%) did not treat for tapeworms, but of those that did 51.9% used inappropriate treatments (MLs levamisole, oxclozanide, closantel and monepantel) (McMahon, Edgar et al. 2017).

## **Other Parasite Zoonoses**

Four clusters of *Trichostrongylus* infection in humans diagnosed in a single centre, in northern Italy were described. Pain and diarrhoea were the most frequent clinical signs, while other were asymptomatic. Possible source of infection was eating vegetables contaminated with fresh sheep faeces. (Buonfrate, Angheben et al. 2017).

A recombinase polymerase amplification (RPA) test to detect *Fasciola hepatica* in human stool samples was described. (Cabada, Malaga et al. 2017).

## **Besnoitiosis**

An unusual case of chronic besnoitiosis in a calf was published. This disease primarily affects adult beef cattle (Diezma-Díaz, Jiménez-Meléndez et al. 2017).

A study was published to determine the epidemiological pattern of besnoitiosis in an endemic herd reared under extensive conditions (Spanish Pyrenees) by identifying main factors associated with infection and clinical disease dynamics. The results supporting horizontal transmission by close contact as one of the most important methods of disease spread. In addition, the risk of developing the clinical course increased with age, and the presence of clinical signs was related to higher antibody response. The ability of *B. besnoiti* to infect and even cause disease in animals less than 6 months old was noted. Finally, the risk of calf seroconversion was positively related to the serological status of the

cows, suggesting postnatal transmission between dams and offspring by contact during the suckling period. (Esteban-Gil, Calvete et al. 2017).

Studies on a new besnoitia ELISA test proposes the use of the BbSALUVET ELISA 2.0 in cattle prior to entry to herds free of the disease and in valuable samples prior to a selective culling without the need of a confirmatory Western Blot test in positive samples due to its excellent specificity. (García-Lunar, Ortega-Mora et al. 2017).

A study of besnoitiosis in breeding bulls from an endemically infected purebred beef herd in Italy confirm that bovine besnoitiosis is a disease with serious economic impact on beef cattle breeding, particularly on bulls in service. Good management practises such as clinical monitoring and serological testing of imported animals should be implemented to control its occurrence. (Gazzonis, Alvarez Garcia et al. 2017).

The dynamics of bovine besnoitiosis were studied in an area where the disease is endemic. A four-year longitudinal study was conducted for the first time in three infected beef cattle herds located in the Urbasa-Andía Mountains (Navarra, Spain). Clinical prevalence rates were slightly higher (62% on average) than the seroprevalence rates (50% on average), and tissue cysts located in the vestibulum vaginae and sclera were the most frequently detected clinical signs.(Gutiérrez-Expósito, Ortega-Mora et al. 2017).

A review of current diagnostic techniques and options for control of bovine besnoitiosis was published. (Gutiérrez-Expósito, Ferre et al. 2017).

## Apicomplexa

4th International Meeting on Apicomplexan Parasites in Farm Animals proceedings was published (Hemphill, Leitão et al. 2017).

## Ectoparasitocides

Studies into the effects of Lotilaner (Credelio™), a novel representative of isoxazolines chemical class was published. (Rufener, Danelli et al. 2017).

A review of chemicals and the properties needed that can be used for blocking transmission of vector-borne diseases was published. (Schorderet-Weber, Noack et al. 2017).

## References and abstracts

Avramenko, R. W., et al. (2017). "The use of nemabiome metabarcoding to explore gastro-intestinal nematode species diversity and anthelmintic treatment effectiveness in beef calves." International Journal for Parasitology **47**(13): 893-902.

Next-generation deep amplicon sequencing, or metabarcoding, has revolutionized the study of microbial communities in humans, animals and the environment. However, such approaches have yet to be applied to parasitic helminth communities. We recently described the first example of such a method – nemabiome sequencing – based on deep-amplicon sequencing of internal transcribed spacer 2 (ITS-2) rDNA, and validated its ability to quantitatively assess the species composition of cattle gastro-intestinal nematode (GIN) communities. Here, we present the first application of this approach to explore GIN species diversity and the impact of anthelmintic drug treatments. First, we investigated GIN species diversity in cow-calf beef cattle herds in several different regions, using coproculture derived L3s. A screen of 50 Canadian beef herds revealed parasite species diversity to be low overall. The majority of parasite communities were comprised of just two species; *Ostertagia ostertagi* and *Cooperia oncophora*. *Cooperia punctata* was present at much lower levels overall, but nevertheless comprised a substantive part of the parasite community of several herds in eastern Canada. In contrast, nemabiome sequencing revealed higher GIN species diversity in beef calves sampled from central/south-eastern USA and Sao Paulo State, Brazil. In these regions *C. punctata* predominated in most herds with *Haemonchus placei* predominating in a few cases. *Ostertagia ostertagi* and *C. oncophora* were relatively minor species in these regions in contrast to the Canadian herds. We also examined the impact of routine macrocyclic lactone pour-on treatments on GIN communities in the Canadian beef herds. Low treatment effectiveness was observed in many cases, and nemabiome sequencing revealed an overall increase in the proportion of *Cooperia* spp. relative to *O. ostertagi* post-treatment. This work demonstrates the power of nemabiome metabarcoding to provide a detailed picture of GIN parasite community structure in large sample sets and illustrates its potential use in research, diagnostics and surveillance. © 2017

Bagnall, N. H., et al. (2017). "Mutations in the Hco-mptl-1 gene in a field-derived monepantel-resistant isolate of *Haemonchus contortus*." International Journal for Parasitology: Drugs and Drug Resistance **7**(2): 236-240.

Resistance to the anthelmintic drug monepantel (Zolvix®) has emerged in parasitic worms infecting sheep and goats. The mechanism of resistance in these cases is unknown. The drug targets nicotinic acetylcholine receptors belonging to the nematode-specific DEG-3 subfamily. We examined the receptor gene, Hco-mptl-1, in a highly Zolvix®-resistant and a -susceptible isolate of the parasitic nematode *Haemonchus contortus*. cDNA coding for the full length receptor protein (Hco-MPTL-1) was present in all clones prepared from a pool of susceptible larvae (21/21 clones) and approximately 50% of those from the resistant isolate (17/33). On the other hand,

the remaining clones from the resistant isolate showed various mutations that resulted in truncated predicted proteins, missing at least one transmembrane domain. The most common mutation (11/33 clones) resulted in the retention of intron 15, a premature stop codon, and a truncated protein. Sequencing of intron 15 genomic DNA showed very few SNPs in susceptible larvae and in 12/18 clones from resistant larvae, alongside the presence of at least 17 SNPs in the remaining resistant clones. The present study shows that the highly resistant isolate has a number of mutations in the drug target gene that would most-likely result in a non-functional receptor, thus rendering the larvae insensitive to the drug. The presence of many wild-type sequences in this highly-resistant population suggests that there was a significant presence of heterozygotes in the survivors of the field drench treatment from which the isolate was derived, and hence that at least some of the mutations may be dominant. Alternatively, their presence may be due to the additional influence of mutations at another locus contributing to the resistance phenotype. The presence of multiple separate mutations in the Hco-mptl-1 gene in this viable field-derived worm isolate may at least partly explain why resistance to Zolvix® has arisen rapidly in the field. © 2017 The Authors

Baines, D., et al. (2017). "Microscopic and molecular tracing of *Cryptosporidium* oocysts: Identifying a possible reservoir of infection in red grouse." *Pathogens* **6**(4).

Infection by *Cryptosporidium baileyi* causes respiratory cryptosporidiosis in red grouse *Lagopus lagopus scotica*. First diagnosed in 2010, it has since been detected across half of moors managed for grouse shooting in northern England. We hypothesised that contaminated grouse faeces within communal trays visited by grouse containing grit coated with flubendazole, provided to control *Trichostrongylus tenuis* parasites of grouse, is a reservoir of infection. To establish the basis to this hypothesis, contents of 23 trays from a grouse moor were examined for *Cryptosporidium* oocysts. Contents were subjected to Immuno Magnetic Separation oocyst concentration techniques prior to examination by Immuno Fluorescence Antibody Test microscopy and molecular analysis on the 18S rRNA gene. Seven of 13 (54%) grit trays known to be used by infected grouse were positive for *Cryptosporidium* by IMS-IFAT, compared to two of 10 (20%) random background trays. Ten of the 13 (77%) trays used by infected birds amplified positive for *Cryptosporidium* by Polymerase Chain Reaction and three of the 10 (30%) random trays. All PCR amplified products sequenced matched with *C. baileyi*, with *C. parvum* also present in one tray. These data suggest that trays used to "worm" grouse may act as reservoirs of *Cryptosporidium* infection and their future design may need to be reconsidered to minimise contamination. © 2017 by the authors. Licensee MDPI, Basel, Switzerland.

Berk, Z., et al. (2017). "Modelling the impacts of pasture contamination and stocking rate for the development of targeted selective treatment strategies for *Ostertagia ostertagi* infection in calves." *Veterinary Parasitology* **238**: 82-86.

