



Animal &  
Plant Health  
Agency

# Parasitology group: Gastro- intestinal parasites and Cestodes

## Annual review of the literature and horizon scanning report 2019

September 2020



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Year of publication: 2020

[www.gov.uk/defra](http://www.gov.uk/defra)

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## Expert review

This document containing abstracts collected by using the following search terms (cestodes) OR "haemonchus contortus" OR "ostertagia ostertagi" OR "teladorsagia circumcincta" OR trichostrongylus OR cooperia OR "dictyocaulus viviparus" OR ectoparasites OR "psoroptes sp" OR "fasciola hepatica" OR "rumen fluke" OR (paramphistome) OR calicophoron OR dicrocoelium AND (control) OR "alternative Forage\*" OR resistance OR diagnosis OR "anthelmintic resistance" OR vaccine\* OR (anthelmintic\*) and published in 2019.

These were then viewed and collated as those of particular interest to APHA and scanning surveillance.

## Gastro-intestinal (GI) Nematodes

- Sheep-Monepantel resistance identified in UK, more work to detect molecular basis of resistance is being undertaken, focusing on mutations in Tci-mptl-1 gene.
- Cattle parasites- ivermectin treatment failure on four cattle farms in Ireland, one farm *O. ostertagi* survived treatment.
- First detection of ivermectin resistance in *Oesophagostomum dentatum* in pigs in the UK.
- Ongoing research into the way ivermectin acts on parasites and molecular markers of resistance, as well as ways to overcome ivermectin resistance in nematodes.
- Levamisole resistance in *H. contortus* associated with a 63bp deletion in the Hco-acr-8b gene that encodes a subunit for a nicotinic acetylcholine receptor.
- Potential diagnostic test developments, amplicon sequencing for the analysis of benzimidazole resistance allele frequencies in field populations of gastrointestinal nematodes published. Could be extended if more molecular markers of resistance become known. Also novel droplet digital PCR approach, both quite expensive for routine use currently. Other methods being looked at e.g. ELISAs , AMLDiTOF
- Recovery of fenbendazole efficacy on resistant *H. contortus* by management of parasite refugia and population replacement (infecting lambs with susceptible population worms).
- Lots of papers on the effect of condensed tannins from various plants, on GI nematodes either *in vitro* or *in vivo*.
- Also work on zinc oxide nanoparticles, copper capsules, various plants, essential oils, fungal proteins and repurposing drugs as well as looking for new anthelmintic chemicals compounds.
- Effects on environmental stages of nematodes with the golden death bacillus *Chryseobacterium nematophagum* and also more work on *Duddingtonia flagrans* (already commercially available in some countries) as an in feed product.
- Continued work on identifying animals resistant to GI nematodes.
- Continued work on *Teladorsagia circumcincta* vaccine development.

## Cestodes

- One of the most important is the development of a multiplex PCR for the simultaneous detection of 4 important *Taenia* species that seriously impact livestock health (*Taenia hydatigena*, *T. multiceps*, *T. pisiformis* and *Dipylidium caninum*) and infect dogs and other canids. This could potentially aid epidemiological studies and prevention and control strategies.
- The molecular detection of *Taenia crassiceps*, a known parasite of foxes, in a captive animal (Cape fur seal) emphasized its zoonotic potential and the regular need of the parasitological testing of pet dogs.
- An important publication molecularly did not differentiate between *Taenia multiceps*, the cause of cerebral *Coenurus cerebralis* in herbivores and *Taenia gaigeri*, a species originally described on morphological grounds.
- A recent publication describes the development of a rapid coenurosis diagnostic method. Based on an ELISA assay it was shown to have 96% sensitivity and 88% specificity using antigen B (AgB) from *T. multiceps* and thus may have good serodiagnostic potential for the infection.
- A study on bovine cysticercosis caused by *Cysticercus bovis* from Iran investigated the use of serology and meat inspection for the detection of this parasite.
- There were several reports on prevalence and importance of *Taenia solium* porcine infection in Africa and its comparison with *Taenia hydatigena* which was found to be as prevalent as the former and the cause of huge economic losses.
- Publications on cystic and alveolar echinococcosis also featured widely in the last year. A study on bovine cystic echinococcosis in northern Italy underlined the importance of surveillance systems that include testing dogs of transhumant flocks thought to be the source of infection in cattle.
- A PCR-RFLP for the differentiation of *Echinococcus granulosus sensu stricto* and *Taenia* species coproDNA has also been published. This method was tested using experimentally and naturally infected faecal samples. The assay is said to be sensitive enough to detect a single egg from patent infections. Difficulties normally associated with dealing with coproDNA and enzyme restriction digestion were not discussed.
- The transmission dynamics and control measures of *Echinococcus multilocularis* in foxes were reassessed within a mathematical framework using a novel approach called petrinet (PN) potentially useful in analysing and controlling parasite dynamics.
- Since 2012 *Echinococcus multilocularis* has been described in southern Ontario, Canada from an area previously thought to be free of this parasite. The use of spatial scan test, allowed the discovery of an infection cluster in densely populated human areas, suggesting zoonotic transmission.
- A systematic review of surveillance methods for the detection of *Echinococcus multilocularis* in humans and animals emphasized the need for International consensus on gold standard methods and harmonization of surveillance data to allow the assessment of the global burden and distribution of this parasite within a One Health Approach.
- Literature reviews on Echinococcus included the Food and Waterborne Parasitology Special issue which compiled aspects of molecular markers of disease

emergence, diagnostics, treatment of *Echinococcus multilocularis* and control strategies of the definitive hosts.

- Another excellent review discussed the advances made in detection and control of Echinococcosis in the 21st century including those in genomics, epidemiology, diagnosis, treatment, control, surgical procedures and vaccine development. There currently is no alternative drug to albendazole for treatment of echinococcosis. However, it is hoped that advances gained in genomic and proteomic information will improve both diagnosis and treatment in the future.

## Ectoparasites

- Research into red mite continues, looking into treatment options and potential vaccination.

## *Fasciola hepatica* (Liver fluke) and paramphistomes (rumen fluke)

### Treatments, resistance and vaccinations

- Due to the emergence of resistance in several countries, new chemical entities with fasciolicidal activity are urgently required. Work included screening multiple plant extracts and the pathogen box for effective, nontoxic products. Products found to have activity against *Paramphistomun cervi* included Chinese water chestnut, fruit peel and honey.
- Novel methods of drug administration such as via feedblocks to cattle on tropical small holder herds were found to have potential and resulted in increased live weight gain.
- Methods of control by controlling infection of the intermediate snail hosts were investigated using nematophagus fungi to reduce snail egg survival time, and monosaccharides to disrupt miracidial invasion of the snail.
- Resistance to triclabendazole (TBZ) was confirmed on multiple holdings across England and Wales using faecal egg count reduction techniques. Resistance was also confirmed in Chile. Work was carried out in India and China looking at effectiveness of treatments, using FECRT, haematology and biochemistry found no evidence of resistance to the products used. It is important that monitoring effectiveness of treatments is encouraged worldwide.
- Precision cut liver sections were found to have potential for studies on the metabolism of TBZ and fenbendazole in cattle with other work looking at egg hatch assay and albendazole resistance.
- There was limited progress on vaccine development with studies working on identifying glycoproteins which are critical for host-parasite interaction as potential vaccine candidates. There was some positive work using chimeric proteins and

development of improved adjuvants, as well as work on understanding the development of immunity following natural challenge.

## Improving diagnostic techniques and applications

- Additional work on preparation of *Fasciola* and paramphistome antigens and secretory/excretory products, such as two dimensional electrophoresis may have potential in development of improved diagnostic techniques.
- Phage display technology was used to produce recombinant monoclonal antibodies to provide understanding of the biology of the parasite and disease progression at a molecular level. This could lead to development of new drug targets, diagnostic antigens or vaccine candidates.
- *Fasciola gigantica* glutathione peroxidases are abundantly expressed by all stages of the parasite and an indirect ELISA developed could be used to identify all stages of the parasite in humans and animals. There was no cross reactivity with other adult trematodes.
- Work on gene expression identified and validated reference genes to provide tools for monitoring cytokines in *Fasciola hepatica* infected sheep which should result in improved diagnostics.
- Work in cattle in Ireland identified variation in the performance of the commercially available ELISA tests.
- Analysis of monitoring protocols in Australia suggested use of coproantigen and FEC in parallel resulted in improved sensitivity. Validation of the composite faecal egg count for cattle adds robustness to this technique for monitoring large herds.
- Histopathology was used to develop a grading system for liver damage to include prognostic indicators and work with magnet particles appeared to be useful in harvesting fluke eggs for research using magnetic materials.
- Using spiked faecal samples LAMP technology was found to be able to identify a single egg in a faeces sample enabling detection of low burdens with similar technology used to detect micro RNA in the blood of infected cattle.

## On farm impact and farmer attitudes

- A survey of six European countries with regard to fluke and worm control on organic cattle farms identified variation between countries and suggested that any management strategies must be country specific and disseminated appropriately.
- A survey on Irish farms via a farmer survey and faecal analysis revealed a prevalence of liver fluke in sheep of 50.4%, with an association with the presence of cattle on the farm. Co infection with rumen fluke was observed on 41% of the studied population and correlated with increased *F. hepatica* egg counts.
- Concerns about the possibility of co- infection with *F. hepatica* and disclosure of bovine TB via skin testing and reactor PME were negated.
- A review of the survival of metacercariae in silage and preserved forages suggested additional work was required.

- Work looking at the impact of the presence of liver fluke and carcass quality found that *F. hepatica* was associated with poorer carcass quality and lower weights with increased significance in the younger animals in Uruguay.
- Dairy farms in Germany found increased calving to first service intervals with no milk production effects in grazed dairy herds when fluke was present.
- Additional work into inherent genetic resistance and gene expression in cattle, identified a number of novel genomic regions for further study.
- Investigation into spatial heterogeneity, habitat and host factors with regard to liver fluke and red deer were carried out in Scotland, and confirmed uniformity in a wide ranging population.

## Zoonoses

- Infection of people with *F. hepatica* can result in significant health issues. Mass drug administration was introduced by the Bolivian government in high prevalence regions from 2008-2016 to persons willing to participate. This resulted in dramatic reduction in prevalence.

## Rumen fluke

- Using deep sequencing on rumen fluke identified in finished cattle in the UK it was noted that multiple introductions onto farms, probably due to cattle movements resulted in genetic diversity on holdings.

# ABSTRACTS

## Gastro-intestinal nematodes in ruminants

**Author:** Hamer, K., McIntyre, J., Morrison, A. A., Jennings, A., Kelly, R. F., Leeson, S., Bartley, D. J., Chaudhry, U., Busin, V. and Sargison, N.

**Title:** The dynamics of ovine gastrointestinal nematode infections within ewe and lamb cohorts on three Scottish sheep farms

**DOI:** 10.1016/j.prevetmed.2019.104752

**Abstract:** Gastrointestinal nematodes (GIN) are a serious concern for sheep producers worldwide. However, there is a paucity of evidence describing the epidemiology of GIN on modern UK sheep farms. The aim of this paper was to understand whether expected seasonal variations of infection are still found in ewes and lambs under varying management strategies in temperate climates. Faecal egg counts (FEC) were conducted on freshly voided samples collected from groups of ewes and lambs every third week for twelve months on three farms in southeast Scotland. The patterns of egg output have



been described here in relation to management practices undertaken on the farms. Despite changes in farming practice and climatic conditions, the findings complement historical studies detailing the epidemiology of GIN. Findings include a periparturient rise in ewe FEC on two of the farms, while lambing time treatment appeared to suppress this on the third farm. On the same two farms lamb FEC increased during the summer, reaching a peak in the autumn. The work also highlights how the ad hoc use of anthelmintics does little to impact these patterns.

**Author:** Takeuchi-Storm, N., Moakes, S., Thüer, S., Grovermann, C., Verwer, C., Verkaik, J., Knubben-Schweizer, G., Höglund, J., Petkevičius, S., Thamsborg, S. and Werne, S.

**Title:** Parasite control in organic cattle farming: Management and farmers' perspectives from six European countries

**DOI:** 10.1016/j.vprsr.2019.100329

**Abstract:** Organic ruminant production is expanding in the EU, but parasite management remains a constant challenge. Mandatory outdoor access for all age groups can increase exposure to pasture borne parasites, whilst restrictions in the prophylactic use of anthelmintics can limit parasite control. The scientific community has been working to deliver effective parasite control strategies and alternative approaches in order to slow down the development of anthelmintic resistance (AR). However, the current parasite control practices and overall awareness with regards to AR and alternative approaches on farms are largely unknown and may be causing a knowledge gap between the scientific and farming communities. Therefore, a structured survey was conducted in six European countries (Switzerland, Germany, Denmark, Netherlands, Lithuania, Sweden) to provide basic data on practices, management and farmers' perspectives for grazing and parasite control (gastrointestinal worms and liver flukes) on organic cattle farms. Overall, 375 surveys were collected (282 dairy and 93 beef farms) in 2015–2016, and analysed descriptively. Additionally, surveys from the 228 dairy farms were assessed using a double-hurdle adoption model to identify the factors involved in the decision to drench against gastrointestinal parasites. Generally, there are prominent differences between countries, with monitoring methods differing especially, which has important implications in terms of knowledge transfer. For example, media warning was the most common method in DE, while antibody testing in bulk tank milk was the common method in NL. In other countries, clinical signs (diarrhoea, hair coat quality, and reduced weight or yield) and liver condemnation data were used frequently. In general, organic farmers from the six participating countries indicated that they would accept alternative approaches despite greater cost and labour. The likelihood of drenching were higher on farms with smaller farm areas, higher number of young stock and total livestock units and farms where faecal egg counts were used to monitor the parasites. In conclusion, it was evident that grazing and parasite management varied between the countries even though they operate under the same basic principles. Parasite management strategies must therefore be country specific and disseminated with appropriate methods.

**Author:** Waghorn, T. S., Bouchet, C. L. G., Bekelaar, K. and Leathwick, D. M.

**Title:** Nematode parasites in young cattle: what role for unexpected species?

**DOI:** 10.1080/00480169.2018.1532849

**Abstract:** AIMS: To investigate the timing of infection of beef calves with sheep nematode species on three sheep and beef farms, and to determine the prevalence of cross-infection in calves before weaning across a larger number of farms. METHODS: Farms in the Far North, Gisborne and Taranaki districts, in the North Island of New Zealand, were enrolled in 2014. Fresh faecal samples were collected from approximately 10 calves on each farm between birth and up to 5 months after weaning. In 2016, faecal samples were collected from calves before weaning from 22 farms across the upper North Island. For both trials faecal samples were assessed for faecal nematode egg counts and cultured to determine parasite genus. For samples from the three farms, larvae were identified to species using a multiplex PCR assay. RESULTS: On the three farms, the median percentage of sheep nematode species detected in faecal cultures was 25 (min 3, max 77)%. The main sheep species detected were *Cooperia curticei* and *Haemonchus* spp. (putatively *contortus*). In faecal samples collected before weaning from 22 farms, *Haemonchus* spp. were present in 19/22 samples, and the median prevalence was 15 (min 0, max 73)% of the total larvae cultured. CONCLUSIONS AND CLINICAL RELEVANCE: The implications of sheep nematode species being present in calves should be considered by farmers and veterinarians when undertaking anthelmintic efficacy testing, as they may contribute to false conclusions regarding anthelmintic efficacy. Pre-weaning calves may also be a possible source of contamination and/or refugia for *Haemonchus* spp. on farms and should be considered when developing parasite control plans for sheep.

**Author:** Moreno Mañas, E., González Juan, M., Ruiz De Ybáñez Carnero, M. D. R., Gilbert, T., Ortiz, J., Espeso, G., Benzal, J., Ibáñez, B. and Valera Hernández, F.

**Title:** Survey of husbandry practices for bovidae in zoos: The importance of parasite management for reintroduction programmes

**DOI:** 10.1136/vr.104985

**Abstract:** Animals from zoological institutions may be used for reintroductions. These individuals are considered healthy, but they are not necessarily free of parasites, despite the minimum husbandry standards required of zoological institutions as described in the European Association of Zoos and Aquaria guidelines. In this sense, parasitism has been identified as the cause of failure, or has added difficulties, in some reintroduction programmes. Here the authors attempt to summarise the risk of parasitism to animals originating from zoological institutions by analysing a questionnaire about parasite prevalence, sampling methods, treatment and control in three ungulates in European zoos. Completed questionnaires were received from 38 institutions (58.5 per cent response rate). Most of the responding institutions (97 per cent) detected the eggs of endoparasites in faeces, but only one reported ectoparasites. Most institutions followed a similar

preventive schedule, with ivermectin as the preferred prophylactic treatment for parasites, commonly administered in food every six months. The frequent use of concentrating flotation techniques as the sole method to evaluate the presence of parasite eggs in faecal samples is not recommended because it fails to detect trematode and lung nematode infections, so it would be better to use flotation techniques together with sedimentation procedures or serological and molecular tests. The results suggest that parasite control in zoological institutions can be complicated, indicating the need to implement a specific management schedule for institutions involved in reintroduction projects.

**Author:** Mardahl, M., Borup, A. and Nejsum, P.

**Title:** A new level of complexity in parasite-host interaction: The role of extracellular vesicles

**DOI:** 10.1016/bs.apar.2019.02.003

**Abstract:** Humans and animals have co-existed with parasites in a battle of constant adaptation to one another. It is becoming increasingly clear that extracellular vesicles (EVs) play important roles in this co-existence and pathology. This chapter reviews the current research on EVs released by protozoa, nematodes, trematodes, and cestodes with a special focus on EVs in parasite life cycles. The environmental changes experienced by the parasite during its life cycle is associated with distinct changes in EV release and content. The function of these EV seems to have a significant influence on parasite pathology and survival in the host by concomitantly modulating host immune responses and triggering parasite differentiation. The role of EVs in communication between the parasites and the host adds a new level of complexity in our understanding of parasite biology, which may be a key to further understand the complexity behind host-parasite interactions and communication. This increased understanding can, in turn, open up new avenues for vaccine, diagnostic, and therapeutic development for a wide variety of diseases such as parasite infection, cancers, and immunological disorders.

**Author:** Wu, Z., Wang, L., Li, J., Wang, L., Wu, Z. and Sun, X.

**Title:** Extracellular vesicle-mediated communication within host-parasite interactions

**DOI:** 10.3389/fimmu.2018.03066

**Abstract:** Extracellular vesicles (EVs) are small membrane-surrounded structures released by different kinds of cells (normal, diseased, and transformed cells) in vivo and in vitro that contain large amounts of important substances (such as lipids, proteins, metabolites, DNA, RNA, and non-coding RNA (ncRNA), including miRNA, lncRNA, tRNA, rRNA, snoRNA, and scaRNA) in an evolutionarily conserved manner. EVs, including exosomes, play a role in the transmission of information, and substances between cells that is increasingly being recognized as important. In some infectious diseases such as parasitic diseases, EVs have emerged as a ubiquitous mechanism for mediating communication during host-parasite interactions. EVs can enable multiple modes to

transfer virulence factors and effector molecules from parasites to hosts, thereby regulating host gene expression, and immune responses and, consequently, mediating the pathogenic process, which has made us rethink our understanding of the host-parasite interface. Thus, here, we review the present findings regarding EVs (especially exosomes) and recognize the role of EVs in host-parasite interactions. We hope that a better understanding of the mechanisms of parasite-derived EVs may provide new insights for further diagnostic biomarker, vaccine, and therapeutic development.

**Author:** Veríssimo, C. D. M., Graeff-Teixeira, C., Jones, M. K. and Morassutti, A. L.

**Title:** Glycans in the roles of parasitological diagnosis and host-parasite interplay

**DOI:** 10.1017/s0031182019000465

**Abstract:** The investigation of the glycan repertoire of several organisms has revealed a wide variation in terms of structures and abundance of glycan moieties. Among the parasites, it is possible to observe different sets of glycoconjugates across taxa and developmental stages within a species. The presence of distinct glycoconjugates throughout the life cycle of a parasite could relate to the ability of that organism to adapt and survive in different hosts and environments. Carbohydrates on the surface, and in excretory-secretory products of parasites, play essential roles in host-parasite interactions. Carbohydrate portions of complex molecules of parasites stimulate and modulate host immune responses, mainly through interactions with specific receptors on the surface of dendritic cells, leading to the generation of a pattern of response that may benefit parasite survival. Available data reviewed here also show the frequent aspect of parasite immunomodulation of mammalian responses through specific glycan interactions, which ultimately makes these molecules promising in the fields of diagnostics and vaccinology.

## Anthelmintic resistance

Deep amplicon sequencing as a powerful new tool to screen for sequence polymorphisms associated with anthelmintic resistance in parasitic nematode populations

Russell W. Avramenko, Elizabeth M. Redman, Lynsey Melville, Yvonne Bartley, Janneke Wit, Camila Queiroz, Dave J. Bartley, John S. Gilleard.

[International Journal for Parasitology](#) Volume 49, Issue 1, January 2019, Pages 13-26

Parasitic gastrointestinal nematodes contribute to significant human morbidity and cause billions of dollars per year in lost agricultural production. Control is dependent on the use of anthelmintic drugs which, in the case of livestock parasites, is severely compromised by the widespread development of drug resistance. There are now concerns regarding the emergence of anthelmintic resistance in parasitic nematodes of humans in response to the selection pressure resulting from mass drug administration programs. Consequently, there is an urgent need for sensitive, scalable and accurate diagnostic tools to detect the emergence of anthelmintic resistance. Detecting and measuring the frequency of

resistance-associated mutations in parasite populations has the potential to provide sensitive and quantitative assessment of resistance emergence from an early stage. Here, we describe the development and validation of deep amplicon sequencing as a powerful new approach to detect and quantify the frequency of single nucleotide polymorphisms associated with benzimidazole resistance. We have used parasite communities in sheep to undertake a proof-of-concept study of this approach. Sheep provide an excellent host system, as there are multiple co-infecting trichostrongylid nematode species, each likely with a varying prevalence of benzimidazole resistance. We demonstrate that the approach provides an accurate measure of resistance allele frequencies, and can reliably detect resistance alleles down to a frequency of 0.1%, making it particularly valuable for screening mutations in the early stages of resistance. We illustrate the power of the technique by screening UK sheep flocks for benzimidazole resistance-associated single nucleotide polymorphisms at three different codons of the  $\beta$ -tubulin gene in seven different parasite species from 164 populations (95 from ewes and 69 from lambs) in a single MiSeq sequencing run. This approach provides a powerful new tool to screen for the emergence of anthelmintic resistance mutations in parasitic nematode populations of both animals and humans.

**Author:** Lambertz, C., Pouloupoulou, I., Wuthijaree, K. and Gauly, M.

**Title:** Anthelmintic resistance in gastrointestinal nematodes in sheep raised under mountain farming conditions in Northern Italy

**DOI:** 10.1136/vetreco-2018-000332

**Abstract:** Anthelmintic resistance (AR) in sheep raised under mountain farming conditions in South Tyrol (Northern Italy) was assessed on eight farms (n=99 animals). A faecal egg count reduction (FECR) test was done after routine anthelmintic treatments. Furthermore, on 27 farms (n=306 animals), a FECR test was conducted after oral formulations of a macrocyclic lactone (ML), benzimidazole (BZ) (partly in combination with salicylanilide (SA)) or a combination of imidazothiazole and SA were applied under controlled conditions on the same farm. Following routine treatments, three of five ML-treated flocks showed an adequate efficacy, while the other two reached a FECR of only around 75 per cent. A wide range of gastrointestinal nematode genera were identified in one flock following the treatment. From the three BZ-treated flocks, only one showed an adequate FECR, both other farms reached 68 per cent and 84 per cent, respectively. Under controlled conditions, FECR ranged between 77 per cent and 81 per cent indicating AR for all the applied anthelmintics. *Trichostrongylus* species, *Teladorsagia* species and *Haemonchus* species were identified after ML treatment, *Teladorsagia* species after BZ treatment and *Trichostrongylus* species and *Haemonchus* species after combined BZ and SA application. Taking into consideration that underdosing might have affected results of the routine treatments, a high prevalence of AR was found in sheep under mountain farming conditions.

**Author:** Lambertz, C., Pouloupoulou, I., Wuthijaree, K. and Gauly, M.

**Title:** Anthelmintic efficacy against gastrointestinal nematodes in goats raised under mountain farming conditions in northern Italy

**DOI:** 10.1186/s12917-019-1968-8

**Abstract:** Background: This study aimed to evaluate the efficacy of anthelmintics in goats raised under mountain farming conditions in northern Italy. On 8 goat farms (n = 143 animals), a faecal egg count reduction (FECR) test was done after farmers conducted their routine anthelmintic treatments. Furthermore, on 5 goat farms (n = 135 animals) a FECR test was done under controlled conditions applying oral formulations of a macrocyclic lactone (ML), benzimidazole (BZ) (partly in combination with salicylanilide (SA)) or a combination of imidazothiazole (IT) and SA on the same farm. AR was assumed if FECR and the upper confidence interval (CI) was < 95% and the lower 95% CI was < 90%. Results: Underdosing was found in 6 of the 8 farms tested after routine treatments. Out of the 6 routinely ML-treated goat flocks, only three were found where ML showed adequate efficacy. FECR in all others ranged between 64 and 93%. In one flock *Trichostrongylus* spp. and in one *Haemonchus* spp. larvae were identified after treatment. BZ-treated flocks had an efficacy of 99 and 37%. Larvae identified after treatment were *Trichostrongylus* spp. in one and *Haemonchus* spp. in the other flock. Under controlled conditions, ML had an adequate efficacy on 4 farms and a FECR of 88% on another one. BZ was effective on all farms. The combination of BZ and SA had a FECR of 99% on the farm it was tested. IT + SA in combination was effective on 2 farms and had a FECR of 91% on a third farm. Larvae identified after treatment were composed of *Haemonchus* spp. (ML and BZ), *Trichostrongylus* spp. (BZ) and *Teladorsagia* spp. (BZ and SA). Conclusions: This first report on the prevalence of AR in goats in the mountainous region of South Tyrol reveals a low efficacy of the most commonly used anthelmintics after routine treatments. This might be explained by a high level of underdosing as observed in the farms. However, results from the controlled FECR tests suggest that the observed level of AR was lower but cannot be solely explained by underdosing.

**Author:** Preston, S., Piedrafita, D., Sandeman, M. and Cotton, S.

**Title:** The current status of anthelmintic resistance in a temperate region of Australia; implications for small ruminant farm management

**DOI:** 10.1016/j.vprsr.2019.100313

**Abstract:** Widespread anthelmintic resistance in small ruminants is a constraint on the profitability of the meat/wool industry. Limited published data is available on the prevalence and efficacy of anthelmintics, particularly in Australia where parasites affecting ruminant systems vary greatly between geographic regions. This paper reports on the anthelmintic resistance status in a temperate region of Victoria, Australia, a major sheep producing state largely affected by *Trichostrongylus* species and *Teladorsagia circumcincta*. The prevalence of anthelmintic resistance to any product was high (71%),

with farms reporting varying levels of drug efficacies (21–100%). Resistance to older chemical groups (i.e. fenbendazole and levamisole) and single active macrocyclic lactone treatments was higher than newer chemical groups and combination treatments. This report provides clarity on anthelmintic resistance in the temperate region of Victoria and more importantly suggests that more comprehensive, regional specific anthelmintic resistance studies are required to understand the real level of chemical resistance threatening the effective control of worms.

## **Monepantel resistance**

**Author:** Bartley, D. J., Hamer, K., Andrews, L., Sargison, N. D. and Morrison, A. A.

**Title:** Multigenic resistance to monepantel on a UK sheep farm

**DOI:** 10.1016/j.vpoa.2019.100003

**Abstract:** The amino acetonitrile derivative, monepantel, represented the first new broad spectrum anthelmintic to be brought to market for use in sheep for over 25 years when it was introduced in 2009. This study characterised monepantel efficacy, using faecal egg count reduction and controlled efficacy tests, against gastrointestinal nematodes following a report of treatment failure in a UK lowland sheep flock. Twelve lambs were each artificially administered 15,000 infective larvae that had been propagated from lamb faeces collected from the farm of interest. The controlled efficacy test showed that a recommended dose rate of monepantel (2.5 mg/kg body weight) administered at day 28 post infection was ineffective at removing the infection in the treated lambs. The result demonstrated simultaneous resistance to monepantel in *Teladorsagia circumcincta*, *Trichostrongylus vitrinus* and *Oesophagostomum venulosum* with efficacies based on adult worm burden reductions, compared to untreated controls, of 78%, 27% and 22% respectively. Monepantel efficacy based on undifferentiated egg count in treated animals, seven day post administration, compared to untreated controls was 85%. The results raise questions about the origins of, and predisposing factors for, resistance development in the three different species, and reinforces the value of differentiating post treatment faecal egg counts to genus or species level.

**Author:** Niciura, S. C. M., Tizioto, P. C., Moraes, C. V., Cruvinel, G. G., De Albuquerque, A. C. A., Santana, R. C. M., Chagas, A. C. D. S., Esteves, S. N., Benavides, M. V. and Do Amarante, A. F. T.

**Title:** Extreme-QTL mapping of monepantel resistance in *Haemonchus contortus*

**DOI:** 10.1186/s13071-019-3663-9

**Abstract:** Background: *Haemonchus contortus*, a gastrointestinal nematode parasite of sheep, is mainly controlled by anthelmintics; the occurrence of anthelmintic resistance leads to treatment failures and increases economic burden. Because molecular mechanisms involved in drug resistance can be elucidated by genomic studies, an

extreme quantitative trait locus (X-QTL) mapping approach was used to identify co-segregation of the resistance phenotype with genetic markers to detect the genome-wide variants associated with monepantel resistance in *H. contortus*. Methods: A cross between *H. contortus* isolates using parental susceptible (Par-S) males and monepantel resistant (Par-R) females resulted in SR progeny, while reciprocal cross resulted in RS progeny. Pools (n = 30,000) of infective larvae (L3) recovered from Par-R, and from SR and RS populations in the F3 generation, collected both before (unselected group) and 7 days after (selected group) selection with monepantel treatment in sheep hosts, were subjected to genome sequencing (Pool-Seq). Pairwise comparisons of allele frequencies between unselected and selected groups were performed for each population by Fisher's exact test (FET) and for both populations combined by a Cochran-Mantel-Haenszel (CMH) test. Results: Mapping rates varied from 80.29 to 81.77% at a 90.4X mean coverage of aligned reads. After correction for multiple testing, significant ( $P < 0.05$ ) changes in allele frequencies were detected by FET for 6 and 57 single nucleotide polymorphisms (SNPs) in the SR and RS populations, respectively, and by the CMH test for 124 SNPs in both populations. The significant variants located on chromosome 2 generated a selection signal in a genomic region harboring the *mptl-1*, *deg-3* and *des-2* genes, previously reported as candidates for monepantel resistance. In addition, three new variants were identified in the *mptl-1* gene. Conclusions: This study expands knowledge on genome-wide molecular events underlying *H. contortus* resistance to monepantel. The identification of a genome region harboring major genes previously associated with monepantel resistance supports the results of the employed X-QTL approach. In addition, a deletion in exon 11 of the *mptl-1* gene should be further investigated as the putative causal mutation leading to monepantel resistance.

**Author:** Turnbull, F., Devaney, E., Morrison, A. A., Laing, R. and Bartley, D. J.

**Title:** Genotypic characterisation of monepantel resistance in historical and newly derived field strains of *Teladorsagia circumcincta*

**DOI:** 10.1016/j.ijpddr.2019.10.002

**Abstract:** Recent reports of monepantel (MPTL) resistance in UK field isolates of *Teladorsagia circumcincta* has highlighted the need for a better understanding of the mechanism of MPTL-resistance in order to preserve its anthelmintic efficacy in this economically important species. Nine discrete populations of *T. circumcincta* were genotypically characterised; three MPTL-susceptible isolates, three experimentally selected MPTL-resistant strains and three field derived populations. Full-length *Tci-mptl-1* gene sequences were generated and comparisons between the MPTL-susceptible isolates, MPTL-resistant strains and one field isolate, showed that different putative MPTL-resistance conferring mutations were present in different resistant isolates. Truncated forms of the *Tci-mptl-1* gene were also observed. The genetic variability of individual larvae, within and between populations, was examined using microsatellite analyses at 10 'neutral' loci (presumed to be unaffected by MPTL). Results confirmed that there was little background genetic variation between the populations, global  $F_{ST} < 0.038$ . Polymorphisms present in exons 7 and 8 of *Tci-mptl-1* enabled genotyping of individual



larvae. A reduction in the number of genotypes was observed in all MPTL-resistant strains compared to the MPTL-susceptible strains that they were derived from, suggesting there was purifying selection at *Tci-mptl-1* as a result of MPTL-treatment. The potential link between benzimidazole (BZ)-resistance and MPTL-resistance was examined by screening individual larvae for the presence of three SNPs associated with BZ-resistance in the  $\beta$ -tubulin isotype-1 gene. The majority of larvae were BZ-susceptible homozygotes at positions 167 and 198. Increased heterozygosity at position 200 was observed in the MPTL-resistant strains compared to their respective MPTL-susceptible population. There was no decrease in the occurrence of BZ-resistant genotypes in larvae from each population. These differences, in light of the purifying selection at this locus in all MPTL-resistant isolates, suggests that *Tci-mptl-1* confers MPTL-resistance in *T. circumcincta*, as in *Haemonchus contortus*, but that different mutations in *Tci-mptl-1* can confer resistance in different populations.

## Ivermectin resistance

**Author:** Michele Macrelli, Susanna Williamson, Sian Mitchell, Richard Pearson, Leigh Andrews, Alison A. Morrison, Mandy Nevel, Richard Smith, Dave J. Bartley

**Title:** First detection of ivermectin resistance in *Oesophagostomum dentatum* in pigs

Veterinary Parasitology 270 (2019) 1–6

**Abstract:** In 2016 suspected reduced ivermectin (IVM) efficacy in *Oesophagostomum* species in pigs was reported in England. Following this initial report, APHA raised awareness amongst private pig veterinary practitioners of the need to monitor the efficacy of the worm control on pig units. In 2017 another veterinary practitioner highlighted a potential in-field lack of IVM efficacy in treating *Oesophagostomum* species in sows on another breeder/finisher unit. In this trial, the efficacy of IVM against *Oesophagostomum* species worms has been investigated to determine whether suspected reduced efficacy (52% reduction in mean faecal egg count 14 days post ivermectin administration) on a mixed indoor and outdoor breeder-finisher pig farm in England reflected true IVM resistance under controlled experimental conditions. On days 0 and 40 of the trial, twenty helminth-naive pigs were artificially infected per os with 5000 *Oesophagostomum* L3 obtained from the farm under investigation. The pigs were allocated to treatment or control groups (n=10 per group). Treatment group pigs received IVM (0.3 mg/kg body weight) by sub-cutaneous injection as per manufacturer's instructions on day 44. Control group animals were left untreated. Faecal worm egg counts were monitored throughout the trial from day 15 post infection to determine time to patency. On day 50 all pigs were euthanased to assess the worm burdens. Resistance to IVM was confirmed in *Oesophagostomum dentatum* based on the results of a faecal egg count reduction test (FECRT) and a controlled efficacy test (CET). Efficacy based on mean reduction in faecal egg count of IVM-treated pigs compared to untreated control pigs was 86%. Mean reduction in IVM-treated pig worm burdens was 5% against an adult worm population and 94% against an L3/L4 population. The apparent discrepancy between FECRT and CET

efficacy results appears to be due to egg development and/or oviposition suppression in IVM-treated female worms. The detection of IVM resistance in *Oesophagostomum* species worms for the first time in UK pigs is particularly important considering the global situation where resistance to pyrantel, levamisole and benzimidazole anthelmintics in *Oesophagostomum* species in pigs have already been reported. The results also provide an opportunity to discuss the wider issue of anthelmintic usage and efficacy on pig farms and highlight the need for wider surveillance for the occurrence of anthelmintic resistance in pigs.

**Author:** Atif, M., Smith, J. J., Estrada-Mondragon, A., Xiao, X., Salim, A. A., Capon, R. J., Lynch, J. W. and Keramidas, A.

**Title:** GluCIR-mediated inhibitory postsynaptic currents reveal targets for ivermectin and potential mechanisms of ivermectin resistance

**DOI:** 10.1371/journal.ppat.1007570

**Abstract:** Glutamate-gated chloride channel receptors (GluCIRs) mediate inhibitory neurotransmission at invertebrate synapses and are primary targets of parasites that impact drastically on agriculture and human health. Ivermectin (IVM) is a broad-spectrum pesticide that binds and potentiates GluCIR activity. Resistance to IVM is a major economic and health concern, but the molecular and synaptic mechanisms of resistance are ill-defined. Here we focus on GluCIRs of the agricultural endoparasite, *Haemonchus contortus*. We demonstrate that IVM potentiates inhibitory input by inducing a tonic current that plateaus over 15 minutes and by enhancing post-synaptic current peak amplitude and decay times. We further demonstrate that IVM greatly enhances the active durations of single receptors. These effects are greatly attenuated when endogenous IVM-insensitive subunits are incorporated into GluCIRs, suggesting a mechanism of IVM resistance that does not affect glutamate sensitivity. We discovered functional groups of IVM that contribute to tuning its potency at different isoforms and show that the dominant mode of access of IVM is via the cell membrane to the receptor.

**Author:** Kellerová, P., Matoušková, P., Lamka, J., Vokřál, I., Szotáková, B., Zajíčková, M., Pasák, M. and Skálová, L.

**Title:** Ivermectin-induced changes in the expression of cytochromes P450 and efflux transporters in *Haemonchus contortus* female and male adults

**DOI:** 10.1016/j.vetpar.2019.07.006

**Abstract:** *Haemonchus contortus*, one of the most pathogenic of all small ruminant parasites, have developed resistance to all used anthelmintics. Detoxification enzymes, e.g. cytochromes P450 (CYPs) and efflux transporters P-glycoproteins (P-gps), which represent the main defense system against harmful xenobiotics, have been suggested to contribute to drug resistance development. The present study was designed to compare the constitutive expression of individual CYPs and P-gps in females and males of H.

contortus adults and to follow up on the changes in expression of these genes in nematodes exposed to sub-lethal concentrations of ivermectin (IVM), which might occur during inaccurate treatment. The adults of inbred susceptible-Edinburgh strain (ISE, MHco3) of *H. contortus* were used for this purpose. The nematodes were incubated *ex vivo* with or without IVM (1, 10 and 100 nM) in culture medium for 4, 12 and 24 h. After incubation, total RNA was isolated and expression levels of individual CYPs and P-gps were analyzed using qPCR. Our results showed a great variability in the constitutive expression of individual CYPs and P-gps in *H. contortus* adults. The constitutive expression as well as the inducibility of CYPs and P-gps significantly differed in males and females. Contact of adult nematodes with sub-lethal IVM concentrations led to only minor changes in expression of CYPs, while expression of several P-gps, particularly *pgp-9.2* in males and *pgp-10*, *pgp-11* in females was increased significantly in IVM-exposed nematodes. In conclusion, inaccurate treatment of sheep with IVM might contribute to drug resistance development via increased expression of efflux transporters in *H. contortus* adults.

**Author:** Ménez, C., Alberich, M., Courtot, E., Guegnard, F., Blanchard, A., Aguilaniu, H. and Lespine, A.

**Title:** The transcription factor NHR-8: A new target to increase ivermectin efficacy in nematodes

**DOI:** 10.1371/journal.ppat.1007598

**Abstract:** Resistance to the anthelmintic macrocyclic lactone ivermectin (IVM) has a great impact on the control of parasitic nematodes. The mechanisms by which nematodes adapt to IVM remain to be deciphered. We have identified NHR-8, a nuclear hormone receptor involved in the xenobiotic response in *Caenorhabditis elegans*, as a new regulator of tolerance to IVM. Loss-of-function *nhr-8(ok186)* *C. elegans* mutants subjected to larval development assays and electropharyngeogram measurements, displayed hypersensitivity to IVM, and silencing of *nhr-8* in IVM-resistant worms increased IVM efficacy. In addition, compared to wild-type worms, *nhr-8* mutants under IVM selection pressure failed to acquire tolerance to the drug. In addition, IVM-hypersensitive *nhr-8(ok186)* worms displayed low transcript levels of several genes from the xenobiotic detoxification network and a concomitant low Pgp-mediated drug efflux activity. Interestingly, some *pgp* and *cyp* genes known to impact IVM tolerance in many nematode species, were down regulated in *nhr-8* mutants and inversely upregulated in IVM-resistant worms. Moreover, *pgp-6* overexpression in *nhr-8(ok186)* *C. elegans* increased tolerance to IVM. Importantly, NHR-8 function was rescued in *nhr-8(ok186)* *C. elegans* with the homolog of the parasitic nematode *Haemonchus contortus*, and silencing of Hco-*nhr-8* by RNAi on L2 *H. contortus* larvae increased IVM susceptibility in both susceptible and resistant *H. contortus* isolates. Thus, our data show that NHR-8 controls the tolerance and development of resistance to IVM in *C. elegans* and the molecular basis for this relates to the NHR-8-mediated upregulation of IVM detoxification genes. Since our results show that Hco-*nhr-8* functions similarly to Cel-*nhr-8*, this study helps to better understand mechanisms underlying failure

in drug efficacy and open perspectives in finding new compounds with NHR-8 antagonist activity to potentiate IVM efficacy.

**Author:** O'Shaughnessy, J., Drought, Y., Lynch, J., Denny, M., Hurley, C., Byrne, W., Casey, M., De Waal, T. and Sheehan, M.

**Title:** Ivermectin treatment failure on four Irish dairy farms

**DOI:** 10.1186/s13620-019-0142-8

**Abstract:** We report on the use of the faecal egg count reduction test to evaluate the performance of ivermectin in treating gastrointestinal nematode infections in first grazing season (FGS) calves on four dairy farms in Co. Kilkenny, Ireland. On each farm, FGS calves were injected subcutaneously with ivermectin in accordance with their live weight (day 0). Calves were individually faecal sampled on both day 0 and day 14. Faecal egg counts were determined using the Mini-FLOTAC technique. Composite faecal cultures for each farm were performed on each sampling occasion. The faecal egg count reductions (mode) ranged from 17.3-80.2% with the lower 95% confidence limit ranging from 3.1-72.3% on the four farms, respectively. Ivermectin-resistant nematodes were detected on all farms, with evidence of *Ostertagia* resistance on one farm. This study highlights the urgent need for Irish producers to reappraise their parasite control practices.

**Author:** Baiak, B. H. B., Lehen, C. R. and Rocha, R. A.

**Title:** Anthelmintic resistance of injectable macrocyclic lactones in cattle: A systematic review and meta-analysis

**DOI:** 10.1590/s1984-296120180093

**Abstract:** The purpose of this work was to identify, critically assess, and summarize available data from primary research about the anthelmintic resistance of injectable macrocyclic lactones in cattle. Meta-analysis was performed to estimate the pooled Odds Ratio and 95% Confidence Intervals. Of the 1504 abstracts screened for eligibility, 80 were deemed relevant for full publication review. Thirteen publications were included in the qualitative synthesis and assessed for systematic bias. Only five studies were included in the quantitative analysis because they showed a low risk of producing biased results in all the parameters. The forest plot indicated four studies that discuss anthelmintic resistance ( $P < 0.05$ ), while only one study did not discuss anthelmintic resistance ( $P > 0.05$ ). The pooled estimate showed 0.59 (95% Confidence intervals: 0.08, 0.47) times higher odds for studies that report anthelmintic resistance than for studies reporting efficacious anthelmintic treatment, with significant and substantially low heterogeneity ( $I^2 = 25\%$ ). Anthelmintic resistance to injectable macrocyclic lactones is a reality. There are need to improve methodological reporting in studies, which is a problem for investigations that involves systematic review and meta-analysis (SR-MA).

**Author:** Mickiewicz, M., Czopowicz, M., Moroz, A., Szaluś-Jordanow, O., Górski, P., Várady, M., Königová, A., Spinu, M., Lefkaditis, M. and Kaba, J.

**Title:** Development of resistance to eprinomectin in gastrointestinal nematodes in a goat herd with pre-existing resistance to benzimidazoles

**DOI:** 10.24425/pjvs.2019.131404

**Abstract:** We described a first case of resistance to eprinomectin in goat herd in Poland in which resistance to benzimidazoles had been previously reported. The herd was established in 2011 by purchasing several goats from a single herd in south-eastern Poland. Resistance to benzimidazoles in the herd was first reported in 2017. Shortly after the owner started to signal low effectiveness of the treatment with eprinomectin. In June 2018 the larval development test from pooled faecal sample was performed and the results indicated the presence of resistance to macrocyclic lactones and levamisole. In July 2018 a faecal egg count (FEC) reduction test was performed in 39 animals with levamisole, eprinomectin and one untreated control group. Drugs were used in doses recommended for goats. Three methods of calculation of FEC reduction were compared. After eprinomectin treatment, FEC reduction ranged from 0 to 20%, depending on the method of calculation. FEC reduction following levamisole treatment was 100%. Main species present in the faecal samples after treatment and in larvicidal concentrations in larval development test was *Haemonchus contortus*. This is the first report of anthelmintic resistance to macrocyclic lactones (eprinomectin) in goats in Poland.

**Author:** Doyle, S. R., Illingworth, C. J. R., Laing, R., Bartley, D. J., Redman, E., Martinelli, A., Holroyd, N., Morrison, A. A., Rezansoff, A., Tracey, A., Devaney, E., Berriman, M., Sargison, N., Cotton, J. A. and Gilleard, J. S.

**Title:** Population genomic and evolutionary modelling analyses reveal a single major QTL for ivermectin drug resistance in the pathogenic nematode, *Haemonchus contortus*

**DOI:** 10.1186/s12864-019-5592-6

**Abstract:** Background: Infections with helminths cause an enormous disease burden in billions of animals and plants worldwide. Large scale use of anthelmintics has driven the evolution of resistance in a number of species that infect livestock and companion animals, and there are growing concerns regarding the reduced efficacy in some human-infective helminths. Understanding the mechanisms by which resistance evolves is the focus of increasing interest; robust genetic analysis of helminths is challenging, and although many candidate genes have been proposed, the genetic basis of resistance remains poorly resolved. Results: Here, we present a genome-wide analysis of two genetic crosses between ivermectin resistant and sensitive isolates of the parasitic nematode *Haemonchus contortus*, an economically important gastrointestinal parasite of small ruminants and a model for anthelmintic research. Whole genome sequencing of parental populations, and key stages throughout the crosses, identified extensive genomic diversity that differentiates populations, but after backcrossing and selection, a single genomic

quantitative trait locus (QTL) localised on chromosome V was revealed to be associated with ivermectin resistance. This QTL was common between the two geographically and genetically divergent resistant populations and did not include any leading candidate genes, suggestive of a previously uncharacterised mechanism and/or driver of resistance. Despite limited resolution due to low recombination in this region, population genetic analyses and novel evolutionary models supported strong selection at this QTL, driven by at least partial dominance of the resistant allele, and that large resistance-associated haplotype blocks were enriched in response to selection. Conclusions: We have described the genetic architecture and mode of ivermectin selection, revealing a major genomic locus associated with ivermectin resistance, the most conclusive evidence to date in any parasitic nematode. This study highlights a novel genome-wide approach to the analysis of a genetic cross in non-model organisms with extreme genetic diversity, and the importance of a high-quality reference genome in interpreting the signals of selection so identified.

## Levamisole resistance

**Author:** Santos, J. M. L. D., Vasconcelos, J. F., Frota, G. A., Freitas, E. P. D., Teixeira, M., Vieira, L. D. S., Bevilaqua, C. M. L. and Monteiro, J. P.

**Title:** Quantitative molecular diagnosis of levamisole resistance in populations of *Haemonchus contortus*

**DOI:** 10.1016/j.exppara.2019.107734

**Abstract:** Parasitism by *Haemonchus contortus* is one of the main limiting factors in small ruminant production around the globe. Although several studies suggest the use of integrated management practices, these parasites have been controlled essentially with synthetic anthelmintic drugs. The resistance mechanism against the imidazothiazole derivative levamisole in *Haemonchus contortus* has not been fully described. Recently, resistance was associated with a 63bp deletion in the *Hco-acr-8b* gene that encodes a subunit for a nicotinic acetylcholine receptor. This study aimed to standardize a real time PCR (qPCR) protocol for levamisole resistance diagnosis in *H. contortus* populations based on this polymorphism and use it to characterize 23 field *H. contortus* populations obtained from different localities of Ceará State, Northeast Brazil. In addition, two populations of *H. contortus* were used as a standard of susceptibility and resistance, Inbred Strain Edinburgh (ISE) and Kokstad, respectively. Larval development tests (LDT) were performed on five field isolates and both EC50 and EC95 were estimated. LDT EC95 values provided a wider interval between susceptible and resistant populations than EC50 values (EC95 = 1.96–57.93  $\mu$ M; EC50 = 0.05–0.39  $\mu$ M), and were found to be more appropriate for differentiating them. Real time PCR results showed resistance allele frequencies ranged from 20.9 to 76.7%. Our results suggest that levamisole resistance may be present in field populations but it is not as widespread as benzimidazole resistance. This methodology may be useful to monitor levamisole resistance in field populations of *H. contortus*.

## Benzimidazole resistance

**Author:** Sargison, N. D., MacLeay, M., Morrison, A. A., Bartley, D. J., Evans, M. and Chaudhry, U.

**Title:** Development of amplicon sequencing for the analysis of benzimidazole resistance allele frequencies in field populations of gastrointestinal nematodes

**DOI:** 10.1016/j.ijpddr.2019.08.003

**Abstract:** Anthelmintic resistant gastrointestinal helminths have become a major cause of poor health in sheep and goats. Sensitive and specific molecular markers are needed to monitor the genotypic frequency of resistance in field parasite populations. Gastrointestinal nematode resistance to benzimidazole is caused by a mutation in one of three positions within the isotype 1  $\beta$ -tubulin gene. In the absence of markers for resistance to the other broad spectrum anthelmintic classes, these provide a relevant study example. Determination of the prevalence of these single nucleotide polymorphisms in field nematode populations can be impractical using conventional molecular methods to examine individual parasites; which can be laborious and lack sensitivity in determining low levels of resistance in parasite populations. Here, we report the development of a novel method based on an Illumina MiSeq deep amplicon sequencing platform to sequence the isotype 1  $\beta$ -tubulin locus of the small ruminant gastrointestinal nematode, *Teladorsagia circumcincta*, and determine the frequency of the benzimidazole resistance mutations. We validated the method by assessing sequence representation bias, comparing the results of Illumina MiSeq and pyrosequencing, and applying the method to populations containing known proportions of resistant and susceptible larvae. We applied the method to field samples collected from ewes and lambs on over a period of one year on three farms, each highlighting different aspects of sheep management and approaches to parasite control. The results show opportunities to build hypotheses with reference to selection pressures leading to differences in resistance allele frequencies between sampling dates, farms and ewes or lambs, and to consider the impact of their genetic fixation or otherwise. This study provides proof of concept of a practical, accurate, sensitive and scalable method to determine frequency of anthelmintic resistance mutations in gastrointestinal nematodes in field studies and as a management tool for livestock farmers.

**Author:** Ali, Q., Rashid, I., Shabbir, M. Z., Aziz UI, Rahman, Shahzad, K., Ashraf, K., Sargison, N. D. and Chaudhry, U.

**Title:** Emergence and the spread of the F200Y benzimidazole resistance mutation in *Haemonchus contortus* and *Haemonchus placei* from buffalo and cattle

**DOI:** 10.1016/j.vetpar.2018.12.001

**Abstract:** Benzimidazoles have been intensively (for over 40 years) used in the livestock sector, particularly in small ruminants. This has been led to the widespread emergence of

resistance in a number of small ruminant parasite species, especially *Haemonchus contortus*. In many countries benzimidazole resistance has severely compromised the control of *H. contortus* in small ruminants; but there is a little information on benzimidazole resistance in *H. contortus* infecting buffalo and cattle. Resistance to benzimidazoles have also been reported in the large ruminant parasite, *Haemonchus placei*, but again there is relatively little information on its prevalence. Hence it is very important to understand how resistance-conferring mutations emerge and spread in both parasites in buffalo and cattle hosts in order to develop approaches for the recognition of the problem at an early stage of its development. The present study suggests that the F200Y (TAC) mutation is common in *H. contortus*, being detected in 5/7 populations at frequencies between 7 and 57%. Furthermore, 6/10 *H. placei* populations contained the F200Y (TAC) mutation, albeit at low frequencies of between 0.4 and 5%. The phylogenetic analysis suggests that the F200Y (TAC) mutation in *H. contortus* has emerged on multiple occasions in the region, with at least three independent emergences across the populations. In contrast, the F200Y (TAC) resistance-conferring mutation in *H. placei* is only seen on a single haplotype. A high level frequency of the resistance haplotypes in the region, suggests that the unique resistance conferring-mutation has spread from a single emergence; likely by anthropogenic animal movement. Overall, these results provide the first clear genetic evidence for the spread of benzimidazole resistance-conferring mutations to multiple different locations from a single emergence in *H. placei*; while being consistent with previous small ruminant-based observations of multiple emergence of resistance mutations in *H. contortus*.

**Author:** Muchiut, S. M., Fernández, A. S., Lloberas, M., Steffan, P. E., Luque, S. E., Cardozo, P. A., Bernat, G. A., Riva, E. and Fiel, C. A.

**Title:** Recovery of fenbendazole efficacy on resistant *Haemonchus contortus* by management of parasite refugia and population replacement

**DOI:** 10.1016/j.vetpar.2019.06.003

**Abstract:** The recovery of fenbendazole efficacy against *Haemonchus contortus* was attempted in a sheep intensive production system, using a strategy of population replacement in which the initial absolute efficacy of fenbendazole was 0%. The strategy was based on managing the parasite populations in refugia. Firstly, the resistant parasite population was reduced by means of anthelmintic treatments with efficacious drugs (Phase I), then a new, susceptible population was introduced in summer by way of artificially infected lambs at weaning, which were left to graze on the experimental pasture for eleven months (Phase II). Lastly, the impact of the replacement strategy, in terms of benzimidazole efficacy, was measured (Phase III). Faecal egg counts from permanent lambs and worm burdens as a measure of pasture infectivity from tracer lambs were determined throughout the study. During Phase I, faecal egg counts diminished from a peak of 2968 (300–7740) epg to 0 epg at the end, while adult worm burdens of *H. contortus* were reduced from 2625 (800–5100) to 0, which showed that the treatment strategy used in Phase I was effective in reducing the resistant population. These parameters also showed that good levels of pasture contamination and infectivity were achieved in Phase II, as faecal egg counts of up to 7275 (3240–13080) epg and adult



worm burdens of 500 (200–800) *H. contortus* were reached. The absolute benzimidazole efficacy on *H. contortus* estimated at 16 months post-population replacement (Phase III) was 97.58%. The results lead to the conclusion that the recovery of anthelmintic efficacy of fenbendazole against a resistant population of *H. contortus* may be achieved by means of a strategy based on management of refugia and a subsequent introduction of a susceptible population. This strategy might be translatable to other resistant nematode genera.

**Author:** Stasiuk, S. J., MacNevin, G., Workentine, M. L., Gray, D., Redman, E., Bartley, D., Morrison, A., Sharma, N., Colwell, D., Ro, D. K. and Gilleard, J. S.

**Title:** Similarities and differences in the biotransformation and transcriptomic responses of *Caenorhabditis elegans* and *Haemonchus contortus* to five different benzimidazole drugs

**DOI:** 10.1016/j.ijpddr.2019.09.001

**Abstract:** We have undertaken a detailed analysis of the biotransformation of five of the most therapeutically important benzimidazole anthelmintics - albendazole (ABZ), mebendazole (MBZ), thiabendazole (TBZ), oxfendazole (OxBZ) and fenbendazole (FBZ) - in *Caenorhabditis elegans* and the ruminant parasite *Haemonchus contortus*. Drug metabolites were detected by LC-MS/MS analysis in supernatants of *C. elegans* cultures with a hexose conjugate, most likely glucose, dominating for all five drugs. This work adds to a growing body of evidence that glucose conjugation is a major pathway of xenobiotic metabolism in nematodes and may be a target for enhancement of anthelmintic potency. Consistent with this, we found that biotransformation of albendazole by *C. elegans* reduced drug potency. Glucose metabolite production by *C. elegans* was reduced in the presence of the pharmacological inhibitor chrysin suggesting that UDP-glucuronosyl/glucosyl transferase (UGT) enzymes may catalyze benzimidazole glucosidation. Similar glucoside metabolites were detected following *ex vivo* culture of adult *Haemonchus contortus*. As a step towards identifying nematode enzymes potentially responsible for benzimidazole biotransformation, we characterised the transcriptomic response to each of the benzimidazole drugs using the *C. elegans* resistant strain CB3474 ben-1(e1880)III. In the case of albendazole, mebendazole, thiabendazole, and oxfendazole the shared transcriptomic response was dominated by the up-regulation of classical xenobiotic response genes including a shared group of UGT enzymes (*ugt-14/25/33/34/37/41/8/9*). In the case of fenbendazole, a much greater number of genes were up-regulated, as well as developmental and brood size effects suggesting the presence of secondary drug targets in addition to BEN-1. The transcriptional xenobiotic response of a multiply resistant *H. contortus* strain UGA/2004 was essentially undetectable in the adult stage but present in the L3 infective stage, albeit more muted than *C. elegans*. This suggests that xenobiotic responses may be less efficient in stages of parasitic nematodes that reside in the host compared with the free-living stages.

## Effect of condensed tannins

**Author:** Awistaros, A. S., Kustantinah, Raden, W. N., Lovy, P. and Melisa, E.

**Title:** Extraction of condensed tannins from tropical plants as affected by leaves maturity, maceration time, and centrifugal force

**DOI:** 10.4028/www.scientific.net/MSF.948.78

**Abstract:** Condensed Tannin (CT) are secondary metabolites of the plant that synthesized along the phenylpropanoid pathway. It is known to suppress CH<sub>4</sub> emission in the rumen through protozoal defaunation as well as direct effects on methanogen bacteria. Recent studies have been reported to have anthelmintics activity to overcome gastrointestinal nematodes, appertain to the *Haemonchus contortus*. To obtain merits of CT, it can be applied in flour or infusion form. Infusion considered as a convenient alternative of CT application. Evaluation method to produce optimum levels of CT needs to be done for the merits of CT obtained optimally. This study aimed to evaluate the effect of leaf maturity, maceration time, and centrifuge force on CT levels produced in infusion leaf as a feed additive. Mature and immature leaves selected from *Morinda citrifolia* (CF), *Muntingia calabura* (CA), *Azadirachta indica* (AZ), *Hibiscus rosa sinensis* (RS) and *Hibiscus tiliaceus* (HT). The results of this research showed that leaf age had a significant effect on CT, except on AZ and RS. Normally, CT content of immature leaf is higher than the mature leaf. CA has highest levels of CT, can be predicted because of lots of glandular trichomes in their mature leaves as a place to store secondary metabolite compounds. Optimum levels of CT from maceration occurring at 2 h of immersion except on HT that requiring 4 h of immersion. Meanwhile, 372 g is the most effective centrifuge force in producing optimum CT, except in CA which requires 2.318 g. Based on this study, it can be concluded that leaf age, maceration time, and centrifuge force have a significant influence in producing an infusion with maximum CT at each stage of extraction. It is important for infusion production to contain CT in optimal amounts. This research is expected to be the first step in providing multifunctional feed additive for livestock.

**Author:** Costes-Thiré, M., Laurent, P., Ginane, C. and Villalba, J. J.

**Title:** Diet selection and trade-offs between condensed tannins and nutrients in parasitized sheep

**DOI:** 10.1016/j.vetpar.2019.05.013

**Abstract:** Foraging behavior by parasitized herbivores can be interpreted as a decision-making process where individuals are faced with trading-off the ingestion of nutrients with the ingestion of potentially medicinal -and toxic- plant secondary compounds. We determined how parasitized sheep prioritize selection of crude protein, energy and a medicinal plant secondary compound (quebracho tannins-QT). Foraging preferences were tested in 40 lambs before experiencing a parasitic infection (Phase 1), during an infection (Phase 2; 10,000 L3 *Haemonchus contortus* per lamb) and after chemotherapy (Phase 3).

Lambs were assigned to four groups (10 lambs/group) such that animals in Group 1 (Control) could choose between foods of high (HEP) or low (LEP) energy to protein ratios. The other groups received the same choice, but QT were added (4%) to HEP (Group 2), to LEP (Group 3) or to both foods (Group 4). All groups under a parasitic infection (Phase 2) increased their preference for HEP (from 0.44 to  $0.66 \pm 0.042$ ;  $P < 0.05$ ) and intake of digestible energy (from 0.106 to  $0.126 \pm 0.007$  Mcal/kg BW;  $P < 0.05$ ) relative to Phase 1, a pattern that remained during Phase 3. Only lambs receiving QT in HEP increased their intake of QT from Phase 1 to Phase 2 ( $P < 0.05$ ). Fecal egg counts and blood parameters revealed a parasitic infection ( $P < 0.05$ ) in Phase 2 that subsided in Phase 3, although no differences were detected among groups ( $P > 0.05$ ). The importance of protein nutrition on parasitized animals has been highlighted before, but these results suggest that lambs prioritized the ingestion of energy-dense over protein-dense foods or medicinal condensed tannins when challenged by gastrointestinal parasitism. Consumption of medicinal tannins represented a side-effect of the preference manifested for energy-dense foods during testing.

**Author:** Lima, P. D. M. T., Cruzoulon, P., Sanches, T. P., Zabré, G., Kabore, A., Niderkorn, V., Hoste, H., Amarante, A. F. T. D., Costa-Júnior, L. M., Abdalla, A. L. and Louvandini, H.

**Title:** Effects of *Acacia mearnsii* supplementation on nutrition, parasitological, blood parameters and methane emissions in Santa Inês sheep infected with *Trichostrongylus colubriformis* and *Haemonchus contortus*

**DOI:** 10.1016/j.exppara.2019.107777

**Abstract:** Gastrointestinal nematodes are a main problem for ruminant production, reducing animal performance and increasing environmental impact per unit of animal product generated. Tannin supplementation may lead to positive results regarding aspects such as parasitic infections and methane (CH<sub>4</sub>) emissions. Therefore, the objective of this experiment was to evaluate the effects of the condensed tannins (CT) extract made of powdered *Acacia mearnsii* bark (PAB) on nutrition, parasitic status and CH<sub>4</sub> emissions in sheep artificially infected with *Trichostrongylus colubriformis* and *Haemonchus contortus*. Twenty 10-month old Santa Inês lambs ( $24.7 \pm 3.14$  kg of initial body weight) were used in a 50-day trial. Animals were divided in four treatment groups according to parasitic infection and PAB supplementation: two control groups without infections, one without PAB (C-) ( $n = 4$ ) and one with PAB (C+) ( $n = 4$ ); two infected groups, one without PAB (I-) ( $n = 6$ ) and another receiving PAB (I+) ( $n = 6$ ). Initially, animals were kept in individual pens where they were fed ad libitum chopped tifton 85 hay (*Cynodon* spp.) and 210 g/animal/day of concentrate. On the first day of experiment, animals of I- and I+ groups were artificially infected with infective larvae (L3) of *T. colubriformis* and *H. contortus*. Lambs were weighed fortnightly to calculate average daily body weight gain (ADG). Blood and faeces samples were also collected in the same moment of weighing for the evaluation of blood parameters and faecal egg count (FEC) respectively. After 40 days of experiment, measurements of CH<sub>4</sub> emissions in small chamber system started and following that, apparent total tract digestibility (ATTD) assay was carried out in metabolic

cages. In the end of experimental period (50 days), lambs were slaughtered and samples of abomasum and small intestine content were collected for worm count, identification, and eggs/female count. No significant ( $p > 0.05$ ) treatment effects were verified for ADG, ATTD and worm count. Blood parameters were affected in both infected groups ( $p < 0.05$ ) from the 28th experimental day onwards, when these animals started to show reduced red blood cells, haemoglobin and packed cell volume when compared to C- and C+. Decreased FEC was verified in I+ when compared to I- and also, *H. contortus* eggs/female worm for I+ was lower than for I- ( $p < 0.05$ ). Both infected groups showed higher CH<sub>4</sub> emissions than the control groups ( $p < 0.05$ ). Results highlighted the anthelmintic potential of PAB and indicated methanogenic effect of parasitic nematode infections.

**Author:** Gaudin, E., Costes-Thiré, M., Villalba, J. J., Hoste, H., Gerfault, V. and Ginane, C.

**Title:** Relative abilities of young sheep and goats to self-medicate with tannin-rich sainfoin when infected with gastrointestinal nematodes

**DOI:** 10.1017/s175173111800304x

**Abstract:** Plant secondary metabolites (PSM) are one of the promising options to control gastrointestinal nematodes in sheep and goats. The objective of this study was to assess the abilities of sheep and goats to self-medicate with tannin-rich sainfoin (*Onobrychis viciifolia*) (SF) when infected with gastrointestinal nematodes, using a cafeteria and an operant conditioning trial. Hypotheses were that parasitized (P) lambs and goat kids would show greater intake and preference for SF than their non-parasitized (NP) counterparts, that kids would eat more SF than lambs (due to their lower resistance against parasites and their greater ability to consume PSM), and that SF intake would increase over time for P animals. We used 20 female kids and 20 ewe lambs aged 3 months. Half of the animals per species ( $n = 10$ ) were experimentally infected with 170 L3 larvae of *Haemonchus contortus*/kg of BW (P). The other half were free from parasites throughout the study (NP). Five weeks after infection, animals were exposed to a 24-day cafeteria trial (three 8-day periods) offering a free choice between two legume pellets: SF (3.8% condensed tannins) and alfalfa (ALF, *Medicago sativa*; no tannin). Subsequently, animals were involved in an operant conditioning trial of two 4-day long sessions, to assess in short-term tests their motivation to walk for a SF reward when offered in choice with freely available ALF. In the cafeteria trial, SF preference was greater in kids than in lambs, particularly in the first two periods. We did not observe a greater preference for SF in P animals, which was even greater in NP animals for periods 1 and 2. Sainfoin intake increased through periods for P animals, which led to similar SF preferences for all groups during period 3. In the operant-conditioning trial, motivation to get the SF reward was similar between P and NP animals. These results support the hypotheses that goats are more willing to consume tanniferous feeds than sheep, and that P animals increased SF intake through time. However, the emergence of a curative self-meditative behaviour was not supported, as P individuals did not show greater SF intake, preference, nor a greater motivation to get SF than NP animals, regardless of animal species. These findings are discussed with previous results and some explanations are presented.

## Other alternative treatments

**Author:** Baghbani, Z., Esmaeilnejad, B. and Asri-Rezaei, S.

**Title:** Assessment of oxidative/nitrosative stress biomarkers and DNA damage in *Teladorsagia circumcincta* following exposure to zinc oxide nanoparticles

**DOI:** 10.1017/s0022149x19001068

**Abstract:** Drug resistance to helminth parasites is one of the most serious problems to threaten the livestock industry. The problem also poses a major threat to public health. Therefore, novel and safe agents should urgently be investigated to control parasitic infections. The current study was conducted to evaluate the possible antiparasitic effects of zinc oxide nanoparticles (ZnO-NPs) on one of the most prevalent gastrointestinal nematodes, *Teladorsagia circumcincta*. The worms were incubated with various concentrations of ZnO-NPs: 1, 4, 8, 12 and 16 ppm for 24 hours. Mobility and mortality of the parasites were recorded at four-hour intervals. At the endpoint, several biomarkers of oxidative/nitrosative stress, including superoxide dismutase, glutathione peroxidase and catalase, as well as lipid peroxidation, protein carbonylation, total antioxidant status, nitric oxide contents and DNA damage, were measured in the homogenized samples. ZnO-NPs showed significant anthelmintic effects, depending on time and concentration. Furthermore, the nanoparticle induced severe oxidative/nitrosative stress and DNA damage. ZnO-NPs could be considered as a novel and potent anthelmintic agent.

**Author:** Campigotto, G., Gebert, R. R., Santos, D. S., dos Reis, J. H., Alba, D. F., Cazarotto, C. J., Leal, M. L. R., Baldissera, M. D., Lopes, T. F., Druzian, L. T., Morsch, V. M., Vedovatto, M. and Da Silva, A. S.

**Title:** Effects of oral administration of copper capsules on helminth control in lactating dairy sheep: An effective alternative to replace conventional antiparasitics during lactation

**DOI:** 10.1016/j.exppara.2019.107735

**Abstract:** Two experiments were performed to determine whether oral administration of copper oxide capsules controlled helminthic infections in Lacaune sheep without acute collateral effects on animal health. In experiment 1, 48 multiparous lactating sheep (60.1 ± 8.5 kg) were stratified according to initial number of eggs (*Haemonchus contortus*) per gram of feces (EPG) and were assigned randomly to 1 of two treatments (24 sheep/treatment): no oral administration (control) or oral administration of two copper capsules (treated; approximately 58 mg copper/kg body weight). Blood and fecal samples were collected on days 0, 15 and 45. Animals treated with copper capsules showed lower of EPG, eosinophils, acetylcholinesterase (AChE) in whole blood, and lower butyrylcholinesterase (BChE) activity in serum. Treated sheep had higher erythrocyte numbers, hemoglobin concentrations, hematocrit, and lymphocyte numbers. In experiment 2, 12 male lambs negative for helminths and coccidia were assigned randomly to one of two treatments (six lambs/treatment): control or treated (one copper capsule;

approximately 58 mg copper/kg body weight); the experiment was designed to determine whether the results of experiment 1 were due to treatment or parasitism. Blood samples were collected on days 0, 5, 10 and 15 and fecal samples were collected on days 0, 7 and 15. Treated animals showed greater concentrations of lymphocytes; however, treatment had no effect on other hemogram variables, AChE and BChE activities, or levels of alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase, creatinine, urea, albumin, total protein, and reactive oxygen species. These data suggest that copper capsules in dairy sheep efficiently controlled *H. contortus* infections. Treatment was not harmful to lambs during the first 15 days, i.e. there were no signs of acute toxicity.

**Author:** Cheraghipour, K., Moridnia, A., Sharifi, M., Mohaghegh, M. A., Khanizadeh, S., Nourmohammadi, M. and Kalani, H.

**Title:** The effect of medicinal plant extracts on helminthes: A systematic review

**DOI:** 10.22122/jjms.v37i525.11631

**Abstract:** Background: Currently, parasitic infections are the most important global health problems. Helminthic infections cause serious damage to the livestock industry. Most importantly, it can cause severe damage in immunocompromised individuals. The aim of this systematic review study was to assess the research on the treatment of helminthic diseases using medicinal plant extracts. Methods: The search was carried out in 7 databases including 4 English databases (Scopus, PubMed, ScienceDirect, and Embase) and 3 Persian databases (Scientific Information Database, Islamic World Science Citation Center, and Magiran) in order to find the studies carried out in relation to the purpose of the current study between 2008 and 2018 in Persian and English languages. Findings: Most studies focused on *Balanites aegyptiaca* (10.71%). (*Balanites aegyptiaca* Del. (*Zygophyllaceae*), known as 'desert date,' is spiny shrub or tree up to 10 m tall, widely distributed in dry land areas of Africa and South Asia. )The most commonly used extraction method was maceration (78.57%) and then sonication (7.14%). Methanol (35.71%) was the most solvent used for extraction, followed by water (17.85%). The most studied parasite was *Haemonchus contortus* (28.57%), followed by *Schistosoma mansoni* (10.71%). Conclusion: Studies have shown that plant extracts can be a good alternative to synthetic drugs in reducing helminthic disease signs; and plant extracts can be used to produce drugs based on natural and effective compounds against helminthes with fewer side effects than synthetic drugs.

**Author:** Dubois, O., Allanic, C., Charvet, C. L., Guégnard, F., Février, H., Théry-Koné, I., Cortet, J., Koch, C., Bouvier, F., Fassier, T., Marcon, D., Magnin-Robert, J. B., Peineau, N., Courtot, E., Huau, C., Meynadier, A., Enguehard-Gueiffier, C., Neveu, C., Boudesocque-Delaye, L. and Sallé, G.

**Title:** Lupin (*Lupinus* spp.) seeds exert anthelmintic activity associated with their alkaloid content

**DOI:** 10.1038/s41598-019-45654-6

**Abstract:** The growing range of drug resistant parasitic nematode populations threatens the sustainability of ruminant farming worldwide. In this context, nutraceuticals, animal feed that provides necessary dietary requirements while ensuring parasite control, could contribute to increase farming sustainability in developed and low resource settings. In this study, we evaluated the anthelmintic potential of lupin seed extracts against the major ruminant trichostrongylids, *Haemonchus contortus* and *Teladorsagia circumcincta*. In vitro observations showed that seed extracts from commercially available lupin varieties could significantly but moderately inhibit larval migration. This anthelmintic effect was mediated by the seed alkaloid content and was potent against both fully susceptible and multidrug resistant *H. contortus* isolates as well as a susceptible *T. circumcincta* isolate. Analytical chemistry revealed a set of four lupanine and sparteine-derivatives with anthelmintic activity, and electrophysiology assays on recombinant nematode acetylcholine receptors suggested an antagonistic mode of action for lupin alkaloids. An in vivo trial in *H. contortus* infected lupin-fed ewes and goats failed to demonstrate any direct anthelmintic effect of crude lupin seeds but infected lupin-fed goats suffered significantly less parasite-mediated blood losses. Altogether, our findings suggest that the anthelmintic potential of lupin remains limited. However, the potent alkaloids identified could lead to the development of novel drugs or may be used in combination with current anthelmintics to improve their efficacy.

**Author:** Escareño-Díaz, S., Alonso-Díaz, M. A., Mendoza de Gives, P., Castillo-Gallegos, E. and von Son-de Fernex, E.

**Title:** Anthelmintic-like activity of polyphenolic compounds and their interactions against the cattle nematode *Cooperia punctata*

**DOI:** 10.1016/j.vetpar.2019.08.003

**Abstract:** Polyphenolic compounds (PCs) have been proposed as one of the most bioactive group of secondary metabolites occurring in nature and have been associated to anthelmintic (AH)-like activity of plants against cattle nematodes. However, little is known regarding their synergetic / antagonistic interactions. This study assessed the in vitro AH-like activity of commercial PCs: quercetin, caffeic acid, rutin and coumarin, and their combinations against the egg hatching and larval exsheathment of *Cooperia punctata*; one of the most prevalent nematodes affecting grazing cattle in tropical regions. The molecules selected for the in vitro analysis were identified as bioactive phytochemicals of plants through bio-guided fractionation in previous studies. To estimate mean effective concentrations (EC<sub>50</sub>) five increasing concentrations were used for both Egg hatching inhibition assay (EHIA) and larval exsheathment inhibition assay (LEIA) (0.6–9.8 mg mL<sup>-1</sup> and 0.15–2.4 mg mL<sup>-1</sup>, respectively). From the four molecules, only rutin did not affect egg hatching; while quercetin, showed no bioactivity against eggs or larvae ( $P > 0.766$  and  $P > 0.621$ , respectively). Best-fit EC<sub>50</sub> estimated through the EHIA was considered for PCs classification as bioactive (coumarin and caffeic acid) and non-bioactive (quercetin and rutin). Phytochemical interactions were subsequently assessed combining bioactive:non-bioactive PCs (8:2 ratio), and the nature of their interaction was classified using the fractional inhibitory concentration index (FICindex). Combinations had a highly

synergistic interaction against larval exsheathment (FICindex < 0.5) except for coumarin:rutin against egg hatching (FICindex > 0.5). Quercetin and rutin acted as PCs AH-like activity enhancers, reducing EC50 of bioactive molecules in a range of 43%–64% and 68%–83% for EHIA and LEIA, respectively. A linear relationship between low molecular weight of molecules and ovicidal activity was observed; where, molecules with lower molecular weight displayed better-fit EC50 for ovicidal activity. Furthermore, coumarin and caffeic acid bioactivity against free-living stages of *C. punctata* makes them suitable candidates as markers for anthelmintic-like activity in bioactive forages. Combinations used through this investigation showed a potent anthelmintic-like activity against free-living forms of *C. punctata*, representing a first step towards the identification of promising alternatives for nematode control.

**Author:** Esteban-Ballesteros, M., Sanchis, J., Gutiérrez-Corbo, C., Balaña-Fouce, R., Rojo-Vázquez, F. A., González-Lanza, C. and Martínez-Valladares, M.

**Title:** In vitro anthelmintic activity and safety of different plant species against the ovine gastrointestinal nematode *Teladorsagia circumcincta*

**DOI:** 10.1016/j.rvsc.2019.01.004

**Abstract:** The present study was conducted to evaluate the in vitro anthelmintic activity and safety of methanolic and aqueous extracts of 9 plants against the ovine GIN *Teladorsagia circumcincta*. Initially the ovicidal efficacy of all extracts was tested at 50 mg/ml and at 1%, this last concentration for the methanolic extract of *Elettaria cardamomum*, by the Egg Hatch Assay. In those extracts with efficacy higher than 95%, the effective concentration required to inhibit 50% of egg hatching (EC 50) and their cytotoxicity, as the lethal dose 50 (LC 50), was also measured. The aqueous extracts of *Aesculus hippocastanum*, *Isatis tinctoria*, *Chelidonium majus*, *E. cardamomum* and *Sisymbrium irio*, with EC 50 values ranging 1.30–2.88 mg/ml, and the aqueous extract of *Jasminum polyanthum* with a value of 6.41 mg/ml, showed the highest activities. The aqueous extract of *J. polyanthum* was the safest extract, followed by methanolic extract of *E. cardamomum* and aqueous extract of *S. irio*, all of them with a Selective Index higher than 1. According to our results, there is no correlation between the amount of total phenols or total tannins with the anthelmintic activity of the plants tested. Although results need to be interpreted with caution, as in vitro activity may not automatically translate into in vivo efficacy, those extracts with SI equal or higher than 1 and EC 50 equal or lower than 6 mg/ml, could be taken into account for being used subsequently as feed or food additives in infected sheep.

**Author:** Herath, H. M. P. D., Preston, S., Jabbar, A., Garcia-Bustos, J., Addison, R. S., Hayes, S., Rali, T., Wang, T., Koehler, A. V., Chang, B. C. H., Hofmann, A., Davis, R. A. and Gasser, R. B.

**Title:** Selected  $\alpha$ -pyrones from the plants *Cryptocarya novoguineensis* (Lauraceae) and *Piper methysticum* (Piperaceae) with activity against *Haemonchus contortus* in vitro



**DOI:** 10.1016/j.ijpddr.2018.12.006

**Abstract:** Due to the widespread occurrence and spread of anthelmintic resistance, there is a need to develop new drugs against resistant parasitic nematodes of livestock animals. The Nobel Prize-winning discovery and development of the anti-parasitic drugs avermectin and artemisinin has renewed the interest in exploring natural products as anthelmintics. In the present study, we screened 7500 plant extracts for in vitro-activity against the barber's pole worm, *Haemonchus contortus*, a highly significant pathogen of ruminants. The anthelmintic extracts from two plants, *Cryptocarya novoguineensis* and *Piper methysticum*, were fractionated by high-performance liquid chromatography (HPLC). Subsequently, compounds were purified from fractions with significant biological activity. Four  $\alpha$ -pyrones, namely goniothalamine (GNT), dihydrokavain (DHK), desmethoxyyangonin (DMY) and yangonin (YGN), were purified from fractions from the two plants, GNT from *C. novoguineensis*, and DHK, DMY and YGN (= kavalactones) from *P. methysticum*. The three kavalactones induced a lethal, eviscerated (Evi) phenotype in treated exsheathed third-stage larvae (xL3s), and DMY and YGN had moderate potencies (IC 50 values of  $31.7 \pm 0.23 \mu\text{M}$  and  $23.7 \pm 2.05 \mu\text{M}$ , respectively) at inhibiting the development of xL3s to fourth-stage larvae (L4s). Although GNT had limited potency (IC 50 of 200–300  $\mu\text{M}$ ) at inhibiting L4 development, it was the only compound that reduced L4 motility (IC 50 of 6.25–12.50  $\mu\text{M}$ ). The compounds purified from each plant affected *H. contortus* in an irreversible manner. These findings suggest that structure-activity relationship studies of  $\alpha$ -pyrones should be pursued to assess their potential as anthelmintics.

**Author:** Oliveira Santos, F., Ponce Morais Cerqueira, A., Branco, A., José Moreira Batatinha, M. and Borges Botura, M.

**Title:** Anthelmintic activity of plants against gastrointestinal nematodes of goats: A review

**DOI:** 10.1017/s0031182019000672

**Abstract:** The gastrointestinal nematodes (GIN) stand out as an important cause of disease in small ruminant, especially on goat farm. Widespread resistance to synthetic anthelmintics has stimulated the research for alternative strategies of parasite control, including the use of medicinal plants. The present work summarizes the in vitro and in vivo studies of plants with activity against GIN of goats, focusing on the description of chemical constituents related to this effect. This review retrieved 56 scientific articles from 2008 to 2018 describing more than 100 different plant species. The most frequently investigated family was Fabaceae (30.7%) (*legume, pea, or bean family*). Most in vitro studies on the activity of plant extracts and fractions were carried out with of free-living stages nematodes. In vivo studies were conducted mainly with the use of plants in animal feed and generally showed lower effectiveness compared to in vitro assays. The main plant secondary metabolites associated with anthelmintic effect are condensed tannins, saponin and flavonoids. However, the studies with compounds isolated from plants and elucidation of their mechanisms of action are scarce. Herbal medicines are thought to be promising sources for the development of effective anthelmintic agents.

**Author:** Page, A. P., Roberts, M., Félix, M. A., Pickard, D., Page, A. and Weir, W.

**Title:** The golden death bacillus *Chryseobacterium nematophagum* is a novel matrix digesting pathogen of nematodes

**DOI:** 10.1186/s12915-019-0632-x

**Abstract:** Background: Nematodes represent important pathogens of humans and farmed animals and cause significant health and economic impacts. The control of nematodes is primarily carried out by applying a limited number of anthelmintic compounds, for which there is now widespread resistance being reported. There is a current unmet need to develop novel control measures including the identification and characterisation of natural pathogens of nematodes. Results: Nematode killing bacilli were isolated from a rotten fruit in association with wild free-living nematodes. These bacteria belong to the *Chryseobacterium* genus (golden bacteria) and represent a new species named *Chryseobacterium nematophagum*. These bacilli are oxidase-positive, flexirubin-pigmented, gram-negative rods that exhibit gelatinase activity. *Caenorhabditis elegans* are attracted to and eat these bacteria. Within 3 h of ingestion, however, the bacilli have degraded the anterior pharyngeal chitinous lining and entered the body cavity, ultimately killing the host. Within 24 h, the internal contents of the worms are digested followed by the final digestion of the remaining cuticle over a 2-3-day period. These bacteria will also infect and kill bacterivorous free-living (L1-L3) stages of all tested parasitic nematodes including the important veterinary Trichostrongylids such as *Haemonchus contortus* and *Ostertagia ostertagi*. The bacteria exhibit potent collagen-digesting properties, and genome sequencing has identified novel metalloprotease, collagenase and chitinase enzymes representing potential virulence factors. Conclusions: *Chryseobacterium nematophagum* is a newly discovered pathogen of nematodes that rapidly kills environmental stages of a wide range of key nematode parasites. These bacilli exhibit a unique invasion process, entering the body via the anterior pharynx through the specific degradation of extracellular matrices. This bacterial pathogen represents a prospective biological control agent for important nematode parasites.

**Author:** Pertiwi, H., Rochmi, S. E. and Dadi, T. B.

**Title:** Blood parameter profile and helminthiasis identification in sheep fed with diets rich in polyunsaturated fatty acid (PUFA)

**Abstract:** The experiment was conducted to investigate the effect of Poly Unsaturated Fatty Acid (PUFA) rich diet on hematological value and helminth infestation of sheep. The experiment was carried with control group fed only with basal ration and other group were fed with basal ration in addition to 4% maize oil and soya oil, and water ad libitum. After eight months treatment, gastro intestinal tract helminthiasis and hematological changes were closely observed. In hematological analysis, erythrocyte and thrombocyte levels were significantly decreased ( $P < 0.05$ ), whereas MCV level was increased significantly ( $P < 0.05$ ). In this experiment during feeding of PUFA rich diets did not exhibit clinical symptoms,

PUFA rich diets has antihelmintic effect against *T.vitulorum*, *T.giardii*, *H.contortus*, *B.phlebotomum* and *T.globulosa*.

**Author:** Saha, S. and Lachance, S.

**Title:** Effect of essential oils on cattle gastrointestinal nematodes assessed by egg hatch, larval migration and mortality testing

**DOI:** 10.1017/s0022149x19001081

**Abstract:** The efficacy of eight essential oils (EOs) (*Solidago canadensis*, *Eucalyptus globulus*, *Pelargonium asperum*, *Ocimum basilicum*, *Thymus vulgaris*, *Mentha piperita*, *Cymbopogon citratus* and *Cymbopogon martinii*) against gastrointestinal nematodes (GINs) was evaluated using eggs collected from naturally infected cattle and cultured infective larvae (L3). The larvae species cultured from the faecal samples and subjected to two in vitro tests were *Haemonchus* spp. (55.5%), *Trichostrongylus* spp. (28.0%), *Cooperia* spp. (15.0%) and *Oesophagostomum* spp. (1.5%). The genus of EO *Cymbopogon* (*C. citratus* and *C. martinii*) showed the highest anthelmintic activity at the dose of 8.75 mg/ml, for the egg hatch, the larval migration and mortality assays. All of the EOs tested reduced egg hatching to rates <19.0%, compared to the controls (water and water + Tween 20) that had rates >92.0%. *Cymbopogon citratus* and *C. martinii* treatments resulted in 11.6 and 8.1% egg hatch, had the lowest migration of larvae through sieves, 60.5 and 54.9%, and the highest mortality rates, 63.3 and 56.3%, respectively. Dose-response tests showed that EO from *C. citratus* had the lowest larval LC50 and migration inhibition concentration (IC50) values of 3.89 and 7.19 mg/ml, respectively, compared to two other EOs (*C. martinii* and *O. basilicum*). The results suggest that EOs from the genus *Cymbopogon* can be interesting candidates for nematode control in cattle, although it may prove challenging to deliver concentrations to the gastrointestinal tract sufficient to effectively manage GINs.

**Author:** Vieira, Í S., Oliveira, I. D. C., Campos, A. K. and Araújo, J. V. D.

**Title:** Association and predatory capacity of fungi *Pochonia chlamydosporia* and *Arthrobotrys cladodes* in the biological control of parasitic helminths of bovines

**DOI:** 10.1017/s003118201900060x

**Abstract:** Nematophagous fungi are used in the biological control of the parasitic helminths of animals and plants. The association of ovicidal and predator nematophagous fungi may present a complementary and increased action on the biological control of helminths. Joint growth compatibility and predation tests were carried out on infective larvae of nematode parasites of bovines with the nematophagous fungus ovicide *Pochonia chlamydosporia* and the nematophagous fungus predator *Arthrobotrys cladodes*. The tests of antagonism in direct confrontation, antibiosis and the effect of volatile metabolites between the isolates of *P. chlamydosporia* and *A. cladodes* indicated the viability of joint growth of these fungi. The association of the fungi *P. chlamydosporia* and *A. cladodes*

presented a higher predatory capacity of infective larvae of the parasitic nematodes of bovines when compared to the predation of the fungi used alone. Therefore, under laboratory conditions, the fungi studied presented growth compatibility and the association of these increased the nematicidal activity against parasitic helminths of cattle.

**Author:** Wahyuni, S., Sunarso, S., Prasetiyono, B. W. H. E. and Satrija, F.

**Title:** Exploration of anthelmintic activity of *Cassia* spp. extracts on gastrointestinal nematodes of sheep

**DOI:** 10.5455/javar.2019.f338

**Abstract:** Objective: This study aimed to explore the phytochemical constituents and anthelmintic activities of four *Cassia* spp. leaves against *Haemonchus contortus*. Materials and Methods: The extracts were prepared from four species of *Cassia* spp. (*C. siamea*, *C. fistula*, *C. surattensis*, and *C. spectabilis*). Phytochemical screening of the extract was done based on the Harborne method. Evaluation of the anthelmintic activities against *H. contortus* was done in vitro using infective larvae (L3) migration inhibition assay (LMIA). Measurement of larvae migrating was conducted through a nylon filter with a pore size of 20 µm. The doses of *Cassia* spp. extract implemented were 25, 50, 100, and 200 mg/ml. Results: Tannins, alkaloids, phenol hydroquinone, flavonoids, steroids, triterpenoids, and saponins were present in all the extracts, whereas alkaloids were absent in *C. fistula*. No triterpenoids were found in *C. surattensis* and *C. spectabilis*. Movement of *H. contortus* larvae was significantly inhibited after exposure to *Cassia* extracts at various dosage levels ( $p < 0.05$ ). The test results using LMIA on L3 *H. contortus* showed the lowest inhibition in the negative control. Among the species of *Cassia*, the *C. surattensis* (at 200 mg/ml) showed the highest ( $p < 0.05$ ) inhibition level on the larvae. The latter result corresponded to the effect of albendazole. Conclusion: Compared to other *Cassia* spp., *C. surattensis* exhibited the highest inhibition against L3 *H. contortus*. However, the inhibition effect of *C. surattensis* was still lower as compared to albendazole.

**Author:** Wang, B. B., Zhang, N., Gong, P. T., Li, J. H., Yang, J., Zhang, H. B., Zhang, X. C. and Cai, K. Z.

**Title:** Morphological variability, molecular phylogeny, and biological characteristics of the nematophagous fungus *Duddingtonia flagrans*

**DOI:** 10.1002/jobm.201800610

**Abstract:** This study aims to investigate the molecular phylogenetic analysis, morphological variability, nematode-capturing ability, and other biological properties of Chinese *Duddingtonia flagrans* isolates. We isolated 13 isolates of *D. flagrans* and found features that have never been reported before, such as two to three septa including club-shaped conidia. Meanwhile, we conducted molecular phylogenetic analysis of the seven isolates and tested the radical growth of the isolates under different pH values, temperatures, and media. The capturing ability against infective larvae (L3) of *Cooperia*

spp. in yak was detected in vitro. Finally, one isolate was selected for scanning electron microscopy (SEM) to investigate the trap formation process. The fungal sequence was obtained and submitted to GenBank (Accession no. KY288614.1, KU881774.1, KP257593.1, KY419119.1, MF488979.1, MF488980.1, and MF488981.1), and the tested isolates were identified as *D. flagrans*. Except for three isolates, the radial growth of the other isolates on 2% corn meal agar and 2% water agar exhibited faster growth than on other media. The fungus could not grow at 10 and 40°C but grew within 11 to 30°C. Moreover, it did not grow at pH 1–3 and 13–14, but instead at pH 4–12. In the in vitro experimental, L3s were reduced by 94.36%, 88.15%, and 91.04% for SDH035, DH055, and F088, respectively. SEM results showed that at 8 hr post addition of nematodes, some of the latter were captured. In the later stages of the interaction of the fungus with nematodes, a large number of chlamydospores were produced, especially on the predation trap. Results of the present study provided information about the molecular phylogenetic analysis, morphological variability, nematode-capturing ability, and other biological properties of Chinese *Arthrobotrys flagrans* isolates before administering them for biocontrol.

**Author:** Zhong, W., Chen, Y., Gong, S., Qiao, J., Meng, Q., Zhang, X., Wang, X., Huang, Y., Tian, L. and Niu, Y.

**Title:** Enzymological properties and nematode-degrading activity of recombinant chitinase AO-379 of *arthrobotrys oligospora*

**DOI:** 10.9775/kvfd.2018.20603

**Abstract:** Chitinase is an important virulence factor produced by nematode trapping fungi in the process of infection, and plays an important role in the cleavage of nematodes and their eggshells. In this study, the cDNA sequence of *Arthrobotrys oligospora* chitinase AO-379 was amplified by RT-PCR and inserted into the vector pPIC9K to induce the expression of AO-379 in *Pichia pastoris* GS115. The recombinant AO-379 (reAO-379) was purified by nickel ion affinity chromatography, and enzymological properties and nematode-degrading activity of reAO-379 was analyzed. SDS-PAGE and Western blot analysis showed that the reAO-379 with molecular weight of about 44 kDa was successfully obtained. The reAO-379 showed strong chitinase activity at pH 5.5 and 30°C. Using reAO-379 to treat *Strongylus equinus*, *Caenorhabditis elegans* and *Haemonchus contortus* for 12, 24, and 36 h, the killing rates of reAO-379 in *S. equinus* were 42%, 89% and 100%; in *C. elegans* were 50%, 90% and 97%; in and *H. contortus* were 53%, 62% and 84%, respectively. Using reA-379 to treat *Fasciola hepatica* and *Dicrocoelium chinensis* eggs for 24, 48 and 72 h, the degradation rates of reAO-379 were 12%, 43% and 65% in *F. hepatica* eggs, and were 15%, 33% and 55% in *D. chinensis* eggs, respectively. Our study suggests that the reAO-379 is potentially valuable for development of biological control agent against digestive tract nematodes in livestock.

**Author:** Salles, H. O., Braga, A. C. L., Nascimento, D. R., Prado, M. S. M., Souza, H. A., Oliveira, E. L. D., Vieira, L. S., Cavalcante, A. C. R., Lima, A. R., Neto, C. S. T., Sousa, A. M. P., Ribeiro, R. P. and Pompeu, R. C. F. F.

**Title:** Crop residues activity against the free-living stages of small ruminant nematodes

**DOI:** 10.1590/s1984-29612019024

**Abstract:** The nematicidal effect of different organic materials was evaluated in order to develop a non-chemical alternative soil treatment for control of the free-living stages of small ruminant gastrointestinal nematodes. The selected organic materials were residues from the juice industry of acerola, cashew, grape, guava, papaya and pineapple, as well as castor residue from the biodiesel industry. LC90 results showed that pineapple residue was the most efficient inhibitor of larval development, followed by castor, grape, cashew, acerola, guava and papaya. Castor residue was also a good source of nitrogen and was used in a greenhouse experiment to prevent larval development in contaminated goat faeces that was deposited in pots containing the grasses *Brachiaria brizantha* (var. Paiaguás) or *Megathyrsus maximus* x *M. infestum* (var. Massai). Castor residue caused a significant ( $P < 0.05$ ) reduction (85.04%) in Paiaguás grass contamination (L3.dry mass-1) and a reduction of 17.35% in Massai grass contamination ( $P > 0.05$ ), with an increase in the biomass production of Massai (251.43%,  $P < 0.05$ ) and Paiaguás (109.19%,  $P > 0.05$ ) grasses. This strategy, called Econemat®, with good results in vitro shows to be promising on pasture increasing phytomass production.

**Author:** Figueroa, C., Mancebo, O., Scribano, V. and Rigonato, T.

**Title:** Effect of feed intake of *Leucaena leucocephala* on gastrointestinal parasitic load of weaned calves

**Abstract:** The present study was carried out during 105 days in Pilagás (Formosa, Argentina) to evaluate the effect of *Leucaena* sp consumed by weaned calves on the gastrointestinal parasitic load. Thirty calves were divided into two groups: grazing *Leucaena* (TREATED with/L;  $n=15$ ) or without consumption of *Leucaena* (TREATED without/L;  $n=15$ ). This essay was performed in a direct grazing system and animals were naturally infected under the conditions of Pilagás forage. Animals in the TREATED with/L group grazed in an interseeding of *Leucaena leucocephala* with *Brachiaria brizantha* while the TREATED without/L group grazed only on *B. brizantha*. Eggs per gram (EPG) were determined from feces. Fecal culture and identification of larvae were also performed for each experimental group. Hemotocrit (Ht) was measured and animals were weight. The main genera recovered in the fecal samples were: *Haemonchus*, *Trichostrongylus*, and *Bunostomum*. *Oesophagostomum*, *Cooperia* and *Strongyloides* were less abundant. Since the second sampling, animals with  $Ht \leq 22\%$  required albendazole administration since health was compromised. During the whole study, only one calf under TREATED with/L required treatment, whereas in the other group (TREATED without/L) nine animals needed treatment. In relation to EPG, significant differences were registered for the second and forth sampling ( $p$  value of 0.3 and 0.006, respectively) in favor of TREATED without/L

group. We conclude that under the conditions for the present study, *L. leucocephala* intake exerts an effect over EPG. Therefore, future assays should be performed for a better understanding regarding an alternative integral control of parasites.

**Author:** Macedo, I. T. F., Oliveira, L. M. B., André, W. P. P., Filho, J. V. A., Santos, J. M. L., Rondon, F. C. M., Ribeiro, W. L. C., Camurça-Vasconcelos, A. L. F., Oliveira, E. F., Paula, H. C. B. and Bevilaqua, C. M. L.

**Title:** Anthelmintic effect of *Cymbopogon citratus* essential oil and its nanoemulsion on sheep gastrointestinal nematodes

**DOI:** 10.1590/s1984-29612019065

**Abstract:** The anthelmintic resistance stimulated the search for strategies for controlling gastrointestinal nematodes, including the use of free essential oils or its nanoemulsion. This study evaluated the anthelmintic efficacy of *Cymbopogon citratus* essential oil (CcEO) and *C. citratus* essential oil nanoemulsion (CcEOn). Physicochemical analyses were performed. The *in vitro* effect was determined using the egg hatch test (EHT) on *Haemonchus contortus* and *in vivo* effect was evaluated in sheep infected with gastrointestinal nematodes. The animals were treated with CcEO (500 mg/kg) or CcEOn (450 mg/kg) for the fecal egg count (FEC) and the determination of worm burden. The main component of CcEO was citral. The CcEO content in the nanoemulsion was 20% (v/v), and the mean particle size was 248 nm. In EHT, CcEO and CcEOn (1.25 mg/mL) inhibited larval hatching by 98.4 and 97.1%, respectively. Three animals treated with CcEO died whereas in the group treated with CcEOn one animal died. The FEC and total worm burden of the treated groups did not differ from the negative control ( $p > 0.05$ ). The CcEOn showed efficacy only on *H. contortus* ( $p < 0.05$ ). In conclusion, nanoencapsulation reduced toxicity and increased efficacy on *H. contortus*.

**Author:** De Castro, L. L. D., Sprenger, L. K., Madrid, I. M., De Oliveira, F. C., De Oliveira, P. A., De Castro, L. M., Berne, M. E. A. and Leite, F. P. L.

**Title:** *In vitro* and *in vivo* effect of *Eugenia uniflora* extracts on gastrointestinal nematode of sheep

**DOI:** 10.1590/1089-6891v20e-49037

**Abstract:** This study evaluated the *in vitro* and *in vivo* action of *Eugenia uniflora* extracts on sheep gastrointestinal nematode. In the egg hatchability inhibition assay, aqueous and hydroalcoholic extracts were tested in the concentrations ranging from 40 to 1.25 mg/mL. The cytotoxic effect was measured using the MTT assay on VERO cells at concentrations ranging from 4000 to 1.95 µg/mL. The chemical composition was analyzed through the qualitative phytochemical screening. In the *in vivo* test, 100 mg/kg of the hydroalcoholic extract was administered orally for three days in sheep naturally infected and fecal collection was performed to estimate the egg count per gram of faeces (EPG). Different extracts of *E. uniflora* inhibited hatchability of eggs with a percentage of inhibition ranging

from 14.56 to 99.75%, being the hydroalcoholic most effective than the aqueous. In phytochemical analysis, compounds with anthelmintic action were observed, such as flavonoids, saponins, tannins and triterpenes. Extracts showed moderate to null cytotoxicity under the conditions tested. Reduction of EPG was observed only in control group (anthelmintic). The extracts of *E. uniflora* proved promising with in vitro action, requiring studies that assess other concentrations and forms of in vivo administration.

**Author:** Soares, A. M. D. S., Wanderley, L. F. and Junior, L. M. C.

**Title:** The potential of plant and fungal proteins in the control of gastrointestinal nematodes from animals

**DOI:** 10.1590/s1984-29612019046

**Abstract:** Gastrointestinal nematode infection is an important cause of high economic losses in livestock production. Nematode control based on a synthetic chemical approach is considered unsustainable due to the increasing incidence of anthelmintic resistance. Control alternatives such as the use of natural products are therefore becoming relevant from an environmental and economic point of view. Proteins are macromolecules with various properties that can be obtained from a wide range of organisms, including plants and fungi. Proteins belonging to different classes have shown great potential for the control of nematodes. The action of proteins can occur at specific stages of the nematode life cycle, depending on the composition of the external layers of the nematode body and the active site of the protein. Advances in biotechnology have resulted in the emergence of numerous protein and peptide therapeutics; however, few have been discussed with a focus on the control of animal nematodes. Here, we discuss the use of exogenous proteins and peptides in the control of gastrointestinal nematodes.

**Author:** Vilela, V. L. R., Da Costa, P. W. L., Da Silva, F. F., Figueiredo Bezerra, H. M. F., Silva De Oliveira, L. V., De Oliveira Dantas, M., Feitosa, T. F., De Araujo, J. V. and Braga, F. R.

**Title:** Predatory viability of the nematophagous fungus *duddingtonia flagrans* on the infective larvae of gastrointestinal nematodes of sheep following storage and refrigeration

**DOI:** 10.11606/issn.1678-4456.bjvras.2019.154252

**Abstract:** The objective of this study was to evaluate the in vivo predatory viability of the nematophagous fungus, *Duddingtonia flagrans*, after storage (36 months) and refrigeration (2-8 °C). This viability was evaluated using the infective larvae of gastrointestinal nematodes of sheep in the Northeastern semi-arid region of Brazil. Sixteen Santa Ines sheep with negative counting of eggs per gram of feces (EPG) were divided into four experimental groups, each group comprised of four animals. The pellets were administered at the dose of 3 g/10 kg of live weight (20% fungal micelyum), and a single administration was performed for each animal. Group I was administered pellets that had been stored for 36 months; Group II, freshly produced pellets; Group III, freshly produced



pellets that did not contain fungi; and Group IV, pellets were not administered, and this was the control group. Feces were collected for 5 days, every 24 h for analysis. There was a significant decrease in the number of infective larvae of sheep nematodes that received *D. flagrans* pellets in a sodium alginate matrix, 82% was observed for Group I and 71% for Group II, compared to the control group. It is therefore concluded that the fungus, *D. flagrans*, pelleted in sodium alginate matrix after 36 months of storage at 2-8 °C, showed efficacy in reducing the number of infective larvae of gastrointestinal nematodes of sheep.

## Host Resistance to infection

**Author:** Ali, A. O. A., Murphy, L., Stear, A., Fairlie-Clarke, K., Nikbakht Brujeni, G., Donskow-Łysoniewska, K., Groth, D., Buitkamp, J. and Stear, M. J.

**Title:** Association of MHC class II haplotypes with reduced faecal nematode egg count and IgA activity in British Texel sheep

**DOI:** 10.1111/pim.12626

**Abstract:** Nematode infection is one of the principal diseases suffered by sheep and the class II region of the MHC has been repeatedly associated with differences in susceptibility and resistance to infection. The aim of this study was to examine the association of MHC class II haplotypes in a flock of Texel sheep with faecal egg counts and antibody responsiveness. Two haplotypes carried the DRB1\*11:01 allele which has previously been associated with reduced egg counts in Scottish Blackface and Suffolk sheep. One of the two haplotypes was associated with reduced egg counts in the Texel breed, and both haplotypes were associated with reduced IgA activity against an extract from fourth-stage larvae. The reduced IgA activity is probably a consequence of reduced numbers of fourth-stage larvae in sheep carrying the resistance allele. The association of specific MHC alleles with reduced egg counts, reduced worm numbers and decreased IgA activity provides a mechanism for the density-dependent regulation of parasite growth and fecundity.

**Author:** Fairlie-Clarke, K., Kaseja, K., Sotomaior, C., Brady, N., Moore, K. and Stear, M.

**Title:** Salivary IgA: A biomarker for resistance to *Teladorsagia circumcincta* and a new estimated breeding value

**DOI:** 10.1016/j.vetpar.2019.04.005

**Abstract:** *Teladorsagia circumcincta* is the dominant nematode of sheep in cool, temperate climates. Faecal nematode egg counts (FEC) are widely used to identify the intensity of infection and as a measure of host resistance to nematodes. However due to density-dependent effects on worm fecundity the relationship between FEC and worm burden is not linear. In addition collecting FEC data is challenging on a practical level and there is a need for more reliable markers of resistance. There are two major known mechanisms of immunity to *T. circumcincta*: IgE against third stage larvae (L3) and IgA

against fourth stage larvae (L4), which inhibits parasite growth. In this study salivary IgA responses were measured in over 5000 animals against L3 antigen by Enzyme Linked Immunosorbent Assay (ELISA). Antigen-specific IgA levels were negatively correlated with FEC ( $r=-0.26$ ,  $SE = 0.02$ ) and were heritable ( $h^2 = 0.16$ ,  $SE = 0.04$ ) indicating that they can be used to identify resistant animals suitable for inclusion in selective breeding programs. Antigen-specific IgA responses were not negatively correlated with muscle depth. Our analyses indicate that selection for *T. circumcincta* L3 antigen-specific IgA is possible without impacting on the production traits for the Lleyn breed.