A simulation study was carried out to assess whether variation in pasture contamination or stocking rate impact upon the optimal design of targeted selective

treatment (TST) strategies. Two methods of TST implementation were considered: 1) treatment of a fixed percentage of a herd according to a given phenotypic trait, or 2) treatment of individuals that exceeded a threshold value for a given phenotypic trait. Four phenotypic traits, on which to base treatment were considered: 1) average daily bodyweight gain, 2) faecal egg count, 3) plasma pepsinogen, or 4) random selection. Each implementation method (fixed percentage or threshold treatment) and determinant criteria (phenotypic trait) was assessed in terms of benefit per R (BPR), the ratio of average benefit in weight gain to change in frequency of resistance alleles R (relative to an untreated population). The impact of pasture contamination on optimal TST strategy design was investigated by setting the initial pasture contamination to 100, 200 or 500 *O. ostertagi* L3/kg DM herbage; stocking rate was investigated at a low (3calves/ha), conventional (5 calves/ha) or high (7 calves/ha) stocking rates. When treating a fixed percentage of the herd, treatments according to plasma pepsinogen or random selection were identified as the most beneficial (i.e. resulted in the greatest BPR) for all levels of initial pasture contamination and all stocking rates. Conversely when treatments were administered according to threshold values ADG was most beneficial, and was identified as the best TST strategy (i.e. resulted in the greatest overall BPR) for all levels of initial pasture contamination and all stocking rates. © 2017 The Authors

Brauneis, M. D., et al. (2017). "The acaricidal speed of kill of orally administered fluralaner against poultry red mites (*Dermanyssus gallinae*) on laying hens and its impact on mite reproduction." Parasites and Vectors **10**(1).

Background: *Dermanyssus gallinae*, the poultry red mite, is a growing threat to chickens in poultry farms. This nocturnal hematophagous ectoparasite has a rapid rate of proliferation with a negative impact on the birds' health, welfare and productivity resulting in severe economic consequences for poultry farmers. A study was performed with fluralaner, a novel systemic ectoparasiticide, to evaluate its effect on mite vitality and reproduction after oral administration to laying hens. Methods: Sixteen healthy hens were randomly allocated to two study groups (n = 8). One group was orally treated with fluralaner by gavage at a dose of 0.5 mg/kg bodyweight twice 7 days apart. The negative control group received no treatment. Hens in each group were repeatedly infested with approximately 200 unfed adult *D. gallinae* at 1, 5, 8, 12, 15, 19, 22 and 26 days after the initial administration. After infestation and feeding for 2.5 h, 25 engorged mites per hen were collected and incubated in tubes. Mites were assessed for vitality (dead/live) at 4, 8, 12, and 24 h after each infestation. Tubes containing eggs and/or living mites were incubated another 8 days for assessment of mite reproductive capacity. Results: Fluralaner demonstrated a fast speed of kill in mites within 4 h post-infestation for 12 days after treatment initiation. An efficacy (mite mortality) of 98.7-100% was achieved. At 15 days after treatment initiation, 100% efficacy was achieved within 24 h post-infestation, and no mite oviposition occurred during this period. Nineteen days after treatment initiation, the mites' ability to generate nymphs was reduced by 90.8%, which decreased to < 24.1% at later infestations. Conclusions: Fluralaner administered orally to hens twice, 7 days apart, provides efficacy against experimental poultry red mite infestation for at least 2 weeks. The

demonstrated rapid speed of kill results in substantial depletion of the mites' oviposition and suggests that fluralaner can be an effective tool in the control of *D. gallinae*, one of the most urgent problems in poultry farms. © 2017 The Author(s).

Buonfrate, D., et al. (2017). "Four clusters of *Trichostrongylus* infection diagnosed in a single center, in Italy." *Infection* **45**(2): 233-236.

*Trichostrongylus* spp. are parasites that are seldom recognized as a cause of eosinophilia and gastroenteric symptoms in industrialized countries. The index of suspicion raises when several members of a same household present eosinophilia. We report four clusters of *Trichostrongylus* infection diagnosed in a single center, in northern Italy. Patients came from four different provinces of three Italian Regions. Some patients presented symptoms (abdominal pain and diarrhea were the most frequent ones, reported by 67 and 42% of our patients, respectively), while other were asymptomatic. All of them presented eosinophilia, that was severe (>5000 eosinophils/mm<sup>3</sup>) in 58% cases. Obtaining an accurate history from patients, investigating possible ingestion of vegetables contaminated by organic manure or sheep dejections, is particularly important to achieve diagnosis, also in light of the low sensitivity of parasitological tests. © 2016, Springer-Verlag Berlin Heidelberg.

Cabada, M. M., et al. (2017). "Recombinase polymerase amplification compared to real-time polymerase chain reaction test for the detection of *Fasciola hepatica* in human stool." *American Journal of Tropical Medicine and Hygiene* **96**(2): 341-346.

*Fasciola hepatica* is the most widely distributed trematode infection in the world. Control efforts may be hindered by the lack of diagnostic capacity especially in remote endemic areas. Polymerase chain reaction (PCR)-based methods offer high sensitivity and specificity but require expensive technology. However, the recombinase polymerase amplification (RPA) is an efficient isothermal method that eliminates the need for a thermal cycler and has a high deployment potential to resource-limited settings. We report on the characterization of RPA and PCR tests to detect *Fasciola* infection in clinical stool samples with low egg burdens. The sensitivity of the RPA and PCR were 87% and 66%, respectively. Both tests were 100% specific showing no cross-reactivity with trematode, cestode, or nematode parasites. In addition, RPA and PCR were able to detect 47% and 26% of infections not detected by microscopy, respectively. The RPA adapted to a lateral flow platform was more sensitive than gel-based detection of the reaction products. In conclusion, the *Fasciola* RPA is a highly sensitive and specific test to diagnose chronic infection using stool samples. The *Fasciola* RPA lateral flow has the potential for deployment to endemic areas after further characterization. © 2017 by The American Society of Tropical Medicine and Hygiene.

Csivincsik, Á., et al. (2017). "Shared pastures and anthelmintic resistance in wildlife and livestock." *Agriculturae Conspectus Scientificus* **82**(2 Special Issue 1): 189-191.

Parasitic diseases are an important threat to grazing livestock. Until recently, the most accepted control methods were regular, herd-level deworming regime and grazing on

“clean” or “safe” pasture. Presence of wild ruminants on pastures was considered as the main risk of parasitic infection. In the last decades, the failure of these conventional attitude was suspected. This study was carried out in Hungary, where springtime, whole-herd deworming is still in practice. Our hypotheses were that the above-mentioned strategy led to high prevalence of anthelmintic resistance; on the other hand, wildlife could not contribute to deleterious parasitosis of livestock. For this, we accomplished an investigation in the close surroundings of typical sheep herds. The aims were to determine the species structure and anthelmintic resistance in the parasite community of the sheep herds and the adjacent roe deer population. As a result, we found that in the roe deer (N=53), a more diverse parasite community exists and the most devastating worm species, *Haemonchus contortus* plays a less important role in it; than in the sheep (N=40). Prevalence of benzimidazole resistance in *H. contortus* was 17.1% and 68.6% in the roe deer and sheep, respectively. Our findings suggest that routine deworming cannot succeed; while presence of roe deer is rather useful, as its parasites attenuate the simplistic, anthelmintic resistant pasture community. © 2017, University of Zagreb. All rights reserved.

de Albuquerque, A. C. A., et al. (2017). "Development of *Haemonchus contortus* resistance in sheep under suppressive or targeted selective treatment with monepantel." *Veterinary Parasitology* 246: 112-117.

This study examined the development of resistance to anthelmintics in *Haemonchus contortus* in lambs under suppressive or selective treatment regimens that included monepantel. Twenty Ile de France and 20 Santa Ines lambs were allocated to two anthelmintic treatment regimens, based on body weight and nematode faecal egg counts (FEC): targeted selective treatment (TST) or suppressive treatment, both with monepantel. Lambs of the TST group were treated individually when they presented with a packed cell volume (PCV)  $\leq 20\%$ . On 7 October 2016, the lambs were allocated to clean pastures, where they grazed in separated paddocks by group until late February 2017. The experimental area was contaminated with nematodes that were introduced with the experimental Ile de France and Santa Ines lambs, naturally infected with gastrointestinal nematodes. To maintain the grazing lambs in the suppressive treatment group and their pasture as free of worms as possible, these lambs were treated with anthelmintics before being allocated to their paddock and then were periodically treated with monepantel. However, the use of a suppressive treatment regimen that included monepantel over a period of 3 months resulted in the emergence of a population of resistant *H. contortus*. In the TST group, there was a rapid and progressive reduction in the efficacy of monepantel, which at the end of the experiment was only 76%. The Ile de France lambs were all treated one or more times during the experiment, whereas only two Santa Ines lambs in the TST required treatment. In conclusion, a population of *H. contortus* resistant to monepantel emerged quickly during the rainy season, even when sheep were submitted to selective treatment. © 2017 Elsevier B.V.

Diezma-Díaz, C., et al. (2017). "Bovine chronic besnoitiosis in a calf: Characterization of a novel *B. besnoiti* isolate from an unusual case report." *Veterinary Parasitology* **247**: 10-18.

Bovine besnoitiosis, caused by the apicomplexan *Besnoitia besnoiti*, is a chronic and debilitating disease characterized by cutaneous and systemic manifestations that primarily affects adult beef cattle. Previous studies have reported that clinical besnoitiosis is rare in calves. However, we isolated *B. besnoiti* from a chronically infected calf for the first time. The identity of the *Besnoitia* species was determined after parasite isolation and molecular genotyping. According to the results obtained in vitro the new isolate, named as Bb-Spain3, was characterized in a reproducible in vitro model and was categorized as a low invader and low prolific isolate with a slower lytic cycle compared to Bb-Spain 1 isolate. Specific traits that differentiate isolates obtained from adult animals from those infecting calves were not found. Next, we described the first case report of chronic besnoitiosis in a female calf less than 6 months-old with a low body condition. The disease was confirmed by the presence of specific anti-*B. besnoiti* antibodies and parasite detection in the skin. At post-mortem examination, tissue samples were collected for histological, immunohistochemical and molecular analyses. DNA-parasite was detected in 31 different calf's tissues, being the most highly parasitized tissues the skin and the respiratory and reproductive tracts. In addition, the parasite was also present in heart, eyes, lymph nodes and brain. The high parasite load, a wide intra-organic parasite distribution and the presence of both viable and degenerated cysts, were indicative of a rapid progression of the disease. This case report underlines the need to include the inspection of young animals in besnoitiosis control. © 2017 Elsevier B.V.

Eckert, J. and R. C. A. Thompson (2017). Historical Aspects of Echinococcosis. *Advances in Parasitology*. **95**: 1-64.

Echinococcosis is a zoonosis whose history dates back to antiquity. This article provides an overview on the general history of echinococcosis, including the elucidation of *Echinococcus* life cycles and the long controversy on the aetiology of the cystic and alveolar forms of echinococcosis (CE and AE), lasting about 100 years since the middle of the 19th century. Furthermore, selected historical aspects of some fields of echinococcosis research are discussed and compared with our current knowledge, such as geographic distribution and epidemiology of CE (*Echinococcus granulosus*) and AE (*Echinococcus multilocularis*), clinical aspects and pathology, diagnosis in humans and animals, treatment (with focus on chemotherapy), control and basic research. A short paragraph is devoted to the neotropical forms of echinococcosis, caused by *Echinococcus vogeli* and *Echinococcus oligarthrus*. In this context the achievements of some ancestral pioneers of echinococcosis research are particularly highlighted and appreciated. Finally, the role of associations, international organizations (World Health Organization and others) and international working groups in echinococcosis research and control is briefly outlined. The retrospective reveals both the admirable achievements of our ancestors and the scientific progress of more recent times. But, it also shows the gaps in our knowledge, skills and resources that we need to control or even eradicate echinococcosis. © 2017 Elsevier Ltd

Esteban-Gil, A., et al. (2017). "Epidemiological patterns of bovine besnoitiosis in an endemic beef cattle herd reared under extensive conditions." Veterinary Parasitology **236**: 14-21.

Bovine besnoitiosis is a parasitic disease caused by the protozoan *Besnoitia besnoiti*. Described many decades ago, recent epidemiological studies reveal its important spread within Europe in the last years. To date, many epidemiological aspects related to life cycle, routes of transmission, incidence rates and associated risk factors are lacking; hence, the establishment of appropriate disease control programmes poses an important challenge. Thus, the aim of the present study was to determine the epidemiological pattern of the disease in an endemic herd reared under extensive conditions (Spanish Pyrenees) by identifying main factors associated with infection and clinical disease dynamics. The study population consisted of 276 Brown Swiss and Pirenaica adult animals and 145 calves born and weaned at the farm during the study. Three sampling time frames were used: January 2010, September 2010 and February 2011, which allowed us to differentiate two periods designated as mountain and valley periods. The data related to animals (breed, sex and age) and herd management (animal grouping and time in housing) were recorded. The data collection methodology was mainly based on clinical examinations and defining the serological status against bovine besnoitiosis by the immunofluorescent antibody testing of blood samples. The total prevalence among adult animals was 38.34% (CI95%: 34.53–42.07), with 18.54% of seropositive animals showing clinical signs. In regard to the cumulative incidence, 34.57% of new infections were detected during the mountain period, in contrast to the 24.59% observed in the valley period. The incidence density was 0.058 and 0.061 new infections per animal-month for the mountain and valley periods, respectively. According to the seroepidemiological study, the seroconversion probability of *B. besnoiti* infection was directly associated with the number of seropositive cows with whom an animal had been stabled as well as the housing period duration, supporting horizontal transmission by close contact as one of the most important methods of disease spread. In addition, the risk of developing the clinical course increased with age, and the presence of clinical signs was related to higher antibody responses. Among calves (from 3.1 to 7.1 months old) sampled once at weaning, the total seroprevalence was 15.17% (CI95%: 9.36–21.04), and the chronic stage was observed in three animals, supporting the ability of *B. besnoiti* to infect and even cause disease in animals less than 6 months old. Finally, the risk of calf seroconversion was positively related to the serological status of the cows, suggesting postnatal transmission between dams and offspring by contact during the suckling period. © 2017 Elsevier B.V.

Fanke, J., et al. (2017). "Economic assessment of *Ostertagia ostertagi* and *Fasciola hepatica* infections in dairy cattle herds in Germany using Paracalc®." Veterinary Parasitology **240**: 39-48.

The aim of the current study was to estimate economic costs of *Ostertagia ostertagi* and *Fasciola hepatica* infections in dairy cattle herds in Germany using the online calculation programme Paracalc®. Following a questionnaire, survey data were

available from 464 farms in 14 federal states. On those farms bulk tank milk (BTM) samples and additionally up to six serum samples collected from first season grazing calves were analysed, using a commercially available ELISA (Boehringer Ingelheim SVANOVA Biotech AB, Uppsala, Sweden), an in-house ELISA (*F. hepatica*) and an in-house serum pepsinogen test. In total, samples obtained from 344 farms were included in the analysis since those were the only farms with complete questionnaires. Median costs per farm and year were estimated for gastrointestinal (GI) nematode infections (€721.38) and *F. hepatica* infection (€565.61). Decreases in milk yield in multiparous cows were the major reason for annual production losses due to GI nematodes (€13.33 per cow) and *F. hepatica* infections (€7.95 per cow), which was followed by annual costs for anthelmintic treatment against GI nematode infections in adult cows (€10.00 per cow) and *F. hepatica* infection associated annual costs due to repeated artificial insemination (€10.13 per cow) and prolonged calving intervals (€9.40 per cow). The study demonstrated that if all required information is provided, the Paracalc® tool can assist to identify production losses in dairy cattle herds due to helminth infections and to optimise farm economics in Germany. © 2017 Elsevier B.V.

Fiel, C. A., et al. (2017). "An attempt to replace an ivermectin-resistant *Cooperia* spp. population by a susceptible one on grazing pastures based on epidemiological principles and refugia management." *Veterinary Parasitology* 246: 53-59.

The maintenance of anthelmintic-susceptible parasite refugia to delay the onset of anthelmintic resistance is an almost impossible effort in many grazing livestock production countries given that current refugia consist of already resistant parasites. Rather, efforts could be focused on replacing the resistant parasite refugia by susceptible parasite ones and implementing sustainable parasite control measures from then on. To this purpose, a trial was conducted to attempt to establish a new population of ivermectin-susceptible *Cooperia* sp. on a beef cattle farm with proven problems of ivermectin-resistant *Cooperia*. During two consecutive years, 82 (Year 1) and 100 (Year 2) recently weaned and parasite-free heifers were inoculated with 40,000 or 30,000 susceptible *Cooperia* L3, respectively, at a time when levels of resistant parasite refugia were normally low. The animals were subsequently allowed to graze on the problem pastures during autumn until the end of spring. Levels of parasitism in the animals and on pasture were monitored monthly and animals were treated with levamisole when needed. The combination of parasitological monitoring and local epidemiological knowledge was essential to determine when treatments were to be administered. No clinical signs of gastrointestinal parasitosis in the herd were observed throughout the study and unnecessary treatments were avoided. Faecal egg counts reduction tests (FECRT) and controlled efficacy tests (CET) employing worm counts were carried out at different times throughout the study to determine the clinical efficacy (FECRT) and the absolute efficacy (CET) of ivermectin, respectively. The clinical efficacy of ivermectin increased from an initial 73% to 99.4%, while the absolute efficacy increased from 54.1% to 87.5% after just two animal production cycles. The switch from a resistant parasite population to a susceptible one requires knowledge of parasitological epidemiology, especially in relation to seasonal variations of parasite populations in both the host and in refugia. © 2017 Elsevier B.V.

García-Lunar, P., et al. (2017). "A new lyophilized tachyzoite based ELISA to diagnose *Besnoitia* spp. infection in bovids and wild ruminants improves specificity." Veterinary Parasitology **244**: 176-182.

Recent studies have reported that routinely used whole or soluble *Besnoitia besnoiti* tachyzoite (TZ) extract-based ELISAs potentially give rise to a high number of false-positive results, which may compromise control and the epidemiological studies of bovine besnoitiosis. Thus, western blot (WB) has been recommended as a confirmatory test. In the present study, a new ELISA test that employs lyophilized tachyzoites for the first time (BbSALUVET ELISA 2.0) was developed and validated with cattle sera (n = 606) under a worst-case scenario. False positive and false negative, soluble TZ extract-based BbSALUVET ELISA 1.0 reactors were overrepresented, and WB was used as the reference test. One commercial test (PrioCHECK *Besnoitia* Ab 2.0, which employs whole TZ extract) and a recently developed membrane-enriched ELISA (APure-BbELISA) were also tested. The three ELISAs showed high AUC values (>0.9). However, the best diagnostic performance corresponded to the BbSALUVET ELISA 2.0 and the APure-BbELISA [(92% sensitivity (Se) and 98% specificity (Sp)] followed by PrioCHECK *Besnoitia* Ab 2.0 (88% Se, 98% Sp, and 4.5% doubtful results). In addition, the BbSALUVET ELISA 2.0 was validated with wild ruminant sera, and excellent performance (96% Se, 97% Sp, and 4% doubtful results) was obtained again. A different antigenic composition of the lyophilized tachyzoites, compared with whole or soluble tachyzoite extracts, may be responsible for the improved diagnostic performance. This study proposes the use of the BbSALUVET ELISA 2.0 in cattle prior to entry to herds free of the disease and in valuable samples prior to a selective culling without the need of a confirmatory Western Blot test in positive samples due to its excellent specificity. © 2017 Elsevier B.V.

Gazzonis, A. L., et al. (2017). "Serological dynamics and risk factors of *Besnoitia besnoiti* infection in breeding bulls from an endemically infected purebred beef herd." Parasitology Research **116**(4): 1383-1393.