**Author:** Stear, A., Ali, A. O. A., Brujeni, G. N., Buitkamp, J., Donskow-Łysoniewska, K., Fairlie-Clarke, K., Groth, D., Isa, N. M. M. and Stear, M. J.

**Title:** Identification of the amino acids in the Major Histocompatibility Complex class II region of Scottish Blackface sheep that are associated with resistance to nematode infection

**DOI:** 10.1016/j.ijpara.2019.05.003

**Abstract:** Lambs with the Major Histocompatibility Complex DRB1\*1101 allele have been shown to produce fewer nematode eggs following natural and deliberate infection. These sheep also possess fewer adult *Teladorsagia circumcincta* than sheep with alternative alleles at the DRB1 locus. However, it is unclear if this allele is responsible for the reduced egg counts or merely acts as a marker for a linked gene. This study defined the MHC haplotypes in a population of naturally infected Scottish Blackface sheep by PCR amplification and sequencing, and examined the associations between MHC haplotypes and faecal egg counts by generalised linear mixed modelling. The DRB1\*1101 allele occurred predominately on one haplotype and a comparison of haplotypes indicated that the causal mutation or mutations occurred in or around this locus. Additional comparisons with another resistant haplotype indicated that mutations in or around the DQB2\*GU191460 allele were also responsible for resistance to nematode infections. Further analyses identified six amino acid substitutions in the antigen binding site of DRB1\*1101 that were significantly associated with reductions in the numbers of adult *T. circumcincta*.

**Author:** Aboshady, H. M., Mandonnet, N., Stear, M. J., Arquet, R., Bederina, M., Sarry, J., Tosser-Klopp, G., Klopp, C., Johansson, A. M., Jonas, E. and Bambou, J. C.

**Title:** Transcriptome variation in response to gastrointestinal nematode infection in goats

**DOI:** 10.1371/journal.pone.0218719

**Abstract:** Gastrointestinal nematodes (GIN) are a major constraint for small ruminant production. Due to the rise of anthelmintic resistance throughout the world, alternative control strategies are needed. The development of GIN resistance breeding programs is a promising strategy. However, a better understanding of the mechanisms underlying genetic resistance might lead to more effective breeding programmes. In this study, we

compare transcriptome profiling of abomasal mucosa and lymph node tissues from non-infected, resistant and susceptible infected Creole goats using RNA-sequencing. A total of 24 kids, 12 susceptible and 12 GIN resistant based on the estimated breeding value, were infected twice with 10,000 L3 *Haemonchus contortus*. Physiological and parasitological parameters were monitored during infection. Seven weeks after the second infection, extreme kids (n = 6 resistant and 6 susceptible), chosen on the basis of the fecal egg counts (FEC), and 3 uninfected control animals were slaughtered. Susceptible kids had significantly higher FEC compared with resistant kids during the second infection with no differences in worm burden, male and female worm count or establishment rate. A higher number of differentially expressed genes (DEG) were identified in infected compared with non-infected animals in both abomasal mucosa (792 DEG) and lymph nodes (1726 DEG). There were fewer DEG in resistant versus susceptible groups (342 and 450 DEG, in abomasal mucosa and lymph nodes respectively). 'Cell cycle' and 'cell death and survival' were the main identified networks in mucosal tissue when comparing infected versus non-infected kids. Antigen processing and presentation of peptide antigen via major histocompatibility complex class I were in the top biological functions for the DEG identified in lymph nodes. The TGF $\beta$ 1 gene was one of the top 5 upstream DEG in mucosal tissue. Our results are one of the first investigating differences in the expression profile induced by GIN infection in goats.

**Author:** Escribano, C., Saravia, A., Costa, M., Castells, D., Ciappesoni, G., Riet-Correa, F. and Freire, T.

**Title:** Resistance to *Haemonchus contortus* in Corriedale sheep is associated to high parasite-specific IgA titer and a systemic Th2 immune response

**DOI:** 10.1038/s41598-019-55447-6

**Abstract:** Gastrointestinal nematode infections, including *Haemonchus contortus*, are one of the main causes of economic losses to ovine farmers worldwide. In order to contribute to the control of nematode infections and avoid parasite spreading we generated divergent resistant and susceptible sheep breeds and evaluated the adaptive immunity of these animals developed upon experimental infection against *H. contortus*. The selection of resistant or susceptible animals from the Corriedale Breed has been based on Expected Progeny Differences for faecal egg counts per gram. Furthermore, animals from the resistant Corriedale line were inseminated with imported semen from Australian Rylington Merino rams. Thus, the objective of this work was to analyze the adaptive immune response in both susceptible and resistant obtained lambs. Our results indicate that there is a potent parasite-specific local and systemic immune response in resistant animals and that although susceptible lambs can produce high levels of IgA antibodies during the infection, their antibody response is delayed which, together with an impaired specific-Th2 response, does not contribute to initial parasite elimination. Our results shed light into the immune mechanisms that mediate resistance to *H. contortus* and could constitute important assets to sheep farmers, not only as a means to detect resistance, but also to enhance the efficiency of selection in stud flocks.

**Author:** Greeff, J. C., Liu, S. M., Palmer, D. G. and Karlsson, L. J. E.

**Title:** Expression of worm resistance in sheep selected for low worm-egg counts fed at maintenance or above-maintenance level

**DOI:** 10.1071/an18552

**Abstract:** The present study reports on changes in faecal worm-egg counts (WEC), larval composition, and the number of worms at different developmental stages in young sheep sourced from a flock selected for reduced faecal worm-egg counts over 15 years. The sheep were individually penned and fed a maintenance (1.0 M) or a 1.5 times maintenance (1.5 M) diet over two periods, namely, worm-free and infection phases. They were dosed weekly with 10 000 *Trichostrongylus colubriformis* and 10 000 *Teladorsagia circumcincta* L3 infective larvae for 11 weeks. Sheep on the 1.5 M diet had lower WEC and higher bodyweights than did sheep on the 1.0 M diet. A significant decline in the percentage *T. colubriformis* occurred during the experiment, but no concomitant change in *T. circumcincta* was noticed. Resistant sheep had significantly ( $P < 0.001$ ) fewer worms at necropsy, and also shed significantly ( $P < 0.001$ ) fewer worm eggs during the experiment. Restricted feeding reduced bodyweight significantly ( $P < 0.001$ ) and had a small but significant ( $P < 0.04$ ) effect on the faecal worm-egg output and on the number of *T. colubriformis* worms ( $P < 0.01$ ) in both the control and resistant sheep. The study showed that sheep selected for low WEC resulted in significantly ( $P < 0.001$ ) lower WEC than in an unselected control line fed at a maintenance (1.0 M) and at an above-maintenance (1.5 M) level. Restricted feeding reduced bodyweight and had a small negative, and inconsistent, effect on the faecal worm-egg output in both the control and resistant lines.

**Author:** Hamie, J. C., McAnulty, R. W., Logan, C. M., Lundberg, S. and Greer, A. W.

**Title:** Comparison of the timing of development of immunity in Romney lambs from resistant and resilient selection lines

**DOI:** 10.1016/j.vetpar.2019.04.014

**Abstract:** Variation in the timing of development of immunity to gastro-intestinal nematode parasites was assessed in resistant and resilient Romney selection lines exposed to mixed natural infection. From weaning, at mean 92 days-of-age, animals ( $n = 53$ ) were sampled for faecal egg count (FEC) expressed as eggs per gram of faeces (epg), saliva for immunoglobulin (IgG and IgA) determination and fasted live weight (LW) every 10 days until 351 days-of-age. Overall, mean back-transformed FEC were consistently low for resistant animals ( $<200$  epg) whereas resilient counterparts' FEC increased with time to reach a peak of 1400 epg at day 230 for females and 1800 epg for males at day 280 before declining to less than 500 epg by day 300, respectively ( $P < 0.001$ ). Resistant lambs reached a threshold for *Trichostrongylus colubriformis* L3-specific IgG which was indicative of the presence of immunity earlier at  $220.6 \pm 8.8$  days-of-age compared with resilient-line animals which reach this threshold 40 days later at  $263.4 \pm 6.9$  days-of-age ( $P < 0.001$ ). In addition, resistant females reached sexual maturity earlier compared with their

resilient counterparts viz.  $263.5 \pm 3.7$  c.f.  $274.4 \pm 3.4$  days-of-age, respectively, ( $P = 0.048$ ). Mean fasted live weight (LW) showed a selection line by time interaction ( $P < 0.001$ ) which reflected greater LW in the early phase of the study in resilient males but increasing for all groups until day 280 before declining and being similar for all groups from day 330. In summary, differences appear to exist in the timing of immune development between these Romney lines, with resistant animals developing immunity earlier and these resistant-line animals also appear to be more physiologically mature at the same chronological age than resilient animals. These observations have implications on the timing of identification and selection of resistant animals.

**Author:** May, K., Weimann, C., Scheper, C., Strube, C. and König, S.

**Title:** Allele substitution and dominance effects of CD166/ALCAM gene polymorphisms for endoparasite resistance and test-day traits in a small cattle population using logistic regression analyses

**DOI:** 10.1007/s00335-019-09818-z

**Abstract:** The study investigated the effects of four single-nucleotide polymorphisms (SNPs) in the activated leukocyte cell adhesion molecule (ALCAM) gene on liver fluke (*Fasciola hepatica*) infections (FH-INF), gastrointestinal nematode infections (GIN-INF) and disease indicator traits [e.g. somatic cell score (SCS), fat-to-protein ratio (FPR)] in German dual-purpose cattle (DSN). A genome-wide association study inferred the chip SNP ALCAMc.73+32791A>G as a candidate for *F. hepatica* resistance in DSN. Because of the crucial function of ALCAM in immune responses, SNPs in the gene might influence further resistance and performance traits. Causal mutations were identified in exon 9 (ALCAMc.1017T>C) and intron 9 (ALCAMc.1104+10T>A, ALCAMc.1104+85T>C) in a selective subset of 94 DSN cows. We applied logistic regression analyses for the association between SNP genotypes with residuals for endoparasite traits (rINF-FH, rGIN-INF) and estimated breeding values (EBVs) for test-day traits. The probability of the heterozygous genotype was estimated in dependency of the target trait. Allele substitution effects for rFH-INF were significant for all four loci. The T allele of the SNPs ALCAMc.1017T>C and ALCAMc.1104+85T>C was the favourable allele when improving resistance against FH-INF. Significant allele substitution for rGIN-INF was only found for the chip SNP ALCAMc.73+32791A>G. We identified significant associations between the SNPs with EBVs for milk fat%, protein% and FPR. Dominance effects for the EBVs of test-day traits ranged from 0.00 to 0.47 SD and were in the direction of improved resistance for rFH-INF. We estimated favourable dominance effects from same genotypes for rFH-INF and FPR, but dominance effects were antagonistic between rFH-INF and SCS.

**Author:** Rupp, R., Huau, C., Caillat, H., Fassier, T., Bouvier, F., Pampouille, E., Clément, V., Palhière, I., Larroque, H., Tosser-Klopp, G., Jacquet, P. and Rainard, P.

**Title:** Divergent selection on milk somatic cell count in goats improves udder health and milk quality with no effect on nematode resistance

**DOI:** 10.3168/jds.2018-15664

**Abstract:** Milk somatic cell count (SCC) is commonly higher in goats than in cattle and sheep. Furthermore, the ability of milk SCC to predict mastitis is considered lower in goats than in cattle and sheep, and the relevance of somatic cell score (SCS)-based selection in this species has been questioned. To address this issue, we created 2 divergent lines of Alpine goats using artificially inseminated bucks with extreme estimated breeding values for SCS. A total of 287 goats, 158 in high- and 129 in low-SCS lines, were scrutinized for mastitis infections. We subjected 2,688 milk samples to conventional bacteriological analyses on agarose and bacterial counts were estimated for positive samples. The SCS, milk yield, fat content, and protein content were recorded every 3 wk. Clinical mastitis was systematically noted. A subset of 40 goats (20 from each line) was subsequently challenged with *Haemonchus contortus* and monitored for anemia (blood packed cell volume) and fecal egg counts to see if SCS-based selection had an indirect effect on resistance to gastrointestinal nematodes. Milk production traits, including milk quantity, fat content, and protein content, were similar in both goat lines. In contrast, the raw milk SCC almost doubled between the lines, with 1,542,000 versus 855,000 cells/mL in the high- and low-SCS lines, respectively. The difference in breeding value for SCS between lines was 1.65 genetic standard deviation equivalents. The *Staphylococcus* spp. most frequently isolated from milk were *S. xylosum*, *S. caprae*, *S. epidermidis*, and *S. aureus*. The frequency of positive bacteriology samples was significantly higher in the high-SCS line (49%) than in the low-SCS line (33%). The highest odds ratio was 3.49 (95% confidence interval: 11.95–6.25) for *S. aureus*. The distribution of bacterial species in positive samples between lines was comparable. The average quantity of bacteria in positive samples was also significantly higher in high-SCS goats ( $69 \pm 80$  growing colonies) than in low-SCS goats ( $38 \pm 62$  growing colonies). Clinical cases were rare and equally distributed between high- ( $n = 4$ ; 2.5%) and low-SCS ( $n = 3$ ; 2.3%) lines. Furthermore, the larger the amounts of bacteria in milk the higher the SCS level. Conversely, goats with repeatedly culture-negative udders exhibited the lowest SCC levels, with an average of below 300,000 cells/mL. We therefore confirmed that SCS is a relevant predictor of intramammary infection and hygienic quality of milk in goats and can be used for prophylactic purposes. After challenge with *H. contortus*, goats were anemic with high fecal egg counts but we found no difference between the genetic lines. This result provides initial evidence that resistance to mastitis or to gastrointestinal nematodes infections is under independent genetic regulation. Altogether, this monitoring of the goat lines indicated that SCS-based selection helps to improve udder health by decreasing milk cell counts and reducing the incidence of infection and related bacterial shedding in milk. Selection for low SCC should not affect a goat's ability to cope with gastrointestinal nematodes.

**Author:** Zhao, Z., Wang, M., Liu, S., Palmer, D., Shaw, R., Karlsson, J., Vercoe, P. E., Martin, G. B. and Greeff, J.

**Title:** Heritabilities of IgA and IgE activities against *Teladorsagia* and *Trichostrongylus* L3 larval antigens correlated with traits for faecal worm egg count, health and productivity in Merino sheep

**DOI:** 10.1071/an18630

**Abstract:** Low faecal worm-egg count (FEC) has been used as a phenotypic trait for breeding sheep for resistance to nematode infection. Application of this trait has resulted in a major reduction in worm-egg output in faeces, but in certain environments some of the resistant sheep develop what is believed to be a hypersensitivity-associated diarrhoea, resulting in soiling of the breech area (dags). To avoid breeding sheep with a propensity to scour, it, therefore, seems logical to combine selection for low FEC with selection for a trait based on the immune response to worm infection. We, therefore, investigated the relationships between immunoglobulin A (IgA) and immunoglobulin E (IgE) activities against L3 larval antigens of *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* and measured their inheritance and their genetic relationships with FEC, dag traits and measures of production. Total plasma IgE concentrations were also determined and included in the study. We used 762 progeny from 23 Merino sires selected for resistance to helminths (the 'Rylington Flock') and to breech flystrike. Total plasma IgE and IgE activities against *T. circumcincta* were negatively correlated with liveweight, longissimus dorsi muscle depth, and fleece weight, but positively correlated with severity of dags, softer faeces and back-fat depth. The IgA activity against *T. circumcincta* was negatively correlated with liveweight and clean fleece weight, but positively correlated with back-fat depth and faecal consistency score. The IgA activity against *T. colubriformis* was not correlated with any measure, except the breeding value for dags around yearling age. The heritability for the IgA activity against *T. circumcincta* and *T. colubriformis* was 0.13 and 0.08 respectively. The heritability for both IgE activity against *T. circumcincta* and total plasma IgE concentration was 0.39. We conclude that it is feasible to combine FEC and dag traits with an immune trait (in this case, helminth-specific IgE activity and total plasma IgE concentration) and, thus, genetically select sheep for resistance to both helminths and diarrhoea. However, under natural worm-challenge conditions, blood sampling protocols need to be developed, on the basis of animal age, season and environment, for optimum estimation of the immune trait and its relationships with other traits.

**Author:** Bell, A., McNally, J., Smith, D. V., Rahman, A., Hunt, P., Kotze, A. C., Dominik, S. and Ingham, A.

**Title:** Quantification of differences in resistance to gastrointestinal nematode infections in sheep using a multivariate blood parameter

**DOI:** 10.1016/j.vetpar.2019.05.007

**Abstract:** Breeding for resistance to gastrointestinal nematodes (GIN) in sheep relies largely on the use of worm egg counts (WEC) to identify animals that are able to resist infection. As an alternative to such measures of parasite load we aimed to develop a method to identify animals showing resistance to GIN infection based on the impact of the infection on blood parameters. We hypothesized that blood parameters may provide a measure of infection level with a blood-feeding parasite through perturbation of red blood cell parameters due to feeding behaviour of the parasite, and white blood cell parameters through the mounting of an immune response in the host animal. We measured a set of blood parameters in 390 sheep that had been exposed to an artificial regime of repeated challenges with *Trichostrongylus colubriformis* followed by *Haemonchus contortus*. A simple analysis revealed strong relationships between single blood parameters and WECs with correlation coefficients  $-0.54$  to  $-0.60$ . We then used more complex multi-variate methods based on supervised classifier models (including Bayesian Network) as well as regression models (Lasso and Elastic Net) to study the relationships between WECs and blood parameters, and derived algorithms describing the relationships. The ability of these algorithms to classify sheep GIN resistance status was tested using the WEC and blood parameters collected from a different group of 418 sheep that had acquired natural infections of *H. contortus* from pasture. We identified the most resistant and most susceptible animals (10% percentiles) of this group based on WECs, and then compared the identities of these animals to the identities of animals that were predicted to be most resistant and most susceptible by our algorithms. The models showed varying abilities to predict susceptible and resistant sheep, with up to 65% of the most susceptible animals and 30% of the most resistant animals identified by the Elastic Net model algorithms. The prediction algorithms derived from female sheep data performed better than those for male sheep in some cases, with the predicted animals accounting for up to 50–60% of the actual resistant and susceptible female animals. Heritability values were calculated for blood parameters and the aggregate trait descriptions defined by the novel prediction algorithms. The aggregate trait descriptions were moderately heritable and may therefore be suitable for use in genetic selection strategies. The present study indicates that multivariate models based on blood parameter data showed some ability to predict the resistance status of sheep to infection with *H. contortus*.

## Diagnosis

**Author:** Baltrušis, P., Halvarsson, P. and Höglund, J.

**Title:** Molecular detection of two major gastrointestinal parasite genera in cattle using a novel droplet digital PCR approach

**DOI:** 10.1007/s00436-019-06414-7

**Abstract:** *Cooperia* sp. and *Ostertagia* sp. are two cosmopolitan parasitic nematodes often found in mixed gastrointestinal infections in cattle across temperate regions. In light of the recent increase in the emergence of anthelmintic resistance in these and other nematodes derived from cattle around the globe, and their negative impact on animal



health and productivity, novel molecular assays need to be put forth in order to facilitate the monitoring of parasite burden in infected herds, using pasture and/or fecal samples. Here, we describe a novel droplet digital PCR platform-based concept for precise identification and quantification of the two most abundant and important parasite genera in grazing western European cattle. By exploiting a single nucleotide difference in the two parasites' ITS2 sequence regions, we have developed two specific hydrolysis probes labeled with FAM™ or HEX™ fluorophores, which can not only distinguish between the DNA sequences of the two, but also quantify them in mixed DNA samples. A third, newly developed universal probe was also tested along the genus-specific probes to provide a robust and accurate reference. It was evident that the universal probe displayed congruent results to those obtained by the genus-specific probes when used with DNA from both parasites in a single sample. All in all, the results of our assay suggest that this novel protocol could be used to distinguish and quantify cattle parasites belonging to the two most important genera (i.e., *Cooperia* and *Ostertagia*) in a single mixed DNA sample.

**Author:** Doyle, S. R., Sankaranarayanan, G., Allan, F., Berger, D., Jimenez Castro, P. D., Collins, J. B., Crellen, T., Duque-Correa, M. A., Ellis, P., Jaleta, T. G., Laing, R., Maitland, K., McCarthy, C., Moundai, T., Softley, B., Thiele, E., Ouakou, P. T., Tushabe, J. V., Webster, J. P., Weiss, A. J., Lok, J., Devaney, E., Kaplan, R. M., Cotton, J. A., Berriman, M. and Holroyd, N.

**Title:** Evaluation of DNA Extraction Methods on Individual Helminth Egg and Larval Stages for Whole-Genome Sequencing

**DOI:** 10.3389/fgene.2019.00826

**Abstract:** Whole-genome sequencing is being rapidly applied to the study of helminth genomes, including de novo genome assembly, population genetics, and diagnostic applications. Although late-stage juvenile and adult parasites typically produce sufficient DNA for molecular analyses, these parasitic stages are almost always inaccessible in the live host; immature life stages found in the environment for which samples can be collected non-invasively offer a potential alternative; however, these samples typically yield very low quantities of DNA, can be environmentally resistant, and are susceptible to contamination, often from bacterial or host DNA. Here, we have tested five low-input DNA extraction protocols together with a low-input sequencing library protocol to assess the feasibility of whole-genome sequencing of individual immature helminth samples. These approaches do not use whole-genome amplification, a common but costly approach to increase the yield of low-input samples. We first tested individual parasites from two species spotted onto FTA cards—egg and L1 stages of *Haemonchus contortus* and miracidia of *Schistosoma mansoni*—before further testing on an additional five species—*Ancylostoma caninum*, *Ascaridia dissimilis*, *Dirofilaria immitis*, *Strongyloides stercoralis*, and *Trichuris muris*—with an optimal protocol. A sixth species—*Dracunculus medinensis*—was included for comparison. Whole-genome sequencing followed by analyses to determine the proportion of on- and off-target mapping revealed successful sample preparations for six of the eight species tested with variation both between species and between different life stages from some species described. These results demonstrate the

feasibility of whole-genome sequencing of individual parasites, and highlight a new avenue toward generating sensitive, specific, and information-rich data for the diagnosis and surveillance of helminths.

**Author:** Feucherolles, M., Poppert, S., Utzinger, J. and Becker, S. L.

**Title:** MALDI-TOF mass spectrometry as a diagnostic tool in human and veterinary helminthology: A systematic review

**DOI:** 10.1186/s13071-019-3493-9

**Abstract:** Background: Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry (MS) has become a widely used technique for the rapid and accurate identification of bacteria, mycobacteria and certain fungal pathogens in the clinical microbiology laboratory. Thus far, only few attempts have been made to apply the technique in clinical parasitology, particularly regarding helminth identification. Methods: We systematically reviewed the scientific literature on studies pertaining to MALDI-TOF MS as a diagnostic technique for helminths (cestodes, nematodes and trematodes) of medical and veterinary importance. Readily available electronic databases (i.e. PubMed/MEDLINE, ScienceDirect, Cochrane Library, Web of Science and Google Scholar) were searched from inception to 10 October 2018, without restriction on year of publication or language. The titles and abstracts of studies were screened for eligibility by two independent reviewers. Relevant articles were read in full and included in the systematic review. Results: A total of 84 peer-reviewed articles were considered for the final analysis. Most papers reported on the application of MALDI-TOF for the study of *Caenorhabditis elegans*, and the technique was primarily used for identification of specific proteins rather than entire pathogens. Since 2015, a small number of studies documented the successful use of MALDI-TOF MS for species-specific identification of nematodes of human and veterinary importance, such as *Trichinella* spp. and *Dirofilaria* spp. However, the quality of available data and the number of examined helminth samples was low. Conclusions: Data on the use of MALDI-TOF MS for the diagnosis of helminths are scarce, but recent evidence suggests a potential role for a reliable identification of nematodes. Future research should explore the diagnostic accuracy of MALDI-TOF MS for identification of (i) adult helminths, larvae and eggs shed in faecal samples; and (ii) helminth-related proteins that are detectable in serum or body fluids of infected individuals.

**Author:** Singh, B., Flampouri, E. and Dempsey, E.

**Title:** Electrochemical enzyme-linked immunosorbent assay (e-ELISA) for parasitic nematode: *Ostertagia ostertagi* (brown stomach worm) infections in dairy cattle

**DOI:** 10.1039/c9an00982e

**Abstract:** A sensitive electrochemical immunoassay (e-ELISA) has been developed for the detection of the gastrointestinal parasitic nematode *Ostertagia ostertagi* (brown stomach worm) in infected and control serum samples. An antigen-indirect immunoassay

format was employed to detect the presence of *O. ostertagi* antibodies, coupled with an anti-species monoclonal horseradish peroxidase (HRP) conjugate. ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) and TMB (3,3',5,5'-tetramethylbenzidine/hydrogen peroxide) were investigated as both chromogenic visualising reagents for optical ELISA and electroactive substrates for electrochemical ELISA in the HRP catalysed oxidation reaction. Coulometry was applied for the detection of *O. ostertagi* antibodies (via TMB electrochemistry) and compared with the commercial optical ELISA (ABTS based SVANOVIR® *O. ostertagi*-Ab ELISA kit). Cost-effective in-house sensors were designed and fabricated using polyester and chemical adhesive materials with the aid of stencil printing and laser machining techniques. The performance of the electrochemical ELISA and sensor was evaluated by investigating redox mediators (ABTS vs. TMB), stop solutions (sodium dodecyl sulfate vs. sulfuric acid) and incubation times (150 min vs. 70 min vs. 25 min). For a total assay incubation time of 70 minutes, the TMB/H<sub>2</sub>SO<sub>4</sub> based e-ELISA was able to differentiate between positive (P) and negative (N) control serum samples, with a P/N<sub>70</sub> control ratio 1.6 times higher than that of optical ELISA (TMB/H<sub>2</sub>SO<sub>4</sub> combination) and 2.9 times higher than that of the commercial ELISA kit (ABTS/SDS combination). Furthermore, the e-ELISA approach is quicker and required only 25 min (total incubation time) with even better response (P/N<sub>25</sub> = 14.7), which is approximately 4-fold higher than the optical immunoassay (P/N<sub>25</sub> = 3.8). The proposed e-ELISA is specific (selective Ab-Ag interactions) and highly sensitive-capable of detecting up to 16-fold dilutions of a positive control serum sample. The electrochemical ELISA approach has the potential for rapid sample screening in a portable, disposable format, contributing to the quest for effective prevention and control of parasitic *Ostertagia ostertagi* infections in cattle.

**Author:** Wong, S. S. Y., Poon, R. W. S., To, K. K. W., Chan, J. F. W., Lu, G., Xing, F., Cheng, V. C. C. and Yuen, K. Y.

**Title:** Improving the specific diagnosis of trematode, cestode and nematode infections by a multiplex single-tube real-time PCR assay

**DOI:** 10.1136/jclinpath-2018-205590

**Abstract:** Aims Helminth infections are becoming uncommon in high-income countries and laboratory staff may lose expertise in their morphological identification, especially in histological sections where speciation of helminths is challenging. Commercially available molecular diagnostic panels for faecal specimens only offer tests for protozoa but not helminths. We aim to improve the identification accuracy of helminths using a multiplex PCR assay. Methods We designed three pairs of PCR primers and probes targeting multicopy genes for a multiplex single-tube real-time PCR assay which covers 16 trematode (28S rRNA gene), 24 cestode (cox1 gene) and 33 nematode (cox1 gene) species. Helminths (n=27) from faecal samples (n=10), fresh parasites (n=11), formalin-fixed specimens (n=4), cerebrospinal fluid (n=1) and bile (n=1) were examined morphologically and tested by PCR. Fifty stool samples negative for parasites by microscopy were also tested. Results The PCR assay correctly identified the genera of all tested helminths. Agarose gel electrophoresis and sequencing of the purified PCR

amplicons confirmed that the PCR products were of correct sizes with 100% correlation with the respective species. Sequencing of the *cox1* gene failed to identify *Capillaria* spp. in one sample owing to the lack of corresponding sequences in GenBank. PCR and sequencing of the nematode 18S rRNA gene using consensus primers showed 100% homology with *Capillaria* spp. sequence. No positive PCR products were found in the negative stool samples. Conclusions The highly specific test correctly identified all helminths in our cohort. It is a useful adjunct to helminth identification in difficult situations such as histological sections.

## Anthelmintics

A qualitative investigation of the attitudes and practices of farmers and veterinarians in Wales regarding anthelmintic resistance in cattle

Klaudya Charlton and Philip A. Robinson

*Veterinaria Italiana* 2019, **55** (4), 327-337. doi: 10.12834/VetIt.1848.9845.3

Despite the importance of stakeholder practices in the potential development of anthelmintic resistance (AR) in livestock, there is a lack of qualitative research examining the attitudes and behaviours of anthelmintic end users and their professional advisors. Given the increasing importance of developing anthelmintic resistance patterns in cattle, and the need to avoid AR in cattle developing to the same extent as it has in sheep, the objective of this qualitative study was therefore to assess the factors affecting anthelmintic product choice and usage, and awareness and attitudes towards AR in cattle in north Wales. Twelve semi-structured face-to-face interviews were conducted with nine cattle farmers and three veterinarians. Farmer knowledge and engagement with the issue of AR in cattle in this study was low. A lack of perceived threat was apparent, with only a demonstrable problem at farm level the likely incentive to change future worming protocols and practice. Cost had a very prominent influence on anthelmintic product choice, but importance was also given to product recommendations from social farming networks and other non-veterinary advisory sources. A more proactive approach should be taken to raise farmers' and veterinarians' awareness of increasing levels of AR in cattle and improve anthelmintic governance.

**Author:** Fazzio, L., Moreno, L., Galvan, W., Canton, C., Alvarez, L., Streitenberger, N., Sánchez, R., Lanusse, C. and Sanabria, R.

**Title:** Pharmacokinetic profile and anthelmintic efficacy of moxidectin administered by different doses and routes to feedlot calves

**DOI:** 10.1016/j.vetpar.2018.12.016

**Abstract:** We evaluated the comparative plasma disposition kinetics and efficacy of moxidectin (MXD), administered by the intraruminal (IR) or subcutaneous (SC) route at two different dosage levels (0.2 and 1 mg/kg) in feedlot calves. Additionally, the efficacy

was compared to an ivermectin (IVM, SC administration) treated group. This study was divided into two separate studies, the “Pharmacokinetic (PK) study” and the “Efficacy study”. The “PK study” involved 24 calves free of gastrointestinal nematodes (GIN), which were allocated into 4 groups (n = 6) and treated with MXD by either the SC or the IR route at the therapeutic (MXD SC0.2, MXD IR0.2, respectively) or at fivefold the therapeutic dose (MXD SC1.0, MXD IR1.0 , respectively). Blood samples were collected from 3 h up to 14 days post-treatment. MXD concentrations in plasma samples were analyzed by HPLC. The “Efficacy study” included 125 calves naturally infected with GIN, which were allocated into five experimental groups (n = 25 each); the same four MXD-treated groups described for the “PK study”, and an additional group treated by the SC route with IVM (IVM SC0.2 ). The efficacy of IVM given at its therapeutic dose and the different MXD groups at the therapeutic and fivefold the therapeutic dose was calculated by analysis of the individual efficacy using the package eggCounts-2.1-1' on the R software environment, version 3.5.0 (R Core Team, 2018). Daily weight gain (DWG) was also measured over the first 47 days of the fattening cycle. Independently of the administration route, MXD peak plasma concentration (C max ) and area under the concentration-time curve (AUC) were higher in groups treated with the higher dose (1.0 mg/kg), whereas a longer time to reach C max (T max ) was observed after the IR treatments. The observed MXD efficacies were 85% (MXD SC0.2 ), 94% (MXD SC1.0 ), 84% (MXD IR0.2 ) and 99% (MXD IR1.0 ), at day +27. At day +27, all MXD-treated groups showed higher efficacies than the group having received IVM (45%). The post-treatment *Cooperia* spp. L 3 counts were particularly low in the groups MXD SC1.0 and MXD IR1.0 . All of the groups treated with MXD showed better DWG than the IVM SC0.2 group (P = 0.01). Dose and administration route modifications effectively improved the anthelmintic and productive performance of MXD. A high dose of MXD improved the control of IVM-resistant GIN in feedlot calves. However, this practice must be taken with caution, since MXD resistance could rapidly emerge, especially in grazing cattle.

**Author:** Gomez-Puerta, L. A., Carrasco, J., Vargas-Calla, A. M., Lopez-Urbina, M. T. and Gonzalez, A. E.

**Title:** Alopecia a potential adverse side effect of albendazole use in alpacas

**DOI:** 10.1016/j.vprsr.2019.100297

**Abstract:** Albendazole is a benzimidazole derivative with anthelmintic activity. It is the treatment of choice for fasciolosis. The use of albendazole in South American camelids is common, however, there are no studies about the pharmacokinetics and pharmacodynamics of albendazole in alpacas and llamas. In the present study, a case of fiber loss (alopecia) in alpacas is described because of the suspected use of a high dose of albendazole. In a fasciolosis control program of an alpaca ranch located in the district of Nuñoa in Puno, Peru, 2184 alpacas were oral treated with albendazole (35–40 mg/kg). After 2 weeks of treatment the alpacas began to show loss of fiber in the abdomen, flanks and neck. The alpacas showed no other sign of disease. The alpacas recovered their fiber after 6 months. We suggest studies are needed to determine the safe dose of albendazole in alpacas.

**Author:** Gonzalez, A. E., Codd, E. E., Horton, J., Garcia, H. H. and Gilman, R. H.

**Title:** Oxfendazole: a promising agent for the treatment and control of helminth infections in humans

**DOI:** 10.1080/14787210.2018.1555241

**Abstract:** Introduction: Oxfendazole (methyl [5-(phenylsulphonyl)-1H benzimidazole-2-yl] carbamate) has a particularly long metabolic half-life in ruminants, and its metabolite fenbendazole also has anthelmintic action. A very limited number of drugs are available for the treatment of some zoonotic helminth infections, such as neurocysticercosis and echinococcosis. More recent work has expanded oxfendazole's nonclinical safety profile and demonstrated its safety and bioavailability in healthy human volunteers, thus advancing the possibility of a new and greatly needed option for antiparasitic treatment of geohelminths and tissue parasites. Areas covered: The present article reviews evidence supporting the safety and efficacy of oxfendazole against both gut and tissue dwelling helminths in animals, as well as more recent safety and pharmacokinetic data supporting its potential for use in human parasitoses. Expert commentary: The pharmacokinetics, safety, and wide spectrum of efficacy of oxfendazole are consistently demonstrated in intestinal helminth infections of animals as well as in tissue dwelling larval cestode and trematode infections in diverse animal species. Now supported by first-in-human safety and pharmacokinetic data, oxfendazole becomes a promising alternative to the limited portfolio of antiparasitic drugs available to treat helminthic diseases of humans.

**Author:** Liu, M., Landuyt, B., Klaassen, H., Geldhof, P. and Luyten, W.

**Title:** Screening of a drug repurposing library with a nematode motility assay identifies promising anthelmintic hits against *Cooperia oncophora* and other ruminant parasites

**DOI:** 10.1016/j.vetpar.2018.11.014

**Abstract:** Parasitic nematodes continue to cause significant economic losses in livestock globally. Given the limited number of anthelmintic drugs on the market and the currently increasing drug resistance, there is an urgent need for novel anthelmintics. Most motility assays of anthelmintic activity for parasitic nematodes are laborious and low throughput, and therefore not suitable for screening large compound libraries. *Cooperia oncophora* accounts for a large proportion of reports on the drug-resistance development of parasites globally. Therefore, using a WMicroTracker instrument, we established a practical, automated and low-cost whole-organism motility assay against exsheathed L3 stages (xL3s) of the ruminant parasite *Cooperia oncophora*, and screened a repurposing library comprising 2745 molecules. Fourteen known anthelmintics contained in this library were picked up in this blind screen, as well as four novel hits: thonzonium bromide, NH125, physostigmine sulfate, and EVP4593. The four hits were also active against xL3s of *Ostertagia ostertagi*, *Haemonchus contortus* and *Teladorsagia circumcincta* using the same assay. Cytotoxicity testing showed that thonzonium bromide and NH125 (1-Benzyl-3-cetyl-2-methylimidazolium iodide) have significant cytotoxicity. EVP4593 (N(4)-(2-(4-

phenoxyphenyl)ethyl)-4,6-quinazolinediamine) demonstrated a potent and broad anthelmintic activity, and a high selectivity index. Moreover, given its novel and unexplored chemical scaffold for anthelmintic activity, EVP4593 is an interesting anthelmintic hit for further optimization.

**Author:** Prichard, R. K. and Geary, T. G.

**Title:** Perspectives on the utility of moxidectin for the control of parasitic nematodes in the face of developing anthelmintic resistance

**DOI:** 10.1016/j.ijpddr.2019.06.002

**Abstract:** Macrocyclic lactone (ML) anthelmintics are the most important class of anthelmintics because of our high dependence on them for the control of nematode parasites and some ectoparasites in livestock, companion animals and in humans. However, resistance to MLs is of increasing concern. Resistance is commonplace throughout the world in nematode parasites of small ruminants and is of increasing concern in horses, cattle, dogs and other animals. It is suspected in *Onchocerca volvulus* in humans. In most animals, resistance first arose to the avermectins, such as ivermectin (IVM), and subsequently to moxidectin (MOX). Usually when parasite populations are ML-resistant, MOX is more effective than avermectins. MOX may have higher intrinsic potency against some parasites, especially filarial nematodes, than the avermectins. However, it clearly has a significantly different pharmacokinetic profile. It is highly distributed to lipid tissues, less likely to be removed by ABC efflux transporters, is poorly metabolized and has a long half-life. This results in effective concentrations persisting for longer in target hosts. It also has a high safety index. Limited data suggest that anthelmintic resistance may be overcome, at least temporarily, if a high concentration can be maintained at the site of the parasites for a prolonged period of time. Because of the properties of MOX, there are reasonable prospects that strains of parasites that are resistant to avermectins at currently recommended doses will be controlled by MOX if it can be administered at sufficiently high doses and in formulations that enhance its persistence in the host. This review examines the properties of MOX that support this contention and compares them with the properties of other MLs. The case for using MOX to better control ML-resistant parasites is summarised and some outstanding research questions are presented.

**Author:** Risi, G., Aguilera, E., Ladós, E., Suárez, G., Carrera, I., Álvarez, G. and Salinas, G.

**Title:** *Caenorhabditis elegans* infrared-based motility assay identified new hits for nematicide drug development

**DOI:** 10.3390/vetsci6010029

**Abstract:** Nematode parasites have a profound impact on humankind, infecting nearly one-quarter of the world's population, as well as livestock. There is a pressing need for discovering nematicides due to the spread of resistance to currently used drugs. The free-

living nematode *Caenorhabditis elegans* is a formidable experimentally tractable model organism that offers key advantages in accelerating nematicide discovery. We report the screening of drug-like libraries using an overnight high-throughput *C. elegans* assay, based on an automated infrared motility reader. As a proof of concept, we screened the "Pathogen Box" library, and identical results to a previous screen using *Haemonchus contortus* were obtained. We then screened an in-house library containing a diversity of compound families. Most active compounds had a conjugation of an unsaturation with an electronegative atom (N, O, or S) and an aromatic ring. Importantly, we identified symmetric arylidene ketones and aryl hydrazine derivatives as novel nematicides. Furthermore, one of these compounds, (1E,2E)-1,2-bis(thiophen-3-ylmethylene)hydrazine, was active as a nematicide at 25  $\mu\text{m}$ , but innocuous to the vertebrate model zebrafish at 50  $\mu\text{m}$ . Our results identified novel nematicidal scaffolds and illustrate the value of *C. elegans* in accelerating nematicide discovery using a nonlabor-intensive automated assay that provides a simple overnight readout.

**Author:** Sharun, K., Shyamkumar, T. S., Aneesha, V. A., Dhama, K., Pawde, A. M. and Pal, A.

**Title:** Current therapeutic applications and pharmacokinetic modulations of ivermectin

**DOI:** 10.14202/vetworld.2019.1204-1211

**Abstract:** Ivermectin is considered to be a wonder drug due to its broad-spectrum antiparasitic activity against both ectoparasites and endoparasites (under class of endectocide) and has multiple applications in both veterinary and human medicine. In particular, ivermectin is commonly used in the treatment of different kinds of infections and infestations. By altering the vehicles used in the formulations, the pharmacokinetic properties of different ivermectin preparations can be altered. Since its development, various vehicles have been evaluated to assess the efficacy, safety, and therapeutic systemic concentrations of ivermectin in different species. A subcutaneous route of administration is preferred over a topical or an oral route for ivermectin due to superior bioavailability. Different formulations of ivermectin have been developed over the years, such as stabilized aqueous formulations, osmotic pumps, controlled release capsules, silicone carriers, zein microspheres, biodegradable microparticulate drug delivery systems, lipid nanocapsules, solid lipid nanoparticles, sustained-release ivermectin varnish, sustained-release ivermectin-loaded solid dispersion suspension, and biodegradable subcutaneous implants. However, several reports of ivermectin resistance have been identified in different parts of the world over the past few years. Continuous use of suboptimal formulations or sub-therapeutic plasma concentrations may predispose an individual to resistance toward ivermectin. The current research trend is focused toward the need for developing ivermectin formulations that are stable, effective, and safe and that reduce the number of doses required for complete clinical cure in different parasitic diseases. Therefore, single-dose long-acting preparations of ivermectin that provide effective therapeutic drug concentrations need to be developed and commercialized, which may revolutionize drug therapy and prophylaxis against various parasitic diseases in the near future. The present review highlights the current advances in pharmacokinetic



modulation of ivermectin formulations and their potent therapeutic applications, issues related to emergence of ivermectin resistance, and future trends of ivermectin usage.

**Author:** Taylor, L. F. and Hodge, A.

**Title:** Impact of a single treatment of injectable doramectin on weight gain post weaning in beef heifers and steers in central Queensland, Australia

**DOI:** 10.1111/avj.12809

**Abstract:** Objectives: To demonstrate the impact of a single drench with a label dose of injectable doramectin subsequent to weaning on the growth and performance of heifers and steers in central Queensland beef herds. Methods: Three studies were undertaken on recently-weaned *Bos indicus*-cross beef calves with  $\geq 75\%$  *B. indicus* content on two farms in central Queensland, just north of the Tropic of Capricorn. Farm 1 was located 50 km north and Farm 2 75 km north-west of Rockhampton. In each study, half of a group of recently-weaned beef calves were treated by random allocation with 0.2 mg/kg of injectable doramectin, and the remainder acting as untreated controls. Study 1 (Farm 1) enrolled 250 heifers, while studies 2 and 3 (Farm 2) both enrolled 200 steers and 200 heifers. The farms involved did not historically use macrocyclic lactone-based drenches on their cattle. There were varying periods of follow-up, with treated and control cattle pastured as one group throughout the study period. Worm burdens were monitored using standard faecal egg counts and larval differentiation procedures. In all studies, the worm genera present were a mix of *Cooperia* spp., *Haemonchus* spp. and *Oesophagostomum* spp. Results: In study 1, conducted on Farm 1 beginning 9 July 2012, doramectin-treated cattle gained an average of 0.27 kg/day while control cattle gained 0.19 kg/day over a monitoring period of 121 days ( $P < 0.0001$ ). In study 2, conducted on Farm 2 beginning 28 July 2015, doramectin-treated cattle gained an average of 0.15 kg/day versus 0.145 kg/day in the control group ( $P = 0.44$ ) over a 231-day study period. In study 3, conducted on Farm 2 beginning 4 August 2016, doramectin-treated steers and heifers gained an average of 0.431 and 0.402 kg/day versus 0.342 and 0.311 kg/day in the control group, respectively, over the first 91 days of the study ( $P < 0.0001$  in both cases). The differences in average daily gain (ADG) in subsequent time periods were not statistically significant for steers or heifers. However, overall differences in ADG from day 0 remained statistically significant out to day 258, when the study ended for the heifers. By day 594, when the study ended for the steers, the difference in ADG was no longer significant. Conclusion: Treatment with injectable doramectin soon after weaning resulted in improved weight gain in the 3 months after weaning in two of the three studies.

**Author:** Termatzidou, S. A., Arsenopoulos, K., Siachos, N., Kazana, P., Papadopoulos, E., Achard, D., Karembe, H., Bramis, G. and Arsenos, G.

**Title:** Anthelmintic activity of injectable eprinomectin (eprecis ® 20 mg/mL) in naturally infected dairy sheep

**DOI:** 10.1016/j.vetpar.2018.12.014

**Abstract:** The anthelmintic activity of an injectable eprinomectin formulation (Eprecis® 20 mg/mL) was evaluated in 150 naturally infected dairy sheep raised in 3 semi-intensive flocks. All ewes were at the same stage of lactation and grazed on natural pastures. Ewes did not receive any anthelmintic treatment for at least 4 months prior to the experiment. In each flock, 50 ewes were selected and randomly allocated to control (C) or treatment (T) groups (n = 25 per group). Groups were balanced according to the ewes' bodyweight (BW) and fecal egg count (FEC) measured seven days before eprinomectin administration (day-7). On study day 0, ewes in group T, received 0.2 mg/kg BW of eprinomectin subcutaneously (Eprecis® 20 mg/mL, Ceva). Ewes in group C were left untreated. Fecal samples were collected on day 0, 7, 14, 21 and 28 post-treatment to assess FEC and for coprocultures. Ewes were weighed on day 0 and 28. Overall and within-flock efficacy of eprinomectin was calculated throughout the experimental period. No local or general adverse reaction after injection was observed. The most prevalent parasite genera were *Teladorsagia*, *Haemonchus* and *Trichostrongylus*. Following treatment, the overall mean FEC of C and T groups differed significantly ( $P < 0.001$ ). Overall and within-flock efficacy of eprinomectin was 99.8%–100.0% and 99.7%–100.0%, respectively. Contrary to C group, ewes treated with injectable eprinomectin increased their BW during the study (–0.5 kg vs. + 1.5 kg,  $P < 0.001$ ). In this field study, a single subcutaneous injection of eprinomectin to dairy sheep, at 0.2 mg/kg BW, resulted in excellent curative anthelmintic activity; egg counts remain low for at least 28 days after treatment.

**Author:** Viviani, P., Lifschitz, A. L., Luque, S. E., Lloberas, M. M., Maté, M. L., Cardozo, P. A., Lanusse, C. E. and Virkel, G. L.

**Title:** Pharmacologic interaction between oxfendazole and triclabendazole: In vitro biotransformation and systemic exposure in sheep

**DOI:** 10.1016/j.exppara.2019.107718

**Abstract:** The aim of the current work was to evaluate a potential pharmacokinetic interaction between the flukicide triclabendazole (TCBZ) and the broad-spectrum benzimidazole (BZD) anthelmintic oxfendazole (OFZ) in sheep. To this end, both an in vitro assay in microsomal fractions and an in vivo trial in lambs parasitized with *Haemonchus contortus* resistant to OFZ and its reduced derivative fenbendazole (FBZ) were carried out. Sheep microsomal fractions were incubated together with OFZ, FBZ, TCBZ, or a combination of either FBZ and TCBZ or OFZ and TCBZ. OFZ production was significantly diminished upon coincubation of FBZ and TCBZ, whereas neither FBZ nor OFZ affected the S-oxidation of TCBZ towards its sulfoxide and sulfone metabolites. For the in vivo trial, lambs were treated with OFZ (Vermox® oral drench at a single dose of 5 mg/kg PO), TCBZ (Fasinex® oral drench at a single dose of 12 mg/kg PO) or both compounds at a single dose of 5 (Vermox®) and 12 mg/kg (Fasinex®) PO. Blood samples were taken to quantify drug and metabolite concentrations, and pharmacokinetic parameters were calculated by means of non-compartmental analysis. Results showed that the pharmacokinetic parameters of active molecules and metabolites were not significantly altered upon coadministration. The sole exception was the increase in the mean residence time (MRT) of OFZ and FBZ sulfone upon coadministration, with no

significant changes in the remaining pharmacokinetic parameters. This research is a further contribution to the study of metabolic drug-drug interactions that may affect anthelmintic efficacies in ruminants.

## Vaccination

**Author:** González, J. F., Hernández, J. N., MacHín, C., Pérez-Hernández, T., Wright, H. W., Corripio-Miyar, Y., Price, D. R. G., Matthews, J. B., McNeilly, T. N. and Nisbet, A. J.

**Title:** Impacts of breed type and vaccination on *Teladorsagia circumcincta* infection in native sheep in Gran Canaria

**DOI:** 10.1186/s13567-019-0646-y

**Abstract:** Vaccines and genetic resistance offer potential future alternatives to the exclusive use of anthelmintics to control gastrointestinal nematodes (GIN). Here, a *Teladorsagia circumcincta* prototype vaccine was administered to two sheep breeds which differ in their relative levels of resistance to infection with GIN. Vaccination of the more susceptible Canaria Sheep (CS) breed induced significant reductions in worm length and numbers of worm eggs in utero (EIU) when compared to control CS sheep. In the more resistant Canaria Hair Breed (CHB), although vaccination induced a reduction in all parasitological parameters analysed, differences between vaccinated and control sheep were not statistically significant. Such interactions between sheep breed and vaccination may allow better integrated control of GIN in future.

**Author:** Nisbet, A. J., McNeilly, T. N., Price, D. R. G., Oliver, E. M., Bartley, Y., Mitchell, M., Palarea-Albaladejo, J. and Matthews, J. B.

**Title:** The rational simplification of a recombinant cocktail vaccine to control the parasitic nematode *Teladorsagia circumcincta*

**DOI:** 10.1016/j.ijpara.2018.10.006

**Abstract:** Using data from five independent vaccine trials, which employed a subunit cocktail vaccine containing eight recombinant proteins to protect sheep against *Teladorsagia circumcincta*, a strategy was developed to simplify antigen complexity of the vaccine. A meta-analysis of data from these five trials demonstrated statistically significant reductions in cumulative faecal egg count and worm burden in vaccinated sheep when compared with those which had received adjuvant only ( $P = 0.009$  and  $P < 0.0001$ , respectively). Relationships between antigen-specific antibody levels, antibody avidity and parasitological parameters of efficacy were analysed for each of the eight proteins in these trials. Of these, the strongest correlations between percentage reduction in cumulative faecal egg count and avidity were obtained for the vaccine antigen *T. circumcincta* apyrase-1 (Tci-APY-1) in relation to either total antigen-specific IgG or IgG1 in sera ( $P = 0.019$  and  $P = 0.030$ , respectively). In addition, IgG and IgA within the serum and abomasal mucus of control (parasite challenged) lambs strongly recognised Tci-APY-1

and *T. circumcincta* metalloproteinase-1 (Tci-MEP-1) but only weakly bound the other six antigens, indicating Tci-APY-1 and Tci-MEP-1 are most effectively recognised by the parasite-induced antibody response. On the basis of these findings, a two-protein vaccine comprising Tci-APY-1 and Tci-MEP-1 was tested in a direct comparison with the original eight-component vaccine. A further group was immunised with Tci-MEP-1 in combination with a mutated form of Tci-APY-1 (mTci-APY-1), which had no enzymatic activity. Across the trial, the mean faecal egg count levels of the eight-antigen recipients were lower than those of the adjuvant only control group ( $P = 0.013$ ) and the mean FEC of the mTci-APY-1 and Tci-MEP-1 recipients was lower, although not statistically significantly, than that of the adjuvant-only control group ( $P = 0.093$ ). Mean cumulative faecal egg count levels were reduced by 43% in lambs immunised with mTci-APY-1 plus Tci-MEP-1 compared with the controls ( $P = 0.079$ ).

## *Haemonchus spp*

**Author:** dos Santos, I. B., Giquelin Maciel, W., Felippelli, G., Barbosa Toscano, J. H., Cayeiro Cruz, B., De Souza Chagas, A. C., Soares, V. E., Zanetti Lopes, W. D., da Costa, A. J. and de Oliveira, G. P.