Bovine besnoitiosis has been deemed a re-emerging disease in Western Europe and considered endemic in Spain, Portugal, France and in some areas of Northern Italy. This report refers to an infection outbreak in a purebred beef herd from Northern Italy involving a large number of bulls. In October 2013, 544 animals were serologically tested with an in-house ELISA followed by a confirmatory Western blot to evaluate *Besnoitia besnoiti* seroprevalence. A year later, 461 animals were then serologically re-tested together with imported animals (n = 268). Overall, 812 animals were involved in the study. Histology and immunohistochemistry were performed on skin biopsies of suspected animals and several tissue samples from a slaughtered bull. In the first sampling, 100 animals were seropositive (18.4%); in the second sampling, prevalence increased up to 36.5%, with incidence calculated at 39.6%. The risk factor analysis revealed that the infection was associated with age (OR = 1.007) and sex, with males presenting a greater risk (OR = 2.006). In fact, prevalence values in bulls increased from 29.6 to 56.7%, with an incidence of infection of 53.3%. Moreover, mating with a

seropositive bull enhanced infection risk for a seronegative cow (OR = 1.678). Clinical signs typical of bovine besnoitiosis were found in seven seropositive animals, with confirmation of *B. besnoiti* through histology and immunohistochemistry. The study outcomes confirm that bovine besnoitiosis is a disease with serious economic impact on beef cattle breeding, particularly on bulls in service. Good management practises such as clinical monitoring and serological testing of imported animals should be implemented to control its occurrence. © 2017, Springer-Verlag Berlin Heidelberg.

Geurden, T., et al. (2017). "A multi-country study to assess the effect of a treatment with moxidectin pour-on during the dry period on milk production in dairy cows." *Veterinary Parasitology* **237**: 104-109.

A randomized clinical study was conducted in a total of 45 commercial dairy farms in France (14 farms), Germany (28 farms) and the UK (3 farms) to evaluate the effect of an anthelmintic treatment on milk yield in the subsequent lactation. A total of 1287 animals with suspected exposure to *Ostertagia ostertagi* were included in the study. Animals were treated during the dry period (7–77 days before parturition) with moxidectin pour-on (Cydectin® 0.5% Pour-On, Zoetis; 638 animals) or left untreated (649 animals) according to a randomized block design. Animals were either heifers (n = 296) or multiparous cows (n = 991). The milk production was monitored at regular intervals after treatment up to 335 days after lactation, and analysed using a general linear mixed model with the milk production as outcome variable and several random effects. The effect on milk yield after anthelmintic treatment over the whole subsequent lactation varied from no effect (–0.43 kg/day; P = 0.35) to an increase of milk yield with 2.35 kg/day (P = 0.01), depending on the study region and parity of the cows. Lactation curve analysis suggested that the treatment effect was mainly caused by a slower decay of the milk production in the treated animals compared to untreated animals. The present study highlights the beneficial effect of a topical treatment with moxidectin before parturition on milk yield in the subsequent lactation, as well as the importance of a careful evaluation of nematode exposure risk based on local grazing management practices to guide and target production-based anthelmintic treatment decisions. © 2017 Elsevier B.V.

Gómez-Morales, M. A., et al. (2017). "Present status of laboratory diagnosis of human taeniosis/cysticercosis in Europe." *European Journal of Clinical Microbiology and Infectious Diseases* **36**(11): 2029-2040.

Human cysticercosis (CC) is a parasitic zoonosis caused by the larval stage (cyst) of the *Taenia solium*. Cysts can establish in the human central nervous system (neurocysticercosis, NCC) and other organs and tissues; they also develop in pigs, the natural intermediate host. Human taeniosis may be caused by *T. solium*, *Taenia saginata* and *Taenia asiatica* tapeworms; these infections are usually asymptomatic, but show a significant relevance as they perpetuate the parasites' life cycle, and, in the case of *T. solium*, they are the origin of (N)CC. In European Union (EU) member states and associated countries, the occurrence of autochthonous *T. solium* cases is debated, and imported cases have significantly increased lately; the status of *T.*

asiatica has been never reported, whereas *T. saginata* is prevalent and causes an economic impact due to condemned carcasses. Based on their effects on the EU society, the specific diagnosis of these pathologies is relevant for their prevention and control. The aims of this study were to know the diagnostic tests used in European laboratories for human taeniosis/cysticercosis by means of a questionnaire, to determine potential gaps in their detection, and to obtain preliminary data on the number of diagnosed taeniosis/CC cases. © 2017, The Author(s).

Gutiérrez-Expósito, D., et al. (2017). "Advances in the diagnosis of bovine besnoitiosis: current options and applications for control." *International Journal for Parasitology* **47**(12): 737-751.

Bovine besnoitiosis, which is caused by the tissue cyst-forming intracellular parasite *Besnoitia besnoiti*, is a chronic and debilitating disease that is responsible for severe economic losses in the cattle raised under extensive husbandry systems. The absence of vaccines, treatments or a health scheme at local, national and international levels has led to a rapid spread of bovine besnoitiosis from western Europe towards eastern countries and northwards. Moreover, this parasitic disease is widely present in many sub-Saharan countries. Thus, bovine besnoitiosis should be included in the animal health scheme of beef cattle herds. Accurate diagnostic tools and common diagnostic procedures are mandatory in any control programme. Relevant advances have been made in this field during the last decade. Succeeding with accurate diagnosis relies on the technique employed and the antibody and parasite kinetics of the infection stage, which may notably influence control programmes and surveillance. Moreover, control programmes should be adapted to the epidemiological status of the disease, as the disease presentation in a herd has important implications for prospective control. Herein, we review the clinical disease presentation of bovine besnoitiosis and the correlation between its clinical course and laboratory parameters. We also provide an update on the available diagnostic tools, discuss their strengths and pitfalls, and provide guidelines for their use in control, surveillance and epidemiological studies. A rational control strategy is also recommended. © 2017 Australian Society for Parasitology

Gutiérrez-Expósito, D., et al. (2017). "Clinical and Serological Dynamics of *Besnoitia besnoiti* Infection in Three Endemically Infected Beef Cattle Herds." *Transboundary and Emerging Diseases* **64**(2): 538-546.

The dynamics of bovine besnoitiosis were studied in an area where the disease is endemic. A four-year longitudinal study was conducted for the first time in three infected beef cattle herds located in the Urbasa-Andía Mountains (Navarra, Spain). Each herd was visited four to seven times, and clinical and serological prevalence rates and incidence rates were estimated. Clinical inspections to identify compatible clinical signs with the disease stages were conducted at the beginning and end of the study. Serological assessment was initially performed by ELISA. Seronegative animals with clinical signs and seropositive animals with relative index per cent (RIPC) values lower than 30 that did not increase during the study period were analysed by Western

blot to optimize the sensitivity and specificity of the ELISA test. Clinical prevalence rates were slightly higher (62% on average) than the seroprevalence rates (50% on average), and tissue cysts located in the vestibulum vaginae and sclera were the most frequently detected clinical signs. The proportion of seropositive animals with clinical signs varied from 16.7% to 73.6% among the herds, and 17% of cattle with clinical signs proved to be seronegative by both serological tests. An average 22% serological incidence rate was also reported in addition to clinical incidence rates that varied from 12.5% to 16.7%. Additionally, parasitemia was investigated in the herd that showed the highest clinical and seroprevalence rates. Only one PCR positive blood sample was detected. Thus, the role that blood may play in parasite transmission needs to be further investigated. Infected herds maintained both high prevalence and incidence rates in the absence of control measures and a high number of parasite carriers. Finally, economic impact studies on reproductive and productive losses associated with besnoitiosis need to be performed to implement a cost–benefit control programme. © 2015 Blackwell Verlag GmbH

Hamel, D., et al. (2017). "Eprinomectin pour-on (EPRINEX® Pour-on, Merial): Efficacy against gastrointestinal and pulmonary nematodes and pharmacokinetics in sheep." BMC Veterinary Research **13**(1).

Background: The anthelmintic efficacy of the 0.5% w/v topical formulation of eprinomectin (EPN), EPRINEX® Pour-on (Merial) when administered at 1 mg/kg body weight was evaluated in sheep in two dose confirmation laboratory studies and one multicenter field study. In addition, the pharmacokinetics of EPN when administered at that dosage to adult sheep was determined. Results: In the two dose confirmation studies, which included 10 sheep each, sheep treated with topical EPN had significantly ( $p < 0.05$ ) fewer of the following nematodes than the untreated sheep with overall reduction of nematode counts by  $>99\%$ : adult *Dictyocaulus filaria*, *Haemonchus contortus*, *Teladorsagia circumcincta* (pinnata/trifurcata), *Trichostrongylus axei*, *T. colubriformis*, *T. vitrinus*, *Cooperia curticei*, *Nematodirus battus*, *Strongyloides papillosus*, *Chabertia ovina* and *Oesophagostomum venulosum*, and inhibited fourth-stage *Teladorsagia* larvae. A total of 196 sheep harboring naturally acquired gastrointestinal nematode infections were included in the field efficacy study at two sites each in Germany (48 Merino x Ile de France lambs, 52 adult Merino females) and in Italy (adult male and female Bagnolese, Lacaune, Lacaune x Bagnolese, Bagnolese x Sarda sheep; 48 animals per site). Animals were blocked on pre-treatment body weight and within each block, one animal was randomly assigned to the control (untreated) group and three animals were randomly assigned to be treated with topical EPN. Examination of feces 14 days after treatment demonstrated that, relative to the controls, topical EPN-treated sheep had significantly ( $p < 0.0001$ ) lower strongylid egg counts. Reduction was  $\geq 97\%$  at each site and 98.6% across all sites. Pharmacokinetics of EPN following single treatment with topical EPN were determined in eight ~4.5 year old female Merino cross sheep based on the analysis of plasma samples which were collected from two hours to 21 days following treatment. The main pharmacokinetic parameters were:  $C_{max}$   $6.20 \pm 1.71$  ng/mL,  $AUC_{last}$   $48.8 \pm 19.2$  day\*ng/mL,  $T_{max}$   $3.13 \pm 2.99$  days and  $T_{1/2}$   $6.40 \pm 2.95$  days. No treatment-

related health problems or adverse drug events were observed in any study. Conclusion: These studies demonstrated 0.5% w/v EPN administered topically at 1 mg/kg body weight to be highly efficacious against a broad range of ovine gastrointestinal nematodes and *D. filaria* lungworms and well tolerated by sheep of different ages, breeds, gender and physiological status. © 2017 The Author(s).

Hemphill, A., et al. (2017). "ApiCOWplexa 2017 – 4th International Meeting on Apicomplexan Parasites in Farm Animals." International Journal for Parasitology **47**(12): 697-699.

Hodgson, B. A. S. and C. J. Mulvaney (2017). "Resistance to a triple-combination anthelmintic in *Trichostrongylus* spp. on a commercial sheep farm in New Zealand." New Zealand Veterinary Journal **65**(5): 277-281.

AIM: To evaluate resistance to anthelmintics containing abamectin, levamisole, and oxfendazole (AB-LEV-OX), derquantal and abamectin (DEQ-AB), moxidectin, and monepantel in naturally acquired gastrointestinal nematodes present on a sheep farm. METHODS: Faecal nematode egg count reduction tests (FECRT) were carried out on lambs that were approximately 7 months-old and infected with naturally acquired nematodes. Lambs were randomly allocated to one of five groups (n=15 per group): treatment with 2 mg/kg derquantel and 0.2 mg/kg abamectin; 0.2 mg/kg abamectin, 8 mg/kg levamisole HCl and 4.5 mg/kg oxfendazole; 2.5 mg/kg monepantel; 0.2 mg/kg moxidectin, or no treatment. Post-treatment samples were collected 12 days later. Abomasa and small intestines were collected from two slaughtered lambs from each of the DEQ-AB, AB-LEV-OX, moxidectin and control groups 15 days after treatment, for nematode counting. RESULTS: The FECRT demonstrated that efficacy was 90.3 (95% CI=84.2–94.1)% for AB-LEV-OX, 54.5 (95% CI=28.4–71.1)% for moxidectin, 99.2 (95% CI=97.4–99.8)% for DEQ-AB and 100% for monepantel, across all genera. For *Trichostrongylus* spp. efficacy was 85.5% for AB-LEV-OX and 46.7% for moxidectin. *Haemonchus* spp. were fully susceptible to all treatments. Post-treatment nematode counts indicated that the resistant *Trichostrongylus* spp. were from the small intestine. CONCLUSIONS: Anthelmintic resistance to both AB-LEV-OX and moxidectin was present in the *Trichostrongylus* genus on a commercial sheep farm. Monepantel and DEQ-AB were both effective against *Trichostrongylus* spp. based on FECRT results. CLINICAL RELEVANCE: This finding of resistance to an AB-LEV-OX triple-combination anthelmintic in the *Trichostrongylus* genus in sheep in New Zealand further limits anthelmintic treatment options available, and calls into question whether this combination is suitable for use as a quarantine treatment. © 2017 New Zealand Veterinary Association.

Holzhauser, M., et al. (2017). "Reduced efficacy of ivermectin against ostertagia in a dutch cattle herd." Veterinary Record Case Reports **5**(1).

A pilot study on reduced ivermectin efficacy against *Ostertagia ostertagi* following the detection of a high number of strongyle-type eggs in a 1.5-year-old bull during the first part of the pasturing period in 2015 was conducted. This finding was remarkable because of the pasturing history, treatment history and time after turnout (June). The

study involved one beef cattle herd and followed as far as possible the World Association for the Advancement of Veterinary Parasitology guidelines regarding the faecal egg count reduction test. We observed poor ivermectin efficacy (65%) compared with the expected efficacy of >95%. This also has consequences for practitioners, who should ensure the correct application of anthelmintics and perform repeated faecal examinations following the use of parasitological agents for persistent problems. Our finding also should have consequences for the pharmaceutical industry: the administration of registered anthelmintics via the most efficacious route is preferable. © British Veterinary Association (unless otherwise stated in the text of the article) 2017.

Jackson, A., et al. (2017). "Targeted anthelmintic treatment of parasitic gastroenteritis in first grazing season dairy calves using daily live weight gain as an indicator." Veterinary Parasitology **244**: 85-90.

Control of parasitic gastroenteritis in cattle is typically based on group treatments with anthelmintics, complemented by grazing management, where feasible. However, the almost inevitable evolution of resistance in parasitic nematodes to anthelmintics over time necessitates a reappraisal of their use in order to reduce selection pressure. One such approach is targeted selective treatment (TST), in which only individual animals that will most benefit are treated, rather than whole groups of at-risk cattle. This study was designed to assess the feasibility of implementing TST on three commercial farms, two of which were organic. A total of 104 first-grazing season (FGS), weaned dairy calves were enrolled in the study; each was weighed at monthly intervals from the start of the grazing season using scales or weigh-bands. At the same time dung and blood samples were collected in order to measure faecal egg counts (FEC) and plasma pepsinogen, respectively. A pre-determined threshold weight gain of 0.75 kg/day was used to determine those animals that would be treated; the anthelmintic used was eprinomectin. No individual animal received more than one treatment during the grazing season and all treatments were given in July or August; five animals were not treated at all because their growth rates consistently exceeded the threshold. Mean daily live weight gain over the entire grazing season ranged between 0.69 and 0.82 kg/day on the three farms. Neither FEC nor pepsinogen values were significantly associated with live weight gain. Implementation of TST at farm level requires regular (monthly) handling of the animals and the use of weigh scales or tape, but can be integrated into farm management practices. This study has shown that acceptable growth rates can be achieved in FGS cattle with modest levels of treatment and correspondingly less exposure of their nematode populations to anthelmintics, which should mitigate selection pressure for resistance by increasing the size of the refugia in both hosts and pasture. © 2017 Elsevier B.V.

Lamb, J., et al. (2017). "Broad spectrum anthelmintic resistance of *Haemonchus contortus* in Northern NSW of Australia." Veterinary Parasitology **241**: 48-51.

On a sheep farm in Northern New South Wales (NSW) of Australia a degree of anthelmintic resistance was suspected. With noticeable clinical signs of infection and

sheep not responding to treatment, a faecal egg count reduction test was conducted to ascertain the broad spectrum of anthelmintic resistance at this farm. A number of classes of anthelmintics were assessed including organophosphate, macrocyclic lactone (ML) and in combination an ML, benzimidazole, levamisole and salicylanilide. In addition, the more recently registered classes of anthelmintics, monepantel (amino-acetonitrile derivative) and derquantel/abamectin combination (spiroindole + ML) were included. Ninety merino sheep naturally infected with a field strain of *Haemonchus contortus* were randomly allocated to 6 treatment groups (15 animals/group). Sheep were subsequently treated based on label recommendations and individual bodyweight. Faecal samples were collected post-treatment on Days 7, 14 and 21 to conduct faecal egg counts and group bulk larval cultures. Broad spectrum anthelmintic resistance was confirmed at this site with treatment efficacies ranging from 21.3% (monepantel) to 93.8% (derquantel/abamectin combination) against the *H. contortus* strain. Furthermore, resistance to the multi-combination anthelmintic containing 4 active ingredients was evident (52.5%). This broad spectrum of resistance highlights the need for integration of alternative sustainable methods in parasite control in order to slow development of resistance and increase the life time effectiveness of anthelmintics. © 2017 Elsevier B.V.

Lifschitz, A., et al. (2017). "Host pharmacokinetics and drug accumulation of anthelmintics within target helminth parasites of ruminants." New Zealand Veterinary Journal **65**(4): 176-184.

Anthelmintic drugs require effective concentrations to be attained at the site of parasite location for a certain period to assure their efficacy. The processes of absorption, distribution, metabolism and excretion (pharmacokinetic phase) directly influence drug concentrations attained at the site of action and the resultant pharmacological effect. The aim of the current review article was to provide an overview of the relationship between the pharmacokinetic features of different anthelmintic drugs, their availability in host tissues, accumulation within target helminths and resulting therapeutic efficacy. It focuses on the anthelmintics used in cattle and sheep for which published information on the overall topic is available; benzimidazoles, macrocyclic lactones and monepantel. Physicochemical properties, such as water solubility and dissolution rate, determine the ability of anthelmintic compounds to accumulate in the target parasites and consequently final clinical efficacy. The transcuticular absorption process is the main route of penetration for different drugs in nematodes and cestodes. However, oral ingestion is a main route of drug entry into adult liver flukes. Among other factors, the route of administration may substantially affect the pharmacokinetic behaviour of anthelmintic molecules and modify their efficacy. Oral administration improves drug efficacy against nematodes located in the gastrointestinal tract especially if parasites have a reduced susceptibility. Partitioning of the drug between gastrointestinal contents, mucosal tissue and the target parasite is important to enhance the drug exposure of the nematodes located in the lumen of the abomasum and/or small intestine. On the other hand, large inter-animal variability in drug exposure and subsequent high variability in efficacy is observed after topical administration of anthelmintic compounds. As it has been extensively demonstrated under experimental

and field conditions, understanding pharmacokinetic behaviour and identification of different factors affecting drug activity is important for achieving optimal parasite control and avoiding selection for drug resistance. The search for novel alternatives to deliver enhanced drug concentrations within target helminth parasites may contribute to avoiding misuse, and prolong the lifespan of existing and novel anthelmintic compounds in the veterinary pharmaceutical market. © 2017 New Zealand Veterinary Association.

Matos, A. F. I. M. D., et al. (2017). "Attempt to control *Haemonchus contortus* in dairy goats with Barbervax®, a vaccine derived from the nematode gut membrane glycoproteins." Small Ruminant Research **151**: 1-4.