**Title:** Viability of *Haemonchus placei* parasitism in experimentally infected young goats

**Short Title:** Viability of *Haemonchus placei* parasitism in experimentally infected young goats

**DOI:** 10.1016/j.vetpar.2019.06.012

**Abstract:** The present study aimed to evaluate the viability of *Haemonchus placei* parasitism in experimentally infected goats. For that, 14 75 days old kids male Saanen kids were placed in one of the four experimental groups: GI – infected with 5000 *H. placei* L3 ( $n = 4$ ); GII – infected with 5000 *H. contortus* L3 ( $n = 4$ ); GIII – infected with 2500 *H. contortus* L3 + 2500 *H. placei* L3 ( $n = 4$ ), and GIV – control, inoculated with distilled water ( $n = 2$ ). Each kid received, orally, the infective dose in a single inoculum. Based on daily fecal egg counts, the average pre-patent period was determined as 24 days for *H. contortus*, and 31 days for *H. placei*. Regarding the *Haemonchus* spp. recovered at necropsy, the experimental groups GI, GII, and GIII had, respectively, an average of 25.5, 619.5, and 724.75 (120 *H. placei*, and 604.75 *H. contortus*) adult specimens, and no immature forms. Under the conditions of this study, the viability of goat infection by *H. placei* was confirmed, although, with low susceptibility. Nevertheless, the parasitism of this helminth species was more intense when associated with *H. contortus*. This fact indicates that in common grazing between cattle and young goats, when the latter end up ingesting both *Haemonchus* species, especially in a mixed infection, *H. placei* may also parasitize them.

**Author:** Flores-Pérez, I., Hallal-Calleros, C., Cervantes-Pacheco, B. J., Alba-Hurtado, F., Orihuela, A., Castro-del-Campo, N., Acevedo-Ramírez, P., Ortiz-López, B., Jimenez-Leyva, D. and Barajas-Cruz, R.

**Title:** Behavioural and productive response to experimental infection with stage 3 larvae of *Haemonchus contortus* in feedlot bull-calves

**DOI:** 10.1016/j.exppara.2018.12.005

**Abstract:** *Haemonchus contortus* is a nematode parasite that establishes in the abomasum of ruminants, it has a cosmopolitan distribution and is a worldwide health problem for livestock. As a first line of defence against parasites, behaviour can help to prevent or fight infection, and may even serve as a method of early presumptive diagnosis. Parasites can affect performance of cattle and cause significant economic losses. The aim of this study was to determine the behavioural and productive changes induced by an experimental infection with *H. contortus* L3 in bovines. We used 32 dewormed bull calves, randomly divided into two groups, 8 no inoculated controls and 24 that were inoculated with 4000 L3 of *H. contortus*. Inoculation did not influence haematocrit or haemoglobin values at 0, 28 and 42 days post infection ( $P > 0.20$ ); however, an increase in the frequency of urination ( $P = 0.0001$ ) and defecation ( $P = 0.0001$ ), number of steps ( $P < 0.001$ ) and self-grooming ( $P < 0.01$ ) events were observed, even in inoculated animals in which not parasite eggs were found in faeces. During the first 28 days post-inoculation with *H. contortus*, feedlot performance was not affected ( $P = 0.16$ ), but during the last 14 days (29–42) inoculated animals gained 15% less weight compared to controls ( $P = 0.04$ ). Over 42 days, inoculated calves showed a 28% poorer feed efficiency during the 42 days post-inoculation than controls ( $P < 0.01$ ). Across the experiment, calves inoculated with *H. contortus* obtained less net energy both for maintenance as for gain from diet ( $P < 0.01$ ). It is concluded that both, performance and behaviour were modified in feedlot bull-calves parasitized by *H. contortus*, supporting the practice of deworming when eggs of this parasite are detected in the faeces even in low amounts.

**Author:** Khan, S., Zhao, X., Hou, Y., Yuan, C., Li, Y., Luo, X., Liu, J. and Feng, X.

**Title:** Analysis of genome-wide SNPs based on 2b-RAD sequencing of pooled samples reveals signature of selection in different populations of *Haemonchus contortus*

**DOI:** 10.1007/s12038-019-9917-z

**Abstract:** The parasitic nematode *Haemonchus contortus* is one of the world's most important parasites of small ruminants that causes significant economic losses to the livestock sector. The population structure and selection in its various strains are poorly understood. No study so far compared its different populations using genome-wide data. Here, we focused on different geographic populations of *H. contortus* from China (Tibet, TB; Hubei, HB; Inner Mongolia, IM; Sichuan, SC), UK and Australia (AS), using genome-wide population-genomic approaches, to explore genetic diversity, population structure and selection. We first performed next-generation high-throughput 2b RAD pool

sequencing using Illumina technology, and identified single-nucleotide polymorphisms (SNPs) in all the strains. We identified 75,187 SNPs for TB, 82,271 for HB, 82,420 for IM, 79,803 for SC, 83,504 for AS and 78,747 for UK strain. The SNPs revealed low-nucleotide diversity ( $\pi = 0.0092\text{--}0.0133$ ) within each strain, and a significant differentiation level (average  $F_{st} = 0.34264$ ) among them. Chinese populations TB and SC, along with the UK strain, were more divergent populations. Chinese populations IM and HB showed affinities to the Australian strain. We then analysed signature of selection and detected 44 (UK) and 03 (AS) private selective sweeps containing 49 and 05 genes, respectively. Finally, we performed the functional annotation of selective sweeps and proposed biological significance to signature of selection. Our data suggest that 2b-RAD pool sequencing can be used to assess the signature of selection in *H. contortus*.

**Author:** Palevich, N., Maclean, P. H., Baten, A., Scott, R. W. and Leathwick, D. M.

**Title:** The Genome Sequence of the Anthelmintic-Susceptible New Zealand *Haemonchus contortus*

**DOI:** 10.1093/gbe/evz141

**Abstract:** Internal parasitic nematodes are a global animal health issue causing drastic losses in livestock. Here, we report a *H. contortus* representative draft genome to serve as a genetic resource to the scientific community and support future experimental research of molecular mechanisms in related parasites. A de novo hybrid assembly was generated from PCR-free whole genome sequence data, resulting in a chromosome-level assembly that is 465 Mb in size encoding 22,341 genes. The genome sequence presented here is consistent with the genome architecture of the existing *Haemonchus* species and is a valuable resource for future studies regarding population genetic structures of parasitic nematodes. Additionally, comparative pan-genomics with other species of economically important parasitic nematodes have revealed highly open genomes and strong collinearities within the phylum Nematoda.

**Author:** Rezansoff, A. M., Laing, R., Martinelli, A., Stasiuk, S., Redman, E., Bartley, D., Holroyd, N., Devaney, E., Sargison, N. D., Doyle, S., Cotton, J. A. and Gilleard, J. S.

**Title:** The confounding effects of high genetic diversity on the determination and interpretation of differential gene expression analysis in the parasitic nematode *Haemonchus contortus*

**DOI:** 10.1016/j.ijpara.2019.05.012

**Abstract:** Differential expression analysis between parasitic nematode strains is commonly used to implicate candidate genes in anthelmintic resistance or other biological functions. We have tested the hypothesis that the high genetic diversity of an organism such as *Haemonchus contortus* could complicate such analyses. First, we investigated the extent to which sequence polymorphism affects the reliability of differential expression analysis between the genetically divergent *H. contortus* strains MHco3(ISE), MHco4(WRS)

and MHco10(CAVR). Using triplicates of 20 adult female worms from each population isolated under parallel experimental conditions, we found that high rates of sequence polymorphism in RNAseq reads were associated with lower efficiency read mapping to gene models under default TopHat2 parameters, leading to biased estimates of inter-strain differential expression. We then showed it is possible to largely compensate for this bias by optimising the read mapping single nucleotide polymorphism (SNP) allowance and filtering out genes with particularly high single nucleotide polymorphism rates. Once the sequence polymorphism biases were removed, we then assessed the genuine transcriptional diversity between the strains, finding  $\geq 824$  differentially expressed genes across all three pairwise strain comparisons. This high level of inter-strain transcriptional diversity not only suggests substantive inter-strain phenotypic variation but also highlights the difficulty in reliably associating differential expression of specific genes with phenotypic differences. To provide a practical example, we analysed two gene families of potential relevance to ivermectin drug resistance; the ABC transporters and the ligand-gated ion channels (LGICs). Over half of genes identified as differentially expressed using default TopHat2 parameters were shown to be an artifact of sequence polymorphism differences. This work illustrates the need to account for sequence polymorphism in differential expression analysis. It also demonstrates that a large number of genuine transcriptional differences can occur between *H. contortus* strains and these must be considered before associating the differential expression of specific genes with phenotypic differences between strains.

**Author:** Ruiz-Huidobro, C., Sagot, L., Lugagne, S., Huang, Y., Milhes, M., Bordes, L., Prévot, F., Grisez, C., Gautier, D., Valadier, C., Sautier, M. and Jacquet, P.

**Title:** Cell grazing and *Haemonchus contortus* control in sheep: lessons from a two-year study in temperate Western Europe

**DOI:** 10.1038/s41598-019-49034-y

**Abstract:** Managing infections of sheep with anthelmintic resistant gastrointestinal nematodes (GIN) is a major challenge for sheep producers in Western Europe. New methods of grazing management have been poorly explored as a component of an integrated and sustainable control of these parasites. Therefore, the purpose of this study was to evaluate the effect of two different types of grazing systems of sheep (intensive cell grazing versus conventional rotational grazing) on GIN infections over two years in a farm located in a temperate environment of Western France. When considering the whole study, the type of grazing system did not influence significantly the intensity of egg excretions of adult ewes even if the proportion of ewes excreting high numbers of GIN eggs was higher in cell grazing system than in rotational grazing system. The most striking result of this survey was the effect of grazing system on the GIN species composition harbored by ewes and by their lambs: with time, the proportions of *H. contortus* infections were lower in cell grazing system than in rotational grazing system. In conclusion, the cell grazing system, as implemented in this study, could limit the importance of this highly pathogenic nematode species in sheep.

**Author:** Sallé, G., Doyle, S. R., Cortet, J., Cabaret, J., Berriman, M., Holroyd, N. and Cotton, J. A.

**Title:** The global diversity of *Haemonchus contortus* is shaped by human intervention and climate

**DOI:** 10.1038/s41467-019-12695-4

**Abstract:** *Haemonchus contortus* is a haematophagous parasitic nematode of veterinary interest. We have performed a survey of its genome-wide diversity using single-worm whole genome sequencing of 223 individuals sampled from 19 isolates spanning five continents. We find an African origin for the species, together with evidence for parasites spreading during the transatlantic slave trade and colonisation of Australia. Strong selective sweeps surrounding the  $\beta$ -tubulin locus, a target of benzimidazole anthelmintic drug, are identified in independent populations. These sweeps are further supported by signals of diversifying selection enriched in genes involved in response to drugs and other anthelmintic-associated biological functions. We also identify some candidate genes that may play a role in ivermectin resistance. Finally, genetic signatures of climate-driven adaptation are described, revealing a gene acting as an epigenetic regulator and components of the dauer pathway. These results begin to define genetic adaptation to climate in a parasitic nematode.

**Author:** Sargison, N. D., Redman, E., Morrison, A. A., Bartley, D. J., Jackson, F., Hoberg, E. and Gilleard, J. S.

**Title:** Mating barriers between genetically divergent strains of the parasitic nematode *Haemonchus contortus* suggest incipient speciation

**DOI:** 10.1016/j.ijpara.2019.02.008

**Abstract:** *Haemonchus contortus*, in common with many nematode species, has extremely high levels of genetic variation within and between field populations derived from distant geographical locations. MHco10(CAVR), MHco3(ISE) and MHco4(WRS) are genetically divergent *H. contortus* strains, originally derived from Australia, Kenya and South Africa, respectively, that have been maintained by numerous rounds of in vivo experimental infection of sheep. In order to explore potential pre-zygotic competition or post-zygotic incompatibility between the strains, we have investigated the ability of MHco10(CAVR) to interbreed with either MHco3(ISE) or MHco4(WRS) during dual strain co-infections. Sheep were experimentally co-infected with 4000 infective larvae (L3) per os of the MHco10(CAVR) strain and an equal number of either the MHco3(ISE) or the MHco4(WRS) strain L3. The adult worm establishment rates and the proportions of F1 progeny resulting from intra- and inter-strain mating events were determined by admixture analysis of microsatellite multi-locus genotypes. Although there was no difference in adult worm establishment rates, the proportions of F1 progeny of both the MHco10(CAVR)  $\times$  MHco3(ISE) and MHco10(CAVR)  $\times$  MHco4(WRS) dual strain co-infections departed from Mendelian expectations. The proportions of inter-strain hybrid F1 progeny were lower than



the expected 50%, suggesting either pre-zygotic competition or post-zygotic incompatibility between the co-infecting strains. To investigate this further, both eggs and hatched L1 of broods from single adult female worms recovered from each dual co-infection were genotyped. Unhatched eggs from the broods revealed no inter-strain hybrid genotype deficit, suggesting there is no pre-zygotic competition between the strains. In contrast, there was a deficit in L1 inter-strain hybrid genotypes in the broods derived from MHco3(ISE) or MHco4(WRS) maternal parents, but not from MHco10(CAVR) maternal parents. This suggests that hybrid progeny of MHco10(CAVR) paternal parents have reduced post-zygotic development and/or viability consistent with incipient speciation of the MHco10(CAVR) strain. The presence of mating barriers between allopatric *H. contortus* strains has important implications for parasite ecology, including the ability of newly introduced anthelmintic-resistant parasite populations to compete and interbreed with populations already established in a region.

**Author:** Wang, T., Ma, G., Ang, C. S., Korhonen, P. K., Koehler, A. V., Young, N. D., Nie, S., Williamson, N. A. and Gasser, R. B.

**Title:** High throughput LC-MS/MS-based proteomic analysis of excretory-secretory products from short-term in vitro culture of *Haemonchus contortus*

**DOI:** 10.1016/j.jprot.2019.05.003

**Abstract:** Parasitic nematodes of humans, animals and plants have a major, adverse impact on global health and agricultural production worldwide. To cope with their surrounding environment in and the immune attack from the host, excretory-secretory (ES) proteins are released by nematodes to orchestrate or regulate parasite-host interactions. In the present study, we characterised the ES products from short-term (12 h) in vitro culture of different developmental stages/sexes of *Haemonchus contortus* (one of the most important parasitic nematodes of livestock animals worldwide) using a high throughput tandem mass-spectrometry, underpinned by the most recent genomic dataset. In total, 878 unique proteins from key developmental stages/sexes (third-stage and fourth-stage larvae, and female and male adults) were identified and quantified with high confidence. Bioinformatic analyses showed noteworthy ES protein alterations during the transition from the free-living to the parasitic phase, especially for proteins which are likely involved in nutrient digestion and acquisition as well as parasite-host interactions, such as proteolytic cascade-related peptidases, glycoside hydrolases, C-type lectins and sperm-coating protein/Tpx/antigen 5/pathogenesis related-1/Sc7 (= SCP/TAPS) proteins. Our findings provide an avenue to better explore interactive processes between the host and this highly significant parasitic nematode, to underpin the search for novel drug and vaccine targets. Significance: The present study represents a comprehensive proteomic analysis of the secretome of key developmental stages/sexes of *H. contortus* maintained in short-term in vitro culture. High throughput LC-MS/MS analysis of ES products allowed the identification of a large repertoire of proteins (secretome) and the establishment of a new proteomic database for *H. contortus*. The secretome of *H. contortus* undergoes substantial changes during the nematode's transition from free-living to parasitic stages, suggesting a constant adaptation to different environments outside of and within the host animal. Understanding

the host-parasite relationship at the molecular level could assist significantly in the development of intervention strategies (i.e. novel drugs and vaccines) against *H. contortus* and related nematodes.

**Author:** Róbert, F., Petra, A. and Mónika, G.

**Title:** Haemonchosis of sheep: Literature review

**Abstract:** *Haemonchus contortus* has been known for a long time in the tropical and subtropical regions of the world. This highly pathogenic blood-feeding nematode species is an especially significant threat to the health and production of sheep and goats. During the last decade the importance of haemonchosis in sheep flocks has increased in many European countries due to the climate change and anthelmintic resistance. Although, the occurrence of this parasite in Hungary was known many years ago there is still scant knowledge about its distribution and economic importance. Recently, haemonchosis has been diagnosed in some local sheep flocks, therefore the authors summarize the knowledge based on the published data. The biology and the geographical distribution of *H. contortus* and its economic importance are mentioned first. The traditional (morphological identification of eggs and third instar larvae) and new diagnostic methods (fluorescein-labelled peanut agglutinin test, LAMP) to identify its eggs, the drugs available to use for treatments and the related anthelmintic resistance problems are discussed. Finally, alternative control possibilities of haemonchosis, such as the role of targeted selective treatments, grazing and nutritional management, genetic selection of sheep, vaccination and biological methods are discussed.

## Diagnosis

**Author:** Abbas, I. and Hildreth, M.

**Title:** Egg autofluorescence and options for detecting peanut agglutinin binding for the identification of *Haemonchus contortus* eggs in fecal samples

**DOI:** 10.1016/j.vetpar.2019.01.009

**Abstract:** Quantifying eggs from *Haemonchus* and other trichostrongyle genera in sheep and goat fecal samples is important for evaluating control and treatment strategies for this family of nematodes with divergent pathologies, capabilities for anthelmintic resistance and environmental susceptibilities. Unfortunately, egg morphology among most of the genera do not differ enough to support the accurate identification of these genera with standard microscopic techniques. Several studies have identified specific lectins which bind selectively to sugars located on the egg surfaces for individual genera among the trichostrongyles. To detect lectins binding to these eggs, they must be directly or indirectly bound to fluorophores, and observed with an epi-fluorescence microscope. The binding of multiple lectins to isolated eggs from a fecal sample can be simultaneously detected if fluorophores are used whose excitation and emission spectra do not overlap, and this would enable the development of a fluorescence-based diagnostic test that identifies

multiple trichostrongyle genera within each sample. The present study compared the usefulness of different, commercially available detection systems for use in detecting lectin binding to trichostrongyle eggs. Comparisons were made using the detection of PNA binding to *H. contortus* eggs with the goal of finding three systems with color spectra that do not overlap. These evaluations included both fluorophores directly conjugated to PNA in a one-step incubation protocol and a two-step incubation protocol involving biotinylated PNA and streptavidin conjugated to different fluorophores. Autofluorescence can affect the efficiency of any fluorescence-based detection system, and significant autofluorescence was observed among the unstained *H. contortus* eggs with the DAPI-type fluorescence filter, but it was significantly lower with the FITC-type filter and was virtually absent with the rhodamine-type filter. This study demonstrated that all the PNA detection methods tested with *H. contortus* eggs generated fluorescence intensities (FIs) that were significantly above the autofluorescence generated by the eggs among the three different fluorescence filters. Fluorescence intensities from PNA directly conjugated to either the FITC or rhodamine fluorophores were not different, but the lower autofluorescence in the rhodamine-type filter will enable this fluorophore to be detected more efficiently. Use of biotinylated PNA combined with streptavidin-conjugated to synthetic fluorophores (Alexa Fluor 405, 488 and 546) significantly increased FIs over that of the directly conjugated PNA, but there were no significant differences in FIs among these three biotin-avidin conjugation fluorophores. This biotin-avidin system required two incubation steps. Doubling the concentration of PNA also provided increased FI, at least for the biotin-avidin system. Adding an additional amplification step to the biotin-avidin system involving biotinylated anti-streptavidin followed by the streptavidin-Alexa Fluor complex also provided additional fluorescence.

**Author:** Hassan, N. M. F., Aboelsoued, D., Farag, T. K., Hassan, S. E. and Abu El Ezz, N. M. T.

**Title:** Assessment of *Haemonchus contortus* larval and adult somatic antigens in sero-diagnosis of haemonchosis in naturally infected sheep and goats

**DOI:** 10.1007/s12639-019-01152-0

**Abstract:** The current work was carried out to evaluate the potency of larval and adult somatic *Haemonchus contortus* (*H. contortus*) antigens in detection of haemonchosis among sheep and goats using ELISA. Two hundred and forty-three fecal and blood samples were randomly collected from small ruminants (107 sheep and 136 goats) in Beni-Suef Governorate, Egypt, during the period from June to August 2018. The fecal analysis exhibited that 26.33% of the small ruminants were infected with gastrointestinal nematodes. The overall prevalence of *H. contortus* was reached 22.22% whereas it was 27.10% and 18.38% among sheep and goats, respectively. The current study elucidated that the larval antigen has claimed more superior diagnostic results compared to the adult somatic *H. contortus* antigen. The apparent overall sero-prevalence among small ruminants was reached 51.85%. Separately, it was 64.48% in sheep and 41.91% in goats. The larval antigen had proved 96.55% sensitivity and 47.43% specificity, for sheep serum samples. Meanwhile, sensitivity and specificity for goats' sera were 100% and 71.17%,

respectively. Diagnostic efficacy of ELISA was recorded 60.74% in sheep and 76.47% in goats. This study deduced that the larval antigen has proved the priority and the potency for diagnosis of *H. contortus* infection. Moreover, haemonchosis is a prevalent disease among the examined sheep and goats.

**Author:** Höglund, J., Elmahalawy, S. T., Halvarsson, P. and Gustafsson, K.

**Title:** Detection of *Haemonchus contortus* on sheep farms increases using an enhanced sampling protocol combined with PCR based diagnostics

**DOI:** 10.1016/j.vpoa.2019.100018

**Abstract:** An enhanced sampling strategy for detection of gastrointestinal parasites of sheep based on faecal sampling covering approximately 10% of the animals in the flock was evaluated with focus on the major sheep pathogen *Haemonchus contortus*. We also compared traditional diagnostics based on faecal eggs counts (FEC) by microscopy with DNA detection on frozen faeces samples using a droplet digital (dd)PCR assay. The investigation was carried out in 2018 in 20 conventional and 19 organic sheep flocks in Sweden with between 70 and 250 production ewes. On 76 different sampling occasions a total of 810 individual faecal samples were collected. Samples were pooled in the laboratory into 270 triplets which were examined both by microscopy and a ddPCR assay. On most farms (95%) a minimum of three triplets were investigated, first from the ewes prior to turn-out and later from the lambs after they had been grazing for at least six weeks. Extra information about the *Haemonchus* status was provided on 48% of the 76 sampling occasions by including more triplets compared with the old sampling strategy applied in Sweden before 2015 based on two triplets per sampling occasion irrespective of flock size. At a farm level *H. contortus* was identified by microscopy in 22 (56%) of the 39 flocks and by ddPCR it was found in 28 (72%) flocks with the enhanced protocol. There was a substantial agreement between the two diagnostic tests (Cohens kappa =  $0.70 \pm 0.087$ ). No significant differences in infection levels were observed between the two production systems (conventional and organic) irrespective of the diagnostic method used. However, samples from the ewes were more often *Haemonchus* positive than those from the lambs indicating that the level of parasite control was in general acceptable. Combined, our results show that *Haemonchus* infection is widespread throughout Sweden. In conclusion, we have validated a practical tool for sheep producers to assess *Haemonchus* infection with high precision.

**Author:** Naqvi, M. A. U. H., Naqvi, S. Z., Ali Memon, M., Aimulajiang, K., Haseeb, M., Xu, L., Song, X., Li, X. and Yan, R.

**Title:** Combined use of indirect ELISA and western blotting with recombinant hepatocellular carcinoma-associated antigen 59 is a potential immunodiagnostic tool for the detection of prepatent *Haemonchus contortus* infection in goat

**DOI:** 10.3390/ani9080548

**Abstract:** *Haemonchus contortus* is recognized as one of the important health problems in small ruminants, leading to reduced production and economic loss for farmers worldwide. Prepatent diagnosis of *H. contortus* infection is crucial to improve control strategies as this helminth may remove up to one-fifth of total erythrocytes and may cause anemia, edema, diarrhea, and ultimately death in young animals. In this study, one of the excretory and secretory products, rHc-HCA59, was purified and used as antigen to detect specific antibodies in *H. contortus* infected goats during prepatent stage of infection using indirect enzyme linked immunosorbent assay (ELISA) as screening test. All goats (n = 38) were housed indoor, experimentally infected with 8000 infective larvae (L3) of *H. contortus*, and serum samples were collected prior to infection and at 14th day of infection. Immunoblotting was performed to confirm the results of indirect ELISA, evaluate the cross reactivity against rHc-HCA59 in sera of most common co-infecting parasites and rectify the false negative samples. Furthermore, three different batches of rHc-HCA59 were produced to evaluate the repeatability of ELISA. No eggs were detected in feces of all goats collected at 7th and 14th day of infection but, *H. contortus* eggs were detected at 21 days post infection in the feces. Indirect ELISA performed in this study showed 87% sensitivity and 100% specificity. The western blot analysis confirmed immunoreactivity in serum samples which scored positive in indirect ELISA and recognized the samples as negative which had OD450 lower than negative cut-off value in indirect ELISA. Furthermore, all false negative sera (n = 5) that had OD450 value between positive and negative cut-off value in rHc-HCA59 based ELISA were clearly positive in western blot. Moreover, no cross-reactivity was detected in ELISA and western blotting against rHc-HCA59 in positive sera of *Toxoplasma gondii*, *Fasciola hepatica*, and *Trichinella spiralis*. The results of this study concluded that combined use of indirect ELISA and western blotting with rHc-HCA59 is a potential immunodiagnostic tool for the detection of *H. contortus* infection during prepatent period in goats.

**Author:** Rizzon Cintra, M. C., Ollhoff, R. D., Weber, S. H. and Santos Sotomaior, C.

**Title:** Is the Famacha © system always the best criterion for targeted selective treatment for the control of haemonchosis in growing lambs?

**DOI:** 10.1016/j.vetpar.2018.12.015

**Abstract:** Targeted selective treatment (TST) aims to aid in the control of gastrointestinal nematodes in small ruminants, identifying animals that require treatment with the purpose of preserving the Refugia population and delaying the buildup of anthelmintic (AH) resistance. The objective of this study was to evaluate the effectiveness of both productive and clinical indicators in the selective treatment of gastrointestinal parasites in growing lambs. Two experiments were carried out between 2015 and 2016. Experiment 1: lambs (n = 70) exclusively raised on pasture were evaluated from the ages of 60 days to 180 days old. Experiment 2: lambs (n = 48) raised on pasture and fed concentrated commercial supplementation were evaluated from the ages of 60 days to 150 days old. Parasitological (eggs per gram of feces - EPG), productive (average daily weight gain - DWG), and clinical (hematocrit - Ht and FAMACHA © system - F) parameters were measured every 14 days. The lambs were divided into 4 experimental groups with different criteria for AH treatment:

the control group (CG), was treated with AH every 30 days; the FAMACHA © group (FG), in which lambs classified as F3, F4, and F5 were treated with AH; Daily weight gain group (DWGG), in which lambs with average DWG  $\leq$  the average DWG of the CG minus one standard deviation were treated with AH; FAMACHA © + daily weight gain group (FDWGG), in which lambs classified as F3, F4 and F5 and/or lambs with average DWG  $\leq$  the average DWG of the CG minus one standard deviation were treated with AH. In experiment 1, no significant differences in the general means of DWG and Ht ( $p > 0.05$ ) were observed between experimental groups, although the lowest mean EPG was observed in the CG ( $p < 0.05$ ). In experiment 2, the lowest mean EPG ( $p < 0.05$ ) was also observed in the CG (1044.5 EPG), and the highest mean ( $p < 0.05$ ) in the FG (4845.5 EPG). There were differences in the mean DWG of the CG (198.4 g) and FDWGG (200.0 g), which were both higher ( $p < 0.05$ ) than that of the FG (90.5 g), and similar ( $p > 0.05$ ) to the DWGG (151.8 g). In both experiments, considering the total mean number of AH treatments, FG had the lowest number of lambs treated with AH ( $p < 0.05$ ), with the DWG and FDWGG being similar to each other ( $p > 0.05$ ). Even when *Haemonchus* is the major parasite, it is not recommended to use the FAMACHA © system as an exclusive criterion for TST in growing lambs. The DWG productive criterion can be used effectively in the TST of growing lambs.

## Resistance of hosts to infection

**Author:** Alam, M. B. B., Omar, A. I., Faruque, M. O., Notter, D. R., Periasamy, K., Mondal, M. M. H., Sarder, M. J. U., Shamsuddin, M., Cao, J., Du, X., Wu, Z. and Zhao, S.

**Title:** Single nucleotide polymorphisms in candidate genes are significantly associated with resistance to *Haemonchus contortus* infection in goats

**DOI:** 10.1186/s40104-019-0327-8

**Abstract:** Background: Haemonchosis is a major economic problem in goat production in humid, tropical and subtropical regions. The disease is caused by an abomasal nematode, *Haemonchus contortus*, which is highly pathogenic in small ruminants. The aim of this study was to identifying single-nucleotide polymorphisms (SNP) that were associated with fecal egg counts (FEC) and could be used as markers to identify resistance to *H. contortus* in goats. Results: Ten novel variants in the *CIITA*, *ATP2A3*, *HSPA8*, *STAT5B*, *ESYT1*, and *SERPING1* genes were associated with FEC in goats with a nominal significance level of  $P < 0.05$ . Two missense mutation in the exon region of the caprine *CIITA* gene resulted in replacement of arginine with cysteine at position 9473550 (R9473550C) and aspartic acid with glutamic acid at position 9473870 (D9473870E). Chinese goat breeds had significantly higher FEC than Bangladeshi goat breeds within their respective genotypes. Polymorphism information content (PIC), effective allele number ( $N_e$ ), and heterozygosity ( $H_e$ ) were greatest for the *STAT5B*-197-A > G SNP locus in all goat breeds. Pairwise coefficients of linkage disequilibrium ( $D$ ,  $r^2$ ) revealed complete LD ( $r^2 = 1$ ) between significant SNP polymorphisms in *CIITA* and *SERPING1* and strong LD ( $r^2 = 0.93$  and  $0.98$ ) between polymorphisms in *HSPA8* and *ATP2A3*, respectively. Correlation coefficient

(r) between FEC and body weight (BW) was significantly positive ( $r = 0.56$ ,  $P < 0.001$ ) but that between FEC and packed cell volume (PCV) was negatively significant ( $r = -0.47$ ,  $P < 0.01$ ) in the total population of goats. On the other hand, correlation coefficient (r) between BW and PCV was not significant in total population of goats. Association analysis revealed that haplotypes within ATP2A3, HSPA8, and SERPING1 were significantly associated with FEC. Quantitative real-time PCR revealed that the relative expression of mRNA was higher ( $P < 0.001$ ) for resistant, compared to susceptible, groups of goats for all candidate genes except CIITA. Conclusions: This study identified SNP markers that can potentially be used in marker-assisted selection programs to develop goat breeds that are resistant to *H. contortus*.

**Author:** Cériac, S., Durbant, P., Godard, X., Barbier, C., Feuillet, D., Félicité, Y., Archimède, H. and Bambou, J. C.

**Title:** Effect of the nutritional status of Creole goats on the density-dependent prolificacy of *Haemonchus contortus*

**DOI:** 10.1016/j.vetpar.2019.108973

**Abstract:** The objective of this study was to measure the effect of the nutritional status of Creole goat kids on the host responses and the nematode population established after an experimental *H. contortus* infection. Fifty six kids were fed with 4 diets corresponding to 2 nutritional statuses: the low nutritional status (HAY, hay ad libitum and HB, Hay ad libitum + banana) and the high nutritional status (HS, hay ad libitum + soya meal and HSB, hay ad libitum + banana + soya meal). For each diet, 8 kids were experimentally infected with 10,000 *H. contortus* infective larvae (L3) and 6 kids were kept as non-infected controls. From the day of infection until 6 weeks post-infection, samples were collected to measure individual intake, total tract digestibility, parasitological and hematological parameters. The dry matter intake (DMI), the average daily gain (ADG), the crude protein (CP) and the digestible CP intake were higher in goats fed the HS and HSB diets, but no statistically significant interaction between the nutritional status and the infection was observed. The packed cell volume (PCV), the red blood cell counts (RBC) and the mean corpuscular volume (MCV) were higher with the HS and the HSB diets. In kids with the high nutritional status the nematode burden and pathophysiological impact of the infection were significantly lower but not the FEC. In conclusion, this reduced establishment rate was associated with an increased production of eggs by the female parasites and suggested a phenomenon of density-dependent prolificacy of *H. contortus* probably inherent to the fitness of the parasite population.

**Author:** Estrada-Reyes, Z. M., Tsukahara, Y., Amadeu, R. R., Goetsch, A. L., Gipson, T. A., Sahl, T., Puchala, R., Wang, Z., Hart, S. P. and Mateescu, R. G.

**Title:** Signatures of selection for resistance to *Haemonchus contortus* in sheep and goats

**DOI:** 10.1186/s12864-019-6150-y

**Abstract:** Background: Gastrointestinal nematode infection (GNI) is the most important disease affecting the small ruminant industry in U.S. The environmental conditions in the southern United States are ideal for the survival of the most pathogenic gastrointestinal nematode, *Haemonchus contortus*. Host genetic variation for resistance to *H. contortus* allows selective breeding for increased resistance of animals. This selection process increases the prevalence of particular alleles in sheep and goats and creates unique genetic patterns in the genome of these species. The aim of this study was to identify loci with divergent allelic frequencies in a candidate gene panel of 100 genes using two different approaches (frequentist and Bayesian) to estimate  $F_{st}$  outliers in three different breeds of sheep and goats exposed to *H. contortus*. Results: Our results for sheep populations showed SNPs under selection in *C3AR1*, *CSF3*, *SOCS2*, *NOS2*, *STAT5B*, *TGFB2* and *IL2RA* genes using frequentist and Bayesian approaches. For goats, SNPs in *CD1D*, *ITGA9*, *IL12A*, *IL13RA1*, *CD86* and *TGFB2* genes were under selection. Common signatures of selection in both species were observed in *NOS2*, *TGFB2* and *TLR4* genes. Directional selection was present in all SNPs evaluated in the present study. Conclusions: A total of 13 SNPs within 7 genes of our candidate gene panel related to *H. contortus* exposure were identified under selection in sheep populations. For goats, 11 SNPs within 7 genes were identified under selection. Results from this study support the hypothesis that resistance to *H. contortus* is likely to be controlled by many loci. Shared signatures of selection related to mechanisms of immune protection against *H. contortus* infection in sheep and goats could be useful targets in breeding programs aimed to produce resistant animals with low FEC.

**Author:** Estrada-Reyes, Z. M., Tsukahara, Y., Goetsch, A. L., Gipson, T. A., Sahl, T., Puchala, R. and Mateescu, R. G.

**Title:** Association analysis of immune response loci related to *Haemonchus contortus* exposure in sheep and goats using a targeted approach

**DOI:** 10.1016/j.livsci.2019.08.005

**Abstract:** *Haemonchus contortus* infections are an important source of animal production loss to livestock industry. Genetics of helminth resistance involves a complex set of factors related to the host immune response. The increasing use of genetic markers such as single nucleotide polymorphisms (SNPs) in genome wide association studies (GWAS) offers the potential to identify loci or regions associated with nematode resistance in small ruminants. The aims of this study were: (1) to use a targeted sequencing approach to identify SNPs in 100 genes related to immune response during *Haemonchus contortus* exposure in growing males of St. Croix, Katahdin and Dorper sheep, and in Kiko, Boer and



Spanish goats, and (2) to perform an association analysis for fecal egg count (FEC), packed cell volume (PCV), immunoglobulin levels (IgA, IgG and IgM) and average daily gain (ADG) in the populations under study. After quality control (call rate < 95%, MAF < 0.05), 1356 SNPs (sheep) and 1,029 SNPs (goats) were used for the association analysis. A mixed model was used to analyze the phenotypic information. To control for population structure, the genomic relationship matrix (G) calculated from marker information was included in the model. Fixed effects included year and breed. Bonferroni correction was used to control for multiple testing. For sheep, SNPs located on OAR1 (42487870, 42489606) and OAR2 (192231080, 26321541) were significantly associated with IgM, ADG, and FEC. For goats, SNPs on CHR3 (42898132) and CHR22 (23066762) were associated with ADG and IgM. In both species, no significant associations were found for IgA, IgG and PCV. The results from this study revealed genes involved in the immune response to *H. contortus* exposure and provide additional SNP marker information that has potential to aid selection of resistance to gastrointestinal parasites in sheep and goats from different breeds. Significant SNPs within IL12RB2, NFIL3 and STAT4 genes could be potential markers for IgM, FEC and ADG in sheep populations. For goats, potential markers for IgM and ADG were identified within IL5RA and IL12RB2 genes. These results could be directly implemented in the populations used in this study, however, they should be validated before using these markers in other sheep and goat populations.

**Author:** Omar, A. I., Alam, M. B. B., Notter, D. R., Zhao, S., Faruque, M. O., Thi, T. N. T., Yin, L., Li, J., Azmal, S. A. and Du, X.

**Title:** Association of single nucleotide polymorphism in NLRC3, NLRC5, HIP1, and LRP8 genes with fecal egg counts in goats naturally infected with *Haemonchus contortus*

**DOI:** 10.1007/s11250-019-02154-z

**Abstract:** *Haemonchus contortus* is a common, intractably pathogenic and economically important gastrointestinal nematode for goat producers worldwide, especially in tropical and subtropical regions. The objective of this study is to identify single nucleotide polymorphisms (SNPs) of 12 candidate goat genes mainly related to the innate immune response associated with fecal egg counts (FECs) of *Haemonchus contortus* in goat as an indicator of the level of parasite infection. Phenotypic data including FEC and blood traits were recorded in 189 native goats from China and 191 ones from Bangladesh, respectively. Bangladeshi goats had significantly ( $P < 0.01$ ) lower FEC compared to that of Chinese goats, suggesting higher susceptible and infection rates in Chinese goat populations. FEC was significantly positive correlated with body weight ( $r = 0.64$ ,  $P < 0.01$ ) and hemoglobin ( $r = 0.49$ ,  $P < 0.01$ ) value, but negative with pack cell volume ( $r = -0.63$ ,  $P < 0.05$ ) in goats. Genotyping of SNPs was performed using a matrix-assisted laser desorption ionization time of flight mass spectrometry assay and a generalized linear model was used to evaluate the association between each SNP and goat FEC trait. Eleven novel SNPs in the NLRC3, NLRC5, HIP1, and LRP8, out of 46 variants from these 12 genes, were significantly associated with FEC of goats with a nominal significance level of  $P < 0.05$ . Of these 11 SNPs, linkage disequilibrium were revealed among SNPs in LRP8 ( $r^2 = 0.87$  to 1), between SNPs in NLRC3, NLRC5, and HIP1 ( $r^2 = 0.96$  to 0.99),

respectively. Further, haplotypes within NLRC3, NLRC5, and HIP1 were significantly associated ( $P < 0.001$ ) with FEC. In artificial challenge trial, quantitative real-time PCR exposed that the relative expression of mRNA was higher in the resistant group for NLRC3 ( $P < 0.01$ ), LRP8 and HIP1 ( $P < 0.001$ ) but lower in the resistant group for NLRC5 ( $P < 0.0001$ ), compared to the susceptible group. The possible SNP markers and genes identified in this study could be potentially used in marker-assisted selection for breeding local goats breeds resistant to gastrointestinal nematode parasite particularly for *Haemonchus contortus*, and then for improving health and productivity of goat.

**Author:** Zhang, R., Liu, F., Hunt, P., Li, C., Zhang, L., Ingham, A. and Li, R. W.

**Title:** Transcriptome analysis unraveled potential mechanisms of resistance to *Haemonchus contortus* infection in Merino sheep populations bred for parasite resistance

**DOI:** 10.1186/s13567-019-0622-6

**Abstract:** *Haemonchus contortus* is one of the most pathogenic gastrointestinal nematodes in small ruminants. To understand molecular mechanisms underlying host resistance to this parasite, we used RNA-sequencing technology to compare the transcriptomic response of the abomasal tissue, the site of the host-parasite interaction, of Merino sheep bred to be either genetically resistant or susceptible to *H. contortus* infection. Two different selection flocks, the *Haemonchus* selection flock (HSF) and the *Trichostrongylus* selection flock (TSF), and each contains a resistant and susceptible line, were studied. The TSF flock was seemingly more responsive to both primary and repeated infections than HSF. A total of 127 and 726 genes displayed a significant difference in abundance between resistant and susceptible animals in response to a primary infection in HSF and TSF, respectively. Among them, 38 genes were significantly affected by infection in both flocks. Gene ontology (GO) enrichment of the differentially expressed genes identified in this study predicted the likely involvement of extracellular exosomes in the immune response to *H. contortus* infection. While the resistant lines in HSF and TSF relied on different mechanisms for the development of host resistance, adhesion and diapedesis of both agranulocytes and granulocytes, coagulation and complement cascades, and multiple pathways related to tissue repair likely played critical roles in the process. Our results offered a quantitative snapshot of changes in the host transcriptome induced by *H. contortus* infection and provided novel insights into molecular mechanisms of host resistance.

## Alternative treatments

**Author:** Acevedo-Ramírez, P. M. D. C., Hallal-Calleros, C., Flores-Pérez, I., Alba-Hurtado, F., Mendoza-Garfías, M. B., Castro del Campo, N. and Barajas, R.

**Title:** Anthelmintic effect and tissue alterations induced in vitro by hydrolysable tannins on the adult stage of the gastrointestinal nematode *Haemonchus contortus*

**DOI:** 10.1016/j.vetpar.2018.12.008

**Abstract:** *Haemonchus contortus* constitutes a severe problem for ruminant's production, it is the most frequent nematode parasite both in temperate and tropical regions, induces economical losses, and shows increasing resistance to currently available anthelmintics. Tannins are secondary metabolites that naturally fulfill defense functions in plants, representing a non-conventional, natural alternative in the treatment of gastrointestinal parasites in sheep. The objective of this work was to explore the in vitro anthelmintic activity of hydrolysable tannins on adult stage of *Haemonchus contortus*. Adult nematodes were obtained directly from the abomasum of ovines, and a dose response curve was performed with tannins extract at 0, 2, 4, 8, 25 and 50 mg/mL, and a time response curve at 0.5, 1, 2 and 24 h at 37 °C. Hydrolysable tannins decreased motility and induced mortality of *H. contortus*. We observed cuticle disruption around the mouth and reproductive organs, as well as evisceration. After 24 h of exposition, 8 mg/mL induced 83% of mortality and with 25 mg/kg 100% of mortality was achieved. The LD 50 was 3.54 mg/mL, while LD 90 was 10 mg/mL. We propose hydrolysable tannins as an alternative to contribute in the nematode control in ruminants.

**Author:** Alowanou, G. G., Olounladé, P. A., Akouèdegni, G. C., Faihun, A. M. L., Koudandé, D. O. and Hounzangbé-Adoté, S.

**Title:** In vitro anthelmintic effects of *Bridelia ferruginea*, *Combretum glutinosum*, and *Mitragyna inermis* leaf extracts on *Haemonchus contortus*, an abomasal nematode of small ruminants

**DOI:** 10.1007/s00436-019-06262-5

**Abstract:** Gastrointestinal nematodes remain a major constraint on the health, welfare, and production of small ruminants. This study was conducted to evaluate three plant extracts (from *Bridelia ferruginea*, *Combretum glutinosum*, and *Mitragyna inermis*) as effective remedies against gastrointestinal parasites of small ruminants. Phytochemical screening was conducted on the plant leaves, and the potential anthelmintic properties of these plants were tested in vitro on *Haemonchus contortus* using the egg hatch, larval migration, and adult worm motility assays. The phytochemical screening of the leaves revealed the presence of several bioactive components in all the plants. The number of eggs that hatched was reduced in a concentration-dependent manner ( $p < 0.01$ ) upon treatment with the methanol extract of *B. ferruginea* and the acetone extracts of *C. glutinosum* and *M. inermis*. The inhibitory effect of the acetone extract of *B. ferruginea* and the methanol extracts of *C. glutinosum* and *M. inermis* was not concentration-dependent ( $p > 0.05$ ). There was a significant difference ( $p < 0.05$ ) in the reduction in larval migration between the lowest concentrations (75 to 150  $\mu\text{g/mL}$ ) and the highest concentrations (300 to 1200  $\mu\text{g/mL}$ ) of plant extracts. The ability of plant extracts to affect the mobility of the adult worms was not concentration-dependent ( $p > 0.05$ ); however, it was dependent on the time of incubation ( $p < 0.01$ ). At the highest concentration (2400  $\mu\text{g/mL}$ ), all adult worms were motionless after 24 h of exposure, while at the lowest concentration ( $< 150 \mu\text{g/mL}$ ), this occurred after 48 h of exposure. *M. inermis* and *C. glutinosum* extracts were more effective than *B. ferruginea* extracts ( $p < 0.05$ ). Overall, these results suggest that these plants used by small-scale farmers possess antiparasitic properties useful for

helminthiasis control. However, the effects of the plants remain to be confirmed via in vivo assays and toxicity tests in further studies.

**Author:** Araujo, A. C. M., Almeida, E. B., Rocha, C. Q., Lima, A. S., Silva, C. R., Tangerina, M. M. P., Lima Neto, J. S. and Costa-Junior, L. M.

**Title:** Antiparasitic activities of hydroethanolic extracts of *Ipomoea imperati* (Vahl) Griseb. (Convolvulaceae)

**DOI:** 10.1371/journal.pone.0211372

**Abstract:** *Ipomoea imperati* is widely used in tropical areas to treat several pathological conditions. The effect of this plant against parasitic species has not been investigated even being used for this purpose in the Brazilian northeastern. This study aimed to evaluate the anthelmintic and acaricide potential of a hydroethanolic extract of *I. imperati* leaves and stolons. *I. imperati* leaves and stolons were crushed and subjected to maceration in ethanol 70% (v/v), after which the solvent was removed using a rotary evaporator. The chromatographic profile of the extract was obtained by UV Spectrum high-performance liquid chromatography and compounds were identified by liquid chromatography/electrospray ionization tandem mass spectrometry. Identification of the compounds present in the extract was achieved by comparing their retention times and UV spectra with data in the literature. Anthelmintic activity was evaluated by larval exsheathment inhibition assays using *Haemonchus contortus* larvae and five concentrations of each extract ranging from 0.07 to 1.2 mg/mL. Acaricide activity was evaluated via larval immersion of *Rhipicephalus microplus* in eight concentrations of each extract ranging from 5.0 to 25.0 mg/mL. Live and dead larvae were counted after 24 hours. The median inhibitory concentration (IC<sub>50</sub>) for *H. contortus* larvae and the median lethal concentration (LC<sub>50</sub>) for *R. microplus* larvae were calculated. Twelve compounds were observed in the hydroethanolic extract of leaves, with a predominance of the aglycone form of flavonoids and tannins. This extract was effective against *H. contortus* larvae, presenting an average inhibitory concentration of 0.22 mg/mL, but showed no activity toward *R. microplus* larvae. The stolon hydroethanolic extract presented 11 compounds, with phenolic acids and glycosylated flavonoids prevailing. This extract showed low activity on *R. microplus* and no effect on inhibiting *H. contortus* larval exsheathment at the concentrations tested. This study is the first to assess the anthelmintic and acaricidal activities of *I. imperati*. Data reported confirm promising potential of *I. imperati* leaves hydroethanolic extract against *H. contortus*. This effect could be due to its secondary compounds presents in this extract, such as procyanidin, kaempferol, isoquercitrin and rutin.

**Author:** Alimi, D., Rekik, M. and Akkari, H.

**Title:** Comparative in vitro efficacy of kefir produced from camel, goat, ewe and cow milk on *Haemonchus contortus*

**DOI:** 10.1017/s0022149x18000378

**Abstract:** One of the great challenges of veterinary parasitology is the search for alternative methods for controlling gastrointestinal parasites in small ruminants. Milk kefir is a traditional source of probiotic, with great therapeutic potential. The objective of this study was to investigate the anthelmintic effects of kefir on the abomasal nematode *Haemonchus contortus* from sheep. The study used camel, goat, ewe and cow milk as a starting material, to produce camel, goat, cow and ewe milk kefir. All kefirs showed a significant concentration-dependent effect on *H. contortus* egg hatching at all tested concentrations. The highest inhibition (100%) of eggs was observed with camel milk kefir at a concentration 0.125 mg/ml. In relation to the effect of kefirs on the survival of adult parasites, all kefirs induced concentration-dependent mortality in adults, with variable results. The complete mortality (100%) of adults of *H. contortus* occurred at concentrations in the range 0.25-2 mg/ml. The highest inhibition of motility (100%) of worms was observed after 8 h post exposure with camel milk kefir at 0.25 mg/ml. These findings indicate that kefir can be considered a potential tool to control haemonchosis in sheep. Further investigations are needed to assess the active molecules in kefir responsible for its anthelmintic properties and to investigate similar in vivo effects.

**Author:** Adamu, M., Mukandiwa, L., Awouafack, M. D., Ahmed, A. S., Eloff, J. N. and Naidoo, V.

**Title:** Ultrastructure changes induced by the phloroglucinol derivative agrimol G isolated from *Leucosidea sericea* in *Haemonchus contortus*

**DOI:** 10.1016/j.exppara.2019.107780

**Abstract:** Plant extracts used for the treatment of helminth infections in sheep are an alternative to chemical anthelmintic drugs. Previous studies have reported the anthelmintic activity of acetone leaf extracts of *Leucosidea sericea*. For this study, we evaluate the ultrastructure changes induced by the acetone leaf extract of *L. sericea* and the component agrimol G (AG) that was isolated for the first time on adult haemonchus parasites. Adult haemonchus parasites harvested from sheep were incubated with the plant extract and AG for 3 h and evaluated by both scanning and transmission electron microscopy in comparison and in combination with albendazole or ivermectin. In all cases the method of evaluation shows ultrastructural changes, with albendazole inducing mitochondrial damage and ivermectin inducing muscle degeneration, both as previously described. Incubation with the plant extract and AG resulted in the formation of numerous non-membrane bound multi-vesicular like bodies and evenly spread disruptions/erosion in the epicuticle. Combining AG with ivermectin or albendazole resulted in an absence of effect of AG. Based on the structural changes induced by AG, together with the absence of

an effect in combination with ivermectin and albendazole would suggest a disrupted microtubular network. The latter does however require biochemical confirmation.

**Author:** Castañeda-Ramírez, G. S., Torres-Acosta, J. F. D. J., Sandoval-Castro, C. A., Borges-Argáez, R., Cáceres-Farfán, M., Mancilla-Montelongo, G. and Mathieu, C.

**Title:** Bio-guided fractionation to identify *Senegalia gaumeri* leaf extract compounds with anthelmintic activity against *Haemonchus contortus* eggs and larvae

**DOI:** 10.1016/j.vetpar.2019.05.001

**Abstract:** Small ruminants browsing in tropical forests readily consume the foliage of *Senegalia gaumeri*. A *S. gaumeri* methanol:water extract was recently shown to have ovicidal activity against *Haemonchus contortus* eggs in vitro. In the present study, the fraction of a *S. gaumeri* methanol:water extract with ovicidal activity against *H. contortus* eggs and the metabolites potentially involved in this activity were identified. Bio-guided fractionation of the *S. gaumeri* methanol:water extract identified high ovicidal activity (80.29%, EC<sub>50</sub> = 58.9 µg/mL) in the non-polar sub-fraction P1. Gas chromatography-mass spectrometry (GC–MS) identified several fatty acids: pentacosane (18.05%), heneicosane (18.05%), triacontane (30.94%), octacosane (18.05%), and hexanedioic acid bis-(2-ethylhexyl) ester (32.72%). Purification of the polar components of sub-fraction P1 led to the identification of *p*-coumaric acid as a major constituent. In egg hatch tests, 400 µg/mL *p*-coumaric acid resulted in an ovicidal effect of 8.7%, a larvae failing eclosion effect of 2.9%, and of the emerged larvae (88.4%), many were damaged. In conclusion, the low AH activity of *p*-coumaric acid against *H. contortus* eggs indicates that it is not solely responsible for the ovicidal activity of sub-fraction P1 but might act in synergy with other compounds in this fraction. However, *p*-coumaric acid showed potential anthelmintic effects against the larval stage of *H. contortus*.

**Author:** Cériac, S., Archimède, H., Feuillet, D., Félicité, Y., Giorgi, M. and Bambou, J. C.

**Title:** Supplementation with rumen-protected proteins induces resistance to *Haemonchus contortus* in goats

**DOI:** 10.1038/s41598-018-37800-3

**Abstract:** Resistance to gastro-intestinal nematode (GIN) in small ruminant is expected to arise from protein-rich rather than from energy-rich feeds. The objective of this study was to investigate the effect of the quality of the dietary proteins on the response of Creole goats to *Haemonchus contortus*. Three diets were compared: no supplementation (Hay: hay ad libitum), Control supplement (CS: hay ad libitum +2% BW of CS at 70 g of by-pass proteins/kg) and supplement enriched in rumen-protected proteins (RPP: hay ad libitum +2% BW of RPP at 139 g of by-pass proteins/kg). The FEC (faecal eggs counts) and the TFEC (total faecal eggs excreted/day) were significantly lower in the RPP. No difference was found between the supplemented diets for the total number of nematodes, but the RPP reduced the parasite prolificacy. The highest IgA responses were observed in

animals with the highest nematode burden (Hay compared with CS diets). However, while the FEC and the TFEC were lower in animals feed with the RPP the IgA response were similar to those of the Hay. The IgA response that control GIN egg production in sheep could be one mediator of the resistance to *H. contortus* induced with by-pass proteins in goats.

**Author:** Cortes-Morales, J. A., Olmedo-Juárez, A., Trejo-Tapia, G., González-Cortazar, M., Domínguez-Mendoza, B. E., Mendoza-de Gives, P. and Zamilpa, A.

**Title:** In vitro ovicidal activity of *Baccharis conferta* Kunth against *Haemonchus contortus*

**DOI:** 10.1016/j.exppara.2019.01.003

**Abstract:** The indiscriminate use of chemical drugs to deworm livestock tends to trigger an anthelmintic resistance problem. In this context, the use of plant extracts rich in secondary metabolites could be an alternative method for the control of gastrointestinal nematodes. *Baccharis conferta* Kunth is a native plant species from Mexico that is widely used by several ethnic groups as forage for farm animals and medicinally to treat gastrointestinal diseases such as acute stomach ache, dysentery, diarrhoea, vomiting, indigestion, colic, intestinal spasms, urinary problems, and cramps. The aim of the present study was to isolate and characterise the ovicidal constituents of *B. conferta* and to determine a possible mode of action against *Haemonchus contortus*. The ovicidal activity was determined using the egg hatching inhibition test (EHI) to assess the methanol extract obtained from *B. conferta* foliage. The dry extract was partitioned (water/ethyl acetate) to obtain an ethyl acetate (BcEtOAc-F) and aqueous fraction. BcEtOAc-F showed an ovicidal activity of 72.32% EHI at 1 mg/mL. The chromatographic fractionation of BcEtOAc-F resulted in three active sub-fractions with higher ovicidal activity: BcC1R4 (99.15% EHI at 1.0 mg/mL); BcC1R5 (92.51% EHI at 0.75 mg/mL); and BcC1R8 (96.8% EHI at 3.0 mg/mL). Chemical analysis of the BcC1R4 fraction allowed the identification of the major active compound, isokaempferide (1, 98.06% EHI at 1 mg/mL). While, 4,5-di-O-acid caffeoylquinic (3; 96.8% EHI at 3 mg/mL) and an inactive flavone (vicenin-2, 2) were identified as the main compounds in BcC1R8. Chemical characterisation of the isolated compounds was performed via spectroscopic (NMR) and spectrometric (UPLC-MS) analyses. Additionally, the environmental and confocal scanning microscopy analyses revealed that isokaempferide was able to cross the eggshell layer without breaking it and attach itself to the embryo, causing its death. The flavonol, isokaempferide, and the hydroxycinnamic acid, 4,5-di-O-caffeoylquinic, displayed powerful ovicidal effects, proving to be a potential alternative for the development of a phytodrug for the control of haemonchosis.

**Author:** Davuluri, T., Chennuru, S., Pathipati, M., Krovvidi, S. and Rao, G. S.

**Title:** In Vitro Anthelmintic Activity of Three Tropical Plant Extracts on *Haemonchus contortus*

**DOI:** 10.2478/s11686-019-00116-x

**Abstract:** Aim: To evaluate the anthelmintic activity of *Anacardium occidentale* shell, *Illicium verum* fruit, and *Artocarpus heterophyllus* seed to substantiate their traditional use against helminths. Materials and methods: In vitro anthelmintic activity of hydroalcoholic extracts of three plants was evaluated against eggs, infective larvae (L3), adult, and lactate dehydrogenase (LDH) of *Haemonchus contortus* of naturally infected sheep. Results: The three extracts exhibited significant ( $P < 0.001$ ) dose-dependent anthelmintic responses by inhibiting egg hatching and causing paralysis of larvae and mortality of worms. The extracts were most effective on egg and adult stage of *H. contortus* than on L3 stage at a higher concentration of 6 mg/mL. *Anacardium occidentale* shell exhibited maximum activity with 100% paralysis of L3 larvae. Probit analysis revealed that the extracts of *A. Occidentale* shell induced 50% egg hatch inhibition ( $LD_{50} = 0.0255$  mg/mL), larval paralysis ( $LD_{50} = 0.196$  mg/mL), and adult worm mortality ( $LD_{50} = 1.0365$  mg/mL) at a lower concentration ( $LD_{50}$ ) compared with those of *I. verum* fruit and *A. heterophyllus* seed extracts. Further, all extracts significantly ( $P < 0.01$ ) inhibited the LDH activity catalyzing the oxidation of lactate in adult *H. contortus*, with maximum level of inhibition caused by *A. occidentale* shell extract. Conclusion: Phytochemical screening of the extracts revealed the presence of alkaloids, flavonoids, tannins, saponins, and amino acids that could be responsible for the anthelmintic effects noticed. The results warrant further in vivo evaluation of these plants for potential use as anthelmintic agents.

**Author:** De Jesús-Martínez, X., Olmedo-Juárez, A., Rojas Hernández, S., Zamilpa, A., de Gives, P. M., Lopez-Arellano, M. E., Villa-Mancera, A., Camacho-Díaz, L. M., Cipriano Salazar, M. and Olivares-Pérez, J.

**Title:** Evaluation of the hydroalcoholic extract elaborated with *Caesalpinia coriaria* Jacq Willd tree fruits in the control of *Haemonchus contortus* Rudolphi

**DOI:** 10.1007/s10457-019-00398-0

**Abstract:** The aim of this study was to evaluate the in vitro lethal effect of a hydroalcoholic extract (HAE) from *C. coriaria* fruit against eggs and larvae of *H. contortus* of domestic ruminants. The HAE was assessed using five concentrations: 50, 25, 12.5, 6.15 and 3.12 mg/mL to eggs and 100, 125, 175, 150 and 200 mg/mL to larvae, respectively; 0.5% ivermectin was used as positive control and 4% methanol in distilled water as negative control. The data of larvicidal and ovicidal effect were analysed with a completely randomised design by ANOVA analysis using the general linear model, while lethal concentrations ( $LC_{50}$  and  $LC_{90}$ ) were estimated by a Probit analysis of the SAS programme. A clear HAE-increased concentration dependence effect was observed on eggs and larvae. The highest activity of the HAE was obtained at the highest concentration ( $P < 0.05$ ) to obtain a similar effect as the positive control (ivermectin), with  $LC_{50} = 22.93$  and  $10.3$  mg/mL and  $LC_{90} = 44.0$  and  $84.18$  mg/mL, respectively, for larvae and eggs. The results indicate that the HAE of *C. coriaria* fruit possesses in vitro ovicidal and larvicidal properties (total phenols methyl gallate) against *H. contortus*, and it becomes an alternative for in vivo research for the control of gastroenteric nematodes in ruminants, either whole fruits or extracts administered orally. In this context, in vivo studies with this legume are thus necessary.



**Author:** Herath, H. M. P. D., Preston, S., Jabbar, A., Garcia-Bustos, J., Taki, A. C., Addison, R. S., Hayes, S., Beattie, K. D., McGee, S. L., Martin, S. D., Ekins, M. G., Hooper, J. N. A., Chang, B. C. H., Hofmann, A., Davis, R. A. and Gasser, R. B.

**Title:** Identification of fromiamycalin and halaminol a from australian marine sponge extracts with anthelmintic activity against haemonchus contortus

**DOI:** 10.3390/md17110598

**Abstract:** There is an urgent need to discover and develop new anthelmintics for the treatment of parasitic nematodes of veterinary importance to circumvent challenges linked to drug resistant parasites. Being one of the most diverse natural ecosystems, the marine environment represents a rich resource of novel chemical entities. This study investigated 2000 extracts from marine invertebrates, collected from Australian waters, for anthelmintic activity. Using a well-established in vitro bioassay, these extracts were screened for nematocidal activity against *Haemonchus contortus*- a socioeconomically important parasitic nematode of livestock animals. Extracts (designated Mu-1, Ha-1 and Ha-2) from two marine sponges (*Monanchora unguiculata* and *Haliclona* sp.) each significantly affected larvae of *H. contortus*. Individual extracts displayed a dose-dependent inhibition of both the motility of exsheathed third-stage larvae (xL3s) and the development of xL3s to fourth-stage larvae (L4s). Active fractions in each of the three extracts were identified using bioassay-guided fractionation. From the active fractions from *Monanchora unguiculata*, a known pentacyclic guanidine alkaloid, fromiamycalin (1), was purified. This alkaloid was shown to be a moderately potent inhibitor of L4 development (half-maximum inhibitory concentration (IC<sub>50</sub>) = 26.6 ± 0.74 µM) and L4 motility (IC<sub>50</sub> = 39.4 ± 4.83 µM), although it had a relatively low potency at inhibiting of xL3 motility (IC<sub>50</sub> ≥ 100 µM). Investigation of the active fractions from the two *Haliclona* collections led to identification of a mixture of amino alcohol lipids, and, subsequently, a known natural product halaminol A (5). Anthelmintic profiling showed that 5 had limited potency at inhibiting larval development and motility. These data indicate that fromiamycalin, other related pentacyclic guanidine alkaloids and/or halaminols could have potential as anthelmintics following future medicinal chemistry efforts.

**Author:** Jiao, Y., Preston, S., Garcia-Bustos, J. F., Baell, J. B., Ventura, S., Le, T., McNamara, N., Nguyen, N., Botteon, A., Skinner, C., Danne, J., Ellis, S., Koehler, A. V., Wang, T., Chang, B. C. H., Hofmann, A., Jabbar, A. and Gasser, R. B.

**Title:** Tetrahydroquinoxalines induce a lethal evisceration phenotype in *Haemonchus contortus* in vitro

**DOI:** 10.1016/j.ijpddr.2018.12.007

**Abstract:** In the present study, the anthelmintic activity of a human tyrosine kinase inhibitor, AG-1295, and 14 related tetrahydroquinoxaline analogues against *Haemonchus contortus* was explored. These compounds were screened against parasitic larvae - exsheathed third-stage (xL3) and fourth-stage (L4) - using a whole-organism screening

assay. All compounds were shown to have inhibitory effects on larval motility, development and growth, and induced evisceration through the excretory pore in xL3s. The estimated IC 50 values ranged from 3.5 to 52.0  $\mu$ M for inhibition of larval motility or development. Cytotoxicity IC 50 against human MCF10A cells was generally higher than 50  $\mu$ M. Microscopic studies revealed that this eviscerated (Evi) phenotype occurs rapidly (<20 min) and relates to a protrusion of internal tissues and organs (evisceration) through the excretory pore in xL3s; severe pathological damage in L4s as well as a suppression of larval growth in both stages were also observed. Using a relatively low concentration (12.5  $\mu$ M) of compound m10, it was established that the inhibitor has to be present for a relatively short time (between 30 h and 42 h) during in vitro development from xL3 to L4, to induce the Evi phenotype. Increasing external osmotic pressure prevented evisceration and moulting, and xL3s remained unaffected by the test compound. These results point to a mode of action involving a dysregulation of morphogenetic processes during a critical time-frame, in agreement with the expected behaviour of a tyrosine kinase inhibitor, and suggest potential for development of this compound class as nematocidal drugs.

**Author:** Katiki, L. M., Araujo, R. C., Ziegelmeyer, L., Gomes, A. C. P., Gutmanis, G., Rodrigues, L., Bueno, M. S., Verissimo, C. J., Louvandini, H., Ferreira, J. F. S. and Amarante, A. F. T.

**Title:** Evaluation of encapsulated anethole and carvone in lambs artificially- and naturally-infected with *Haemonchus contortus*

**DOI:** 10.1016/j.exppara.2019.01.002

**Abstract:** Molecules from natural sources, such as essential oils, have shown activity against parasites in vitro, but have not yet been explored extensively in vivo. Anethole and carvone (10% each), encapsulated with 80% of a solid matrix, referred to as EO (encapsulated oils), were tested in vivo in 2 experiments. In Experiment 1: Lambs were artificially infected with multidrug resistant *Haemonchus contortus*, or left uninfected, and treated (or not) with 50 mg/kg bw (body weight) of EO in a controlled environment. Thirty-two male lambs were kept in individual cages for a period of 45 days, after which animals were evaluated for parasitological, hematological, toxicological, and nutritional parameters. After 45 days of treatment, EO at 50 mg/kg bw provided a significant ( $P \leq 0.05$ ) reduction in fecal egg count (FEC). Although FEC was reduced, animals from both treatments had similar counts of total adult worms. The low FEC was caused probably by a significant reduction ( $P \leq 0.05$ ) in both male worm size and female fecundity. Dry matter intake of uninfected controls was significantly ( $P \leq 0.05$ ) reduced, although no toxicity was observed in treated animals. Thus, in Experiment 2, conducted for five months we used an EO dose of 20 mg/kg bw. Thirty-four weaned lambs, free of parasites, were divided in two groups and kept in collective pens. One group received EO at 20 mg/kg bw mixed with concentrate for 5 months and the other was kept as a control group (CTL). Parasitological and hematological parameters as well as body weight were evaluated. In the first 2.5 months, CTL and EO groups were confined, and both presented similar clinical parameters. Then, animals were allotted to graze on contaminated pastures to acquire natural infection for the next 2.5 months. The infection was patent after 25 days and both

groups had similar decreases in weight gain, increases in FEC, and decreases in blood parameters. Coprocultures from CTL and EO groups established that parasite population was 90% *Haemonchus* sp. We concluded that the technology of encapsulation is safe and practical to deliver to lambs at the farm level and anethole and carvone at 50 mg/kg bw caused a significant decrease in FEC and, consequently, in pasture contamination by free living stages of *H. contortus*. However, EO at 20 mg/kg bw was not effective to prevent or treat sheep naturally-infected with gastrointestinal nematodes.

**Author:** Le, T. G., Kundu, A., Ghoshal, A., Nguyen, N. H., Preston, S., Jiao, Y., Ruan, B., Xue, L., Huang, F., Keiser, J., Hofmann, A., Chang, B. C. H., Garcia-Bustos, J., Wells, T. N. C., Palmer, M. J., Jabbar, A., Gasser, R. B. and Baell, J. B.

**Title:** Novel 1-Methyl-1 H-pyrazole-5-carboxamide Derivatives with Potent Anthelmintic Activity

**DOI:** 10.1021/acs.jmedchem.8b01790

**Abstract:** A phenotypic screen of two different libraries of small molecules against the motility and development of the parasitic nematode *Haemonchus contortus* led to the identification of two 1-methyl-1H-pyrazole-5-carboxamide derivatives. Medicinal chemistry optimization targeted modifications of the left-hand side, middle section, and right-hand side of the hybrid structure of these two hits to elucidate the structure-activity relationship (SAR). Initial SAR around these hits allowed for the iterative and directed assembly of a focused set of 30 analogues of their hybrid structure. Compounds 10, 17, 20, and 22 were identified as the most potent compounds, inhibiting the development of the fourth larval (L4) stage of *H. contortus* at sub-nanomolar potencies while displaying strong selectivity toward the parasite when tested in vitro against the human MCF10A cell line. In addition, compounds 9 and 27 showed promising activity against a panel of other parasitic nematodes, including hookworms and whipworms.

**Author:** Le, T. G., Kundu, A., Ghoshal, A., Nguyen, N. H., Preston, S., Jiao, Y., Ruan, B., Xue, L., Huang, F., Keiser, J., Hofmann, A., Chang, B. C. H., Garcia-Bustos, J., Wells, T. N. C., Palmer, M. J., Jabbar, A., Gasser, R. B. and Baell, J. B.

**Title:** Structure-Activity Relationship Studies of Tolfenpyrad Reveal Subnanomolar Inhibitors of *Haemonchus contortus* Development

**DOI:** 10.1021/acs.jmedchem.8b01789

**Abstract:** Recently, we have discovered that the registered pesticide, tolfenpyrad, unexpectedly and potently inhibits the development of the L4 larval stage of the parasitic nematode *Haemonchus contortus* with an IC<sub>50</sub> value of 0.03  $\mu$ M while displaying good selectivity, with an IC<sub>50</sub> of 37.9  $\mu$ M for cytotoxicity. As a promising molecular template for medicinal chemistry optimization, we undertook anthelmintic structure-activity relationships for this chemical. Modifications of the left-hand side (LHS), right-hand side (RHS), and middle section of the scaffold were explored to produce a set of 57 analogues. Analogues

25, 29, and 33 were shown to be the most potent compounds of the series, with IC 50 values at a subnanomolar level of potency against the chemotherapeutically relevant fourth larval (L4) stage of *H. contortus*. Selected compounds from the series also showed promising activity against a panel of other different parasitic nematodes, such as hookworms and whipworms.

**Author:** Mancilla-Montelongo, G., Castañeda-Ramírez, G. S., Torres-Acosta, J. F. D. J., Sandoval-Castro, C. A. and Borges-Argáez, R.

**Title:** Evaluation of cinnamic acid and six analogues against eggs and larvae of *Haemonchus contortus*

**DOI:** 10.1016/j.vetpar.2019.05.009

**Abstract:** This study evaluated the *in vitro* anthelmintic (AH) activity of cinnamic acid and six analogues against eggs and larvae of *Haemonchus contortus*. Stock solutions of each compound (trans-cinnamic acid, p-coumaric acid, caffeic acid, trans-ferulic acid, trans-sinapic acid, 3,4-dimethoxycinnamic acid, and chlorogenic acid) were prepared in PBS:Tween-20 (1%) for use in the egg hatch test (EHT) and larval exsheathment inhibition test (LEIT) at different concentrations (25–400 µg/mL). The respective effective concentration 50% (EC<sub>50</sub>) values with 95% confidence intervals were estimated. Mixtures made of all cinnamic acid and its analogues as well as some selected individual compounds were also tested in the EHT. Only ferulic and chlorogenic acids showed AH activity in the EHT (EC<sub>50</sub>: 245.2 µg/mL (1.26 mM) and 520.8 µg/mL (1.47 mM), respectively) (*P* < 0.05). A higher EC<sub>50</sub> (1628.10 µg/mL) of the mixture of cinnamic acid and its analogues was required to observe activity against eggs mostly blocking the larvae hatching. The analogues' mixtures tested were less active than ferulic or chlorogenic acid alone. The activity of ferulic and chlorogenic acids against eggs was associated with larvae failing to hatch, and the two compounds exhibited antagonistic effects when evaluated together. All standards had an EC<sub>50</sub> lower than 0.42 mM in the LEIT. Caffeic acid had the best activity in the LEIT (EC<sub>50</sub> 0.04 mM), followed by ferulic acid (EC<sub>50</sub> 0.11 mM) (*P* < 0.05). There was no clear, definitive structure-activity relationship for these non-flavonoid polyphenols against eggs or larvae of *H. contortus* *in vitro*. This study is the first to directly evaluate cinnamic acid and its derivatives as active compounds against eggs and larvae of *H. contortus*.

**Author:** Martínez-Ortiz-De-Montellano, C., Torres-Acosta, J. F. D. J., Fourquaux, I., Sandoval-Castro, C. A. and Hoste, H.

**Title:** Ultrastructural study of adult *Haemonchus contortus* exposed to polyphenol-rich materials under *in vivo* conditions in goats

**DOI:** 10.1051/parasite/2019065

**Abstract:** This study assessed the ultrastructural changes caused in adult *Haemonchus contortus* obtained from goats fed fodder based on polyphenol-rich plants *Lysiloma*

latisiliquum or *Onobrychis viciifolia* or from goats drenched with quebracho extract, *Schinopsis* spp. The *H. contortus* were obtained from artificially infected goats used as models to investigate the anthelmintic effect of feeding or drenching with the polyphenol-rich materials. Nematode populations were exposed to polyphenol-rich plant materials by feeding host goats for 8 consecutive days (D28 to D35 post-infection) with (a) *L. latisiliquum* fodder at 800 g fresh basis/day, (b) *O. viciifolia* fodder offered ad libitum, and (c) drenched with a solution containing quebracho extract (90 g/day). Meanwhile, control *H. contortus* were obtained from goats fed polyphenol-free diets. The *H. contortus* specimens were recovered from the goats on D36 post-infection, and transmission electron microscopy (TEM) was used to identify ultrastructural changes. In vivo exposure to different polyphenol-rich plant materials caused vacuolization of the nematodes' intestinal, muscular and hypodermal cells. These alterations represent the first evidence of cell damage caused in *H. contortus* when hosts were fed or drenched with polyphenol-rich materials. Ultrastructural changes affecting several types of cells could explain modifications in worm motility and nutrition, eventually affecting *H. contortus* reproductive success. This study contributes to our understanding of the mechanisms of action of polyphenol-rich plants against *H. contortus* when given as nutraceuticals to goats.

**Author:** Mata-Padrino, D. J., Belesky, D. P., Crawford, C. D., Walsh, B., MacAdam, J. W. and Bowdridge, S. A.

**Title:** Effects of grazing birdsfoot trefoil-enriched pasture on managing *Haemonchus contortus* infection in Suffolk crossbred lambs

**DOI:** 10.1093/jas/sky405

**Abstract:** High-tannin forages can be used to help mitigate the serious limitations associated with gastrointestinal nematode (GIN) infections on efficient small ruminant production. The objective of this experiment was to determine how grazing a GIN-free, established stand of a high-tannin cultivar of birdsfoot trefoil (*Lotus corniculatus* L.) influenced the prevention or treatment of *Haemonchus contortus* (Hc) infection in lambs. A birdsfoot trefoil-enriched pasture was established on an area that was previously row cropped and not grazed for at least 15 yr. Treatments included preventative (PREV) with parasite-naïve lambs transitioned onto pasture 1 wk prior to receiving an infection of 10,000 Hc larvae, therapy (THER) with parasite-naïve lambs infected with 10,000 Hc larvae 4 wk prior to the start of grazing, and control (CONT) with naïve, uninfected lambs to verify that natural infection did not occur on pasture. Each treatment group of 12 Suffolk crossbred lambs was divided into 3 replicates per treatment, and all were supplemented with a grain mix to provide 16% CP. Fecal egg count (FEC, eggs/g wet feces) in THER lambs peaked 1 wk after the start of grazing (9,404) and after 4 wk fell to 1,068, equivalent to a FEC reduction of 88.6%. Lambs in PREV had a peak FEC of 4872 at 4 wk after infection where peak FEC was 48% less in PREV than THER lambs. Lambs in CONT did not have measurable FEC for the duration of this study. Packed cell volume (PCV, %) reflected infection status of the lambs in each group, where CONT (32%) had the highest ( $P < 0.05$ ) PCV followed by THER (29%) and PREV (26%). Total weight gained in CONT lambs was greatest at 5.51 kg ( $P < 0.01$ ), whereas THER and PREV (2.68 and 2.97 kg,

respectively) did not differ. Grazing birdsfoot trefoil-enriched pasture can have both therapeutic and preventative effects on Hc infection in lambs and can be used in a systems approach to control GIN parasites in grazing sheep.

**Author:** Mendonça Soares, S., Domingues, R., Baldo Gaspar, E., Azevedo Dos Santos, P., Marques Canuto, K., Pelegrine Minho, A. and Botelho Vieira, M. I.

**Title:** In vitro ovicidal effect of a *Senecio brasiliensis* extract and its fractions on *Haemonchus contortus*

**DOI:** 10.1186/s12917-019-1843-7

**Abstract:** Background: Haemonchosis affects sheep husbandry and its treatment is often compromised due to the development of anthelmintic resistance. Plant-derived bioactive compounds have been studied as alternative to control *Haemonchus contortus*. The objective of this study was to evaluate the effect of *Senecio brasiliensis* extracts on *H. contortus* egg hatching and infective larvae migration. Results: The aqueous extract from dried and fresh plant and alkaloid-enriched fraction of the previously dried leaves of *S. brasiliensis* inhibited *H. contortus* egg hatching. The main plant compound in alkaloid fraction was integerrimine, a pyrrolizidine alkaloid (PA). However, the aqueous extract from dried plant displayed higher efficacy when compared to their alkaloid enriched or non-polar fractions, meaning that, although PAs contributed to the ovicidal effect, other compounds in the plant can also contribute to their effect. Furthermore, the aqueous extract from dried plant also had higher efficacy than aqueous extract from fresh plant in larvae migration inhibition. Finally, extract from dried plant presented low in vitro cytotoxic effect. Conclusion: Taken together our results suggest a good anthelmintic effect of *S. brasiliensis*, especially when aqueous extract is prepared from dried plant. Further in vivo studies should be performed focused on forms of administration of this extract in rearing sheep.

**Author:** Min, B. R., Frank, A., Gurung, N., Lee, J. H., Joo, J. W. and Pacheco, W.

**Title:** Peanut skin in diet alters average daily gain, ruminal and blood metabolites, and carcass traits associated with *Haemonchus contortus* infection in meat goats

**DOI:** 10.1016/j.aninu.2019.05.006

**Abstract:** The aim of this study was to determine the effects of tannin-rich peanut skin (PS) supplementation on growth performance, ruminal and blood metabolites, and carcass traits associated with internal parasite infection in meat goats under confined conditions. Twenty-one Kiko crossbred male goats were blocked by body weight (BW) and randomly assigned to one of 3 treatment groups. Experimental diets contained different levels of peanut (*Arachis hypogaea*) skin replacing alfalfa (*Medicago sativa*) pellets (ALP) in a control diet. Experimental treatments included: 30% ALP (control), 15% PS and 15% ALP, and 30% PS. Peanut skin was incorporated in the grain mix portion of the diets. Animals were fed once daily, and the intake was adjusted every 3 to 4 d. Each animal was each

artificially infected with 5,000 larvae of the 3rd stage of barber's pole worm (*Haemonchus contortus*). Body weights, dry matter intake (DMI), and fecal samples for fecal egg counts (FEC) were taken at d 0, 12, 23, and 41. Rumen fluid and blood samples were collected at d 45. The performance period lasted 45 d and at the completion of the study, goats were harvested, and carcass characteristics, abomasal worm counts were measured. The results showed that DMI, BW, carcass traits, and meat color were not affected by PS supplementation, whereas average daily gain (ADG,  $P < 0.01$ ), blood glucose ( $P < 0.001$ ), phosphorus ( $P < 0.05$ ), and cholesterol levels ( $P < 0.001$ ) significantly increased with increasing levels of PS supplementation. There was a linear ( $P < 0.01$ ) reduction in rumen acetate to propionate ratio, ammonia-nitrogen, FEC, and *H. contortus* worm counts, with increasing levels of PS supplementation. This study shows that PS supplementation up to 30% of the diet can improve ADG and rumen fermentation while reducing gastrointestinal parasite infection in meat goats.

**Author:** Mravčáková, D., Váradyová, Z., Kopčáková, A., Čobanová, K., Grešáková, L., Kišidayová, S., Babják, M., Dolinská, M. U., Dvorožňáková, E., Königová, A., Vadlejch, J., Cieslak, A., Ślusarczyk, S. and Várady, M.

**Title:** Natural chemotherapeutic alternatives for controlling of haemonchosis in sheep

**DOI:** 10.1186/s12917-019-2050-2

**Abstract:** Background: Parallel in vitro and in vivo experiments were designed to evaluate promising chemotherapeutic alternatives for controlling haemonchosis in ruminants. In vitro anthelmintic activities (egg hatch test - EHT; larval development test - LDT) of aqueous and methanolic herbal extracts Mix1 and Mix2 were investigated. The in vivo effects of dietary supplementation with Mix1 and Mix2 on the parasitological status, inflammatory response, antioxidant parameters and microbial community of the lambs infected experimentally with *Haemonchus contortus* were investigated. Lambs were divided into four groups for the in vivo study: uninfected control lambs (C), infected lambs (I), infected lambs supplemented with Mix1 (I + Mix1) and infected lambs supplemented with Mix2 (I + Mix2). The experimental period was 70 days. Results: The number of eggs per gram (EPG) of feces was quantified 22, 30, 37, 44, 51, 58, 65 and 70 days post-infection, and mean abomasal worm counts were assessed 70 days post-infection. Quantitative analyses identified 57.3 and 22.2 mg/g phenolic acids, 41.5 and 29.5 mg/g flavonoids and 1.4 and 1.33 mg/g protoberberine-type alkaloids in Mix1 and Mix2, respectively. The methanolic extracts of the herbal mixtures in both in vitro tests had higher anthelmintic effects ( $P < 0.01$ ) than the aqueous extracts, but the effects did not differ significantly between Mix1 and Mix2 ( $P > 0.05$ ). I + Mix1 and I + Mix2 lowered mean EPGs between 44 and 70 d by 58.1 and 51.6%, respectively. The level of IgG antibodies against *H. contortus* increased significantly after infection in each infected group. Conclusion: These results represent the first monitoring of the in vitro anthelmintic effects of herbal mixtures on *H. contortus*. The in vivo experiment indicated that the anthelmintic effect was not sufficient for the elimination of parasites, but this herbal treatment may affect the host over a longer term, reducing the parasitic infection in the host.

**Author:** Mustabi, J., Prahesti, K. I. and Nurpaidah

**Title:** Efficacy of calliandra (*Calliandra calothyrsus*) leaf extract on *Haemonchus contortus* mortality in vitro

**DOI:** 10.1088/1755-1315/343/1/012032

**Abstract:** Calliandra (*Calliandra calothyrsus*) is a feed plant that contains high tannins. Tanin in calliandra leaves can cause death in nematodes. The purpose of this study was to determine the effectiveness of calliandra leaf extract, comprising optimum concentration and time, on mortality of *Haemonchus contortus* nematode. Calliandra leaves were extracted by 70% ethanol then analyzed for the tannin content. Twenty-five adult *H. contortus* nematodes were used for mortality test in vitro with a gradual concentration of calliandra leaf extract. The study used a completely randomized design with a factorial pattern consisting of six treatments and four replications. The treatment groups were as follow: R0 as negative control group given normal saline (0.9% NaCl), R1 as positive control (albendazole 10 mg/ml), R2 (10% calliandra leaf extract), R3 (25% calliandra leaf extract), R4 (50% calliandra leaf extract), and R5 (100% calliandra leaf extract). Extraction of calliandra leaves using 70% ethanol produced a tannin content of 5%. In vitro examination showed that administration of the calliandra extract had a significant effect ( $P < 0.01$ ) on mortality of *H. contortus* and showed an interaction between treatment and mortality time. A higher concentration of calliandra extract caused faster mortality of *H. contortus*. The concentration of 100% calliandra extract caused 100% mortality after 3 hours, while the concentration of 10%, 25%, and 50% caused 100% mortality after 4 hours. This study showed that calliandra leaf extract had an anthelmintic efficacy on *H. contortus*.

**Author:** Nixon, S. A., Saez, N. J., Herzig, V., King, G. F. and Kotze, A. C.

**Title:** The antitrypanosomal diarylamidines, diminazene and pentamidine, show anthelmintic activity against *Haemonchus contortus* in vitro

**DOI:** 10.1016/j.vetpar.2019.05.008

**Abstract:** Parasitic nematodes pose a major threat to livestock production worldwide. The blood-feeding parasite *Haemonchus contortus* is a key small-ruminant pathogen that causes anaemia, and thereby seriously impacts animal health and production. Control of this parasite relies largely upon broad-spectrum anthelmintics, but new drugs are urgently needed to combat the threat of widespread multidrug resistance. Repurposing drugs can accelerate the development pipeline by reducing costs and risks, and can be an effective way of quickly bringing new antiparasitic drugs to market. Diarylamidine compounds such as pentamidine and diminazene have been employed in the treatment of trypanosomiasis and leishmaniasis in both human and veterinary settings, but their activity against parasitic worms has not yet been reported. We screened a small panel of diarylamidine compounds against *H. contortus* to assess their potential to be repurposed as anthelmintic drugs. Pentamidine and diminazene inhibited *H. contortus* larval development at low micromolar



concentrations (IC<sub>50</sub> 4.9 μM and 16.1 μM, respectively, in a drug-susceptible isolate) with no existing cross-resistance in two multidrug resistant isolates and a monepantel-resistant isolate. Combinations of pentamidine with commercial anthelmintics showed additive activity, with no significant synergism detected. Pentamidine and diminazene showed different life-stage patterns of activity; both were active against early stage larvae in development assays, but only diminazene was active against the infective L3 stage in migration assays. This suggests some differences in uptake of the two drugs across the nematode cuticle, or differences in the nature and expression patterns of their molecular targets. As pentamidine and diminazene have been reported to be potent inhibitors of mammalian acid-sensing ion channels (ASIC), we tested the activity of known ASIC inhibitors against *H. contortus* to probe whether these channels may represent potential anthelmintic targets in nematodes. Remarkably, the spider-venom peptide Hi1a, a potent inhibitor of ASIC1a, inhibited *H. contortus* larval development with an IC<sub>50</sub> of 22.9 ± 1.9 μM. This study highlights the potential use of diarylamidines as anthelmintics, although their activity needs to be confirmed in vivo. In addition, our demonstration that ASIC inhibitors have anthelmintic activity raises the possibility that this family of ion channels may represent a novel anthelmintic target.

**Author:** Robson Duarte, E., Duarte Matias, A., Almeida Bastos, G., Cordeiro Maia, R., Soares Martins, V. Júnior, Maia Soares, A. C., dos Santos Magaço, F., de Oliveira, N. J. F. and dos Santos, T. A. X.

**Title:** Anthelmintic efficacy of trichlorfon and blood parameters of young lambs infected with *Haemonchus contortus*

**DOI:** 10.1016/j.vetpar.2019.06.015

**Abstract:** In this study we evaluated the efficacy of trichlorfon against *Haemonchus contortus*, monitoring its influence on blood parameters and plasma enzymes of lambs with haemonchosis. A lamb group was orally treated with trichlorfon at 100 mg kg<sup>-1</sup> while the other group was untreated. Split-plot design analysis was performed with the lamb groups defined as plots while the subplots were the four periods (weeks) of collection. The trichlorfon treatment promoted a significant and effective reduction of fecal egg counts after one week, with efficacies > 99%. After 21 days of treatment, detected blood parameters and serum levels of plasma enzymes were normal. Additionally, serum albumin and urea concentrations increased to normal values, which were not observed in untreated lambs. The treatment with this organophosphate, using a correct oral administration, may represent an effective therapeutic alternative for sheep infected with multi resistant strain of *H. contortus*.

**Author:** Rojo-Rubio, R., González-Cortazar, M., Olmedo-Juárez, A., Zamilpa, A., Arece-García, J., Mendoza-Martínez, G. D., Lee, H. A., Vázquez-Armijo, J. F. and Gives, P. M.

**Title:** *Caesalpinia coriaria* fruits and leaves extracts possess in vitro ovicidal activity against *haemonchus contortus* and *haemonchus placei*

**DOI:** 10.22201/fmvz.24486760e.2019.4.601

**Abstract:** The ovicidal effect of hydro-alcoholic extracts from mature fruits and dried leaves of the *Caesalpinia coriaria* tree, against *Haemonchus contortus* and *H. placei* was evaluated by the egg hatching inhibition test (EHIT). Secondary compounds within the extracts were identified by high performance liquid chromatography. Five extract concentrations were assessed in the inhibition tests (25.0, 12.5, 6.2, 3.1 and 1.5 mg/mL), using distilled water and Thiabendazole (0.5 mg/mL) as negative and positive controls, respectively. The results were analysed under a 2 × 2 × 6 completely randomized factorial design, and mean differences were established by the Tukey's post hoc test. The 50% (EC50) and 90% (EC90) effective concentrations for each extract were determined using a Probit analysis. A concentration-dependent ovicidal effect of hydro-alcoholic extracts from both leaves and fruits was observed, reaching a 100% efficacy against both nematode species with the 25.0 mg/mL concentration ( $p < 0.05$ ). The EC50 for the fruit and leaves extracts were established as 1.63 and 3.91 mg/mL, and as 3.98 and 11.68 mg/mL, against *H. contortus* and *H. placei*, respectively. The most important secondary compounds identified in extracts were free-condensed tannins, gallic acid and methyl-gallate. In conclusion, hydro-alcoholic extracts from *C. coriaria* mature fruits and dried leaves inhibit *in vitro* egg hatching of *H. contortus* and *H. placei*.

**Author:** Salvadori Schafer, A., D. Baldissera, M., Bagolin da Silva, C., Sorraia de Oliveira, J., Igor Magalhães de Matos, A. F., Lopes Dornelles, G., Grando, T. H., Trevisan Gressler, L., Stefanello, S., Santi, E., Pelegrine Minho, A., Rodrigues, D., F. Souza, C., L. R. Leal, M., G. Monteiro, S., T.A. Lopes, S. and Melazzo de Andrade, C.

**Title:** Copper oxide and closantel prevent alterations in hepatic energetic metabolism and reduce inflammation in *Haemonchus contortus* infection

**DOI:** 10.1016/j.exppara.2019.107726

**Abstract:** The aims of this study were to evaluate if the use of copper oxide wire particles, isolated or in association with closantel, in lambs infected with *Haemonchus contortus* enhances the anthelmintic efficacy of closantel, as well as to evaluate the effects of treatment in hepatic energy metabolism, inflammatory markers and hematological and biochemical tests. The lambs were randomly divided into five groups (6 animals each), as follows: uninfected animals (Control); animals infected with *H. contortus* (HC); infected and treated with closantel (HC + CL); infected and treated with copper oxide wire particles (HC + Cu); and infected and treated with closantel plus copper oxide wire particles (HC + CL + Cu). The animals of infected groups were infected orally with *H. contortus* (5,000 L3 - larvae) and on day 14 post infection (p.i) the treatments were initiated. The egg per gram of feces (EPG), butyrylcholinesterase (BuChE), myeloperoxidase (MPO), adenylate kinase (AK) and pyruvate kinase (PK) activities and hematological and biochemical tests were evaluated. Treatments with copper oxide (isolated and associated) were able to reduce the EPG count on days 28, 35, 42 and 49 p.i when compared to HC group, while closantel was able to reduce EPG only from day 35 p.i. Moreover, treatment with closantel (isolated or associated) was able to prevent the inhibition of hepatic AK and PK activities caused by *H. contortus* infection, which may contribute to efficient intracellular energetic communication in order to maintain the balance between cellular ATP consumption and

production. Butyrylcholinesterase and MPO activities were higher in infected lambs compared to uninfected, while treated groups showed lower enzymatic activity compared to the group HC. The use of all therapeutic protocols was able to reduce the EPG count. Based on these evidences, the use of copper oxide plus closantel may be considered an alternative to treat lambs infected by *H. contortus*.

**Author:** Silva, R. R. S., Silva, C. R., Santos, V. F., Barbosa, C. R. S., Muniz, D. F., Santos, A. L. E., Santos, M. H. C., Rocha, B. A. M., Batista, K. L. R., Costa-Júnior, L. M., Coutinho, H. D. M. and Teixeira, C. S.

**Title:** *Parkia platycephala* lectin enhances the antibiotic activity against multi-resistant bacterial strains and inhibits the development of *Haemonchus contortus*

**DOI:** 10.1016/j.micpath.2019.103629

**Abstract:** Lectins have been studied in the past few years as an alternative to inhibit the development of pathogenic bacteria and gastrointestinal nematodes of small ruminants. The development of new antibacterial and anthelmintic compounds is necessary owing to the increase in drug resistance among important pathogens. Therefore, this study aimed to evaluate the capacity of a glucose/mannose-binding lectin from *Parkia platycephala* seeds (PPL) to inhibit the development of *Haemonchus contortus* and to modulate antibiotic activity against multi-resistant bacterial strains, thereby confirming its efficacy when used in combination with gentamicin. PPL at the concentration of 1.2 mg/mL did not show inhibitory activity on *H. contortus* in the egg hatch test or the exsheathment assay. However, it did show significant inhibition of *H. contortus* larval development with an IC<sub>50</sub> of 0.31 mg/mL. The minimum inhibitory concentration (MIC) obtained for PPL against all tested bacterial strains was not clinically relevant (MIC ≥ 1024 µg/mL). However, when PPL was combined with gentamicin, a significant increase in antibiotic activity was observed against *S. aureus* and *E. coli* multi-resistant strains. The inhibition of hemagglutinating activity by gentamicin (MIC = 50 mM) revealed that it may be interacting with the carbohydrate-binding site of PPL. It is this interaction between the antibiotic and lectin carbohydrate-binding site that may be responsible for the enhanced activity of gentamicin against multi-resistant strains. It can be concluded that PPL showed selective anthelmintic effect, inhibiting the development of *H. contortus* larvae and that it increased the effect of the antibiotic gentamicin against multi-resistant bacterial strains, thus constituting a potential therapeutic resource against resistant bacterial strains and *H. contortus*.

**Author:** Zaheer, S., Hussain, A., Khalil, A., Mansha, M. and Lateef, M.

**Title:** In vitro anthelmintic activity of ethanolic extracts of *Camellia sinensis* L. and *Albizia lebbek* L. against *Haemonchus contortus*

**DOI:** 10.17582/journal.pujz/2019.34.1.41.45

**Abstract:** Helminths are familiar as a major problem to livestock production. Helminths cause multiple infections in animals of significant economic importance. *Haemonchus contortus*, (helminths worm), is highly infectious parasite of small ruminants, and is a major cause of diseases and significant mortality in various cattle, sheep, deer, buffalo and goat, etc. Various synthetic anthelmintics have been used, on wide scale, to control the helminths infections. But the parasite worms have developed multiple resistances against the synthetic anthelmintics. Hence, various biological controls, vaccines and local medicinal plants are being explored to control the parasitic worms. The aim of this study was to evaluate the anthelmintic potential of two local plants, *Albizia lebbek* L. and *Camellia sinensis* L against *H. contortus* using Adult Motility Assay (AMA). Crude Ethanolic Extracts (CEE) of dried leaves of *A. lebbek* L. and whole plant of *C. sinensis* L. were prepared by cold maceration method. The anthelmintic activity was analyzed by adult motility assay (AMA). Levamisole was used as positive control to compare the efficacy of CEE of both plants. Adult worms were treated with different concentrations i.e., 2, 4, 6 and 8 mg/ml of ethanolic extract and motility of the worms was examined after every two to eight hours. Both *A. lebbek* and *C. sinensis* exhibited 88% and 95% mortality at 6 & 8 mg/ml after 8 hrs of treatment. In conclusion, the ethanolic extracts of *A. lebbek* L. and *C. sinensis* L. showed in vitro anthelmintic activity against *H. contortus* at concentrations tested.

**Author:** Zamilpa, A., García-Alanís, C., López-Arellano, M. E., Hernández-Velázquez, V. M., Valladares-Cisneros, M. G., Salinas-Sánchez, D. O. and Mendoza-De Gives, P.

**Title:** In vitro nematicidal effect of *Chenopodium ambrosioides* and *Castela tortuosa* n-hexane extracts against *Haemonchus contortus* (Nematoda) and their anthelmintic effect in gerbils

**DOI:** 10.1017/s0022149x18000433

**Abstract:** The in vitro nematicidal effect of *Chenopodium ambrosioides* and *Castela tortuosa* n-hexane extracts (E-Cham and E-Cato, respectively) on *Haemonchus contortus* infective larvae (L3) and the anthelmintic effect of these extracts against the pre-adult stage of the parasite in gerbils were evaluated using both individual and combined extracts. The in vitro confrontation between larvae and extracts was performed in 24-well micro-titration plates. The results were considered 24 and 72 h post confrontation. The in vivo nematicidal effect was examined using gerbils as a study model. The extracts from the two assessed plants were obtained through maceration using n-hexane as an organic agent. Gerbils artificially infected with *H. contortus* L3 were treated intraperitoneally with the corresponding extract either individually or in combination. The results showed that the highest individual lethal in vitro effect (96.3%) was obtained with the E-Cham extract at 72 h post confrontation at 40 mg/ml, followed by E-Cato (78.9%) at 20 mg/ml after 72 h. The highest combined effect (98.7%) was obtained after 72 h at 40 mg/ml. The in vivo assay showed that the individual administration of the E-Cato and E-Cham extracts reduced the parasitic burden in gerbils by 27.1% and 45.8%, respectively. Furthermore, the anthelmintic efficacy increased to 57.3% when both extracts were administered in

combination. The results of the present study show an important combined nematicidal effect of the two plant extracts assessed against L3 in gerbils.

**Author:** De Araújo-Filho, J. V., Ribeiro, W. L. C., André, W. P. P., Cavalcante, G. S., Rios, T. T., Schwinden, G. M., Da Rocha, L. O., Macedo, I. T. F., de Moraes, S. M., Bevilaqua, C. M. L. and De Oliveira, L. M. B.

**Title:** Anthelmintic activity of *Eucalyptus citriodora* essential oil and its major component, citronellal, on sheep gastrointestinal nematodes

**DOI:** 10.1590/s1984-29612019090

**Abstract:** This study aimed to evaluate the anthelmintic activity of *Eucalyptus citriodora* essential oil and citronellal on sheep gastrointestinal nematodes. Essential oil composition was determined by gas chromatography mass spectrometry. The substances were evaluated in vitro using adult worm motility test (AWMT) and transmission electron microscopy (TEM). The acute toxicity test in mice and the fecal egg count reduction test (FECRT) in sheep were performed. Citronellal was confirmed as the essential oil major constituent (63.9%). According to the AWMT, 2 mg/mL of essential oil and citronellal completely inhibited *Haemonchus contortus* motility at 6 h post exposure. *H. contortus* exposed to essential oil and citronellal exhibited internal ultrastructural modifications. The lethal dose 50 values in mice were 5,000 and 2,609 mg/kg for essential oil and citronellal, respectively. *E. citriodora* essential oil reduced sheep epg at 14 days post treatment by 69.5% ( $P < 0.05$ ). No significant differences were observed in epg between the citronellal and negative control groups ( $P > 0.05$ ). The interaction between citronellal and other constituents in the essential oil may be relevant for its in vivo anthelmintic activity. Thus, *E. citriodora* essential oil and citronellal pharmacokinetic studies may help elucidate the anthelmintic activity of these compounds.

**Author:** Ojeda-Robertos, N. F., Aguilar-Marcelino, L., Olmedo-Juárez, A., Luna-Palomera, C., Peralta-Torres, J. A., López-Arellano, M. E. and Mendoza-De-gives, P.

**Title:** In vitro predatory activity of nematophagous fungi isolated from water buffalo feces and from soil in the Mexican southeastern

**DOI:** 10.1590/s1984-29612019011

**Abstract:** Nematophagous fungi from the feces of water buffalo and soil from southeastern Mexico were isolated, and their in vitro predatory activity against *Haemonchus contortus* infective larvae (L3) (HcL3) was assessed. The fungi were isolated by sprinkling soil or feces on water agar plates. Six series of 10 Petri dishes containing a 7-day-old culture of each fungus and a series without fungi as the control were prepared. Five hundred HcL3 were added to each plate. The plates were incubated at room temperature. The average of recovered HcL3 was considered to estimate the larval reduction rate. Four nematophagous fungi isolates corresponding to *Arthrobotrys oligospora*, var *microspora* (strains 4-276, 269 and 50-80) and one identified as *A.*

oligospora, var. oligospora (isolates 48-80) were obtained from water buffalo feces. From the soil, five isolates were isolated; three corresponded to *A. musiformis* (Bajío, Yumca and Macuspana isolates), and two isolates were identified as *A. oligospora* (Comalcalco and Jalapa de Méndez isolates). The predatory activity of isolates from water buffalo feces ranged between 85.9 and 100%. Meanwhile, the fungi from the soil ranged between 55.5 and 100% ( $p \leq 0.05$ ). The nematophagous fungi obtained could have important implications in the control of parasites of importance in the livestock industry.

**Author:** Fayaz, M. R., Abbas, R. Z., Abbas, A., Khan, M. K., Raza, M. A., Israr, M., Khan, J. A., Mahmood, M. S., Saleemi, M. K., Rehman, T. U., Zaman, M. A. and Sindhu, Z. U. D.

**Title:** Potential of botanical driven essential oils against haemonchus contortus in small ruminants

**DOI:** 10.35588/blacpma.19.18.6.36

**Abstract:** The livestock sector is continuously facing problems in controlling parasitic diseases especially Haemonchosis due to emergence of anthelmintic resistance and failure in vaccination control programmes. Therefore, to increase milk and meat production and emerging demand of meat free from drug residues development of new alternative approaches are appealing for prevention and control of Haemonchosis in small ruminants. Among alternatives, plants driven essentials oils have shown promising results in control of Haemonchus contortus infection at various concentrations by different assays including egg hatch assay, larval development assay, larval exsheathment assay and adult motility assay. Essential oils are complex mixtures of various impulsive or volatile compounds which have potential to control Haemonchosis. The current study reviews the therapeutic effects of essential oils of plants against Haemonchus contortus and to be used them against Haemonchus contortus for future perspectives.

**Author:** Nguyen, L. T., Kurz, T., Preston, S., Brueckmann, H., Lungerich, B., Herath, H. M. P. D., Koehler, A. V., Wang, T., Skálová, L., Jabbar, A. and Gasser, R. B.

**Title:** Phenotypic screening of the 'Kurz-box' of chemicals identifies two compounds (BLK127 and HBK4) with anthelmintic activity in vitro against parasitic larval stages of Haemonchus contortus

**DOI:** 10.1186/s13071-019-3426-7

**Abstract:** Background: Due to anthelmintic resistance problems, there is a need to discover and develop new drugs for the treatment and control of economically important and pathogenic nematodes of livestock animals. With this focus in mind, we screened 236 compounds from a library (called the 'Kurz-box') representing chemically diverse classes such as heterocyclic compounds (e.g. thiazoles, pyrroles, quinolines, pyrimidines, benzo[1,4]diazepines), hydroxamic acid-based metalloenzyme inhibitors, peptidomimetics (bis- and tris-pyrimidoneamides, alkoxyamides) and various intermediates on Haemonchus contortus, one of the most important parasitic nematodes of ruminants.

**Methods:** In the present study, we tested these compounds, and measured the inhibition of larval motility and development of exsheathed third-stage (xL3) and fourth-stage (L4) larvae of *H. contortus* using an optimised, whole-organism phenotypic screening assay. **Results:** Of the 236 compounds, we identified two active compounds (called BLK127 and HBK4) that induced marked phenotypic changes in the worm in vitro. Compound BLK127 induced an 'eviscerated' phenotype in the xL3 stage and also inhibited L4 development. Compound HBK4 exerted a 'curved' phenotype in both xL3s and L4s. **Conclusions:** The findings from this study provide a basis for future work on the chemical optimisation of these compounds, on assessing the activity of optimised compounds on adult stages of *H. contortus* both in vitro and in vivo (in the host animal) and against other parasitic worms of veterinary and medical importance.

## Vaccination

**Author:** González-Sánchez, M. E., Ndombasi-Bokuy, M., Cuquerella, M. and Alunda, J. M.

**Title:** Immunization with recombinant rHc23 partially protects lambs against trickle infections by *Haemonchus contortus*

**DOI:** 10.1186/s12917-019-2084-5

**Abstract:** Background: Haemonchosis is one of the most economically important parasitic diseases affecting small ruminants all over the world. Chemotherapeutic control has several shortcomings (limited anthelmintic arsenal, frequent resistance) and is hardly affordable by many farm economies. A recombinant antigen (rHc23) was shown to induce significant protection in vaccination trials with single dose challenges and different adjuvants. Results: Lambs were vaccinated with 100 µg rHc23/dose + bacterial immunostimulant (BI) (LPS from *Escherichia coli* + *Propionibacterium acnes* extract) (days -2, 0, 7 and 14) and subjected to a trickle infection with two dosages [6x, 1000 infective larvae (L3) or 6x, 2000 L3]. Vaccinated lambs showed a significant antibody response against rHc23 and *Haemonchus contortus* soluble extract as assessed by ELISA and Western blot (WB). Fecal egg counts (epg) along the experiment of vaccinated and BI treated lambs were significantly reduced. All vaccinated animals showed total egg output and abomasal helminth burdens (median, average) lower than those from unvaccinated or BI-treated animals lambs although differences were not statistically significant. Conclusions: Vaccination with 100 µg rHc23/dose + BI against *H. contortus* trickle infections apparently induced lower epg values and helminth burdens at the end of the experiment. Intragroup individual variations did not allow to obtain conclusive results and more research is needed including adjuvants and larger groups of animals to validate the potential value of rHc23 as candidate to develop a recombinant vaccine for lambs haemonchosis.