The nematode *Haemonchus contortus* is the most important parasite of small ruminants around the world and the major cause of mortality in tropical regions. Goats are putatively more susceptible than sheep to this parasite and alternatives to anthelmintic utilization are constantly being sought especially after the rise of multiple anthelmintic resistance. Here we present results of a trial designed to evaluate Barbervax®, a vaccine containing gut proteins from *Haemonchus contortus*, in the control of haemonchosis in dairy goats. In this study female Saanen (n = 20) and Anglo Nubian (n = 20) goats aged six months were assigned by breed, body weight and fecal egg counts into four experimental groups (n = 10) (vaccinated or non-vaccinated). Barbervax® was injected subcutaneously, starting with three priming doses 21 days apart followed by boosts every 6 weeks. All animals grazed on the same bushland area and were subjected to natural and artificial worm infection. Mean efficacy over the trial period was  $69.8 \pm 2.11\%$  and  $57.4 \pm 2.76\%$  for the Anglo Nubians and Saanens, respectively. Significant differences in packed cell volume (PCV) and total plasma proteins (TPP) values were also observed but only in the Anglo Nubians. The protection afforded by vaccination was considered useful for management of *Haemonchus* infection in dairy goats by reducing egg counts. Furthermore, the vaccine does not generate chemical residues and could be very interesting for *Haemonchus* control during milk production. © 2017 Elsevier B.V.

McMahon, C., et al. (2017). "Control of *Nematodirus* spp. infection by sheep flock owners in Northern Ireland." Irish Veterinary Journal **70**(1).

Background: To address a lack of information on the control of ovine helminth parasites in Northern Ireland (NI), a number of research projects have been undertaken, dealing with gastrointestinal nematodes, tapeworms and liver fluke. This investigation concerns *Nematodirus* and concentrates on three aspects of disease: farm management strategies for its control, derived from the results of a Questionnaire; the efficacy of treatment used by farmers, as determined by a coprological survey; and the hatching requirements of *Nematodirus* eggs, that is, whether prolonged chilling is a pre-requisite for hatching. Results: A Questionnaire was sent to 252 sheep farmers in NI in March 2012 (covering the years 2009-2012) and replies were received from 228 farmers. Under-dosing, inaccurate calibration of equipment and inappropriate product choice were poor practices identified. Following

this survey, the efficacy of treatment of *Nematodirus* spp. in sheep flocks was evaluated in April and May 2012. Sampling kits were sent to 51 flock owners, all of whom returned pre- and post-anthelmintic dosing faecal samples to the laboratory for analysis. At the time of treatment, 41 flocks were positive for *Nematodirus* (as diagnosed by the presence of eggs). Reduced benzimidazole efficacy was detected in 35.7% of flocks tested (n = 28). Although only involving a small number of flocks, reduced efficacy of levamisole treatment was detected in 50%, of avermectins in 33% and of moxidectin in 75% of flocks tested (n = 2, 6 and 4, respectively). In the egg hatch experiment, carried out under "chilled" and "non-chilled" conditions, 43% of the eggs in the "non-chilled" group were able to hatch, compared to 100% in the "chilled" group. Conclusions: The identification of inefficient control strategies argues for continued education of stockholders, in order to improve their management programmes. This is particularly important where the practices might impact on the development of anthelmintic resistance, which has been shown to exist on NI farms. The appropriate choice of anthelmintic is a vital part of this plan. The ability of eggs to hatch under non-chilled conditions demonstrates a flexibility in hatching behaviour. This may represent an adaptation to climate change and account for the recent emergence of a second, autumnal peak of infection. © 2017 The Author(s).

McMahon, C., et al. (2017). "Tapeworm control practices by sheep farmers in Northern Ireland." *Veterinary Parasitology: Regional Studies and Reports* 7: 14-18.

A questionnaire to obtain information on tapeworm control practices was sent to 252 sheep farmers in Northern Ireland (NI) in 2012. Replies were received from 228 flock owners. Most farmers considered that tapeworm infections had less impact on productivity than gastrointestinal nematodes, flukes and ectoparasites. The majority of respondents (61.8%) did not treat for tapeworms. Of those that did, the average number of treatments given per year was 2.3, with some owners treating up to 6 times a year. The highest percentages of treatments were given over the period May–July. Benzimidazole compounds were the predominant class of drugs used (48.2%), followed by macrocyclic lactones (MLs) (31.2%). Levamisole, oxclozanide, closantel and Monepantel were also used; together with MLs, their combined use accounted for 51.9% of all treatments given, and represents inappropriate product choice. Diagnostic data for tapeworm infections in NI over the period 2007–2014 was retrieved from the database held by the Veterinary Sciences Division at Stormont. Positive diagnoses remained low throughout this period: the highest recorded figure was 3.1%, in 2007. Despite there being little-to-no justification for treating sheep for *M. expansa* on the basis of any likely benefit to the health or production of the animals, many farmers in NI do treat for tapeworm and often with ineffective products. This is of concern, in that it could lead to the inadvertent development of anthelmintic resistance in nematode and trematode parasites. © 2016 Elsevier B.V.

Merlin, A., et al. (2017). "Mid-season targeted selective anthelmintic treatment based on flexible weight gain threshold for nematode infection control in dairy calves." *Animal*: 1-11.

The suitability of a single mid-season targeted selective treatment (TST) for gastrointestinal nematodes control, based on flexible average daily weight gain (ADWG) thresholds, was investigated in 23 groups of first grazing season calves. In each group, animals were weighed three times: before turnout, at mid-season and at housing. Just after the first weighing, each group was divided in two homogenous sub-groups in terms of age, breed and weight, and randomly allocated to one of two sub-groups intended for two different mid-season anthelmintic treatment strategies: (1) a treatment of all calves composing the sub-group (whole-group treatment (WT)) or (2) a targeted selective weight gain-based treatment (TST) of the animals showing an individual pre-treatment ADWG inferior to the mean pre-treatment ADWG of the corresponding WT sub-group. Anthelmintic treatment (levamisole 7.5 mg/kg BW) was performed 3 to 4 months after turnout. At housing, two parasitological parameters (the anti-Ostertagia ostertagi antibody level-Ostertagia optical density ratio (ODR) and the pepsinogen level) and a clinical parameter (the breech soiling score) were assessed at individual level in each group. Then, the high exposed groups to gastrointestinal nematode (GIN) were defined as groups for which untreated animals exhibited a mean Ostertagia ODR  $\geq 0.7$  and among these groups, the ones characterized by high abomasal damage due to Ostertagia for which untreated animals exhibited a mean pepsinogen level  $\geq 2.5$  U Tyr were also identified. Among TST sub-groups, the treatment ADWG thresholds varied from 338 to 941 g/day and the percentage of treated animals from 28% to 75%. Pre- and post-treatment ADWG as well as parasitological and clinical parameters measured at housing were similar between TST and WT sub-groups including the 17 high exposed groups to GIN. Within these 17 groups, the treatment allowed to significantly improve post-treatment ADWG compared with untreated animals. In the six high exposed groups showing mean pepsinogen level  $\geq 2.5$  U Tyr, the average effect of treatment on post-treatment ADWG was the highest and estimated up to 14 kg after a grazing duration of 4 months. In contrast, in six other groups showing mean Ostertagia ODR  $< 0.7$  in untreated animals, no effect of treatment was seen suggesting an absence of production losses related to a low level of GIN infection. This study highlighted the suitability of a convenient mid-season TST strategy for first grazing season calves, based on the use of flexible thresholds of ADWG, allowing similar growth compared with a whole-group treatment while keeping a GIN population in refugia. © The Animal Consortium 2017

Mullens, B. A., et al. (2017). "Comparative in vitro evaluation of contact activity of fluralaner, spinosad, phoxim, propoxur, permethrin and deltamethrin against the northern fowl mite, *Ornithonyssus sylviarum*." Parasites and Vectors **10**(1).

Background: Northern fowl mites (*Ornithonyssus sylviarum*) are obligate hematophagous ectoparasites of both feral birds and poultry, particularly chicken layers and breeders. They complete their entire life-cycle on infested birds while feeding on blood. Infestations of *O. sylviarum* are difficult to control and resistance to some chemical classes of acaricides is a growing concern. The contact susceptibility of *O. sylviarum* to a new active ingredient, fluralaner, was evaluated, as well as other compounds representative of the main chemical classes commonly used to control poultry mite infestations in Europe and the USA. Methods: Six acaricides (fluralaner,

spinosad, phoxim, propoxur, permethrin, deltamethrin) were dissolved and serially diluted in butanol:olive oil (1:1) to obtain test solutions used for impregnation of filter paper packets. A carrier-only control was included. Thirty adult northern fowl mites, freshly collected from untreated host chickens, were inserted into each packet for continuous compound exposure. Mite mortality was assessed after incubation of the test packets for 48 h at 75% relative humidity and a temperature of 22 °C. Results: Adult mite LC50 /LC99 values were 2.95/8.09 ppm for fluralaner, 1587/3123 ppm for spinosad, 420/750 ppm for phoxim and 86/181 ppm for propoxur. Permethrin and deltamethrin LC values could not be calculated due to lack of mortality observed even at 1000 ppm. Conclusions: Northern fowl mites were highly sensitive to fluralaner after contact exposure. They were moderately sensitive to phoxim and propoxur, and less sensitive to spinosad. Furthermore, the tested mite population appeared to be resistant to the pyrethroids, permethrin and deltamethrin, despite not being exposed to acaricides for at least 10 years. © 2017 The Author(s).

Murillo, A. C. and B. A. Mullens (2017). "A review of the biology, ecology, and control of the northern fowl mite, *Ornithonyssus sylviarum* (Acari: Macronyssidae)." Veterinary Parasitology **246**: 30-37.

The northern fowl mite, *Ornithonyssus sylviarum* (Canestrini & Fanzago, 1877), is found on several continents and has been a major pest of poultry in the United States for nearly a century. Lack of earlier USA reports in the United States suggests an introduction or change to pest status in domestic poultry systems occurred in the early 1900s. Though predominantly a nest-parasite of wild birds, this obligate hematophagous mite is a permanent ectoparasite on domestic birds, especially egg-laying chickens. Economic damage is incurred by direct blood feeding and activation of the of host's immune responses. This in turn causes decreased egg production and feed conversion efficiency, and severe infestations can cause anemia or death to birds. Here we review the biology, ecology, and recent control measures for the northern fowl mite. Photomicrographs are included of adult males and females, protonymphs, and larvae with key characters indicated. Special emphasis is placed on current knowledge gaps of basic and applied science importance. © 2017 Elsevier B.V.

Nagy, G., et al. (2017). "Benzimidazole resistance within red deer, roe deer and sheep populations within a joint habitat in Hungary." Small Ruminant Research **149**: 172-175.

The anthelmintic resistance of gastrointestinal nematodes is one of the most important, economic risk factors in grazing ruminant systems, all over the world. We have infinitesimal information about the resistance status of nematodes in deer species. Our aim was to determine the presence of BZ resistance in the generalist worm, *Haemonchus contortus* in pastured sheep and free ranging red and roe deer by RFLP-PCR method based on the detection Phe200Tyr single nucleotide polymorphism. By investigation of 70 worms from each host species, the homozygous susceptible genotype was the most representative in the red deer (100%), the homozygous resistant genotype was most prevalent in the sheep (68.6%) and

moderate in the roe deer (17.1%), while the heterozygous genotype was observed in equal proportion in the sheep and roe deer (28.6%). Our results suggest that overlapping habitats of sheep flocks and roe deer could contribute to the occurrence and spread of resistant allele within wildlife. © 2017 Elsevier B.V.

Otero-Abad, B., et al. (2017). "Latent class models for *Echinococcus multilocularis* diagnosis in foxes in Switzerland in the absence of a gold standard." Parasites and Vectors **10**(1).

Background: In Europe the principal definitive host for *Echinococcus multilocularis*, causing alveolar echinococcosis in humans, is the red fox (*Vulpes vulpes*). Obtaining reliable estimates of the prevalence of *E. multilocularis* and relevant risk factors for infection in foxes can be difficult if diagnostic tests with unknown test accuracies are used. Latent-class analysis can be used to obtain estimates of diagnostic test sensitivities and specificities in the absence of a perfect gold standard. Samples from 300 foxes in Switzerland were assessed by four different diagnostic tests including necropsy followed by sedimentation and counting technique (SCT), an egg-PCR, a monoclonal and a polyclonal copro-antigen ELISA. Information on sex, age and presence of other cestode species was assessed as potential covariates in the Bayesian latent class models. Different Bayesian latent-class models were run, considering dichotomized test results and, additionally, continuous readings resulting in empirical ROC curves. Results: The model without covariates estimated a true parasite prevalence of 59.5% (95% CI: 43.1-66.4%). SCT, assuming a specificity of 100%, performed best among the four tests with a sensitivity of 88.5% (95% CI: 82.7-93.4%). The egg-PCR showed a specificity of 93.4% (95% CI: 87.3-99.1%), although its sensitivity of 54.8% was found moderately low (95% CI: 48.5-61.0%). Relatively higher sensitivity (63.2%, 95% CI: 55.3-70.8%) and specificity (70.0%, 95% CI: 60.1-79.4%) were estimated for the monoclonal ELISA compared to the polyclonal ELISA with a sensitivity and specificity of 56.0% (95% CI: 48.0-63.9%) and 65.9% (95% CI: 55.8-75.6%), respectively. In the Bayesian models, adult foxes were found to be less likely infected than juveniles. Foxes with a concomitant cestode infection had double the odds of an *E. multilocularis* infection. ROC curves following a Bayesian approach enabled the empirical determination of the best cut-off point. While varying the cut-offs of both ELISAs, sensitivity and specificity of the egg-PCR and SCT remained constant in the Bayesian latent class models. Conclusions: Adoption of a Bayesian latent class approach helps to overcome the absence of a perfectly accurate diagnostic test and gives a more reliable indication of the test performance and the impact of covariates on the prevalence adjusted for diagnostic uncertainty. © 2017 The Author(s).

Preston, S., et al. (2017). "Screening of the 'Open Scaffolds' collection from Compounds Australia identifies a new chemical entity with anthelmintic activities against different developmental stages of the barber's pole worm and other parasitic nematodes." International Journal for Parasitology: Drugs and Drug Resistance **7**(3): 286-294.

The discovery and development of novel anthelmintic classes is essential to sustain the control of socioeconomically important parasitic worms of humans and animals.

With the aim of offering novel, lead-like scaffolds for drug discovery, Compounds Australia released the 'Open Scaffolds' collection containing 33,999 compounds, with extensive information available on the physicochemical properties of these chemicals. In the present study, we screened 14,464 prioritised compounds from the 'Open Scaffolds' collection against the exsheathed third-stage larvae (xL3s) of *Haemonchus contortus* using recently developed whole-organism screening assays. We identified a hit compound, called SN00797439, which was shown to reproducibly reduce xL3 motility by  $\geq 70\%$ ; this compound induced a characteristic, "coiled" xL3 phenotype ( $IC_{50} = 3.46\text{--}5.93 \mu\text{M}$ ), inhibited motility of fourth-stage larvae (L4s;  $IC_{50} = 0.31\text{--}12.5 \mu\text{M}$ ) and caused considerable cuticular damage to L4s in vitro. When tested on other parasitic nematodes in vitro, SN00797439 was shown to inhibit ( $IC_{50} = 3\text{--}50 \mu\text{M}$ ) adults of *Ancylostoma ceylanicum* (hookworm) and first-stage larvae of *Trichuris muris* (whipworm) and eventually kill ( $>90\%$ ) these stages. Furthermore, this compound completely inhibited the motility of female and male adults of *Brugia malayi* ( $50\text{--}100 \mu\text{M}$ ) as well as microfilariae of both *B. malayi* and *Dirofilaria immitis* (heartworm). Overall, these results show that SN00797439 acts against genetically (evolutionarily) distant parasitic nematodes i.e. *H. contortus* and *A. ceylanicum* [strongyloids] vs. *B. malayi* and *D. immitis* [filaroids] vs. *T. muris* [enoplid], and, thus, might offer a novel, lead-like scaffold for the development of a relatively broad-spectrum anthelmintic. Our future work will focus on assessing the activity of SN00797439 against other pathogens that cause neglected tropical diseases, optimising analogs with improved biological activities and characterising their targets. © 2017 The Authors

Roeber, F., et al. (2017). "Multiplexed-tandem PCR for the specific diagnosis of gastrointestinal nematode infections in sheep: an European validation study." *Parasites and Vectors* **10**(1).

Background: Traditional methods of detecting and identifying gastrointestinal nematode infections in small ruminants, including sheep and goats, are time-consuming and lack in sensitivity and specificity. Recently, we developed an automated multiplexed-tandem (MT)-PCR platform for the diagnosis and identification patent infections with key genera/species of gastrointestinal nematodes of sheep and validated this approach in detailed experiments carried out in Australia. In the present study, we deployed this diagnostic platform in Scotland and Belgium to test samples from naturally infected sheep in these environments and to validate the MT-PCR platform relative to traditional diagnostic methods routinely used by local laboratories. Results: MT-PCR detected all microscopy positive samples and there was a significant agreement between the results of the different test methods in terms of the species detected and their relative proportion in a test sample, however, for some samples, there were discrepancies between the results of the different test methods. Selective sequencing of purified MT-PCR products demonstrated the results to be 100% specific. Conclusions: The MT-PCR platform is an advanced method for the species/genus-specific diagnosis of gastrointestinal nematode infections in small ruminants and has demonstrated utility when deployed in different countries and climatic zones. The platform is user-friendly due to the largely automated procedure and has high versatility in that it can achieve a specific diagnosis from different types

of sample templates, including larval culture and faecal samples. With appropriate modifications of the primers used, the MT-PCR platform also provides potential for the diagnosis of a variety of other pathogens of veterinary or medical importance. © 2017 The Author(s).

Rufener, L., et al. (2017). "The novel isoxazoline ectoparasiticide lotilaner (Credelio™): A non-competitive antagonist specific to invertebrates  $\gamma$ -aminobutyric acid-gated chloride channels (GABACIs)." Parasites and Vectors **10**(1).

Background: The isoxazolines are a novel class of parasiticides that are potent inhibitors of  $\gamma$ -aminobutyric acid (GABA)-gated chloride channels (GABACIs) and, to a lesser extent, of inhibitory glutamate-gated chloride channels (GluCl<sub>s</sub>). Lotilaner (Credelio™), a novel representative of this chemical class, is currently evaluated for its excellent ectoparasiticide properties. Methods: In this study, we investigated the molecular mode of action and pharmacology of lotilaner. We report the successful gene identification, cDNA cloning and functional expression in *Xenopus* oocytes of *Drosophila melanogaster* (wild type and dieldrin/fipronil-resistant forms), *Lepeophtheirus salmonis* (an ectoparasite copepod crustacean of salmon), *Rhipicephalus microplus* and *Canis lupus familiaris* GABACIs. Automated *Xenopus* oocyte two-electrode voltage clamp electrophysiology was used to assess GABACIs functionality and to compare ion channel inhibition by lotilaner with that of established insecticides addressing GABACIs as targets. Results: In these assays, we demonstrated that lotilaner is a potent non-competitive antagonist of insects (fly) GABACIs. No cross-resistance with dieldrin or fipronil resistance mutations was detected, suggesting that lotilaner might bind to a site at least partly different from the one bound by known GABACI blockers. Using co-application experiments, we observed that lotilaner antagonism differs significantly from the classical open channel blocker fipronil. We finally confirmed for the first time that isoxazoline compounds are not only powerful antagonists of GABACIs of acari (ticks) but also of crustaceans (sea lice), while no activity on a dog GABAA receptor was observed up to a concentration of 10  $\mu$ M. Conclusions: Together, these results demonstrate that lotilaner is a non-competitive antagonist specific to invertebrate's  $\gamma$ -aminobutyric acid-gated chloride channels (GABACIs). They contribute to our understanding of the mode of action of this new ectoparasiticide compound. © 2017 The Author(s).