**Author:** Teixeira, M., Matos, A. F. I. M., Albuquerque, F. H. M. A., Bassetto, C. C., Smith, W. D. and Monteiro, J. P.

**Title:** Strategic vaccination of hair sheep against *Haemonchus contortus*

**DOI:** 10.1007/s00436-019-06367-x

**Abstract:** In this study, we evaluated in two trials a protocol designed to protect hair sheep using Barbervax®, a vaccine containing *Haemonchus contortus* gut membrane glycoprotein antigens. Results indicated that naturally infected vaccinated sheep had significant egg count reductions ( $90.2 \pm 4.03\%$ ) compared with controls, although blood parameters remained relatively unchanged probably because the level of challenge was low. Vaccination prevented the periparturient rise in egg shedding of ewes, as well as egg shedding in lambs (37.1%). In the second trial, sheep which were experimentally exposed to higher artificial challenge also showed an efficient response to the vaccine as confirmed by high antibody levels and reduced egg counts and worm burdens ( $87 \pm 5.4\%$  and 79%) respectively. Thus, we believe that the vaccine should be integrated with other management practices for meat hair sheep as it has the advantages of adequate efficacy, reducing anthelmintic utilization and avoiding milk and environmental contamination with chemical residues.

## GI nematodes in South American camelids

**Author:** Kultscher, L., Hinney, B., Schmäscke, R., Joachim, A. and Wittek, T.

**Title:** Current anthelmintic treatment is not always effective at controlling strongylid infections in German alpaca herds

**DOI:** 10.1186/s13071-019-3588-3

**Abstract:** Background: Endoparasites are considered a major health problem of South American camelids as shown in a recent survey among German and Austrian camelid owners. Although prophylactic and therapeutic measures such as application of anthelmintics are commonly used, treatment efficacy is usually not assessed. Owners have expressed significant concerns regarding the effect of antiparasitic therapy, so this study aimed to evaluate the outcome of anthelmintic treatment in German alpaca herds with different drugs. Results: Overall, 617 samples from 538 clinically healthy alpacas > 1 year-old from 27 farms (n = 11-157 animals/herd) were examined. The most common parasites detected by flotation were *Eimeria* spp. (75.1%) followed by strongylids (55.0%), *Nematodirus* spp. (19.3%), cestodes (3.1%) and *Trichuris* (2.7%). After initial coproscopical examination by flotation and strongylid egg quantification by the McMaster technique, positive animals excreting at least 150 eggs per gram of faeces were included in a faecal egg count reduction test (FECRT) using fenbendazole (n = 71 samples), moxidectin (n = 71) or monepantel (n = 66). Pre-treatment larval cultures (n = 23 positive pooled farm samples) revealed *Haemonchus* (87% of the farms), *Cooperia* (43.5%),



Trichostrongylus (21.7%), Ostertagia (13.0%), Nematodirus and Oesophagostomum (4.3% each). Fenbendazole treatment reduced egg excretion by 45%, moxidectin by 91% and monepantel by 96%. On the farm level, 13/18 farms that used fenbendazole, 6/6 farms that used moxidectin and 2/5 farms that used monepantel had individual FECR values < 90% (fenbendazole) or < 95% (moxidectin, monepantel). Haemonchus and Cooperia were overrepresented on the farms with reduced treatment efficacy. Conclusions:

Gastrointestinal strongylids are common in German alpacas and fenbendazole in particular was not sufficiently effective to reduce strongylid egg excretion. Although the FECRT could not unambiguously determine anthelmintic resistance in the present study, the finding that small ruminant strongylids, especially Haemonchus, are common in alpacas indicates that determination of effective anthelmintic doses, monitoring of efficacy and adapted (selective) treatment regimens must be implemented as part of sustainable deworming practices in this species in accordance with recommendations for ruminants.

**Author:** Rashid, M. H., Stevenson, M. A., Campbell, A. J. D., Vaughan, J. L., Beveridge, I. and Jabbar, A.

**Title:** An assessment of worm control practices used by alpaca farmers in Australia

**DOI:** 10.1016/j.vetpar.2018.12.006

**Abstract:** This study aimed to assess current worm control practices used by Australian alpaca farmers with an online questionnaire survey. The questionnaire contained questions about farm demography and general husbandry practices, farmers' knowledge about gastrointestinal nematodes (GINs) and their importance, the use of worm control strategies and anthelmintics, and grazing management. A link for the questionnaire survey was sent to all (n = 954) registered members of the Australian Alpaca Association in July 2015. The response rate for the questionnaire was 25% (239/954). The majority of respondents were from small ( $\leq 50$  alpacas; 64%, 153/239) followed by medium (50–100 alpacas; 24%, 57/239) and large (>100 alpacas; 12%, 29/239) farms. Findings revealed that the majority of respondents kept Huacaya alpacas to produce high-quality fibre and alpacas were usually kept with other domestic ruminants (e.g. cattle and sheep). Although half of alpaca farmers (114/220) perceived that GINs were an important health problem of alpacas, with Haemonchus spp. being the most common nematode, the majority of them (174/220) used anthelmintics for nematode control. Macrocytic lactones, a commercial combination of four anthelmintics (abamectin, albendazole, closantel and levamisole) and monepantel were the three most commonly used dewormers by Australian alpaca farmers. Although a significant proportion (166/213) of respondents used a quarantine drench for alpacas, very few respondents were aware of strategic deworming and the issue of anthelmintic resistance. Alpaca farmers mostly used anthelmintics at the dose rate recommended for sheep (47%, 79/167) and cattle (9%, 15/167), though some used 1.5 (31%, 51/167) and 2 (13%, 22/167) times the dose rate recommended for sheep. The majority of small herds used anthelmintics at the dose rate recommended for sheep and cattle while medium and large herds used anthelmintics at 1.5 to 2 times the dose rate recommended for sheep. This study provides invaluable insights into the demography of alpaca farms in Australia, husbandry practices used by alpaca farmers and their

knowledge about worms and their control, thereby paving the way for developing guidelines for the control of GINs of alpacas.

**Author:** Rashid, M. H., Stevenson, M. A., Vaughan, J. L., Saeed, M. A., Campbell, A. J. D., Beveridge, I. and Jabbar, A.

**Title:** Epidemiology of gastrointestinal nematodes of alpacas in Australia: II. A longitudinal study

**DOI:** 10.1007/s00436-019-06236-7

**Abstract:** We conducted a longitudinal survey on 13 alpaca farms in four climatic zones of Australia to understand the epidemiology of gastrointestinal nematodes (GINs) of alpacas. A total of 1688 fresh faecal samples were collected from both sexes of alpacas from May 2015 to April 2016 and processed for faecal egg counts (FEC) and molecular identification of eggs using the multiplexed-tandem PCR assay. Based on egg morphology, the overall prevalence of GINs was 61% while that for strongyles was 53%. The overall mean FEC was 168 eggs per gram (EPG) of faeces, with the highest count of 15,540 EPG. Weaners had the highest prevalence (73%) and mean FEC (295 EPG) of GINs followed by tuis, crias and adults. Alpacas in the winter rainfall zone had the highest prevalence (68%) as well as FEC (266 EPG) followed by Mediterranean-type, non-seasonal and summer rainfall zones. *Trichostrongylus* spp. (83%, 89/107), *Haemonchus* spp. (71%, 76/107) and *Camelostrongylus mentulatus* (63%, 67/107) were the three most common GINs of alpacas across all climatic zones. The mixed-effects zero-inflated negative binomial regression model used in this study showed that it could help to design parasite control interventions targeted at both the herd level and the individual alpaca level. The findings of this study showed that the epidemiology of GINs of alpacas is very similar to those of cattle and sheep, and careful attention should be paid when designing control strategies for domestic ruminants co-grazing with alpacas.

**Author:** Rashid, M. H., Vaughan, J. L., Stevenson, M. A., Campbell, A. J. D., Saeed, M. A., Indjein, L., Beveridge, I. and Jabbar, A.

**Title:** Epidemiology of gastrointestinal nematodes of alpacas in Australia: I. A cross-sectional study

**DOI:** 10.1007/s00436-019-06235-8

**Abstract:** This study involved a national cross-sectional survey of gastrointestinal nematodes (GINs) of alpacas in Australia. A total of 1545 fresh faecal samples were collected from both sexes of alpacas and processed for faecal egg counts (FEC) and molecular identification of nematodes using the multiplexed tandem PCR assay. Based on egg morphology, the overall prevalence of GINs was 66% while that for strongyles was 59%. The overall mean FEC was 276 eggs per gram (EPG) of faeces, with the highest count of 17,415 EPG. Male alpacas had a higher prevalence (68%, 334/490) as well as mean FEC ( $328 \pm 60$  EPG) of GINs than females (63%, 602/954;  $227 \pm 26$ , respectively).

Weaners had the highest prevalence (80%) whereas tuis had the highest FEC (402 EPG) of nematodes. The highest prevalence (77%, 293/383) and FEC (630 EPG) of GINs were observed in the summer rainfall zone followed by the Mediterranean-type rainfall, non-seasonal rainfall and winter rainfall zones. The characterisation of nematode DNA isolated from faeces revealed the occurrence of seven different GINs, including *Camelostrongylus mentulatus*, *Cooperia* spp., *Haemonchus* spp., *Oesophagostomum* spp., *Ostertagia ostertagi*, *Teladorsagia circumcincta* and *Trichostrongylus* spp. Besides, *Nematodirus* spp. and *Trichuris* spp. were also found during FECs. The prevalence of *Haemonchus* spp. was highest in the summer rainfall zone while that of *C. mentulatus* was highest in the Mediterranean-type rainfall, non-seasonal rainfall and winter rainfall zones. The findings of this study revealed that alpacas harbour many of the same nematodes as sheep and cattle.

## Cestodes

**Author:** Assana, E., Awah-Ndukum, J., Djonmaïla, J. D., Djatche, H. D., Awé, C., Manchang, T. K. and Zoli, A. P.

**Title:** A comparison of *Taenia solium* and *Taenia hydatigena* infection in pigs using serological diagnosis and post-mortem inspection methods in Benoué division, North Cameroon

**DOI:** 10.1016/j.vprsr.2019.100306

**Abstract:** The metacestodes of *Taenia solium* and *Taenia hydatigena* are the cause of cysticercosis in pigs. *T. solium* is also responsible of the taeniosis/neurocysticercosis complex in humans, constituting a main cause of epilepsy cases across endemic countries. *T. hydatigena* is non-zoonotic, but its occurrence in pigs contributes significantly to false positive reactions should genus-species serological methods be used for diagnosis of *T. solium* porcine cysticercosis. *T. hydatigena* is often considered not common in pigs in Africa compared to *T. solium*. On the basis of the evidence that these two cestodes coexist in Cameroon, we examined the viscera of 305 pigs for the identification of the metacestodes of *T. hydatigena* in Bénoué division, North Region of Cameroon. Tongue, masticatory muscles and heart were sliced for the identification of *T. solium* cysticerci (TMH dissection test). Twenty seven (8.85%) and 16 (5.24%) pigs were found infected with the metacestodes of *T. solium* and *T. hydatigena*, respectively. The difference between the two rates of infection was not statistically significant ( $P > 0.05$ ). Serum samples were also collected for the evaluation of an inhibition ELISA (i-ELISA) specific to antibodies anti- *T. solium* or anti-*T. hydatigena* cysticerci. After incubation of these sera with cyst fluid of *T. solium*, *T. hydatigena*, *T. multiceps multiceps*, *T. multiceps gaigeri* and *T. saginata* to eliminate cross-reactions among cestodes parasites, the i-ELISA indicated that 26.56% and 28.52% slaughtered pigs had predominant specific antibodies to cyst fluid of *T. solium* and *T. hydatigena*, respectively. Combination of TMH dissection test, i-ELISA and a standard indirect ELISA in a Bayesian simulation approach revealed a true prevalence of 19.27% (0.7–49.27, CI 95%) and 24.85% (5.17–48.34, CI 95%) of porcine

cysticercosis due to *T. solium* and *T. hydatigena*, respectively. These results indicated that *T. hydatigena* is as prevalent as *T. solium* in pigs in the North of Cameroon.

**Author:** Assana, E., Awah-Ndukum, J., Djonmaïla, J. D. and Zoli, A. P.

**Title:** Prevalence of porcine *Taenia solium* and *Taenia hydatigena* cysticercosis in Cameroon

**DOI:** 10.1016/j.prevetmed.2019.104690

**Abstract:** *Taenia solium*, a zoonotic tapeworm species of human and pigs, has been targeted by the World Health Organisation (WHO) for elimination in endemic areas of Africa, Latin America and some parts of Asia. Unfortunately, the assessment of control measures against *T. solium* is complicated by the lack of specificity of current immunodiagnostic techniques for diagnosis of porcine cysticercosis. Many authors have concluded that the prevalence of *T. hydatigena* in pigs in Africa is low and extensive epidemiological studies on *T. solium* cysticercosis in pigs have been carried out using serological tests that cannot differentiate the taeniid species. To estimate and compare the prevalence of *T. solium* and *T. hydatigena* in pigs in Cameroon, we examined 416 slaughtered pigs at a pig abattoir in Yaoundé city with the animals originating from several parts of the country. At post-mortem inspection, 35 pigs (8.4%; 95% Confidence Interval (CI), 5.9–11.5 %) were found infected exclusively with *T. solium* and 27 (6.5%; 95% CI, 4.3–9.3 %) infected exclusively with *T. hydatigena* metacestodes. One animal was infected with *T. solium* and *T. hydatigena*. A stochastic simulation model which took into account the sensitivity of dissection of the tongue, external masseters and heart for diagnosis of *T. solium* porcine cysticercosis (TMH dissection test) and examination of mesentery, surfaces of the intestines and liver to identify *T. hydatigena* cysticerci in slaughtered pigs was developed to estimate the prevalence of these taeniid species. The results revealed that the actual prevalence of *T. solium* and *T. hydatigena* in Cameroon were 24.8% (95% CI, 20.1–30.5%) and 19.2% (95% CI, 15.1–24.1%), respectively. This study reports the highest porcine *T. hydatigena* prevalence ever in Africa and adjusted the prevalence of porcine *T. solium* infection obtained with genus-specific Ag-ELISA (Dorny et al., 2000) in Cameroon due to the presence of *T. hydatigena*.

**Author:** Amrabadi, O., Oryan, A., Moazeni, M., Shari-Fiyazdi, H. and Akbari, M.

**Title:** Histopathological and molecular evaluation of the experimentally infected goats by the larval forms of *taenia multiceps*

**Abstract:** Background: Introduction of *Taenia multiceps* and *T. gaigeri* as two separate species have been recognized mainly on morphological grounds. This experimental study was conducted to test whether cerebral and non-cerebral forms of *Coenurus cerebralis* belong to one origin or they are originated from two different tape worms. Methods: Two groups of dogs were infected with the cerebral and muscular sources of the coenuri cysts. About two months later the eggs were collected from the fecal samples to be used to experimentally infect other healthy goats. Histopathological and molecular evaluation was

conducted in two groups of goats that were challenged with *T. multiceps* eggs obtained from the infected dogs by brain and muscular sources of coenuri cysts in School of Veterinary Medicine of Shiraz University, Shiraz, Iran in 2015. All aberrant sites of predilection of the metacestode in goats were muscles, heart, diaphragm and lungs. The brain and spinal cord were carefully dissected and examined but the cysts were not found in these locations. In addition, the molecular genetic markers of mitochondrial DNA (CO1 and ND1) were applied to resolve the questionable relationship between *T. multiceps* and *T. gaigeri*. Results: The larval stages of *T. multiceps* in brain and in other aberrant sites, which showed similar morphological criteria, were monophyletic species. Conclusion: Therefore, *T. gaigeri* must be considered taxonomically invalid.

**Author:** Cassini, R., Simonato, G., Mulatti, P., Ravagnan, S., Danesi, P., Pascotto, E., Breda, T., Bricchese, M., Pietrobelli, M. and Capelli, G.

**Title:** A new approach to outbreak management for bovine Cystic Echinococcosis cases in hypo-endemic areas

**DOI:** 10.1016/j.vprsr.2019.100269

**Abstract:** Cystic Echinococcosis (CE) surveillance in Italy is based on detection of its larval stage (hydatid cysts) at the slaughterhouse. In northern Italy, a hypo-endemic area, local health authorities investigate each individual farm with positive animals to treat their dogs with cestocidal drugs, but this system is time-consuming and poorly effective for bovine farms. The study applied a new approach based on targeted epidemiological surveys in areas with aggregation of bovine CE cases, and compared the outcome with that of two control areas with farms individually investigated. The presence of territorial cluster of bovine farms with CE cases was investigated for 3 consecutive years (2013–2014–2015) in a high-risk area of Veneto Region (north-eastern Italy), using a spatial scan statistic. Epidemiological investigations, consisting of a questionnaire survey and canine faecal samples collection, were conducted in cluster and control areas. All faecal samples were analyzed for taeniids eggs and positive samples were sequenced and identified. In total, 99 farms were surveyed and 208 faecal samples were retrieved from dogs. Sixty-two farms (42 bovine and 20 sheep) were investigated in cluster areas and 37 farms (33 bovine and 4 sheep) in control areas. Based on the results of the cestode egg isolation procedure, 14 animals (6.7%) were positive to taeniid eggs. For molecular analysis, two dogs resulted positive to *Echinococcus granulosus*, and seven to *Taenia hydatigena*. Twelve positive dogs were found in targeted survey areas and ten of these dogs were shepherd dogs, belonging to transhumant sheep flocks known to pass in cluster areas. The new approach demonstrated to successfully identify the probable source of infection of CE positive bovines. Most of positive dogs belonged to transhumant flocks, underlining the importance to include shepherd dogs in the surveillance system for CE.

**Author:** Delling, C., Böttcher, D., Schiffbauer, V., Bernhard, A. and Schmäschke, R.

**Title:** First report of pulmonary cysticercosis caused by *Taenia crassiceps* in a Cape fur seal (*Arctocephalus pusillus*)

**DOI:** 10.1016/j.ijppaw.2019.07.006

**Abstract:** The cestode *Taenia crassiceps* parasitizes in the intestine of domestic and wild carnivores, especially in red foxes. Usually, the metacestode stage, also known as *Cysticercus longicollis*, is located in muscles, peritoneal and pleural cavity of wild rodents. In this case, larval stages were found in a female Cape fur seal, which lived in a German zoo since June 1998. In January 2019, the animal presented clinical signs in terms of inappetence and reduced mobility and, within a short time, it developed dyspnoea and died. Pathological and parasitological examinations were performed. In a large mass of the right thoracic wall and in nodular lung lesions, metacestodes with numerous protoscoleces were identified. Morphological and molecular analyses led to the diagnosis of a *Taenia crassiceps* infection. Probably, the urban fox population was the source of infection. Thus, regarding the zoonotic potential of this cestode, a regularly performed parasitological examination of pet dogs is recommended.

**Author:** Dixon, M. A., Braae, U. C., Winskill, P., Walker, M., Devleeschauwer, B., Gabriël, S. and Basáñez, M. G.

**Title:** Strategies for tackling *Taenia solium* taeniosis/ cysticercosis: A systematic review and comparison of transmission models, including an assessment of the wider Taeniidae family transmission models

**DOI:** 10.1371/journal.pntd.0007301

**Abstract:** Background The cestode *Taenia solium* causes the neglected (zoonotic) tropical disease cysticercosis, a leading cause of preventable epilepsy in endemic low and middle-income countries. Transmission models can inform current scaling-up of control efforts by helping to identify, validate and optimise control and elimination strategies as proposed by the World Health Organization (WHO). Methodology/Principal findings A systematic literature search was conducted using the PRISMA approach to identify and compare existing *T. solium* transmission models, and related Taeniidae infection transmission models. In total, 28 modelling papers were identified, of which four modelled *T. solium* exclusively. Different modelling approaches for *T. solium* included deterministic, Reed-Frost, individual-based, decision-tree, and conceptual frameworks. Simulated interventions across models agreed on the importance of coverage for impactful effectiveness to be achieved. Other Taeniidae infection transmission models comprised force-of-infection (FoI), population-based (mainly *Echinococcus granulosus*) and individual-based (mainly *E. multilocularis*) modelling approaches. Spatial structure has also been incorporated (*E. multilocularis* and *Taenia ovis*) in recognition of spatial aggregation of parasite eggs in the environment and movement of wild animal host populations. Conclusions/Significance Gaps identified from examining the wider Taeniidae

family models highlighted the potential role of FoI modelling to inform model parameterisation, as well as the need for spatial modelling and suitable structuring of interventions as key areas for future T. solium model development. We conclude that working with field partners to address data gaps and conducting cross-model validation with baseline and longitudinal data will be critical to building consensus-led and epidemiological setting-appropriate intervention strategies to help fulfil the WHO targets.

**Author:** Escolà-Vergé, L., Salvador, F., Sánchez-Montalvá, A., Escudero-Fernández, J. M., Sulleiro, E., Rando, A., Bilbao, I., Lázaro, J. L., Serres, X., Salvador, J. and Molina, I.

**Title:** Retrospective Study of Cystic Echinococcosis in a Recent Cohort of a Referral Center for Liver Surgery

**DOI:** 10.1007/s11605-018-3971-y

**Abstract:** Background: Cystic echinococcosis (CE) is a zoonosis endemic in Spain caused by the larval stage of the cestode *Echinococcus granulosus* and is one of the 18 neglected tropical diseases recognized by the WHO. The aim of this study was to describe the epidemiological and clinical data of CE in a surgical referral hospital. Methods: A retrospective descriptive study of all adults' patients diagnosed with CE and followed at Vall d'Hebron University Hospital in Barcelona, Spain, between 2000 and 2015. Results: We found 151 cases, 78 (51.7%) women, and median age at diagnosis was 68 (range, 15–92) years. Diagnosis was a radiological finding in 97 (64.2%) and the most frequent location was the liver [135 (89.4%) patients]. Nearly 80% of the cysts were calcified and serology was positive in 48 (51.6%). The WHO-IWGE classification was only available in 70 of the 104 (67.3%) cases of liver cysts that had an ultrasound. First therapeutic plan was “watch and wait” followed by surgery. International recommendations were not always followed, particularly in CE4 and CE5 stages, and 20% needed a change of treatment because of progression or recurrence. Patients treated surgically were younger, more symptomatic, and had larger and less calcified cysts in multiple sites. Serology was not useful for CE diagnosis and neither serology nor calcification of the cyst helped to predict viability. Conclusions: The formation of multidisciplinary teams in reference hospitals could help to improve CE diagnosis, its management, and follow-up, since international recommendations are not usually followed.

**Author:** Frey, C. F., Jenkins, E. and Lundström-Stadelmann, B.

**Title:** Editorial overview: From farms and forests to forks? A review of diagnosis and management of globally important zoonotic *Echinococcus* spp. cestodes

**DOI:** 10.1016/j.fawpar.2019.e00061

**Abstract:** This FAWPAR Special Issue is dedicated to zoonotic *Echinococcus* species. It is a compilation of invited papers that spans important aspects from molecular markers of emergence, diagnostics in both definitive and intermediate hosts, treatment of human alveolar echinococcosis, to control strategies in definitive hosts.

**Author:** Gholami, N., Mosayebi, M., Dehghan Rahim Abadi, P., Rasmi Atigh, H., Sedaghat, R., Naji Zadeh, M. H. and Farahani, M.

**Title:** Bovine cysticercosis in feedlot cattle in central region of Iran

**DOI:** 10.1007/s12639-019-01157-9

**Abstract:** The cestode, *Taenia saginata* is a zoonotic tapeworm that its larval stage which known as *Cysticercus bovis* cause cyst formation in cattle's organs such as heart, lung, liver, tongue, esophagus and diaphragm muscle, despite the infected cattle may show no clinical signs. Antemortem diagnosis of bovine cysticercosis can be made by antigen detecting ELISA. In a feedlot near city of Arak, beef cattle had different degrees of lethargy, dullness, unthriftiness and were reluctant to move. In postmortem examination of cattle, samples were collected from heart tissue and stained by H&E method for light microscopic examination. 10 ml of blood samples were taken from jugular veins of 90 cattle that were going to be sent to slaughterhouse. Serums obtained from blood samples were investigated for presence of *C. bovis* antigen by ELISA assay. Soils and dusts from farm yard, pen's floor, feed store and both toilets of workers and employer were sampled and evaluated for presence of parasite eggs by floating method. *Cysticercus bovis* antigen were identified in serums of 18 cattle; and also, samples from workers toilet was contaminated by eggs of *T. saginata*. This study showed that serologic methods in conjunction with meat inspection can be used for diagnosis of bovine cysticercosis. The aim of this study is to identify infected cattle with *C. bovis* by serologic methods before slaughter and determine microscopic characteristics of lesions on postmortem examination in central area of Iran.

**Author:** Hidalgo, A., Melo, A., Romero, F., Villanueva, J., Carrasco, C., Jara, P., Venegas, J. and Fonseca-Salamanca, F.

**Title:** A PCR-RFLP assay for discrimination of *Echinococcus granulosus sensu stricto* and *Taenia* spp. in dogs stool

**DOI:** 10.1016/j.exppara.2019.03.015

**Abstract:** In order to develop a method of identification and discrimination of *Echinococcus granulosus sensu stricto* from faecal samples of dogs infected with taeniid eggs (*Echinococcus* spp., *Taenia* spp.), a combined strategy of Polymerase Chain Reaction (PCR) and Restriction Fragments of Length Polymorphisms (RFLP) was proposed. Initially, a pair of primers was designed to amplify a fragment of the 12S subunit of ribosomal RNA gene (12S rRNA) from mitochondrial DNA. The amplified product was digested by *SspI* restriction enzyme, which in *E. granulosus* kept the intact fragment of 160 basis pairs (bp), while in *Taenia* spp. produced two fragments (62 bp and another of 98 bp). The method was tested using positive controls of DNA, in faecal samples experimentally contaminated with eggs of *E. granulosus* and *Taenia* spp. and in dogs naturally infected. In all of them, reproducible results were obtained and the primers were specific to amplify only Taeniidae DNA. The sensitivity of the technique was tested,



achieving amplification of DNA extractions with a single egg. In conclusion, the technique developed was optimal and easy to identify patent infections by *E. granulosus* s.s., constituting a possible alternative for epidemiological studies in dogs, especially in endemic areas where this infection occurs.

**Author:** Khan, A., Ahmed, H., Sohail, A., Alam, F. and Simsek, S.

**Title:** A Mathematical Modelling Approach for Treatment and Control of *Echinococcus multilocularis*

**DOI:** 10.1017/s0031182019001720

**Abstract:** Alveolar echinococcosis (AE) is a zoonotic parasitic diseases caused by a cestode parasite known as *Echinococcus multilocularis*. The parasite has a wildlife cycle with definitive hosts (foxes) and small mammals as intermediate hosts (rodents) while human are the accidental hosts. Parasite infection pressure relation to time of the year and age dependent infection pressure for parasite abundance also depends on the urbanization. The aim of current work is forecasting the thresholds via the computational analysis of the disease spread is a useful approach since it can help to design the experimental settings with better planning and efficiency. Network analysis when interlinked with the computational techniques provides better insight of the spatial and temporal heterogeneities. In the present study, a mathematical framework that describes the transmission dynamics and control measures of *Echinococcus multilocularis* in foxes is documented. We used treatment of foxes with baits for the prevention of the *E. multilocularis* infection. A novel approach of networking, called petrinet (PN), based on density dependent differential equations, is utilized during this research. The accurate description of the transmission of the parasite and the effect of drug on it is provided to the readers in this article. The transitions, which are difficult to analyze theoretically, are presented with the aid of discrete approach of networking. Discrete mathematical framework can prove to be accurate and robust tool to analyze and control the parasite dynamics.

**Author:** Kotwa, J. D., Isaksson, M., Jardine, C. M., Campbell, G. D., Berke, O., Pearl, D. L., Mercer, N. J., Osterman-Lind, E. and Peregrine, A. S.

**Title:** *Echinococcus multilocularis* infection, Southern Ontario, Canada

**DOI:** 10.3201/eid2502.180299

**Abstract:** Alveolar echinococcosis, the disease caused by infection with the intermediate stage of the *Echinococcus multilocularis* tapeworm, is typically fatal in humans and dogs when left untreated. Since 2012, alveolar echinococcosis has been diagnosed in 5 dogs, 3 lemurs, and 1 chipmunk in southern Ontario, Canada, a region previously considered free of these tapeworms. Because of human and animal health concerns, we estimated prevalence of infection in wild canids across southern Ontario. During 2015–2017, we collected fecal samples from 460 wild canids (416 coyotes, 44 foxes) during postmortem

examination and analyzed them by using a semiautomated magnetic capture probe DNA extraction and real-time PCR method for *E. multilocularis* DNA. Surprisingly, 23% (95% CI 20%–27%) of samples tested positive. By using a spatial scan test, we identified an infection cluster (relative risk 2.26;  $p = 0.002$ ) in the western-central region of the province. The cluster encompasses areas of dense human population, suggesting zoonotic transmission.

**Author:** Larrieu, E., Gavidia, C. M. and Lightowlers, M. W.

**Title:** Control of cystic echinococcosis: Background and prospects

**DOI:** 10.1111/zph.12649

**Abstract:** Cystic echinococcosis (CE), caused by *Echinococcus granulosus*, is a chronic and debilitating zoonotic larval cestode infection in humans, which is principally transmitted between dogs and domestic livestock, particularly sheep. Human CE occurs in almost all pastoral communities and rangeland areas of the underdeveloped and developed world. Control programmes against CE have been implemented in several endemic countries to reduce or eliminate the disease. New Zealand and Tasmania are examples of some of the first programmes to be undertaken (in insular territories) and which were very successful in the elimination of CE. The advent and proven effectiveness of praziquantel, plus the experience of insular models, produced high expectations for rapid advances in a second generation of control programmes undertaken in continental areas (Argentina, Uruguay and Chile). Nevertheless, only moderate gains in CE control have been made and the impact on prevalence among humans has been slight. A major impediment to the adoption of procedures that were successful in New Zealand and Tasmania has been the requirement to administer praziquantel to dogs in rural areas eight times per year over numerous years. In addition, there have been clear technological improvements made in the diagnosis of canine echinococcosis for surveillance, the genetic characterization of parasite strains and in vaccination against CE infection in livestock. In order to establish new paradigms and appropriate combinations of control strategies, we have carried out a review and discussion of the available control tools and control models. Control strategies must be suitable and sustainable to benefit the Echinococcosis–endemic areas primarily, which at the same time are the poorest regions of the world.

**Author:** Lima, N. F., Picanço, G. A., Alves, D. D. S. M. M., Silva, L. D., Isac, E., Costa, T. L., Lino Junior, R. D. S. and Vinaud, M. C.

**Title:** Oral nitazoxanide treatment of experimental neurocysticercosis induces gluconeogenesis in *Taenia crassiceps* cysticerci

**DOI:** 10.1016/j.actatropica.2018.12.017

**Abstract:** Neurocysticercosis is the most frequent helminthiasis of the central nervous system and is caused by the presence of *Taenia solium* cysticerci. Nitazoxanide (NTZ) is an antifolate containing the pyrrolopyrimidine-based nucleus that exerts its antiprotozoal

activity due to interference with the pyruvate:ferredoxin oxidoreductase (PFOR) enzyme which is essential to anaerobic energy metabolism. The aim of this work was to determine the effect of NTZ on the energetic metabolism of *Taenia crassiceps cysticerci* intracranially inoculated BALB /c mice. The infected animals were treated with a single oral dose of NTZ 30 days after the inoculation. Analysis of the organic acids was performed through high performance liquid chromatography. Glucose was detected only in the treated groups, alongside with a significant decrease in lactate, pyruvate and oxaloacetate concentrations which indicate an increase in gluconeogenesis. The non-detection of alpha-ketoglutarate indicated the use of the fumarate reductase pathway in all groups. It was possible to confirm the drugs mode of action due to the non-detection of acetate in the treated groups. There was an increase in the fatty acids oxidation. Therefore it was possible to observe that NTZ induces gluconeogenesis as well as the increase of alternative energetic pathways such as fatty acids oxidation in *T. crassiceps cysticerci*.

**Author:** Liu, Y., Yang, Y., Xu, J., Dong, X., Gu, X., Xie, Y., Lai, W., Jing, B., Peng, X. and Yang, G.

**Title:** Expression and serodiagnostic potential of antigen B and thioredoxin peroxidase from *Taenia multiceps*

**DOI:** 10.1016/j.vetpar.2019.07.002

**Abstract:** Coenurosis is a serious parasitic disease of herbivorous animals caused by the metacestode of *Taenia multiceps* (*Coenurus cerebralis*). Accordingly, a significant amount of research is currently dedicated to the development of appropriate antigens for use in rapid and accurate coenurosis diagnosis kits. In the present study, antigen B (AgB) and thioredoxin peroxidase (TPx) from *T. multiceps* were cloned and expressed using a prokaryotic system, molecular characterization of Tm-AgB was determined by bioinformatical analyses. The serological diagnostic potentials of rTm-AgB and rTm-TPx were evaluated by indirect ELISA and compared with those of previously reported rTm-AnxB2, rTm-HSP70, and rTm-GST. The results showed that Tm-AgB is a specific lipoprotein of cestodes with good thermal stability. The ELISA assay showed that rTm-AgB exhibited a sensitivity of 95.8% and a specificity of 87.5%, indicating its strong potential for serological diagnosis of *T. multiceps*.

**Author:** Picanço, G. D. A., De Lima, N. F., Gomes, T. C., Fraga, C. M., Alves, D. D. S. M. M., Castillo, R., Da Costa, T. L., Lino-Junior, R. D. S., Ambrosio, J. and Vinaud, M. C.

**Title:** Partial inhibition of the main energetic pathways and its metabolic consequences after in vivo treatment with benzimidazole derivatives in experimental neurocysticercosis

**DOI:** 10.1017/s0031182019000933

**Abstract:** Benzimidazole derivatives such as albendazole (ABZ) and mebendazole are important molecules used in helminthic treatment. Neurocysticercosis is the main cause of acquired epilepsy throughout the world and is currently treated with ABZ. New molecules

have been studied in order to aid in the treatment of this neglected tropical disease, among them RCB15 and RCB20. The aim of this study was to evaluate the metabolic impact of RCB15 and RCB20 on *Taenia crassiceps cysticerci* intracranially inoculated in Balb/c mice. Thirty days after the inoculation the mice were treated with 50 mg kg<sup>-1</sup> of RCB15, RCB20, ABZ or NaCl 0.9%. The euthanasia and cysticerci removal were performed 24 h after the treatment. The cysticerci were analysed through high performance liquid chromatography. After the treatments, there was an impairment in the main energetic pathways such as glycolytic pathway, homolactic fermentation or in mitochondrion energy production detected through the decrease in pyruvate, lactate, oxaloacetate, malate and fumarate concentrations. This induced the parasite to resort to alternative energetic pathways such as proteins catabolism, propionate fermentation and fatty acids oxidation. Therefore, benzimidazole derivatives are a promising alternative to ABZ use as they also reach the brain tissue and induce a metabolic stress in the cysticerci.

**Author:** Schurer, J. M., Nishimwe, A., Hakizimana, D., Li, H., Huang, Y., Musabyimana, J. P., Tuyishime, E. and MacDonald, L. E.

**Title:** A One Health systematic review of diagnostic tools for *Echinococcus multilocularis* surveillance: Towards equity in global detection

**DOI:** 10.1016/j.fawpar.2019.e00048

**Abstract:** *Echinococcus multilocularis* is a zoonotic cestode of canid definitive hosts that is emerging as a parasite of medical and veterinary concern in regions of North America, Europe and Asia. Infection with the metacestode stage (alveolar echinococcosis – AE) is life-threatening, especially for patients who reside in low resource countries and lack access to modern diagnostic tests and treatments. The overall objectives of this One Health review were to systematically describe the diagnostic tests currently employed in endemic countries to detect *E. multilocularis* in people, canids and the environment, and to report the test characteristics of new diagnostic techniques for population surveillance. In this systematic review of English and Chinese language databases, we identified 92 primary records of *E. multilocularis* surveillance in canids (N = 75), humans (N = 20) and/or the environment (food, soil; N = 3) and 12 grey literature records that reported *E. multilocularis* surveillance or health systems protocols between 2008 and 2018. Surveillance for *E. multilocularis* was conducted using a broad range of combined morphological, molecular, immunological and imaging techniques. Nine studies reporting diagnostic evaluations for cestode or metacestode detection were identified, including studies on copro-antigen ELISA, copro-PCR, intestinal examination, Western Blot, magnetic capture RT-PCR and immunochromatography. Our dataset includes prevalence estimates for *E. multilocularis* in canids, people, or environment in 27 of the 43 endemic countries and reports data gaps in surveillance, laboratory methods, and diagnostic sensitivity. International consensus on gold standard diagnostic techniques and harmonization of human, canid and environmental surveillance data across political boundaries are needed to comprehensively assess the global burden and distribution of this parasite.

**Author:** Tabari, M. A., Youssefi, M. R., Nasiri, M., Hamidi, M., Kiani, K., Alian Samakkhah, S. and Maggi, F.

**Title:** Towards green drugs against cestodes: Effectiveness of *Pelargonium roseum* and *Ferula gummosa* essential oils and their main component on *Echinococcus granulosus* protoscoleces

**DOI:** 10.1016/j.vetpar.2018.12.019

**Abstract:** Cystic echinococcosis is of great public health importance. Surgery is the efficient treatment for this infection. To minimize the risk of cyst intraoperative leakage, using scolicides is crucial. To date, any efficacious scolicide without side effect has not been introduced. Since essential oils of *Pelargonium roseum* and *Ferula gummosa* have shown several bioactivities, we evaluated their potential against protoscoleces of *E. granulosus* using albendazole as positive control. Furthermore, chemical composition of both essential oils was analyzed by gas chromatography-mass spectrometry (GC-MS) analyses, and their main constituents were also evaluated for scolicide activity. Different concentrations of essential oils and their two main constituents were tested for scolicide activity. Mortality rate was measured by eosin staining. Results of GC-MS revealed citronellol and  $\beta$ -pinene as the main constituents of *P. roseum* and *F. gummosa* essential oils, respectively. After 60 min of exposure to 50  $\mu\text{g/mL}$  of *P. roseum* and *F. gummosa*, mean mortality rate of protoscoleces was 100%. However,  $\beta$ -pinene and citronellol at the same time point with only 10  $\mu\text{g/mL}$  concentrations resulted in approximately higher than 80% mortality. Essential oils of *P. roseum* and *F. gummosa* showed significant toxic effect on *E. granulosus* with 50% lethal concentration (LC 50) values of 8.52 and 17.18  $\mu\text{g/mL}$ , respectively. Based on the LC 50 values,  $\beta$ -pinene (2.20  $\mu\text{g/mL}$ ) was the most potent scolicide agent in the present study. The overall toxicity of  $\beta$ -pinene and citronellol was significantly higher than the whole essential oils of *F. gummosa* and *P. roseum*. Based on these results,  $\beta$ -pinene and citronellol can be considered as candidate ingredients for the development of green scolicides.

**Author:** Vaca, H. R., Celentano, A. M., Macchiaroli, N., Kamenetzky, L., Camicia, F. and Rosenzvit, M. C.

**Title:** Histone deacetylase enzymes as potential drug targets of Neglected Tropical Diseases caused by cestodes

**DOI:** 10.1016/j.ijpddr.2019.02.003

**Abstract:** Cestode parasites cause neglected diseases, such as echinococcosis and cysticercosis, which represent a significant problem in human and animal health. Benzimidazoles and praziquantel are the only available drugs for chemotherapy and it is therefore important to identify new alternative drugs against cestode parasites. Histone deacetylases (HDACs) are validated drug targets for the treatment of cancer and other diseases, including neglected diseases. However, knowledge of HDACs in cestodes is very scarce. In this work, we investigated cestode HDACs as potential drug targets to

develop new therapies against neglected diseases caused by cestodes. Here we showed the full repertoire of HDAC coding genes in several members of the class Cestoda. Between 6 and 7 zinc-dependent HDAC coding genes were identified in the genomes of species from *Echinococcus*, *Taenia*, *Mesocestoides* and *Hymenolepis* genera. We classified them as Class I and II HDACs and analyzed their transcriptional expression levels throughout developmental stages of *Echinococcus* spp. We confirmed for the first time the complete HDAC8 nucleotide sequences from *Echinococcus canadensis* G7 and *Mesocestoides corti*. Homology models for these proteins showed particular structural features which differentiate them from HDAC8 from *Homo sapiens*. Furthermore, we showed that Trichostatin A (TSA), a pan-HDAC inhibitor, decreases the viability of *M. corti*, alters its tegument and morphology and produces an increment of the total amount of acetylated proteins, including acetylated histone H4. These results suggest that HDAC from cestodes are functional and might play important roles on survival and development. The particular structural features observed in cestode HDAC8 proteins suggest that these enzymes could be selectively targeted. This report provides the basis for further studies on cestode HDAC enzymes and for discovery of new HDAC inhibitors for the treatment of neglected diseases caused by cestode parasites.

**Author:** Wen, H., Vuitton, L., Tuxun, T., Li, J., Vuitton, D. A., Zhang, W. and McManus, D. P.

**Title:** Echinococcosis: Advances in the 21st century

**DOI:** 10.1128/cmr.00075-18

**Abstract:** Echinococcosis is a zoonosis caused by cestodes of the genus *Echinococcus* (family Taeniidae). This serious and near-cosmopolitan disease continues to be a significant public health issue, with western China being the area of highest endemicity for both the cystic (CE) and alveolar (AE) forms of echinococcosis. Considerable advances have been made in the 21st century on the genetics, genomics, and molecular epidemiology of the causative parasites, on diagnostic tools, and on treatment techniques and control strategies, including the development and deployment of vaccines. In terms of surgery, new procedures have superseded traditional techniques, and total cystectomy in CE, ex vivo resection with autotransplantation in AE, and percutaneous and perendoscopic procedures in both diseases have improved treatment efficacy and the quality of life of patients. In this review, we summarize recent progress on the biology, epidemiology, diagnosis, management, control, and prevention of CE and AE. Currently there is no alternative drug to albendazole to treat echinococcosis, and new compounds are required urgently. Recently acquired genomic and proteomic information can provide a platform for improving diagnosis and for finding new drug and vaccine targets, with direct impact in the future on the control of echinococcosis, which continues to be a global challenge.

**Author:** Zhu, G. Q., Li, L., Ohiolei, J. A., Wu, Y. T., Li, W. H., Zhang, N. Z., Fu, B. Q., Yan, H. B. and Jia, W. Z.

**Title:** A multiplex PCR assay for the simultaneous detection of *Taenia hydatigena*, *T. multiceps*, *T. pisiformis*, and *Dipylidium caninum* infections

**DOI:** 10.1186/s12879-019-4512-3

**Abstract:** Background: *Taenia hydatigena*, *T. multiceps*, *T. pisiformis*, and *Dipylidium caninum* are four common large and medium-sized tapeworms parasitizing the small intestine of dogs and other canids. These parasites cause serious impact on the health and development of livestock. However, there are, so far, no commercially available molecular diagnostic kits capable of simultaneously detecting all four parasites in dogs. The aim of the study was therefore to develop a multiplex PCR assay that will accurately detect all four cestode infections in one reaction. Methods: Specific primers for a multiplex PCR were designed based on corresponding mitochondrial genome sequences, and its detection limit was assessed by serial dilutions of the genomic DNAs of tapeworms examined. Furthermore, field samples of dog feces were tested using the developed assay. Results: A multiplex polymerase chain reaction (PCR) assay was developed based on mitochondrial DNA (mtDNA) that accurately and simultaneously identify four cestode species in one reaction using specific fragment sizes of 592, 385, 283, and 190 bp for *T. hydatigena*, *T. multiceps*, *T. pisiformis*, and *D. caninum*, respectively. The lowest DNA concentration detected was 1 ng for *T. hydatigena*, *T. multiceps* and *T. pisiformis*, and 0.1 ng for *D. caninum* in a 25 µl reaction system. This assay offers high potential for the rapid detection of these four tapeworms in host feces simultaneously. Conclusions: This study provides an efficient tool for the simultaneous detection of *T. hydatigena*, *T. multiceps*, *T. pisiformis*, and *D. caninum*. The assay will be potentially useful in epidemiological studies, diagnosis, and treatment of these four cestodes infections during prevention and control program.

## Ectoparasites

**Author:** de la Fuente, J. and Estrada-Peña, A.

**Title:** Why new vaccines for the control of ectoparasite vectors have not been registered and commercialized?

**DOI:** 10.3390/vaccines7030075

**Abstract:** The prevention and control of vector-borne diseases is a priority for improving global health. Despite recent advances in the characterization of ectoparasite-host-pathogen molecular interactions, vaccines are not available for most ectoparasites and vector-borne diseases that cause millions of deaths yearly. In this paper, in response to the question of why new vaccines for the control of ectoparasite vectors have not been registered and commercialized, and to contribute developing new effective vaccines

against ectoparasite vectors, we propose challenges and approaches to be addressed.

**Author:** Lihou, K. and Wall, R.

**Title:** Sheep blowfly strike: The cost of control in relation to risk

**DOI:** 10.1017/s1751731119000831

**Abstract:** Sheep blowfly strike (ovine cutaneous myiasis) is a widespread economic and welfare problem in sheep husbandry in many parts of the world. Strike incidence is determined by a complex interaction of fly abundance, host susceptibility and climate, combined with farmer husbandry and intervention strategies. Sheep farmers adopt a range of approaches to the type and timing of the management used for the control of blowfly strike, the rational basis for which is often not robust. Here a deterministic model, based on existing data relating to fly abundance, seasonal risk and strike incidence, is used to compare the variable costs associated with different strike management strategies. The model shows that not employing prophylactic treatment is the lowest cost strategy only where strike risk is low. In all other circumstances, prophylactic treatment incurs lower costs than not doing so, because the deaths associated with strike outweigh the costs of prophylactic treatment. Lamb treatment, in particular, has a substantial effect on strike and cost reduction, since lambs are the most abundant age-class of animals and are at the highest risk over the period when fly abundance is the greatest. Early-season treatment of ewes before shearing is also an important component of the lowest cost strategies, particularly when the blowfly season is extended. While the rational choice of the most appropriate strike management strategy is essential in the context of farm economics, welfare considerations lend added importance to treatment decisions that reduce strike incidence.

**Author:** Showler, A. T., Harlien, J. L. and Perez de León, A. A.

**Title:** Effects of Laboratory Grade Limonene and a Commercial Limonene-Based Insecticide on *Haematobia irritans irritans* (Muscidae: Diptera): Deterrence, Mortality, and Reproduction

**DOI:** 10.1093/jme/tjz020

**Abstract:** The horn fly, *Haematobia irritans irritans* (L.) (Diptera: Muscidae), is an important and cosmopolitan blood feeding ectoparasite of cattle. Resistance to conventional insecticides is increasingly problematic and alternative pesticides, including natural products, are being investigated. Limonene is a cyclic monoterpene repellent to some insects that occurs in citrus fruit rinds and in other plants. We assessed laboratory grade limonene and a commercial product, Orange Guard (5.8% AI limonene), against *H. irritans irritans* in terms of their contact effects upon contact on egg mortality, adults, and larval and pupal development; adult repellency as well as sublethal and fumigation effects. Egg viability declined when they were exposed to Orange Guard at concentrations of 1.45%, 2.9%, and 5.8% whereas laboratory grade limonene at 5.8% and 11.6% was



ovicidal. Contact exposure of adult *H. irritans irritans* to 5.8% laboratory grade limonene and 2.9% Orange Guard caused up to 100 and 88% knockdown (immobilization), respectively. At higher concentrations, laboratory grade limonene and Orange Guard resulted in less, and often shorter periods of knockdown. Although direct contact of 2.9 and 5.8% laboratory grade limonene caused mortality it was negligible when flies were sprayed directly with undiluted Orange Guard. Female *H. irritans irritans* exposed to sublethal concentrations of Orange Guard did not reduce the numbers of eggs produced, but the undiluted product reduced egg hatchability. Interestingly, limonene and Orange Guard attracted adult *H. irritans irritans* at concentrations <0.1%. We suggest that the attractancy of unformulated pure limonene might be useful for trapping *H. irritans irritans* adults.

## Mites of poultry

**Author:** Alves, L. F. A., Oliveira, D. G. P., Kasburg, C. R. and Nardelli, M. S.

**Title:** Acaricidal activity of inert powders against the poultry red mite *Dermanyssus gallinae* (De Geer, 1778) (Mesostigmata: Dermanyssidae)

**DOI:** 10.5380/avs.v24i2.62775

**Abstract:** *Dermanyssus gallinae* is one of the most important ectoparasite of laying hens. The intensive use of chemicals has led to the selection of resistant mite populations. The difficulty in controlling the mites has promoted the search for alternative products. Thereby, the aim of this study was to assess in laboratory the acaricidal activity of inert mineral powders against *D. gallinae*, in dust and water suspensions (5 and 10%) treatments. The products were tested against eggs, nymphs and adults of the mite. There were differences in the activity of tested products. Some products based on diatomaceous earth, kaolin and talc, applied in powder form on adults of the mite were more efficient and selected for subsequent tests. In liquid suspension bioassay it was observed no ovicidal activity of any of the tested products. However, the products were efficient on the nymphs with no difference between both products and concentrations. The activity of diatomaceous earth and Kaolin 2120 in 5 and 10% water suspension on adults was significantly higher than other treatments against adults. There was no difference between the treatments in residual activity. The morphological characterization and identification of diatom frustules present in the product PosturaSec® indicated prevalence of the genus *Brachysira* Kützing, density of  $5.7 \times 10^7$  valves/g, 200 µm size particles and 86.2% of silicon.

**Author:** Cafiero, M. A., Barlaam, A., Camarda, A., Radeski, M., Mul, M., Sparagano, O. and Giangaspero, A.

**Title:** *Dermanyssus gallinae* attacks humans. Mind the gap!

**DOI:** 10.1080/03079457.2019.1633010

**Abstract:** *Dermanyssus gallinae* is a haematophagous ectoparasite primarily known as a pest of domestic and wild birds. It occasionally feeds on a range of mammals, and, more importantly, is of growing concern in human medicine. This review highlights mite attacks on people working with poultry, and updates the increasing incidence of dermanyssosis in urban environments in Europe. Although several cases of dermanyssosis have been documented, there are a number of reasons why diagnosis of *D. gallinae* infestations in humans is likely to be underestimated. Firstly, medical specialists are not well aware of *D. gallinae* infestations in humans. There is also a lack of collaboration with specialists from other disciplines. The problem is compounded by misdiagnoses and by the lack of diagnostic tools. We review the literature on human dermanyssosis cases in Europe, and also provide information on the epidemiology, clinical, histo-pathological and immunological aspects of dermanyssosis. We stress the need for improved recognition of this challenging infestation in humans, and provide straightforward recommendations for health practitioners, starting with collection of the correct anamnestic information and including appropriate management methods for case recognition and resolution. Finally, we indicate the most urgent areas to be addressed by future research. **RESEARCH HIGHLIGHTS** *Dermanyssus gallinae* is of growing concern in human medicine. Most physicians are not well aware of dermanyssosis in humans. Bio-epidemiological and clinical aspects of this ectoparasitosis are highlighted. Practical key actions for diagnosis and correct management of infestation in humans are provided.

**Author:** Lima-Barbero, J. F., Contreras, M., Mateos-Hernández, L., Mata-Lorenzo, F. M., Triguero-Ocaña, R., Sparagano, O., Finn, R. D., Strube, C., Price, D. R. G., Nunn, F., Bartley, K., Höfle, U., Boadella, M., Nisbet, A. J., de la Fuente, J. and Villar, M.

**Title:** A vaccinology approach to the identification and characterization of *Dermanyssus gallinae* candidate protective antigens for the control of poultry red mite infestations

**DOI:** 10.3390/vaccines7040190

**Abstract:** The poultry red mite (PRM), *Dermanyssus gallinae*, is a hematophagous ectoparasite considered as the major pest in the egg-laying industry. Its pesticide-based control is only partially successful and requires the development of new control interventions such as vaccines. In this study, we follow a vaccinology approach to identify PRM candidate protective antigens. Based on proteomic data from fed and unfed nymph and adult mites, we selected a novel PRM protein, calumenin (Deg-CALU), which is tested as a vaccine candidate on an on-hen trial. *Rhipicephalus microplus* Subolesin (Rhm-SUB) was chosen as a positive control. Deg-CALU and Rhm-SUB reduced the mite oviposition by 35 and 44%, respectively. These results support Deg-CALU and Rhm-SUB as candidate protective antigens for the PRM control.

**Author:** Lima-Barbero, J. F., Díaz-Sánchez, S., Sparagano, O., Finn, R. D., de la Fuente, J. and Villar, M.

**Title:** Metaproteomics characterization of the alphaproteobacteria microbiome in different developmental and feeding stages of the poultry red mite *Dermanyssus gallinae* (De Geer, 1778)

**DOI:** 10.1080/03079457.2019.1635679

**Abstract:** The poultry red mite (PRM), *Dermanyssus gallinae* (De Geer, 1778), is a worldwide distributed ectoparasite and considered a major pest affecting the laying hen industry in Europe. Based on available information in other ectoparasites, the mite microbiome might participate in several biological processes and the acquisition, maintenance and transmission of pathogens. However, little is known about the role of PRM as a mechanical carrier or a biological vector in the transmission of pathogenic bacteria. Herein, we used a metaproteomics approach to characterize the alphaproteobacteria in the microbiota of PRM, and variations in its profile with ectoparasite development (nymphs vs. adults) and feeding (unfed vs. fed). The results showed that the bacterial community associated with *D. gallinae* was mainly composed of environmental and commensal bacteria. Putative symbiotic bacteria of the genera *Wolbachia*, *C. Tokpelaia* and *Sphingomonas* were identified, together with potential pathogenic bacteria of the genera *Inquilinus*, *Neorickettsia* and *Roseomonas*. Significant differences in the composition of alphaproteobacterial microbiota were associated with mite development and feeding, suggesting that bacteria have functional implications in metabolic pathways associated with blood feeding. These results support the use of metaproteomics for the characterization of alphaproteobacteria associated with the *D. gallinae* microbiota that could provide relevant information for the understanding of mite-host interactions and the development of potential control interventions. Research highlights Metaproteomics is a valid approach for microbiome characterization in ectoparasites. Alphaproteobacteria putative bacterial symbionts were identified in *D. gallinae*. Mite development and feeding were related to variations in bacterial community. Potentially pathogenic bacteria were identified in mite microbiota.

**Author:** Price, D. R. G., Küster, T., Øines, Ø, Oliver, E. M., Bartley, K., Nunn, F., Lima Barbero, J. F., Pritchard, J., Karp-Tatham, E., Hauge, H., Blake, D. P., Tomley, F. M. and Nisbet, A. J.

**Title:** Evaluation of vaccine delivery systems for inducing long-lived antibody responses to *Dermanyssus gallinae* antigen in laying hens

**DOI:** 10.1080/03079457.2019.1612514

**Abstract:** *Dermanyssus gallinae*, the poultry red mite, is a global threat to the commercial egg-laying industry. Control of *D. gallinae* is difficult, with only a limited number of effective pesticides and non-chemical treatments available. Here, we characterize the candidate vaccine antigen *D. gallinae* cathepsin D-1 (Dg-CatD-1) and demonstrate that purified

refolded recombinant Dg-Cat-D1 (rDg-CatD-1) is an active aspartyl proteinase which digests haemoglobin with a pH optimum of pH 4. Soluble protein extracts from *D. gallinae* also have haemoglobinase activity, with a pH optimum comparable to the recombinant protein, and both proteinase activities were inhibited by the aspartyl proteinase inhibitor Pepstatin A. Enzyme activity and the ubiquitous localization of Dg-CatD-1 protein in sections of adult female mites is consistent with Dg-CatD-1 being a lysosomal proteinase. Using Dg-CatD-1 as a model vaccine antigen, we compared vaccine delivery methods in laying hens via vaccination with: (i) purified rDg-CatD-1 with Montanide™ ISA 71 VG adjuvant; (ii) recombinant DNA vaccines for expression of rDg-CatD-1 and (iii) transgenic coccidial parasite *Eimeria tenella* expressing rDg-CatD-1. In two independent trials, only birds vaccinated with rDg-CatD-1 with Montanide™ ISA 71 VG produced a strong and long-lasting serum anti-rDg-Cat-D1 IgY response, which was significantly higher than that in control birds vaccinated with adjuvant only. Furthermore, we showed that egg-laying rates of *D. gallinae* mites fed on birds vaccinated with rDg-CatD-1 in Montanide™ ISA 71 VG was reduced significantly compared with mites fed on unvaccinated birds. RESEARCH HIGHLIGHTS *Dermanyssus gallinae* cathepsin D-1 (Dg-CatD-1) digests haemoglobin Vaccination of hens with rDg-CatD-1 in Montanide™ ISA 71 VG results in long-lasting IgY levels Serum anti-rDg-CatD-1 antibodies reduce egg laying in *D. gallinae* after a single blood meal.

**Author:** Puvača, N., Čabarkapa, I., Petrović, A., Bursić, V., Prodanović, R., Soleša, D. and Lević, J.

**Title:** Tea tree (*Melaleuca alternifolia*) and its essential oil: Antimicrobial, antioxidant and acaricidal effects in poultry production

**DOI:** 10.1017/s0043933919000229

**Abstract:** The aim of this review paper is to review the data on tea tree (*Melaleuca alternifolia*) as an antimicrobial, antioxidant and acaricidal in poultry production. Tea tree exhibits a wide spectrum of antimicrobial activities with minimal inhibitory concentrations between 0.12 and 4 mg/ml. Its modes of action against Gram-negative bacterium *Escherichia coli* (0.12 to 1.5 mg/ml), Gram-positive bacterium *Staphylococcus aureus* (0.12 to 1 mg/ml), *Aspergillus fumigatus* (1.78 mg/ml) and yeast, *Candida albicans* (0.05 to 0.5 mg/ml) have been investigated using a range of different methods. As an antimicrobial, tea tree has high antifungal, bacteriostatic and germicidal activity (e.g. a decrease of 73.8% in *Candida* sp.), because of its components such as terpinen-4-ol,  $\alpha$ -terpineol, linalool,  $\alpha$ -pinene,  $\beta$ -pinene,  $\beta$ -myrcene and 1,8-cineole. Its bioactive compounds such as  $\alpha$ -terpinene,  $\alpha$ -terpinolene and  $\gamma$ -terpinene show high antioxidant activity when applied in concentrations of 100 and 200  $\mu$ l/ml, while its essential oils demonstrated free radical scavenging activity of 60 to 80%. Tea trees insecticidal and acaricidal properties have been tested for tick control. The mortality of ticks (*Ixodes ricinus*) and poultry red mites (*Dermanyssus gallinae*) have been recorded at levels over 60% and 80%, respectively, when used in concentrations of 0.15 to 0.30 mg/cm<sup>2</sup> during in vitro testing, and in vivo, when sprayed in poultry houses. When tea tree was used in a form of essential oils as a dietary supplement in concentrations of 50 to 150 mg/kg in broiler chicken diets, a

significant increase in daily weight (by around 7%) and decrease in morbidity and mortality were seen. Additionally, when applied in laying hen nutrition, a significant increase in daily egg production has been recorded. Tea tree essential oils when supplemented in poultry diets have high positive effects regarding productivity performance, but this requires further field experiments to clarify standardisation of the material and effective inclusion levels.

**Author:** Wang, C., Huang, Y., Zhao, J., Ma, Y., Xu, X., Wan, Q., Li, H., Yu, H. and Pan, B.

**Title:** First record of *Aspergillus oryzae* as an entomopathogenic fungus against the poultry red mite *Dermanyssus gallinae*

**DOI:** 10.1016/j.vetpar.2019.06.011

**Abstract:** The poultry red mite, *Dermanyssus gallinae*, is a blood-feeding ectoparasite that affects egg-laying hens worldwide. Strategies to control this parasite have focused in the use of entomopathogenic fungi, such as *Metarhizium anisopliae*. However, only a few studies have evaluated the use of *Aspergillus oryzae* to control *D. gallinae* and none of them have employed native strains. In the work presented here, a novel entomopathogenic fungus was isolated from a dead *D. gallinae*. The results of phylogenetic analysis showed 100% similarity between the isolated strain and those of two species, *A. oryzae* and *Aspergillus flavus*, and 99.82% similarity with *A. parvisclerotigenus*, which were in the same branch of the Flavi section of the genus *Aspergillus*. This entomopathogenic fungus was a non-aflatoxin B1 producer, as shown by the presence of aflatoxin B1 in the conidial infection suspension. Morphological features of fungus in comparison with *A. oryzae* and *A. flavus* indicated that the isolated strain belonged to *A. oryzae*, and was named *Aspergillus* sp. Dg-1. The pathogenicity of *Aspergillus* sp. Dg-1 on *D. gallinae* at different life stages was then assessed under laboratory conditions. The experiments showed that the isolated strain significantly increased the mortality rate in adult mites, up to  $24.83 \pm 2.25$ , compared to the mortality rates in the control group, which were  $15.17 \pm 2.75$  ( $P < 0.05$ ). However, *Aspergillus* sp. Dg-1 did not have pathogenic effects on the second nymph stage of *D. gallinae*. Our findings demonstrate that *Aspergillus* sp. Dg-1 has pathogenic effects on *D. gallinae* in their adult stage, presenting biocontrol potential against *D. gallinae*.

**Author:** Wang, C., Ma, Y., Huang, Y., Su, S., Wang, L., Sun, Y., Wan, Q., Li, H., Zhang, S., Øines, Ø and Pan, B.

**Title:** Darkness increases the population growth rate of the poultry red mite *Dermanyssus gallinae*

**DOI:** 10.1186/s13071-019-3456-1

**Abstract:** Background: The poultry red mite (PRM), *Dermanyssus gallinae*, is one of the most economically deleterious ectoparasites affecting egg-laying hens worldwide. It may be possible to control *D. gallinae* populations by manipulating lighting regimes within

poultry units. However, no studies have clearly shown the effects of darkness on the population growth rate of *D. gallinae*. Methods: The effect of darkness on the population growth rate of *D. gallinae* was investigated, together with the first description of the molecular identity of the mite from China. Mite variables under two lighting regimens (1:23 h L:D and 12:12 h L:D) were compared, including number of mites and eggs, survival and feeding rates, engorgement, oviposition, hatchability and the life-cycle of *D. gallinae*. Results: The results showed that the number of mites ( $13,763 \pm 956$ ) and eggs ( $5424 \pm 317$ ) in the rearing system with prolonged darkness of 1:23 h L:D at 4th week were 2.4- and 3.6-fold higher than those under a conventional lighting regimen of 12:12 h L:D, respectively. The feeding rates of mites under prolonged darkness ranged from  $36.7 \pm 1.1\%$  to  $52.0 \pm 7.0\%$ , which were significantly higher than those under conventional lighting regimen (ranging from  $22.6 \pm 1.9\%$  to  $37.3 \pm 1.6\%$ ). The mean weight of engorged females ( $0.26 \pm 0.01$  mg) and the mean number of eggs per female (on average  $5.87 \pm 0.36$ ) under prolonged darkness were significantly higher than those under conventional lighting regimen ( $0.22 \pm 0.01$  mg and  $3.62 \pm 0.31$ , respectively). However, the survival rate ranging from  $98.07 \pm 0.10\%$  to  $98.93 \pm 0.19\%$ , hatchability of  $97.93 \pm 0.01\%$  and the life-cycle of *D. gallinae* (9 days) was not affected by the lighting period. Conclusions: Our findings demonstrated that prolonged darkness significantly promoted the proliferation levels of *D. gallinae*, resulting in increased number of mites and eggs in the rearing system. The promoted population growth of *D. gallinae* was found to be related to the increased feeding rate, engorgement level and oviposition level of mites under prolonged darkness. The egg hatchability, the survival rates and the duration of life-cycle of *D. gallinae* were not affected by the light regimes.

## ***Trichostrongylus* spp of grouse**

**Author:** Baines, D., Newborn, D. and Richardson, M.

**Title:** Are *Trichostrongylus tenuis* control and resistance avoidance simultaneously manageable by reducing anthelmintic intake by grouse?

**DOI:** 10.1136/vr.105029

**Abstract:** Benzimidazole-based anthelmintics bound to grit (medicated grit) are annually prescribed on request by veterinary practices to grouse managers to control *Trichostrongylus tenuis* an intestinal parasite of red grouse *Lagopus lagopus scotica*. Those prescribing medication typically do without knowledge of parasite loads and hence often prescribe when loads are low and unlikely to impact the host. Inappropriate use of anthelmintics in livestock has led to development of parasite resistance to anthelmintics. To encourage grouse managers to reduce anthelmintic use, the authors experimentally withdrew medication from parts of eight moors. The authors monitored parasite and grouse responses by counting eggs and adult worms and grouse mortality and breeding success. Rapid increases in parasite egg counts in early spring culminated in resuming medication at three wet, blanket-peat sites; one in the first spring and two in the second. Medication was restored, despite low parasite counts, at a fourth moor. On the remaining

four moors, drier heaths in the east, parasite levels remained low, were not associated with grouse mortality, but breeding success was 16 per cent lower in years without medication. Better parasite monitoring by grouse managers and vets alike may reduce anthelmintic use, helping prevent drug resistance, but this may be off-set by reduced grouse productivity.

## Zoonotic nematode parasites

**Author:** Angora, K. E., Bosson-Vanga, A. H., Tuo, K., Ako, B., Ira-Bonouman, A., Beourou, S., Tiacoh, L., Dou, S. G., Koffi, A. C., Soumahoro, A. and Offianan, A. T.

**Title:** Intestinal helminthiasis: Retrospective study at parasitology laboratory from Pasteur Institute of Côte d'Ivoire

**DOI:** 10.4314/njpar.v40i1.14

**Abstract:** The intestinal helminthiasis due to soil-transmitted helminths (STH) is a cause of morbidity in the world, especially in the poor and tropical regions of sub-Saharan Africa. It leads to real problem of global public health importance. In order to update data about the intestinal helminthiasis, an 11 year retrospective study has been carried out. This work precisely run from 2005 to 2016 and was based on the data about intestinal helminthiasis parasitological diagnosis fulfilled in the Parasitology Unit of Pasteur Institute of Côte d'Ivoire. The data used for this study were obtained from an office access computer designed database. All the sample results recorded from the diagnosis were analyzed. Of 3,600 patients received 36 were positive to intestinal helminths (1.1%). Sex ratio was 0.83 (45.3% males, 54.7% females) with an average age of 33.8 (standard deviation = 19.1 years). The patients aged from 30 to 45 were the most infected ( $p = 0.011$ ). The helminth species identified were of *Trichuris trichiura* (17.1%), hookworms (19.5%), *Strongyloides stercoralis* (21.9%) and *Dicrocoelium dendriticum* (19.5%). This study shows that the intestinal helminthiasis has just recently decreased in Côte d'Ivoire. The health system authorities must raise more awareness campaigns of the albendazole systematic use in massive treatment in order to reach the utter eradication of these parasitic diseases.

**Author:** Hung, T. Y., Janson, S., Smith, P., Legg, A. and Baird, R. W.

**Title:** Declining soil transmitted helminth detections in an Australian tropical region

**DOI:** 10.1016/j.pathol.2019.09.002

**Abstract:** Soil-transmitted helminths (STHs), are recognised neglected tropical diseases and have been endemic in patients in tropical Northern Australia. We reviewed the temporal trends in detections of STHs and *Hymenolepis nana* in faecal samples from Northern Territory (NT) Government Health facilities, representing patients with acute illnesses and comorbidities between 2008 and 2018. *Ascaris lumbricoides* is not detected in patients in the NT. The number of faecal samples examined yearly was relatively constant with a median of 4458 (range 4246–4933). Faecal samples from patients under

the age of 5 years declined by 45% over the 11 years of the study. Detections of *Trichuris trichiura*, *Strongyloides* spp., and hookworm ova fell significantly by 89% ( $p < 0.001$ ), 71% ( $p < 0.001$ ), and 43% ( $p < 0.01$ ), respectively, over the 11 years. Detections of *H. nana* declined by 33% absolutely, but not significantly, when assessed relative to the reduction in faecal samples from patients under the age of 5 years. The marked reduction in STH numbers coincided with a 10-fold increase in NT dispensing of ivermectin, predominantly used for scabies control, in widely geographically spaced locations throughout the NT, over the 11 years of the study. Our data support previous findings of the beneficial collateral effects of ivermectin therapy. Ivermectin is not recognised as having anti-cestode activity, hence the continued presence of *H. nana* endemically in the NT, suggests declines in STHs are not related to other changes in health hardware or existing mass drug administration programs. The reduction in *T. trichiura* detections may not be explained by this association, as unlike *Strongyloides* spp., the anti-helminthic effect of ivermectin has been less marked.

**Author:** Mubanga, C., Mwape, K. E., Phiri, I. K., Trevisan, C., Zulu, G., Chabala, C., van Damme, I., Schmidt, V., Dorny, P. and Gabriël, S.

**Title:** Progress on the development of rapid diagnostic tests for foodborne neglected zoonotic helminthiases: A systematic review

**DOI:** 10.1016/j.actatropica.2019.03.030

**Abstract:** Background: Foodborne Neglected Zoonotic Helminths (FNZH) are parasites of both economic and public health importance. They include *Taenia solium*, *Echinococcus granulosus sensu lato*, *Echinococcus multilocularis* and Foodborne trematodes (FBT). FNZH are earmarked for major interventions for control, elimination and eradication. This systematic review highlights the progress towards development of rapid tests for the diagnosis of FNZH since 2010 when they were listed as neglected tropical diseases. Methodology: A systematic search was conducted in three databases, World of Science, Embase and PubMed using the same search phrase. The search produced 480 hits. Three studies from back referencing were included. Only 22 of these met the inclusion criteria. Data was extracted from these and presented qualitatively. Results: Twenty-five rapid diagnostic tests were found to have been developed since 2010, eight for diagnosis of *T. solium* infections, eight for echinococcosis and nine for FBT infections. The rapid tests for diagnosing *T. solium* infections included six antibody detecting and two antigen detecting tests. They constitute a combination among them, with some tests providing qualitative, others quantitative results. Similarly, seven out of the eight rapid tests developed for *Echinococcus* infections were antibody detecting tests save for one loop mediated isothermal amplification test. All of them were qualitative tests. For FBT infections, nine rapid tests were described; two antibody and one nucleic acid detecting test for diagnosis of Fascioliasis; three nucleic acid detecting tests for Opisthorchiasis; one antibody detecting test for Paragonimiasis; and for Clonorchiasis, one antibody and one nucleic acid detecting test. The FBT infection rapid tests were all qualitative in nature. Most of these tests have not undergone field evaluation in endemic areas where they will be used most. Conclusion: This review describes the development and evaluation of rapid



diagnostic tests, while highlighting the need for in depth validations of the tools to determine how well they can perform in endemic areas.

**Author:** Oleaga, A., Rey, O., Polack, B., Grech-Angelini, S., Quilichini, Y., Pérez-Sánchez, R., Boireau, P., Mulero, S., Brunet, A., Rogon, A., Vallée, I., Kincaid-Smith, J., Allienne, J. F. and Boissier, J.

**Title:** Epidemiological surveillance of schistosomiasis outbreak in Corsica (France): Are animal reservoir hosts implicated in local transmission?

**DOI:** 10.1371/journal.pntd.0007543

**Abstract:** Environmental and anthropogenic changes are expected to promote emergence and spread of pathogens worldwide. Since 2013, human urogenital schistosomiasis is established in Corsica island (France). Schistosomiasis is a parasitic disease affecting both humans and animals. The parasite involved in the Corsican outbreak is a hybrid form between *Schistosoma haematobium*, a human parasite, and *Schistosoma bovis*, a livestock parasite. *S. bovis* has been detected in Corsican livestock few decades ago raising the questions whether hybridization occurred in Corsica and if animals could behave as a reservoir for the recently established parasite lineage. The latter hypothesis has huge epidemiological outcomes since the emergence of a zoonotic lineage of schistosomes would be considerably harder to control and eradicate the disease locally and definitively needs to be verified. In this study we combined a sero-epidemiological survey on ruminants and a rodent trapping campaign to check whether schistosomes could shift on vertebrate hosts other than humans. A total of 3,519 domesticated animals (1,147 cattle; 671 goats and 1,701 sheep) from 160 farms established in 14 municipalities were sampled. From these 3,519 screened animals, 17 were found to be serologically positive but were ultimately considered as false positive after complementary analyses. Additionally, our 7-day extensive rodent trapping (i.e. 1,949 traps placed) resulted in the capture of a total of 34 rats (*Rattus rattus*) and 4 mice (*Mus musculus*). Despite the low number of rodents captured, molecular diagnostic tests showed that two of them have been found to be infected by schistosomes. Given the low abundance of rodents and the low parasitic prevalence and intensity among rodents, it is unlikely that neither rats nor ruminants play a significant role in the maintenance of schistosomiasis outbreak in Corsica. Finally, the most likely hypothesis is that local people initially infected in 2013 re-contaminated the river during subsequent summers, however we cannot definitively rule out the possibility of an animal species acting as reservoir host.

**Author:** Zhang, H., Liu, C. and Zheng, Q.

**Title:** Development and application of anthelmintic drugs in China

**Short Title:** Development and application of anthelmintic drugs in China

**DOI:** 10.1016/j.actatropica.2019.105181

**Abstract:** China was once a country plagued by parasitic diseases. At the beginning of the founding of the People's Republic of China, nearly 80% of the population suffered from parasitic diseases because of poverty and poor sanitary conditions. After nearly 70 years of development, China has made remarkable achievements in the prevention and control of parasitic diseases, and the prevalence of parasitic diseases has been greatly reduced. In addition to organizational leadership from the government and various preventive measures, drug treatment and drug research & development are important and irreplaceable links in prevention and control work. Since the 1950s, China has begun to introduce, produce and imitate antiparasitic drugs from abroad, such as santonin, benzimidazole, and praziquantel. Chinese scientists have also contributed to the optimization of production techniques, improvements in drug formulation, the application in the clinic and the mechanisms of actions of generic drugs. At the same time, China has independently developed tribendimidine (TrBD, a broad spectrum anthelmintic), and its anthelmintic spectrum has been comprehensively studied. It is active against almost 20 parasites, is especially superior to benzimidazoles against *Necator americanus*, and surpasses the effectiveness of praziquantel against *Clonorchis sinensis*. In the treatment of tapeworm disease, the traditional Chinese medicines pumpkin seeds and betel nuts have good curative effects for taeniasis. Chinese scientists have explored the action modes and clinical administration methods of pumpkin seeds and betel nuts, which is still the main clinical regimen for the disease. This paper reviews the history and progress of the study of anthelmintics in intestinal helminth infections since the founding of the People's Republic of China and aiming to support clinicians and drug researchers in China and other countries.

## Diagnostic techniques for other parasites

**Author:** Bal, M. S., Sagar, R., Kaur, P., Mahajan, V., Singla, L. D. and Singh, C. K.

**Title:** Foldscope as a diagnostic tool for identification of parasites of domesticated animals

**Abstract:** The present study was carried out to evaluate the effectiveness of foldscope (portable paper microscope) attached with smartphone as a novel, valuable low cost tool for quick diagnosis of animal parasitic diseases under field condition so as to manage the infection at an early stage. To fulfil the objective of the study, specimens of ectoparasites, helminth parasites, haemoparasites, faecal and skin scrapings from animals/clinical cases were examined by foldscope and pictures were taken. All the parasitic slides were also examined by conventional light microscope for comparison purpose and to validate the results. From the results it was observed that foldscope (with magnification of 140×) can be used as a cheap, effective and reliable diagnostic tool to identify helminth and arthropod parasites with comparable efficacy to that of expensive light microscope. Faecal samples with heavy infection of parasitic eggs/ova/cysts and *Trypanosoma evansi* were detectable by foldscope with comparable efficacy with optical microscope. However, low grade gastrointestinal parasitic infections and intracellular haemoparasites were not detected using foldscope. This is the first report on evaluation of diagnostic efficacy of

foldoscope to detect economically important parasitic infections of livestock and companion animals in India and its field application. Mobile phone enabled imaging and diagnostics technologies have the potential to bring revolutionary changes in the field of animal disease diagnosis.

**Author:** Attia, M. M., Ismael, E. and Saleh, N. M. K.

**Title:** A sensitive serodiagnostic tool for the detection of active infection of zoonotic visceral and nasopharyngeal linguatulus

**DOI:** 10.14202/vetworld.2019.883-889

**Abstract:** Aim: This study aimed to evaluate the different serological techniques for early diagnosis of acute concurrent infections with linguatulus in the definitive host (dogs) and an intermediate host (goats). This evaluation compared between the gold standard (GS) test (GS; examination of nasal and fecal samples in dogs and examination of lymph nodes in goats), sandwich enzyme-linked immunosorbent assay (S-ELISA), and indirect ELISA. Materials and Methods: Fifty goats and fifty dogs were examined for the presence of *Linguatula serrata* nymphs and adults, respectively, besides the collection of blood samples from the examined animals for serologic testing. Results: In goats; GS, S-ELISA, and indirect ELISA showed positivity in 32 (64%), 28 (56%), and 39 (78%) samples, respectively. In dogs; GS, S-ELISA, and indirect ELISA showed positivity in 25 (50%), 25 (50%), and 30 (60%) samples, respectively. S-ELISA displayed significant higher agreement with the GS test ( $\geq 0.83$ ) than indirect ELISA ( $\leq 0.67$ ) in both hosts. Infection with linguatulus showed significant relation with the age of goats and dogs and the sex of goats ( $p < 0.05$ ). Conclusion: S-ELISA displayed more sensitivity and specificity for the detection of concurrent infections with linguatulus in both dogs and goats than indirect ELISA, which could detect the prior infections. Similarly, these assays could be used for diagnosis of concurrent infections with linguatulus in human, especially the chronic ones.

**Author:** Attia, M. M., Soliman, S. M. and Saleh, N. M. K.

**Title:** Advanced and rapid serodiagnosis of oestrosis (*Oestrus ovis*; Diptera: Oestridae) in sheep using indirect and dot-ELISA

**Abstract:** This study is aimed at evaluating performance of the indirect ELISA for the diagnosis of oestrosis versus the rapid easy assay of Dot-ELISA which could be directly used in the field. Two hundred and forty head of sheep were examined in a Cairo abattoir over the period from May 2017 to May 2018. Sera were collected from each examined sheep and preserved in  $-20\text{ }^{\circ}\text{C}$ . The Anterior cone (AC) of third-stage larvae of *Oestrus ovis* were prepared. Hyperimmune sera were processed in rats using the anterior part of the larvae (AC); ELISA and Dot-ELISA tests were done after checkerboard titration. The lowest antigen concentration which gives positive results in ELISA was  $10\text{ }\mu\text{g}$  protein, whereas the concentration used in the Dot-ELISA was  $200\text{ ng}/\mu\text{L}$ , with the sera dilution being 1:100 in the two tests. The two tests were performed with known *O. ovis*-positive and negative sheep sera and known hyperimmunized rat sera against AC of *O. ovis*, as

well as known sera for *Coenurus cerebralis*, *Dictyocaulus filaria* and *Haemonchus contortus* without an *O. ovis* infection. Two hundred out of the two-hundred and forty examined sheep were positive for *O. ovis* larvae at post mortem. High optical density (O.D.) values ranging from 0.9- 2 were estimated in the sheep infected with the second and third-stage larvae. On the other hand, O.D. values varying from 0.9 to 1.6 were recorded when sheep (95 sheep) were infected only with the third-stage larvae of *O. ovis*. The results of the current study confirm that Dot-ELISA had similar sensitivity and specificity to those by the indirect ELISA but is more rapid and has an easy assay. So, it could be applicable in the field directly for diagnosing oestrosis.

## ***Fasciola* spp and paramphistomes**

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Do triclabendazole medicated molasses blocks have a role in control of *Fasciola gigantica* in smallholder cattle production in Lao PDR?

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**DOI:** 10.1071/AN17255

**Abstract:** Smallholder cattle production in many developing tropical countries including Laos is compromised by widespread endoparasitism, with limited farmer knowledge of parasites and facilities for administration of therapeutics. We report a pilot study examining the potential for triclabendazole provided in medicated molasses blocks offered to control *Fasciola gigantica* in smallholder cattle production. This study involved 241 cattle allocated into three groups: (1) triclabendazole (as Fasinex<sup>®</sup>, Novartis Animal Health Australia, Pty Ltd) medicated molasses blocks (MMB) with each tonne of MMB containing 0.5 kg triclabendazole; (2) unmedicated molasses blocks; and (3) a Control group. Data and faecal samples were obtained at Weeks 1, 4, 8 and 12 for faecal egg counts (FEC) determination. Reductions in FEC in the MMB group of 90.48% and a mean FEC of  $4 \pm 17$  eggs per gram of faeces at 12 weeks post-treatment was observed, with liveweight increasing from 174.60 ( $\pm 3.35$ ) kg to 191.50 ( $\pm 3.69$ ) kg in Weeks 1 and 12, respectively ( $P = 0.001$ ) and an average daily weight gain of 201 g/day. Reduction in FEC in the unmedicated molasses blocks group was also observed, by 28.78% and 18.96%, with liveweight increasing from 179.50 ( $\pm 3.35$ ) kg to 189.90 ( $\pm 6.05$ ) kg in Weeks 1 and 12 respectively ( $P = 0.3$ ), with an average daily gain of 124 g/day. This study suggests that productivity was enhanced when triclabendazole was added to the blocks, delivering parasite suppression or potentially therapeutic doses on ad libitum feeding of the MMB. Although further work is required to establish the therapeutic potential of MMB, the use of MMB may offer a parasite management and nutritional supplementation strategy for smallholder farmers, particularly in Laos and other countries where unmanaged *Fasciola* spp. infestations reduce ruminant productivity and facilities for animal restraint to enable delivery of oral anthelmintics, are largely non-existent. © 2019 CSIRO.

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<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85068194502&doi=10.1016%2fj.jip.2019.107212&partnerID=40&md5=b511605e46820509721d46433345f6f5>

**DOI:** 10.1016/j.jip.2019.107212

**Abstract:** Snails of the species *Pseudosuccinea columella* are considered intermediate hosts of *Fasciola hepatica*, a digenetic trematode that infects bile ducts of ruminants and humans, causing economic damage and serious problems for public health. These gastropods inhabit ponds, have high reproductive capacity, and lay their egg masses in submerged substrates on pond edges where they are exposed to desiccation and microbes, including fungi that may exert pathogenic effects on the snail and its embryos. This information is relevant for control of the intermediate host and therefore of fasciolosis. With the objective of evaluating ovicidal potential of *Pochonia chlamydosporia* (Pc-10 isolate), a nematophagous fungus used as antagonistic agent for a wide variety of helminths of medical and veterinary importance, on egg masses of *P. columella*, we compared a treated group, where the egg masses were exposed to Pc-10 for a period of 25 days, and a control group, in which there was no exposure to the fungus. The results indicated that the embryogenesis process was significantly inhibited (93.15%) by Pc-10, suggesting its applicability in biological control programs of lymnaeid snails. In addition, ultrastructure showed the occurrence of different types of interactions between the egg masses with the mycelia of Pc-10: type 1, biochemical effects by the adherence of hyphae; type 2, morphological alterations, but without hyphal penetration; and type 3, lytic effect, morphological damage caused by penetration of hyphae by the fungus, resulting in some important structural modifications, thus compromising the viability of the eggs. The results demonstrate the susceptibility of *P. columella* egg masses to an isolate of *P. chlamydosporia* under laboratory conditions, providing valuable information for the biological control of this intermediate host. © 2019 Elsevier Inc.

Georgieva, K., Hristov, P., Tsocheva-Gaytandzhieva, N., Nanev, V.

Inhibition of *Fasciola hepatica* infection in *Galba truncatula* snails by application of monosaccharides to the aquatic environment

(2019) *Biologia*, . Article in Press.

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85059516132&doi=10.2478%2fs11756-018-00182-y&partnerID=40&md5=b242a1c40fd500d309a9eba3873418e3>

**DOI:** 10.2478/s11756-018-00182-y

**Abstract:** *Fasciola hepatica* is one of the etiological agents of fasciolosis, a widespread disease in domestic animals and occasionally in humans. Fasciolosis may be reduced by blocking the parasite transmission through its intermediate hosts. In the present work, different monosaccharides have been tested for their ability to impact on ligand/receptor interactions at the interface between the parasite and the intermediate host. Laboratory snails were subjected to miracidia in the presence of methyl- $\alpha$ -D-mannopyranoside (MetMan),  $\alpha$ -D-glucose (Glc), N-acetyl-D-glucosamine (GlcNAc), D-(+)-galactose (Gal), N-acetyl-D-galactosamine (GalNAc) or L-(-)-fucose (Fuc), in 10 mM concentration. The snail survival rates and the prevalence of infection were determined after 50 days. Survived snails in the study groups varied from 78% to 97%. A remarkable reduction in the number of parasite-infected snails was observed in groups subjected to MetMan, Glc, or GlcNAc – 36.9%, 10.9%, and 11.9%, respectively, compared to 92.7% in the control group. Other tested monosaccharides had a low impact on snail infection. The results point to the implication that surface carbohydrate/receptor interactions are among the determining factors concerning the transmission of *F. hepatica* by the specific vector *Galba truncatula*. Biological recognition between the two organisms can be interfered with appropriate monosaccharides and this can be used to develop an alternative method for control of fasciolosis at the intermediate host level. © 2019, Institute of Zoology, Slovak Academy of Sciences.

Alba, A., Tetreau, G., Chaparro, C., Sánchez, J., Vázquez, A.A., Gourbal, B.

Natural resistance to *Fasciola hepatica* (Trematoda) in *Pseudosuccinea columella* snails: A review from literature and insights from comparative “omic” analyses

(2019) *Developmental and Comparative Immunology*, 101, art. no. 103463, .

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85071402595&doi=10.1016%2fj.dci.2019.103463&partnerID=40&md5=36f958caedcfa21d19a073fd44ced156>

**DOI:** 10.1016/j.dci.2019.103463

**Abstract:** The snail *Pseudosuccinea columella* is one of the main vectors of the medically-important trematode *Fasciola hepatica*. In Cuba, the existence of natural *P. columella* populations that are either susceptible or resistant to *F. hepatica* infection offers a unique snail-parasite for study of parasite-host compatibility and immune function in gastropods. Here, we review all previous literature on this system and present new “omic” data that provide a molecular baseline of both *P. columella* phenotypes from naïve snails. Comparison of whole snail transcriptomes (RNAseq) and the proteomes of the albumen

gland (2D-electrophoresis, MS) revealed that resistant and susceptible strains differed mainly in an enrichment of particular biological processes/functions and a greater abundance of proteins/transcripts associated with immune defense/stress response in resistant snails. These results indicate a differential allocation of molecular resources to self-maintenance and survival in resistant *P. columella* that may cause enhanced responsiveness to stressors (i.e. *F. hepatica* infection or tolerance to variations in environmental pH/total water hardness), possibly as trade-off against reproduction and the ecological cost of resistance previously suggested in resistant populations of *P. columella*.  
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Zhong, W., Chen, Y., Gong, S., Qiao, J., Meng, Q., Zhang, X., Wang, X., Huang, Y., Tian, L., Niu, Y.

Enzymological properties and nematode-degrading activity of recombinant chitinase AO-379 of *Arthrobotrys oligospora* [*Arthrobotrys oligospora* rekombinant çitinaz ao-379'un enzim özellikleri ve nematod indirgeyici aktivitesi]

(2019) Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 25 (4), pp. 435-444.

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85067482723&doi=10.9775%2fkvfd.2018.20603&partnerID=40&md5=52cb5d4bd7c0e07508dac51ca099029a>

**DOI:** 10.9775/kvfd.2018.20603

**Abstract:** Chitinase is an important virulence factor produced by nematode trapping fungi in the process of infection, and plays an important role in the cleavage of nematodes and their eggshells. In this study, the cDNA sequence of *Arthrobotrys oligospora* chitinase AO-379 was amplified by RT-PCR and inserted into the vector pPIC9K to induce the expression of AO-379 in *Pichia pastoris* GS115. The recombinant AO-379 (reAO-379) was purified by nickel ion affinity chromatography, and enzymological properties and nematode-degrading activity of reAO-379 was analyzed. SDS-PAGE and Western blot analysis showed that the reAO-379 with molecular weight of about 44 kDa was successfully obtained. The reAO-379 showed strong chitinase activity at pH 5.5 and 30°C. Using reAO-379 to treat *Strongylus equinus*, *Caenorhabditis elegans* and *Haemonchus contortus* for 12, 24, and 36 h, the killing rates of reAO-379 in *S. equinus* were 42%, 89% and 100%; in *C. elegans* were 50%, 90% and 97%; in and *H. contortus* were 53%, 62% and 84%, respectively. Using reA-379 to treat *Fasciola hepatica* and *Dicrocoelium chinensis* eggs for 24, 48 and 72 h, the degradation rates of reAO-379 were 12%, 43% and 65% in *F. hepatica* eggs, and were 15%, 33% and 55% in *D. chinensis* eggs, respectively. Our study suggests that the reAO-379 is potentially valuable for development of biological control agent against digestive tract nematodes in livestock. © 2019, Veteriner Fakültesi Dergisi. All rights reserved.

Kamaludeen, J., Graham-Brown, J., Stephens, N., Miller, J., Howell, A., Beesley, N.J., Hodgkinson, J., Learmount, J., Williams, D.



Lack of efficacy of triclabendazole against *Fasciola hepatica* is present on sheep farms in three regions of England, and Wales

(2019) *Veterinary Record*, 184 (16)

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85062323934&doi=10.1136%2fvr.105209&partnerID=40&md5=77797e56752b2b8038c3a5e4176ab5ac>

**DOI:** 10.1136/vr.105209

**Abstract:** The liver fluke *Fasciola hepatica* is a parasitic trematode that has a major impact on livestock production and human health. Control of *F hepatica* is difficult and relies on anthelmintics, particularly triclabendazole, due to its efficacy against both adult and juvenile stages of the parasite. Emergence of triclabendazole-resistant *F hepatica* populations has been reported in a number of countries, including the UK, but the overall prevalence and distribution of triclabendazole resistance is unknown. In this study, the authors established the presence of reduced efficacy of triclabendazole in sheep flocks in England and Wales, using a validated composite faecal egg count reduction test. Seventy-four sheep farms were sampled from Wales, southwest, northwest and northeast England between Autumn 2013 and Spring 2015. *F hepatica* eggs were detected in samples from 42/74 farms. Evidence of a lack of efficacy of triclabendazole was detected on 21/26 farms on which the faecal egg count reduction test was completed, with faecal egg count reductions ranging from 89 per cent to 0 per cent. Regression analysis suggested that both prevalence of *F hepatica* and lack of efficacy of triclabendazole were spatially correlated, with higher faecal egg counts and lower percentage reductions on farms located in the northwest of England, and Wales. Overall, the results show that reduced efficacy of triclabendazole is present across England and Wales, with a complete lack of therapeutic efficacy observed on 9/26 farms. © British Veterinary Association 2019. Re-use permitted under CC BY. Published by BMJ.

Zhang, J.-L., Si, H.-F., Zhou, X.-Z., Shang, X.-F., Li, B., Zhang, J.-Y.

High prevalence of fasciolosis and evaluation of the efficacy of anthelmintics against *Fasciola hepatica* in buffaloes in Guangxi, China

(2019) *International Journal for Parasitology: Parasites and Wildlife*, 8, pp. 82-87.

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85059742207&doi=10.1016%2fj.ijppaw.2018.12.010&partnerID=40&md5=d6b1976a6125347a6c4d7807bf15dd53>

Romero, J., Villaguala, C., Quiroz, F., Landaeta-Aqueveque, C., Alfaro, G., Pérez Fernández, R.

Flukicide efficacy against *Fasciola hepatica* of Triclabendazole and Nitroxynil in cattle of the central valley of Chile [Avaliação da eficácia de triclabendazol e nitroxinil para o tratamento da infecção por *Fasciola hepatica* em bovinos do Vale Central do Chile]

(2019) Revista Brasileira de Parasitologia Veterinaria, 28 (1), pp. 164-167.

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85063926435&doi=10.1590%2fs1984-296120180089&partnerID=40&md5=a3c4dfa71cff7cb8041a338d44d37581>

**DOI:** 10.1590/s1984-296120180089

**Abstract:** On a farm with permanent history of fasciolosis a study was performed aimed to know the efficacy of triclabendazole (TCBZ) and then to contrast with that of nitroxynil. Thirty-nine cattle naturally infected with *Fasciola hepatica* were randomly allocated into 4 experimental groups: Group 1 (control) was left untreated. Group 2 was treated with of 12 mg/kg body weight (bw) of TCBZ by oral route. Group 3 treated with 24 mg/kg bw TCBZ orally. Group 4 was treated with 10 mg/kg bw of nitroxynil subcutaneously. The anthelmintic efficacy was calculated as the percentage of reduction in faecal egg count (FEC) at 14 and 28 d post-treatment. Results indicated that there were no significant differences in the percentage of FEC reduction between control group and the groups treated with 12 or 24 mg/kg of TCBZ. On the contrary, the treatment with nitroxynil significantly reduced the FEC and decreased the percentage of positive animals. In conclusion, *Fasciola hepatica* is reported for first time as resistant to TCBZ in Chile, which highlights the need of rotating drugs and assessing the efficacy of the administered drug in order to avoid the selection of resistant worms. © 2019, Brazilain Coll Veterinary Parasitology. All rights reserved.

Bushra, M., Shahardar, R.A., Allaie, I.M., Wani, Z.A.

Efficacy of closantel, fenbendazole and ivermectin against GI helminths of cattle in central Kashmir

(2019) Journal of Parasitic Diseases, 43 (2), pp. 289-293.

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85061389779&doi=10.1007%2fs12639-019-01091-w&partnerID=40&md5=0e9cb5b44edf4bdb09caa1d7cf74104b>

**DOI:** 10.1007/s12639-019-01091-w

**Abstract:** Three trials were conducted to evaluate the therapeutic efficacy of closantel (@ 7.5 mg/kg body weight, orally), fenbendazole (@ 7.5 mg/kg body weight, orally) and ivermectin bolus (@ 0.2 mg/kg body weight, orally) against gastrointestinal (GI) helminth parasites of cattle in central zone of Kashmir valley. Twenty cattle suffering from GI helminth infection were randomly selected and divided into two groups in each trial. In each trial, one group comprising of fifteen animals were treated with anthelmintic and

second group comprising of five animals were kept as infected untreated control. To determine the therapeutic efficacy of different anthelmintic drugs against GI helminths, faecal samples were directly collected from rectum before treatment and on 7th, 14th and 28th day post treatment and eggs per gram of faeces was determined by using Stoll's method. Then the efficacy of different anthelmintics was calculated by employing Faecal Egg Count Reduction Test. On 14th day post treatment closantel was found to be 100 and 94.44% effective against *Fasciola* spp. and strongyle worms, respectively, however, it was not found effective against *Trichuris* spp. The efficacy of fenbendazole was 100% against *Fasciola* spp., paramphistomes, *Moniezia* spp., *Trichuris* spp. and strongyle worms on 14th day post treatment. Ivermectin was found to be 100% effective against *Trichuris* spp. and strongyle worms on 14th day post treatment, however, it was not effective against *Fasciola* spp. and paramphistomes. © 2019, Indian Society for Parasitology.

Maté, L., Giantin, M., Viviani, P., Ballent, M., Tolosi, R., Lifschitz, A., Lanusse, C., Dacasto, M., Virkel, G.

Effects of fenbendazole and triclabendazole on the expression of cytochrome P450 1A and flavin-monooxygenase isozymes in bovine precision-cut liver slices

(2019) Veterinary Journal, 245, pp. 61-69.

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85060161877&doi=10.1016%2fj.tvjl.2019.01.001&partnerID=40&md5=cfbcb06687fc505f4d29e4c17ac54c3a>

**DOI:** 10.1016/j.tvjl.2019.01.001

**Abstract:** Combinations of the anthelmintics fenbendazole (FBZ) and triclabendazole (TCBZ) have shown enhanced efficacy against the liver fluke *Fasciola hepatica*. This study aimed to measuring the constitutive expression of CYP1A1, CYP1A2, FMO1 and FMO3, thought to be involved in the metabolism of those compounds, by using an absolute quantitative real time (RT)-PCR approach in bovine precision-cut liver slices (PCLS). It also aimed to characterize the effects of FBZ and TCBZ (alone and in combination) on the expression and activity of the aforementioned isozymes. Both FMO1 and FMO3 were equally represented in control PCLS, whereas CYP1A2 was expressed more than CYP1A1 ( $P < 0.05$ ). PCLS cultured in the presence of beta naphthoflavone ( $\beta$ -NF; CYP1A inducer) had higher mRNA levels of CYP1A1, CYP1A2, FMO1 and FMO3 ( $P < 0.05$ ). No clear-cut evidence of transcriptional effects of the anthelmintics were recorded. After incubation of PCLS with FBZ, there was a significant increase ( $P < 0.05$ ) vs. controls and TBCZ was observed for CYP1A1. PCLS treated with FBZ showed a higher ( $P < 0.05$ ) expression of CYP1A2 compared to controls, TCBZ alone, and the combination FBZ + TCBZ. The gene expression profiles of FMO1 and FMO3 were not affected by the presence of the anthelmintics; the only exception was an upregulation of FMO3 by TCBZ alone. The observed transcriptional effects of the xenobiotics were not mirrored by increased enzyme activities using prototypical substrates of the isozymes under study. Although further confirmatory studies are needed, these results suggest that PCLS

represent an alternative in vitro tool for studies on the expression, regulation and function of relevant xenobiotic-metabolizing enzymes in cattle. © 2019 Elsevier Ltd

Ceballos, L., Canton, C., Pruzzo, C., Sanabria, R., Moreno, L., Sanchis, J., Suarez, G., Ortiz, P., Fairweather, I., Lanusse, C., Alvarez, L., Valladares, M.M.

The egg hatch test: A useful tool for albendazole resistance diagnosis in *Fasciola hepatica* (2019) *Veterinary Parasitology*, 271, pp. 7-13.

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85066794065&doi=10.1016%2fj.vetpar.2019.06.001&partnerID=40&md5=3861cc9c96709ad82b0ae937b3bfec1d>

**DOI:** 10.1016/j.vetpar.2019.06.001

**Abstract:** In the current study, the egg hatch test (EHT) has been evaluated as an in vitro technique to detect albendazole (ABZ) resistance in *Fasciola hepatica*. The intra- and inter-assay variations of the EHT were measured by means of the coefficient of variation in different fluke isolates and over time; then, the results of the EHT were compared with the “gold standard” controlled efficacy test, which assesses the in vivo anthelmintic efficacy. The EHT was used later to evaluate the intra-herd variability regarding the level of ABZ resistance in calves infected by the same fluke isolate. Finally, several factors of the initial protocol were modified to improve the simplicity of the assay, including the incubation time of eggs with the drug and the use of eggs collected from faeces. The greatest uniformity between results within the assay and over time until 8 weeks after gallbladder collection (the deadline proposed for egg analysis) was obtained with an ABZ concentration of 0.5 µM. The length of exposure to ABZ was shown to be critical, as prolonged incubation (15 days) led to a change of ovicidal activity. The ABZ concentration of 0.5 µM is suggested as a possible discriminating dose to predict ABZ resistance, due to the close agreement between the results of the EHT at an ABZ concentration of 0.5 µM and those of the in vivo assays. © 2019 Elsevier B.V.

Wesołowska, A., Basałaj, K., Zawistowska-Deniziak, A., Januszkiewicz, K., Kozak Ljunggren, M., Jedlina, L., Wędrychowicz, H.

The failure of a DNA prime/protein boost regime and CTLA-4 mediated targeting to improve the potency of a DNA vaccine encoding *Fasciola hepatica* phosphoglycerate kinase in sheep

(2019) *Veterinary Immunology and Immunopathology*, 217, art. no. 109941, .

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Proteomic identification of galectin-11 and -14 ligands from *Fasciola hepatica*

(2019) *International Journal for Parasitology*, 49 (12), pp. 921-932.

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Protective efficacy of liver fluke DNA vaccines: A systematic review and meta-analysis: Guiding novel vaccine development

(2019) *Veterinary Parasitology*, 267, pp. 90-98.

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Garcia-Campos, A., Correia, C.N., Naranjo-Lucena, A., Garza-Cuartero, L., Farries, G., Browne, J.A., Machugh, D.E., Mulcahy, G.

*Fasciola hepatica* infection in cattle: Analyzing responses of peripheral blood mononuclear cells (PBMC) using a transcriptomics approach

(2019) *Frontiers in Immunology*, 10 (AUG), art. no. Article 2081, .

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85072010155&doi=10.3389%2ffimmu.2019.02081&partnerID=40&md5=6db03d8702da03a7fb99341b3603d8c5>

**DOI:** 10.3389/fimmu.2019.02081

**Abstract:** The parasitic helminth *Fasciola hepatica* (liver fluke) causes economic loss to the livestock industry globally and also causes zoonotic disease. New control strategies such as vaccines are urgently needed, due to the rise of drug resistance in parasite populations. Vaccine development requires a comprehensive understanding of the immunological events during infection. Previous *in vivo* studies by our group have investigated global differentially expressed genes (DEGs) in ovine peripheral blood mononuclear cells (PBMC) in response to both acute and chronic *F. hepatica* infection. This work demonstrated that pathways involved in the pathogenesis of ovine fasciolosis included fibrosis, inhibition of macrophage nitric oxide production, and antibody isotype switching, among others. Transcriptomic changes in PBMC populations following *F. hepatica* infection in cattle, in which the disease phenotype is quite different, have not yet been examined. Using RNA sequencing we investigated gene expression changes in PBMC isolated from 9 non-infected and 11 *F. hepatica*-experimentally-infected calves immediately before infection, at 1 and at 14 weeks post-infection. Longitudinal time-course comparisons between groups revealed 21 and 1,624 DEGs driven exclusively by *F.*

hepatica infection in cattle at acute and chronic stages, respectively. These results show that fewer DEGs at the acute stage of infection can be identified in cattle, as compared with sheep. In addition, the log<sub>2</sub> fold-changes of these DEGs were relatively low (−1 to 3) reflecting the different clinical presentation of *F. hepatica* infection in cattle. Gene pathways for hepatic fibrosis and hepatic cholestasis along with apoptosis of antigen-presenting cells were enriched at chronic stages. Our results reflect the major differences in the disease phenotype between cattle and sheep and may indicate pathways to target in vaccine development. © 2019 Garcia-Campos, Correia, Naranjo-Lucena, Garza-Cuartero, Farries, Browne, MacHugh and Mulcahy.

Azizi, H., Mirzaeei, H., Bagheri, A., Bazi, A., Khamesipour, A., Yaghoobi, H., Mirzapour, A., Khatami, M., Elikae, S.

A Survey on the Adjuvant Role of Naloxone Alone or Combined with Alum in Vaccination Against Fasciolosis in BALB/c Mice

(2019) *Acta Parasitologica*, 64 (2), pp. 236-245.

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85069899605&doi=10.2478%2fs11686-019-00030-2&partnerID=40&md5=b80e30d6143bea48cfd0c8a13030312>

**DOI:** 10.2478/s11686-019-00030-2

**Abstract:** Background: Fasciolosis is a zoonotic parasitic disease imposing a heavy load of livestock losses worldwide. Purpose: We aimed to evaluate immune-stimulatory effects of naloxone (NLX), an opioid receptor antagonist, in combination with alum in mice vaccinated with excretory–secretory antigens (E/S) of *Fasciola hepatica*. Methods: 8-week-old female BALB/c mice were subcutaneously vaccinated using E/S antigens of *F. hepatica*. Experimental groups (14 mice per group) included: vaccine (E/S antigen), alum vaccine (E/S antigen plus alum), NLX vaccine (E/S antigen plus NLX), and alum–NLX vaccine (E/S antigen plus a mixture of alum–NLX). The control group was infused with PBS. Lymphocyte proliferation and the levels of IFN- $\gamma$ , IL-4, IgG2a, IgG1, and total IgG were measured. Results: Mice vaccinated with NLX or alum–NLX adjuvants showed significantly higher rates of lymphocyte proliferation, IFN- $\gamma$ , total IgG, and IgG2a levels. The mice that were injected with alum showed a significantly higher concentration of IL-4. Ratios of IFN- $\gamma$ /IL-4 and IgG2a/IgG1 were significantly higher in the NLX and alum–NLX groups in comparison with the groups vaccinated either with alum or without any adjuvant. A significantly higher protection rate (62.5%) was seen in mice vaccinated with the alum–NLX adjuvant compared to the other groups. Conclusion: NLX can be effective in conferring cellular immunity and protection against *F. hepatica*. It is recommended to consider this agent as a potential adjuvant in vaccines against fasciolosis. © 2019, Witold Stefański Institute of Parasitology, Polish Academy of Sciences.