Santos, J. M. L. D., et al. (2017). "Haemonchus contortus  $\beta$ -tubulin isotype 1 gene F200Y and F167Y SNPs are both selected by ivermectin and oxfendazole treatments with differing impacts on anthelmintic resistance." Veterinary Parasitology **248**: 90-95.

Parasitism by *Haemonchus contortus* is one of the main limiting factors in small ruminant production in tropical areas. Benzimidazoles (BZ) and macrocyclic lactones (ML) are the most used anthelmintic classes in gastrointestinal nematodes control. There is considerable scientific evidence of a possible relation between the anthelmintic resistance to BZ and ML. This study aimed to characterize the dynamics of anthelmintic resistance in an *H. contortus* susceptible isolate under selection pressure for BZ and ML alone or in combination and the role of isotype 1  $\beta$ -tubulin

gene SNPs in these situations. A total of 12 Somali sheep were infected with 5000 third stage larvae of *H. contortus* Inbred-Susceptible Edinburgh (ISE) isolate. Once infection was established, animals were distributed in three groups (n = 4), each treated with crescent doses of oxfendazole (OXF), ivermectin (IVM) and oxfendazole plus ivermectin (IVMOXF). An additional control group with untreated animals was maintained during the entire experiment. After each treatment, eggs were collected and real-time PCR was performed to identify single nucleotide polymorphisms (SNPs) F167Y, F200Y and E198A, in addition to egg hatch test (EHT) for BZ and larval development test (LDT) for ivermectin resistance. All treatments led to increased resistance allelic frequencies at SNPs F200Y and F167Y ( $p < 0.05$ ). In vitro results showed increased phenotypic resistance against both anthelmintic classes in groups IVM and IVMOXF while group OXF only developed resistance against BZ. Finally, we provide evidence that while isotype 1  $\beta$ -tubulin gene SNPs may have some involvement with ML resistance, the presence of these  $\beta$ -tubulin SNPs alone are not sufficient to develop ML resistance. © 2017 Elsevier B.V.

Schorderet-Weber, S., et al. (2017). "Blocking transmission of vector-borne diseases." International Journal for Parasitology: Drugs and Drug Resistance **7**(1): 90-109.

Vector-borne diseases are responsible for significant health problems in humans, as well as in companion and farm animals. Killing the vectors with ectoparasitic drugs before they have the opportunity to pass on their pathogens could be the ideal way to prevent vector borne diseases. Blocking of transmission might work when transmission is delayed during blood meal, as often happens in ticks. The recently described systemic isoxazolines have been shown to successfully prevent disease transmission under conditions of delayed pathogen transfer. However, if the pathogen is transmitted immediately at bite as it is the case with most insects, blocking transmission becomes only possible if ectoparasitocides prevent the vector from landing on or, at least, from biting the host. Chemical entities exhibiting repellent activity in addition to fast killing, like pyrethroids, could prevent pathogen transmission even in cases of immediate transfer. Successful blocking depends on effective action in the context of the extremely diverse life-cycles of vectors and vector-borne pathogens of medical and veterinary importance which are summarized in this review. This complexity leads to important parameters to consider for ectoparasiticide research and when considering the ideal drug profile for preventing disease transmission. © 2017 The Authors

Sigognault Flochlay, A., et al. (2017). "Poultry red mite (*Dermanyssus gallinae*) infestation: A broad impact parasitological disease that still remains a significant challenge for the egg-laying industry in Europe." Parasites and Vectors **10**(1).

The poultry red mite, *Dermanyssus gallinae*, has been described for decades as a threat to the egg production industry, posing serious animal health and welfare concerns, adversely affecting productivity, and impacting public health. Research activities dedicated to controlling this parasite have increased significantly. Their veterinary and human medical impact, more particularly their role as a disease vector,

is better understood. Nevertheless, red mite infestation remains a serious concern, particularly in Europe, where the prevalence of red mites is expected to increase, as a result of recent hen husbandry legislation changes, increased acaricide resistance, climate warming, and the lack of a sustainable approach to control infestations. The main objective of the current work was to review the factors contributing to this growing threat and to discuss their recent development in Europe. We conclude that effective and sustainable treatment approach to control poultry red mite infestation is urgently required, included integrated pest management. © 2017 The Author(s).

Storey, B. E., et al. (2017). "Validation of the FAMACHA© system in South American camelids." Veterinary Parasitology **243**: 85-91.

*Haemonchus contortus* resistant to multiple anthelmintics threaten the viability of the small ruminant industry in areas where this parasite is prevalent. In response to this situation, the FAMACHA© system was developed and validated for use with small ruminants as a way to detect clinical anemia associated with haemonchosis. Given that *H. contortus* and multiple anthelmintic resistance is a similar problem in camelids, the FAMACHA© system might also provide the same benefits. To address this need, a validation study of the FAMACHA© system was conducted on 21 alpaca and llama farms over a 2-year period. *H. contortus* was the predominant nematode parasite on 17 of the 21 farms (10 alpaca and 7 llama farms) enrolled in the study, based on fecal culture results. The FAMACHA© card was used to score the color of the lower palpebral (lower eye lid) conjunctiva on a 1–5 scale. Packed cell volume (PCV) values were measured and compared to FAMACHA© scores using FAMACHA© score cutoffs of  $\geq 3$  or  $\geq 4$  and with anemia defined as a PCV  $\leq 15\%$ ,  $\leq 17\%$ , or  $\leq 20\%$ . PCV was significantly associated with FAMACHA© score, fecal egg count (FEC), and body condition score (BCS), regardless of the FAMACHA© cutoff score or the PCV% chosen to define clinical anemia ( $p < 0.01$  in all cases). The use of FAMACHA© scores  $\geq 3$  and PCV  $\geq 15\%$  indicating anemia provided the best sensitivity (96.4% vs 92.9% for FAMACHA©  $\geq 4$ ), whereas FAMACHA scores  $\geq 4$  and PCV  $\leq 20\%$  provided the best specificity (94.2% vs 69.1% for FAMACHA©  $\geq 3$ ). The data from this study support the FAMACHA© system as a useful tool for detecting clinical anemia in camelids suffering from haemonchosis. Parameters for making treatment decisions based on FAMACHA© score in camelids should mirror those established for small ruminants. © 2017 Elsevier B.V.

van der Voort, M., et al. (2017). "Economic modelling of grazing management against gastrointestinal nematodes in dairy cattle." Veterinary Parasitology **236**: 68-75.

Grazing management (GM) interventions, such as reducing the grazing time or mowing pasture before grazing, have been proposed to limit the exposure to gastrointestinal (GI) nematode infections in grazed livestock. However, the farm-level economic effects of these interventions have not yet been assessed so far. In this paper, the economic effects of three GM interventions in adult dairy cattle were modelled for a set of Flemish farms for which data were available: later turnout on pasture (GM1), earlier housing near the end of the grazing season (GM2), and

reducing the daily grazing time (GM3). Farm accountancy data were linked to *Ostertagia ostertagi* bulk tank milk ELISA results and GM data for 137 farms. The economic effects of the GM interventions were investigated through a combination of efficiency analysis and a whole-farm simulation model. Modelling of GM1, GM2 and GM3 resulted in a marginal economic effect [5th; 95th percentiles] of € 8.36 [-222; 88], € -9.05 [-143; 38] and € -53.37 [-301; 87] per cow per year, respectively. The results suggest that the dairy farms modelled can improve their economic performance by postponing the turnout date, but that advancing the housing date or reducing daily grazing time mostly leads to a lower net economic farm performance. Overall, the GM interventions resulted in a higher technical efficiency and milk production but these benefits were offset by increased feed costs as a result of higher maintenance and cultivation costs. However, results highly differ between farms, indicating the need to evaluate GM interventions at the individual farm level for appropriate decision support. © 2017 Elsevier B.V.

Yang, X., et al. (2017). "Development and evaluation of a loop-mediated isothermal amplification (Lamp) assay for the detection of *haemonchus contortus* in goat fecal samples." Journal of Parasitology **103**(2): 161-167.

*Haemonchus contortus* is one of the most significant strongylid nematodes infecting small ruminants, and it causes great economic losses to the livestock industry worldwide. Accurate diagnosis of *H. contortus* is crucial to control strategies. Traditional microscopic examinations are the most common methods for the diagnosis of *H. contortus*, but they are time-consuming and inaccurate. Molecular methods based on PCR are more accurate, but need expensive machines usually only used in the laboratory. Loop-mediated isothermal amplification (LAMP) is a rapid, simple, specific, and sensitive method that has been widely used to detect viruses, bacteria, and parasites. In the present study, a LAMP method targeting ribosomal ITS-2 gene for detection of the *H. contortus* in goat fecal samples has been established. The established LAMP method was *H. contortus* specific, and the sensitivity of LAMP was the same as that of the *H. contortus* species-specific PCR, with the lowest DNA level detected as being 1 pg. Examination of the clinical samples indicated that the positive rate of LAMP was higher than that of PCR, but no statistical difference was observed between LAMP and PCR ( $\chi^2 = 17.991$ ,  $P = 0.053$ ). In conclusion, a LAMP assay with a high specificity and a good sensitivity has been developed to detect *H. contortus* infection in goats. The established LAMP assay is useful for clinical diagnosis of *H. contortus*. © American Society of Parasitologists 2017.