Ortega-Vargas, S., Espitia, C., Sahagún-Ruiz, A., Parada, C., Balderas-Loeza, A., Villamancera, A., Quiroz-Romero, H.

Moderate protection is induced by a chimeric protein composed of leucine aminopeptidase

and cathepsin L1 against *Fasciola hepatica* challenge in sheep

(2019) *Vaccine*, 37 (24), pp. 3234-3240.

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85064651088&doi=10.1016%2fj.vaccine.2019.04.067&partnerID=40&md5=ed32f739297982f9219b2574137fb844>

**DOI:** 10.1016/j.vaccine.2019.04.067

**Abstract:** Leucine aminopeptidase (FhLAP) and cathepsin L1 (FhCL1) of *Fasciola hepatica* play a critical role in parasite feeding, migration through host tissue, and immune evasion. These antigens have been tested for immune protection as single components with variable degrees of success. The chimeric-protein approach could improve protection levels against fasciolosis. Previously, we reported the design and construction of a chimeric protein composed of antigenic sequences of FhLAP and FhCL1 of *F. hepatica*. The goal of the present study was to express and evaluate the immune-protective capacity of this chimeric protein (rFhLAP-CL1) in sheep. Animals were randomly allocated into five groups with five animals in each group. Groups 1, 2 and 3 were immunized twice with 100 µg, 200 µg and 400 µg of rFhLAP-CL1 emulsified with Quil A adjuvant, whereas groups 4 and 5 were the adjuvant control and infection control groups, respectively. The animals were then challenged with 200 metacercariae two weeks after the rFhLAP-CL1 booster. The fluke burden was reduced by 25.5%, 30.7% ( $p < 0.05$ ) and 46.5% ( $p < 0.01$ ) in sheep immunized with 100 µg, 200 µg and 400 µg of chimeric protein, respectively, in comparison to the infection control group. There was a reduction of 22.7% ( $p < 0.05$ ) and 24.4% ( $p < 0.01$ ) in fecal egg count in groups 2 and 3, respectively, compared to the infection control group. Sheep immunized with chimeric protein produced *F. hepatica* excretion-secretion product-specific total IgG antibody, which were increased after challenge. Moreover, the levels of rFhLAP-CL1-specific IgG1 and IgG2 isotypes in immunized sheep increased rapidly two weeks after the first immunization and were significantly more elevated than those of the control groups, indicating a mixed Th1/Th2 response. This is a preliminary evaluation of the chimeric protein rFhLAP-CL1 as a possible immunogen against *F. hepatica* infection in sheep. © 2019 Elsevier Ltd

Ferraro, F., Merlino, A., Gil, J., Cerecetto, H., Corvo, I., Cabrera, M.

Cathepsin L inhibitors with activity against the liver fluke identified from a focus library of quinoxaline 1,4-di-N-Oxide derivatives

(2019) *Molecules*, 24 (13), art. no. 2348, .

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85068959255&doi=10.3390%2fmolecules24132348&partnerID=40&md5=a2a30c33cc5ebfc604794a6ec3746246>

**DOI:** 10.3390/molecules24132348

**Abstract:** Infections caused by *Fasciola* species are widely distributed in cattle and sheep causing significant economic losses, and are emerging as human zoonosis with increasing reports of human cases, especially in children in endemic areas. The current treatment is chemotherapeutic, triclabendazole being the drug of preference since it is active against all parasite stages. Due to the emergence of resistance in several countries, the discovery of new chemical entities with fasciolicidal activity is urgently needed. In our continuous search for new fasciolicide compounds, we identified and characterized six quinoxaline 1,4-di-N-oxide derivatives from our in-house library. We selected them from a screening of novel inhibitors against FhCL1 and FhCL3 proteases, two essential enzymes secreted by juvenile and adult flukes. We report compounds C7, C17, C18, C19, C23, and C24 with an IC<sub>50</sub> of less than 10 µM in at least one cathepsin. We studied their binding kinetics in vitro and their enzyme-ligand interactions in silico by molecular docking and molecular dynamic (MD) simulations. These compounds readily kill newly excysted juveniles in vitro and have low cytotoxicity in a Hep-G2 cell line and bovine spermatozoa. Our findings are valuable for the development of new chemotherapeutic approaches against fascioliasis, and other pathologies involving cysteine proteases. © 2019 by the authors. Licensee MDPI, Basel, Switzerland.

Rasouli, A., Farahnak, A., Zali, H., Rezaeian, M., Golestani, A., Molaei Rad, M.B.

Protein detection of excretory-secretory products and somatic extracts from *Fasciola hepatica* and *F. gigantica* using two-dimensional electrophoresis

(2019) Iranian Journal of Parasitology, 14 (3), pp. 379-386.

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85073480212&doi=10.18502%2fijpa.v14i3.1476&partnerID=40&md5=a791028a0e61ed4e54488a3089f446db>

**DOI:** 10.18502/ijpa.v14i3.1476

**Abstract:** Background: The aim of this research was to compare excretory-secretory and somatic extract materials of *Fasciola hepatica* and *F. gigantica* to detect protein maps of two species. Methods: Twenty infected livers were collected from sheep in industrial slaughter-house in Tehran, 2017-2019. Worms were detached from bile ducts, then recognized according to morphologic and morphometric criteria. After three times wash-ing, worms were incubated in RPMI culture media and excretory-secretory products were collected. Worms were crushed and homogenized for preparation of somatic extract. Two Dimensional Electrophoresis gels were accomplished for both excretory-secretory material and somatic extracts. Gels were scanned with densitometer and analyzed with Same Spots software and protein spots were identified with ExPasy database. Results: For both excretory-secretory products and somatic extract, protein spots were appeared with two-dimensional electrophoresis technique. Quantitative analysis showed 40 and 28 protein spots for excretory-secretory of *F. hepatica* and *F. gigantica* respectively. For somatic extract 19 and 12 protein spots were recognized for *F. hepatica* and *F. gigantica* in that order. Conclusion: The rate of expression of some proteins were more in *F. hepatica* while



expression of other proteins was high in *F. gigantica*. The expression of protease enzyme was higher in *F. gigantica* than *F. hepatica*. These data could be considered for biochemical differentiation of *Fasciola* species and subsequently to design and prepare of antigens for diagnosis/vaccine development. © 2019, Tehran University of Medical Sciences (TUMS). All rights reserved.

Vijayashanthi, R., Soundararajan, C., Raman, M., Senthil Kumar, T.M.A.

Antigenic profile of excretory / secretory antigens of immature paramphistomes\*

(2019) Indian Veterinary Journal, 96 (6), pp. 29-31.

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85071673498&partnerID=40&md5=14577b6271688a745c16cf752bfcff0a>

**Abstract:** The present study focused on characterization of excretory/secretory antigens of pathogenic immature paramphistomes which could be used for immunodiagnosis of immature paramphistomosis in sheep. The immature flukes were collected from the duodenum of sheep at Perambur slaughter house, Chennai. The flukes were incubated in 10 mM phosphate buffered saline (10 flukes/6ml) at 28.3°C for overnight. The protein concentration varied from 0.236 mg/ml to 0.314 mg/ml using bicinchoninic acid (BCA) method. SDS-PAGE (12%) analysis of the total excretory/secretory antigen of immature flukes revealed 4 immunodominant bands in the range of 10 to 20 kDa which might be used for immunodiagnosis of immature paramphistomosis in sheep. © 2019 Indian Veterinary Association. All rights reserved.

Norbury, L.J., Basalaj, K., Bańska, P., Kalinowska, A., Zawistowska-Deniziak, A., Yap, H.Y., Wilkowski, P., Wesółowska, A., Wędrychowicz, H.

Construction of a novel phage display antibody library against *Fasciola hepatica*, and generation of a single-chain variable fragment specific for *F. hepatica* cathepsin L1

(2019) Experimental Parasitology, 198, pp. 87-94.

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85062382687&doi=10.1016%2fj.exppara.2019.02.001&partnerID=40&md5=e157947fee05382bfa5d7cdd971285c6>

**DOI:** 10.1016/j.exppara.2019.02.001

**Abstract:** Phage display technology to produce recombinant monoclonal antibodies or antibody fragments permits the identification of sought after antibodies in short time frames at low cost along with direct and rapid selection for antibody characteristics. Monoclonal antibodies can facilitate the identification and characterisation of parasite molecules that function at the host-parasite interface to help understand at the molecular level the biology of the parasite and disease progression, which often leads to new drug targets, diagnostic antigens or vaccine candidates. The trematode *Fasciola hepatica* is an important veterinary and human parasite. In this work, we infected rats with *F. hepatica* and

amplified the generated antibody repertoire to produce a single-chain variable fragment (scFv) phage display library. The library was used to identify a scFv that recognises cathepsin L1, a major component of the adult parasites excretory/secretory material and an important vaccine candidate. This is the first report of the construction of a phage display antibody library from a *F. hepatica* infected host, and also the first instance such a library has been used to identify an affinity-matured monoclonal antibody fragment that binds to a *F. hepatica* antigen. The scFv library and methods detailed should facilitate future research characterising *F. hepatica* antigens. © 2019 Elsevier Inc.

Di Maggio, L.S., Tirloni, L., Pinto, A.F.M., Diedrich, J.K., Yates, J.R., III, Carmona, C., Berasain, P., da Silva Vaz, I., Jr.

A proteomic comparison of excretion/secretion products in *Fasciola hepatica* newly excysted juveniles (NEJ) derived from *Lymnaea viatrix* or *Pseudosuccinea columella*

(2019) *Experimental Parasitology*, 201, pp. 11-20.

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85064671047&doi=10.1016%2fj.exppara.2019.04.004&partnerID=40&md5=e80a40e9b4045e4b01c8d0b13b8d1dfd>

**DOI:** 10.1016/j.exppara.2019.04.004

**Abstract:** The characteristics of parasitic infections are often tied to host behavior. Although most studies have investigated definitive hosts, intermediate hosts can also play a role in shaping the distribution and accumulation of parasites. This is particularly relevant in larval stages, where intermediate host's behavior could potentially interfere in the molecules secreted by the parasite into the next host during infection. To investigate this hypothesis, we used a proteomic approach to analyze excretion/secretion products (ESP) from *Fasciola hepatica* newly excysted juveniles (NEJ) derived from two intermediate host species, *Lymnaea viatrix* and *Pseudosuccinea columella*. The two analyzed proteomes showed differences in identity, abundance, and functional classification of the proteins. This observation could be due to differences in the biological cycle of the parasite in the host, environmental aspects, and/or host-dependent factors. Categories such as protein modification machinery, protease inhibitors, signal transduction, and cysteine-rich proteins showed different abundance between samples. More specifically, differences in abundance of individual proteins such as peptidyl-prolyl cis-trans isomerase, thioredoxin, cathepsin B, cathepsin L, and Kunitz-type inhibitors were identified. Based on the differences identified between NEJ ESP samples, we can conclude that the intermediate host is a factor influencing the proteomic profile of ESP in *F. hepatica*. © 2019 Elsevier Inc.

Brecht, K., Kirchhofer, C., Bouitbir, J., Trapani, F., Keiser, J., Krähenbühl, S.

Exogenous iron increases fasciocidal activity and hepatocellular toxicity of the synthetic endoperoxides OZ78 and MT04

(2019) *International Journal of Molecular Sciences*, 20 (19), art. no. 4880,

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85072929856&doi=10.3390%2fijms20194880&partnerID=40&md5=69c4b04fb6526436edfd001f718da3e3>

**DOI:** 10.3390/ijms20194880

**Abstract:** The synthetic peroxides OZ78 and MT04 recently emerged as fasciocidal drug candidates. However, the effect of iron on fasciocidal activity and hepatocellular toxicity of these compounds is unknown. We investigated the in vitro fasciocidal activity and hepatocellular toxicity of OZ78 and MT04 in absence and presence of Fe(II)chloride and hemin, and conducted a toxicological study in mice. Studies were performed in comparison with the antimalarial artesunate (AS), a semisynthetic peroxide. Fasciocidal effects of OZ78 and MT04 were confirmed and enhanced by Fe<sup>2+</sup> or hemin. In HepG2 cells, AS reduced cellular ATP and impaired membrane integrity concentration-dependently. In comparison, OZ78 or MT04 were not toxic at 100 µM and reduced the cellular ATP by 13% and 19%, respectively, but were not membrane-toxic at 500 µM. The addition of Fe<sup>2+</sup> or hemin increased the toxicity of OZ78 and MT04 significantly. AS inhibited complex I, II, and IV of the mitochondrial electron transport chain, and MT04 impaired complex I and II, whereas OZ78 was not toxic. All three compounds increased cellular reactive oxygen species (ROS) concentration-dependently, with a further increase by Fe<sup>2+</sup> or hemin. Mice treated orally with up to 800 mg OZ78, or MT04 showed no relevant hepatotoxicity. In conclusion, we confirmed fasciocidal activity of OZ78 and MT04, which was increased by Fe<sup>2+</sup> or hemin. OZ78 and MT04 were toxic to HepG2 cells, which was explained by mitochondrial damage associated with ROS generation in the presence of iron. No relevant hepatotoxicity was observed in mice in vivo, possibly due to limited exposure and/or high antioxidative hepatic capacity. © 2019 by the authors. Licensee MDPI, Basel, Switzerland.

Orbegozo-Medina, R.A., Martínez-Sernández, V., Folgueira, I., Mezo, M., González-Warleta, M., Perteguer, M.J., Romarís, F., Leiro, J.M., Ubeira, F.M.

Antibody responses to chimeric peptides derived from parasite antigens in mice and other animal species

(2019) *Molecular Immunology*, 106, pp. 1-11.

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85058435329&doi=10.1016%2fj.molimm.2018.11.019&partnerID=40&md5=4f27d1128c23255896fa12f08fcc32d6>

**DOI:** 10.1016/j.molimm.2018.11.019

**Abstract:** Peptide vaccines constitute an interesting alternative to classical vaccines due to the possibility of selecting specific epitopes, easy of production and safety. However, an inadequate design may render these peptides poorly immunogenic or lead to undesirable outcomes (e.g., formation of B neoepitopes). As an approach to vaccine development, we evaluated the antibody response to chimeras composed of two or three known B epitopes

from *Trichinella* and *Fasciola*, and several linkers (GSGSG, GP GPG and KK) in species as different as mice, sheep and turbot. All these species could mount an effective immune response to the short chimeric peptides. Nevertheless, this response depended on several factors including a favorable orientation of B-cell epitopes, adequateness of linkers and/or probability of formation of T neoepitopes. We also observed that, at least in mice, the inclusion of a decoy epitope may have favorable consequences on the antibody response to other epitopes in the chimera. © 2018 Elsevier Ltd

Celias, D.P., Corvo, I., Silvane, L., Tort, J.F., Chiapello, L.S., Fresno, M., Arranz, A., Motrán, C.C., Cervi, L.

Cathepsin L3 from *fasciola hepatica* induces NLRP3 inflammasome alternative activation in murine dendritic cells

(2019) *Frontiers in Immunology*, 10 (MAR), art. no. 552, .

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85064722721&doi=10.3389%2ffimmu.2019.00552&partnerID=40&md5=4669782959b62ae43ee7d79b017e7790>

**DOI:** 10.3389/fimmu.2019.00552

**Abstract:** The production of IL-1-family cytokines such as IL-1 $\beta$  and IL-18 is finely regulated by inflammasome activation after the recognition of pathogens associated molecular pattern (PAMPs) and danger associated molecular patterns (DAMPs). However, little is known about the helminth-derived molecules capable of activating the inflammasome. In the case of the helminth trematode *Fasciola hepatica*, the secretion of different cathepsin L cysteine peptidases (FhCL) is crucial for the parasite survival. Among these enzymes, cathepsin L3 (FhCL3) is expressed mainly in the juvenile or invasive stage. The ability of FhCL3 to digest collagen has demonstrated to be critical for intestinal tissue invasion during juvenile larvae migration. However, there is no information about the interaction of FhCL3 with the immune system. It has been shown here that FhCL3 induces a non-canonical inflammasome activation in dendritic cells (DCs), leading to IL-1 $\beta$  and IL-18 production without a previous microbial priming. Interestingly, this activation was depending on the cysteine protease activity of FhCL3 and the NLRP3 receptor, but independent of caspase activation. We also show that FhCL3 is internalized by DCs, promoting pro-IL-1 $\beta$  cleavage to its mature and biologically active form IL-1 $\beta$ , which is released to the extracellular environment. The FhCL3-induced NLRP3 inflammasome activation conditions DCs to promote a singular adaptive immune response, characterized by increased production of IFN- $\gamma$  and IL-13. These data reveal an unexpected ability of FhCL3, a helminth-derived molecule, to activate the NLRP3 inflammasome, which is independent of the classical mechanism involving caspase activation. Copyright © 2019 Celas, Corvo, Silvane, Tort, Chiapello, Fresno, Arranz, Motrán and Cervi. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication

in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Creedon, N., Robinson, C., Kennedy, E., Riordan, A.O.

Agriculture 4.0: Development of Seriological on-Farm Immunosensor for Animal Health Applications

(2019) Proceedings of IEEE Sensors, 2019-October, art. no. 8956677

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85078697694&doi=10.1109%2fSENSORS43011.2019.8956677&partnerID=40&md5=7fa8f0afef615400794c7cd5f050a135>

**DOI:** 10.1109/SENSORS43011.2019.8956677

**Abstract:** With expanding global population, there is now an urgent need to produce more food, more efficiently with the available existing finite resources. Concerning dairy production, bovine diseases negatively impact animal welfare, while decreasing farm efficiency and profitability through wasted feed, reduced milk production and increased veterinary costs. New digital technologies that provide veterinarians with immediate test results on-farm will enable early clinical interventions, thereby ensuring food security and reduce losses. To address this challenge, we present an impedimetric biosensor platform that enables rapid identification of bovine immunoglobulin G and bovine Liver Fluke antibodies in complex blood plasma with time to result of 15 mins. © 2019 IEEE.

Al-Mahmood, S.S., Al-Sabaawy, H.B.

Fasciolosis: Grading the histopathological lesions in naturally infected bovine liver in Mosul city

(2019) Iraqi Journal of Veterinary Sciences, 33 (2), pp. 379-387.

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85074063190&doi=10.33899%2fijvs.2019.163083&partnerID=40&md5=abd04163840ee00954c3b6ea8ed6fbcd>

**DOI:** 10.33899/ijvs.2019.163083

**Abstract:** Fasciolosis cause economic losses in cattle that breed in Iraq and the world. About 4% of bovine liver's samples included in the current study exhibited classical pathological lesions of fasciolosis. Samples of cattle livers infected with fasciolosis were taken for histopathology. Eighteen grading criteria with four scoring level have been chosen to grading the microscopic lesions caused by *Fasciola hepatica* into a mild infection (grade I), moderate infection (grade II) and severe infection (grade III). The type of hepatic degeneration or necrosis, cloudy cell swelling, coagulative necrosis, infiltration of inflammatory cells, with patterns of infiltration, also type of infiltrated cells, fibrosis between hepatic cells or in portal area, affection to hepatic cords arrangement, hepatic

sinusoids, extensions of hemorrhage, pigment deposition, hyperplasia of bile duct, thickness of hepatic capsule and presence of liver fluke were the main grading levels. In grade, I the microscopic lesions were characterized by simple or mild in their nature with very good reversible prognosis, while grade II characterized by moderate severity of the lesions with a good reversible prognosis, while grade III characterized by hostile severity with bad irreversible prognosis as a result of architecture changes in liver histology. In conclusion, we believed that this grading system could be used as a guide when examining histopathological liver's samples infected with *F. hepatica* to identify the stage of infection and proposed an accurate prognosis. © 2019, University of Mosul - College of Veterinary Medicine. All rights reserved.

Munita, M.P., Rea, R., Martinez-Ibeas, A.M., Byrne, N., Kennedy, A., Sekiya, M., Mulcahy, G., Sayers, R.

Comparison of four commercially available ELISA kits for diagnosis of *Fasciola hepatica* in Irish cattle

(2019) BMC Veterinary Research, 15 (1), art. no. 414, .

Graham-Brown, J., Williams, D.J.L., Skuce, P., Zadoks, R.N., Dawes, S., Swales, H., Van Dijk, J.

Composite *Fasciola hepatica* faecal egg sedimentation test for cattle

(2019) Veterinary Record, 184 (19), p. 589.

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85061103792&doi=10.1136%2fvr.105128&partnerID=40&md5=9a358774777f7e584bf5b0c697477811>

**DOI:** 10.1136/vr.105128

**Abstract:** Options for diagnosing *Fasciola hepatica* infection in groups of cattle are limited. Increasing the opportunities for herd-level diagnosis is important for disease monitoring, making informed treatment decisions and for flukicide efficacy testing. The sensitivity of a simple sedimentation method based on composite faecal samples for the detection of fluke eggs in cattle was assessed through a combination of experimental and statistical modelling techniques. Initially, a composite sample method previously developed for sheep was used to investigate the sensitivity of composite sample testing compared with individual counts on the same samples in cattle. Following this, an optimised, validated, qualitative (presence-absence) composite sample field test was developed for cattle. Results showed that fluke egg counts obtained from a composite sample are representative of those expected from individual counts. The optimal sampling strategy was determined to be 10 individual 10 g samples (100 g composite sample) from which a 10 g subsample is taken for sedimentation. This method yielded a diagnostic sensitivity of 0.69 (95 per cent CI 0.5 to 0.85). These results demonstrate the validity and usefulness of a composite faecal egg sedimentation method for use in the diagnosis and control of *F.*

hepatica in groups of cattle, with the caveat that a negative test should be followed up with a second test due to limitations relating to test sensitivity. © British Veterinary Association 2019.

George, S.D., George, A.J., Rolfe, P.F., Emery, D.L.

Comparative assessment of faecal diagnostics for detection and predictive modelling of endemic *Fasciola hepatica* infection in sheep and cattle on Australian farms

(2019) *Veterinary Parasitology*: X, 1, art. no. 100001, .

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85060643318&doi=10.1016%2fj.vpoa.2018.100001&partnerID=40&md5=0e8770199fc6b1bfa1841f46b4355090>

**DOI:** 10.1016/j.vpoa.2018.100001

**Abstract:** The diagnosis, monitoring and flukicide efficacy testing of fasciolosis on-farm is reliant on non-terminal methods. The coproantigen ELISA (cELISA) has been recommended for diagnosis of fasciolosis and associated flukicide efficacy testing as an alternative to fluke egg counts for monitoring parasitism. Recently experimental multi-age infections have suggested that the reliability of efficacy results can be improved by a second cELISA testing at 6 weeks post-treatment (wpt) in addition to the generally accepted 1 wpt. A field study was conducted to determine the suitability of faecal fluke egg counts (FFEC) and cELISA as diagnostic, drug efficacy testing and epidemiological tools on Australian sheep and cattle farms. Faecal samples from sheep and/or cattle on three endemic farms were taken at monthly intervals for 12 months and examined by both methods. Normal farm management was maintained during the study period and opportunistic efficacy testing, in line with each farm's normal flukicide management was undertaken. Additionally, the suitability of the Ollerenshaw Index as a predictive model for fasciolosis under Australian conditions was examined. While both diagnostics demonstrated their value in the farm environment, the current data demonstrate a distinct and significant increase in diagnostic sensitivity for epidemiological studies by using the two tests in parallel. The agreement between the two diagnostics was found to be higher in cattle, despite the poor sensitivity of FFEC in this species. Similar levels of agreement between the two tests were demonstrated at both sheep properties, regardless of the marked difference in the intensity of *F. hepatica* challenge. Linear regression models demonstrated the results of the two diagnostics utilized in parallel were explained substantially ( $R^2 = 0.91$ ) as were series data ( $R^2 = 0.88$ ) when the respective models were fitted. In contrast, the fitted models for FFEC ( $R^2 = 0.54$ ) and cELISA ( $R^2 = 0.58$ ) were poor explanations for test outcomes. The outcomes of these models support previous findings that suggest that the two diagnostic tests are best utilized together, particularly in parallel. The application of the Ollerenshaw Index to Australian conditions requires further investigation. © 2018

Ghodsian, S., Rouhani, S., Fallahi, S., Seyyed-Tabaei, S.J., Taghipour, N.

Detection of spiked *Fasciola hepatica* eggs in stool specimens using LAMP technique

(2019) Iranian Journal of Parasitology, 14 (3), pp. 387-393.

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85073411706&doi=10.18502%2fijpa.v14i3.1477&partnerID=40&md5=6ee72daca0afee547fb7917f1bfe8a79>

**DOI:** 10.18502/ijpa.v14i3.1477

**Abstract:** Background: Fascioliasis is one of the most important food-borne worm disease caused by *Fasciola* sp. Parasitological diagnosis is more difficult due to the low para-site burden and a few eggs shedding of helminths. Therefore, it will be valuable to development of simple, fast and reliable diagnostic tests for detection of human and animal fascioliasis. Methods: Infected liver collected from abattoir in Tehran, Iran in 2017. *F. hepatica* eggs were detached from the uterus of worms under a stereo microscope. Various numbers of eggs were spiked to 200 mgr. of negative feces samples. DNA was ex-tracted and then target regions (nuclear IGS) were amplified by LAMP assay using six primers. Fecal specimens without egg and DNA of other helminths were used as negative controls. *F. hepatica* sample which confirmed by morphologic criteria and PCR- RFLP was used as positive control. Results: LAMP products by using SYBR Green I could detect even a single egg in fecal samples which was visible by change of color from orange to green. There was no cross amplification by other helminths including; *Taenia saginata*, *Dicrosolium dendriticum* and *F. gigantica*. Conclusion: LAMP seems a rapid, sensitive, cost-effective technique for detection of human fascioliasis. © 2019, Tehran University of Medical Sciences (TUMS). All rights reserved.

Tran, D.H., Phung, H.T.T.

Detecting *Fasciola hepatica* and *Fasciola gigantica* microRNAs with loop-mediated isothermal amplification (LAMP)

(2019) Journal of Parasitic Diseases, .

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85074005717&doi=10.1007%2fs12639-019-01164-w&partnerID=40&md5=5022e2692bad7280e11214c55e7e1124>

**DOI:** 10.1007/s12639-019-01164-w

**Abstract:** Fascioliasis is a parasitic infection typically caused by two common parasites of class Trematodo, genus *Fasciola*, namely *Fasciola hepatica* and *Fasciola gigantica*. The widespread of these species in water and food makes fascioliasis become a global zoonotic disease that affects 2.4 million people in more than 75 countries worldwide. Typically, *F. hepatica* and *F. gigantica* can be recognized by parasitological techniques to detect *Fasciola* spp. eggs, immunological techniques to detect worm-specific antibodies, or by molecular techniques such as PCR to detect parasitic genomic DNA. Recently, miRNAs have been raised as a key regulator and potential diagnostic biomarkers of



diseases, including parasitic infection. An isothermal PCR called loop-mediated isothermal amplification (LAMP) is rapid, sensitive, and its amplification process is so extensive that making LAMP well-suited for field diagnostics. LAMP reactions for miRNA detection have been introduced and were able to detect the target miRNA amounts in the wide range of 1.0 amol to 1.0 pmol, exhibiting high selectivity to differentiate one-base between miRNA sequences. Here, we introduced a modified LAMP to detect a species-specific miRNA of *F. hepatica* and *F. gigantica*. Our method did not demand an initial heating step and the reactions had a high sensitivity that greater than 1000 times in comparison to that reported in previous studies. Most importantly, the technique could perform well with parasitic miRNA presenting in bovine serum samples without sophisticated equipment required. These results create a promising technique basis for some novel and simple device to diagnose fascioliasis and other parasitic infection diseases at point-of-care. © 2019, Indian Society for Parasitology.

Raineri, M., Lima, E., Jr., Larroza, M., Moreno, M.S., Mansilla, M.V., Pappalardo, J.S., Zysler, R.D.

Interaction between natural magnetite sub-micrometric particles and the *Fasciola hepatica* egg: The role of the exposed surface area

(2019) *Experimental Parasitology*, 199, pp. 59-66.

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85062478057&doi=10.1016%2fj.exppara.2019.02.006&partnerID=40&md5=d1c960304d56797667e4973046f806d0>

**DOI:** 10.1016/j.exppara.2019.02.006

**Abstract:** Fasciolosis is a zoonotic world widely distributed disease caused by the liver fluke *Fasciola hepatica*, which affects animals and occasionally humans. On the other hand, natural iron oxide particles like magnetite are commonly found in soils where they participate in a wide range of environmental processes like organic matter decomposition, the adsorption of ions and molecules, and chemical reactions that involve the participation of soil living microorganisms. Since *Fasciola* eggs become soil components after being released with the infected animal faeces, this study focused on the characterization of the natural interaction between natural sub-micrometric magnetite particles and *F. hepatica* eggs. Our results indicate that particle binding to the *F. hepatica* egg depends on the particle size and it is also related to the exposed surface area since any condition that favors particle agglomeration leads to the reduction of the particle-eggshell binding intensity. Interestingly, this binding was avoided when proteins or phosphate were incorporated to the incubation solution, but not after formaldehyde fixation of eggs. Finally, when eggs were exposed to an external magnet after being incubated with magnetite particles, they were attracted to it without particles being detached, indicating a strong type of bonding between them. Therefore, the results presented here give new insights in order to improve the possibility of harvesting *F. hepatica* eggs by using magnetic materials. © 2019 Elsevier Inc.

Pacheco, I.L., Abril, N., Zafra, R., Morales-Prieto, N., Hernández, V.M., Ruiz, M.T., Perez-Caballero, R., Martínez-Moreno, A., Pérez, J.

Identification of reference genes for real-time PCR cytokine gene expression studies in sheep experimentally infected with *Fasciola hepatica*

(2019) Scientific Reports, 9 (1), art. no. 1485.

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85061146505&doi=10.1038%2fs41598-018-37672-7&partnerID=40&md5=9eda92576e97d4a05edadc73eaf6f07a>

**DOI:** 10.1038/s41598-018-37672-7

**Abstract:** The aim of this study was to validate reference genes for gene normalisation using qRT-PCR in hepatic lymph nodes (HLN) and livers from sheep infected with *Fasciola hepatica* during early and late stages of infection. To this end, a comprehensive statistical approach (RefFinder) encompassing four different methods of analysis (geNorm, BestKeeper,  $\Delta$ Ct method and NormFinder) was used to validate ten candidate reference genes. Stability analysis of gene expression followed by pairwise variation ( $V_n/V_{n+1}$ ) analysis revealed that PGK1, HSP90AA1 and GYPC were the most stable reference genes and suitable for qRT-PCR normalisation in both HLN and liver tissues. These three genes were validated against FoxP3, IL-10, TGF- $\beta$ , TNF- $\alpha$  and IL-1 $\beta$  genes in the HLN tissue of sheep vaccinated with Cathepsin L1 from *F. hepatica* and unvaccinated infected and uninfected controls during early stages of infection. In the liver, the three reference genes were validated against TNF- $\alpha$  and IL-1 $\beta$  during chronic stages of infection with *F. hepatica* and in uninfected controls. Our study is the first to evaluate and validate sheep reference genes in order to provide tools for monitoring cytokines in *Fasciola hepatica* infected sheep target organs. Our results present an approach to elucidate the role of different cytokines in *F. hepatica* vaccinated and infected sheep. © 2019, The Author(s).

Xifeng, W., Mengfan, Q., Kai, Z., Guowu, Z., Jing, L., Lixia, W., Jun, Q., Qingling, M., Shasha, G., Yunfu, H., Xuepeng, C.

Development and evaluation of a colloidal gold immunochromatographic assay based on recombinant protein CatL1D for serodiagnosis of sheep fasciolosis

(2019) Journal of helminthology, 94, p. e98.

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85074400096&doi=10.1017%2fS0022149X19000919&partnerID=40&md5=b94c825fbc12519a7f60123e94f03fb7>

**DOI:** 10.1017/S0022149X19000919

**Abstract:** Fasciolosis is a zoonotic parasitic disease that seriously endangers the development of animal husbandry and human health. In order to develop a rapid serological diagnostic method for fasciolosis in ruminants, the CatL1D and CatB4 genes of *Fasciola hepatica* were amplified by reverse transcription polymerase chain reaction

(PCR) and cloned, respectively, and then the CatL-B fusion gene (MeCatL-B) was constructed by gene splicing by overlap extension PCR technique. The recombinant rCatL1D, rCatB4 and rMeCatL-B proteins were then prepared by prokaryotic expression, respectively, and the recombinant protein with high specificity and sensitivity was screened via indirect enzyme-linked immunosorbent assay. Using the selected recombinant protein rCatL1D as a diagnostic antigen, we developed a colloidal gold immunochromatographic assay (CGIA) for detecting *F. hepatica*-specific antibodies, and 426 serum samples of slaughtered sheep were used to evaluate the sensitivity and specificity of *F. hepatica* CGIA assay. The results showed that the sensitivity and specificity of rCatL1D protein (100%, 96.67%) were higher than those of rCatB4 (94.29%, 80%) and rMeCatL-B (91.43%, 90%). Compared with the gold standard post-mortem inspection, the specificity and sensitivity of the CGIA method was 100% and 97%, respectively, and the consistency rate between these two methods was 99.3%. These results confirmed that the CGIA method based on rCatL1D protein could be a promising approach for rapid diagnosis of sheep fasciolosis because of its high sensitivity and specificity.

Byrne, A.W., McBride, S., Graham, J., Lahuerta-Marin, A., McNair, J., Skuce, R.A., McDowell, S.W.

Liver fluke (*Fasciola hepatica*) co-infection with bovine tuberculosis (bTB) in cattle: A retrospective animal-level assessment of bTB risk in dairy and beef cattle

(2019) *Transboundary and Emerging Diseases*, 66 (2), pp. 785-796.

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85059018865&doi=10.1111/tbed.13083&partnerID=40&md5=7217caeb6314284b6929380c503ab061>

**DOI:** 10.1111/tbed.13083

**Abstract:** Bovine tuberculosis (bTB), caused by *Mycobacterium bovis*, remains a persistent problem for cattle industries in endemic countries. The frequency, quality, and performance of tests, and the presence of wildlife reservoirs, have been identified as impediments to eradication. Recently, exposure to helminth infection (*Fasciola hepatica*) has been associated negatively with the disclosure of bTB. Here, for the first time, we assess impact of concurrent infections of *Fasciola hepatica* and the disclosure of bTB at the animal-level using large surveillance datasets. We utilized a dataset of 138,566 animal records from an abattoir from Northern Ireland (2011–2013). The presence of *F. hepatica* infection was assessed from macroscopic tissue inspection at abattoir. Multivariable models were developed to assess co-infection associations with bTB status based on: Single Intradermal Comparative Tuberculin Test (SICTT), lesion, bacteriological confirmation, including either all animals, or only skin-test negative animals (lesions at routine slaughter; LRS; confirmed nonreactors at routine slaughter; cNRs) or positive (reactors) animals alone, respectively. The relationship between skin tuberculin reaction sizes and fluke status was also explored for a subset of animals with field recordings (n = 24,680). Controlling for known risk factors (e.g., climatic, herd, and individual level

characteristics), we did not find significant associations between the SICTT (standard or severe interpretation), lesion, nor confirmation status of animals and their liver fluke status. The only exception was a negative association between liver fluke positivity, and LRS or cNRs, respectively; though effect-sizes were small (e.g., LRS Odds-Ratio: 0.87; 95% CI: 0.76–1.00). There was limited evidence of a relationship between tuberculin reaction sizes during SICTT testing and liver fluke infection status. These data do not support the contention that the detection of bTB using skin-tests or reactor postmortem follow-up may be compromised by co-infection at a population level, but the relationship with lesion formation (pathogenesis) may indicate an impact for postmortem surveillance. © 2018 Crown copyright. Transboundary and Emerging Diseases © 2018 2018 Blackwell Verlag GmbH

Byrne, A.W., Graham, J., McConville, J., Milne, G., Guelbenzu-Gonzalo, M., McDowell, S.

Liver fluke (*Fasciola hepatica*) co-infection with bovine tuberculosis in cattle: A prospective herd-level assessment of herd bTB risk in dairy enterprises

(2019) Transboundary and Emerging Diseases, 66 (4), pp. 1727-1736.

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85065737384&doi=10.1111/tbed.13209&partnerID=40&md5=c98ade1a7a17609ccb10017ec2f92ef5>

**DOI:** 10.1111/tbed.13209

**Abstract:** Co-infection of tuberculosis (TB) and helminths is recognized as a significant problem in regions where such pathogens are endemic and chronic cases exist. Co-infection can modulate the immune system leading to interference with diagnostic tests, increased pathological impacts and pathogen persistence. However, research has found that such interactions between pathogens can be context and species specific. Recent studies have suggested that liver fluke, *Fasciola hepatica*, infection may impact on immunological responses and diagnostics for bovine tuberculosis (bTB; caused by *Mycobacterium bovis*) in cattle. Where evidence of such interaction exists, there would be an onus on policy makers to adjust eradication programs to minimize impacts. We assessed the association between herd-level bTB breakdown risk and seasonal variation in liver fluke exposure based on 5,753 bulk tank milk (BTM) samples from 1,494 dairy herds across Northern Ireland. BTM was tested by an IDEXX antibody specific enzyme-linked immunosorbent assay (ELISA) using the 'f2' antigen as a detection agent. The ELISA determined the result based on a sample to (known) positive ratio (S/P%) from which binary status and categories of exposure were derived. Associations were tested using multivariable random effects models. Models predicting bTB risk were not improved with the inclusion of liver fluke exposure levels. Variations in modelling liver fluke exposure (S/P%, binary, categories of exposure) and bTB risk (skin test breakdowns, post-mortem confirmed breakdowns, breakdown size and lag effects) also failed to support associations (neither positive nor negative) between the pathogens at herd-level. These results, along with previously published animal-level data from Northern Ireland, suggest that the nexus

between bTB and *F. hepatica* may have small size effects at the population-level. However, our results also highlight the high prevalence of *F. hepatica* in cattle in our study population, and therefore we cannot fully discount the potential hypothesis of population-level depression of immune response to *M. bovis* due to co-infection. © 2019 Blackwell Verlag GmbH

Munita, M.P., Rea, R., Martinez-Ibeas, A.M., Byrne, N., McGrath, G., Munita-Corbalan, L.E., Sekiya, M., Mulcahy, G., Sayers, R.G.

Liver fluke in Irish sheep: Prevalence and associations with management practices and co-infection with rumen fluke

(2019) *Parasites and Vectors*, 12 (1), art. no. 525, .

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85074622358&doi=10.1186%2fs13071-019-3779-y&partnerID=40&md5=d74f2ab2fb2081d96ab338742bf59572>

**DOI:** 10.1186/s13071-019-3779-y

**Abstract:** Background: The present study aimed to identify the national prevalence of *Fasciola hepatica* in Irish sheep and to conduct a risk analysis assessment based on management and treatment practices in participating flocks. Also, co-infection with rumen fluke was quantified and its association with liver fluke and management practices was assessed. Methods: A total of 305 sheep flocks were selected ensuring even national representation of the sheep population. Participating farms were asked to complete a survey questionnaire on farm management practices and submit faecal samples during the winter of 2014-2015. Pooled faecal samples were analysed for the presence of *F. hepatica* and co-infection with rumen fluke. Apparent and true prevalence were calculated, additionally, the rate of co-infection with rumen fluke was also obtained. Correlation and regression analyses were used for assessing associations between management practices, liver fluke infection and co-infection with rumen fluke. Results: The national true prevalence of *F. hepatica* was 50.4% (n = 305). Regional prevalence varied from 41% in the east to 52% in the south. Co-infection with rumen fluke was observed in 40% of the studied population and correlated with increased *F. hepatica* egg counts (OR = 2.9; P ≤ 0.001). Predominant breeds were Suffolk, Texel and Horned Mountain breeds. Beef cattle were the most frequent type of other livestock present on farms and mixed species grazing was frequently reported (73%). More than half of the flocks reported a mid-to-late lambing period (March-April). Use of mountain land for grazing was of 32%. Flukicides were most commonly used twice over the autumn-winter period. Regression analyses highlighted significant association of *F. hepatica* status, with the presence of other livestock on farm, frequency of flukicides used during the winter and clinical presentation of liver fluke. A significant increase in eggs per gram of faeces was observed in Charollais sheep in comparison with all other breeds. Co-infection with *F. hepatica* and *Calicophoron daubneyi* was also significantly associated with the presence of other livestock on the farm, type of flukicide used and clinical fasciolosis. Conclusions: The present study provides up-to-date information on the prevalence of *F. hepatica* in Irish sheep and adds insight to the

epidemiology of the disease. These findings will be useful for designing new holistic control measures for *F. hepatica* infection. © 2019 The Author(s).

Takeuchi-Storm, N., Moakes, S., Thüer, S., Grovermann, C., Verwer, C., Verkaik, J., Knubben-Schweizer, G., Höglund, J., Petkevičius, S., Thamsborg, S., Werne, S.

Parasite control in organic cattle farming: Management and farmers' perspectives from six European countries

(2019) *Veterinary Parasitology: Regional Studies and Reports*, 18, art. no. 100329, .

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85071875206&doi=10.1016%2fj.vprsr.2019.100329&partnerID=40&md5=84a30612d10ecde82a883f789a6f2dd2>

**DOI:** 10.1016/j.vprsr.2019.100329

**Abstract:** Organic ruminant production is expanding in the EU, but parasite management remains a constant challenge. Mandatory outdoor access for all age groups can increase exposure to pasture borne parasites, whilst restrictions in the prophylactic use of anthelmintics can limit parasite control. The scientific community has been working to deliver effective parasite control strategies and alternative approaches in order to slow down the development of anthelmintic resistance (AR). However, the current parasite control practices and overall awareness with regards to AR and alternative approaches on farms are largely unknown and may be causing a knowledge gap between the scientific and farming communities. Therefore, a structured survey was conducted in six European countries (Switzerland, Germany, Denmark, Netherlands, Lithuania, Sweden) to provide basic data on practices, management and farmers' perspectives for grazing and parasite control (gastrointestinal worms and liver flukes) on organic cattle farms. Overall, 375 surveys were collected (282 dairy and 93 beef farms) in 2015–2016, and analysed descriptively. Additionally, surveys from the 228 dairy farms were assessed using a double-hurdle adoption model to identify the factors involved in the decision to drench against gastrointestinal parasites. Generally, there are prominent differences between countries, with monitoring methods differing especially, which has important implications in terms of knowledge transfer. For example, media warning was the most common method in DE, while antibody testing in bulk tank milk was the common method in NL. In other countries, clinical signs (diarrhoea, hair coat quality, and reduced weight or yield) and liver condemnation data were used frequently. In general, organic farmers from the six participating countries indicated that they would accept alternative approaches despite greater cost and labour. The likelihood of drenching were higher on farms with smaller farm areas, higher number of young stock and total livestock units and farms where faecal egg counts were used to monitor the parasites. In conclusion, it was evident that grazing and parasite management varied between the countries even though they operate under the same basic principles. Parasite management strategies must therefore be country specific and disseminated with appropriate methods. © 2019 Elsevier B.V.

da Costa, R.A., Corbellini, L.G., Castro-Janer, E., Riet-Correa, F.

Evaluation of losses in carcasses of cattle naturally infected with *Fasciola hepatica*: effects on weight by age range and on carcass quality parameters

(2019) *International Journal for Parasitology*, 49 (11), pp. 867-872.

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85072596440&doi=10.1016%2fj.ijpara.2019.06.005&partnerID=40&md5=dd1da4aa26930972015b6a23fc67ea41>

**DOI:** 10.1016/j.ijpara.2019.06.005

**Abstract:** Although fasciolosis is a relatively common disease, the productive and economic losses resulting from cattle with chronic fasciolosis are unclear. This paper aims to investigate the effect of fasciolosis on the parameters of carcass quality and discuss the hypothesis that the effects on weight differ among age ranges of cattle. For this, we analysed abattoir data of 30,151 bovines, from 928 farms, slaughtered in Uruguay in 2016, of which 33.9% (95% confidence interval (CI): 27.3–41.1%) had *Fasciola hepatica* (liver fluke). A mixed model was built to assess whether the effect of fasciolosis on weight differs depending on the age range, using the interaction term 'age\*F. hepatica'. The effect on the carcass parameters was tested using a proportional logistic regression. The interaction of age and F. hepatica was statistically significant ( $P < 0.001$ ). Differences in carcass weights between infected and non-infected animals were observed mostly at younger ages (up to 30 months), with the highest difference observed in the 23–30 months age range (estimated marginal mean difference of 6.34 kg). Overall, the presence of F. hepatica was positively associated with poor conformations and lower fat scores of carcasses ( $P < 0.001$ ). The carcasses of cattle infected with F. hepatica had 0.16 times greater odds of having worse conformation scores than carcasses of cattle without F. hepatica (proportional odds ratio (POR) = 1.16; 95% CI: 1.07–1.26). Similarly, carcasses of cattle with F. hepatica had 0.30 times (POR = 1.30, 95% CI: 1.23–1.39) greater odds of having poorer fat scores than carcasses of cattle without F. hepatica. Therefore, infection with F. hepatica is associated with poorer carcass quality parameters and lower weights, and the effect on weight differs across age ranges. © 2019

May, K., Brügemann, K., König, S., Strube, C.

Patent infections with *Fasciola hepatica* and paramphistomes (*Calicophoron daubneyi*) in dairy cows and association of fasciolosis with individual milk production and fertility parameters

(2019) *Veterinary Parasitology*, 267, pp. 32-41.

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85061559207&doi=10.1016%2fj.vetpar.2019.01.012&partnerID=40&md5=a2fa4c2d7c09a72ce9175fe4313576f7>

**DOI:** 10.1016/j.vetpar.2019.01.012

**Abstract:** Infections with the liver fluke *Fasciola hepatica* may result in considerable economic losses in the dairy livestock industry worldwide. Infections have been associated with detrimental impacts on milk production and milk quality as well as reduced fertility. However, most related data rely on examinations on herd level and the rather few studies on individual cow level are based solely on antibodies as measure for *F. hepatica* infections. This entails the risk of including false-positives as anti-*F. hepatica* antibodies persist for months even if the infection is cleared. Therefore, the presented study aimed to overcome this issue by assessing the association between *F. hepatica* infections measured via faecal egg counts (FEC) and milk production as well as fertility parameters in individual dairy cows. In total, 2006 faecal samples from 1166 Black and White dairy cows from 17 small and medium-sized German grassland farms were examined in July and September 2015. The relationship between patent *F. hepatica* infections and the milk production parameters milk yield, milk protein content, milk fat content and somatic cell score (SCS) was assessed in a linear mixed model using test-day records of individual cows. Patent *F. hepatica* infections were found on 35.3% (7/17) of farms with an individual cow prevalence of 10.1% (97/963) in July and 9.1% (95/1036) in September. Patent rumen fluke infections were detected on 17.6% (3/17) farms with an individual cow prevalence of 0.4% (4/963) in July and 0.7% (9/1036) in September. No significant association was found between *F. hepatica* infection status and either SCS as an indicator of udder health or milk production parameters, despite 0.06 and 0.10% lower values for milk protein and fat content in patently infected cows. Linear mixed models and generalized linear mixed models were established to estimate the impact of fasciolosis on the fertility parameters calving to first service (CTFS), calving interval (CI), success in first insemination (SFI) and 56-day nonreturn rate (NRR56). A significantly higher average CTFS of 4.69 days was detected in *F. hepatica* infected cows ( $P = 0.025$ ), but no significant relationship was found for the other fertility parameters. © 2019 Elsevier B.V.

Twomey, A.J., Berry, D.P., Evans, R.D., Doherty, M.L., Graham, D.A., Purfield, D.C.

Genome-wide association study of endo-parasite phenotypes using imputed whole-genome sequence data in dairy and beef cattle

(2019) *Genetics Selection Evolution*, 51 (1), art. no. 15, .

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85064492714&doi=10.1186%2fs12711-019-0457-7&partnerID=40&md5=2d023ebdd5018c815336e7c82cf576e9>

**DOI:** 10.1186/s12711-019-0457-7

**Abstract:** Background: Quantitative genetic studies suggest the existence of variation at the genome level that affects the ability of cattle to resist to parasitic diseases. The objective of the current study was to identify regions of the bovine genome that are associated with resistance to endo-parasites. Methods: Individual cattle records were available for *Fasciola hepatica*-damaged liver from 18 abattoirs. Deregressed estimated



breeding values (EBV) for *F. hepatica*-damaged liver were generated for genotyped animals with a record for *F. hepatica*-damaged liver and for genotyped sires with a least one progeny record for *F. hepatica*-damaged liver; 3702 animals were available. In addition, individual cow records for antibody response to *F. hepatica* on 6388 genotyped dairy cows, antibody response to *Ostertagia ostertagi* on 8334 genotyped dairy cows and antibody response to *Neospora caninum* on 4597 genotyped dairy cows were adjusted for non-genetic effects. Genotypes were imputed to whole-sequence; after edits, 14,190,141 single nucleotide polymorphisms (SNPs) and 16,603,644 SNPs were available for cattle with deregressed EBV for *F. hepatica*-damaged liver and cows with an antibody response to a parasitic disease, respectively. Association analyses were undertaken using linear regression on one SNP at a time, in which a genomic relationship matrix accounted for the relationships between animals. Results: Genomic regions for *F. hepatica*-damaged liver were located on *Bos taurus* autosomes (BTA) 1, 8, 11, 16, 17 and 18; each region included at least one SNP with a p value lower than  $10^{-6}$ . Five SNPs were identified as significant (q value  $\leq 0.05$ ) for antibody response to *N. caninum* and were located on BTA21 or 25. For antibody response to *F. hepatica* and *O. ostertagi*, six and nine quantitative trait loci (QTL) regions that included at least one SNP with a p value lower than  $10^{-6}$  were identified, respectively. Gene set enrichment analysis revealed a significant association between functional annotations related to the olfactory system and QTL that were suggestively associated with endo-parasite phenotypes. Conclusions: A number of novel genomic regions were suggestively associated with endo-parasite phenotypes across the bovine genome and two genomic regions on BTA21 and 25 were associated with antibody response to *N. caninum*. © 2019 The Author(s).

May, K., Weimann, C., Scheper, C., Strube, C., König, S.

Allele substitution and dominance effects of CD166/ALCAM gene polymorphisms for endoparasite resistance and test-day traits in a small cattle population using logistic regression analyses

(2019) Mammalian Genome, 30 (9-10), pp. 301-317.

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85074646081&doi=10.1007%2fs00335-019-09818-z&partnerID=40&md5=76af32fe95c3cc6108a4846cadd341de>

DOI: 10.1007/s00335-019-09818-z

**Abstract:** The study investigated the effects of four single-nucleotide polymorphisms (SNPs) in the activated leukocyte cell adhesion molecule (ALCAM) gene on liver fluke (*Fasciola hepatica*) infections (FH-INF), gastrointestinal nematode infections (GIN-INF) and disease indicator traits [e.g. somatic cell score (SCS), fat-to-protein ratio (FPR)] in German dual-purpose cattle (DSN). A genome-wide association study inferred the chip SNP ALCAMc.73+32791A>G as a candidate for *F. hepatica* resistance in DSN. Because of the crucial function of ALCAM in immune responses, SNPs in the gene might influence further resistance and performance traits. Causal mutations were identified in exon 9 (ALCAMc.1017T>C) and intron 9 (ALCAMc.1104+10T>A, ALCAMc.1104+85T>C) in a

selective subset of 94 DSN cows. We applied logistic regression analyses for the association between SNP genotypes with residuals for endoparasite traits (rINF-FH, rGIN-INF) and estimated breeding values (EBVs) for test-day traits. The probability of the heterozygous genotype was estimated in dependency of the target trait. Allele substitution effects for rFH-INF were significant for all four loci. The T allele of the SNPs ALCAMc.1017T>C and ALCAMc.1104+85T>C was the favourable allele when improving resistance against FH-INF. Significant allele substitution for rGIN-INF was only found for the chip SNP ALCAMc.73+32791A>G. We identified significant associations between the SNPs with EBVs for milk fat%, protein% and FPR. Dominance effects for the EBVs of test-day traits ranged from 0.00 to 0.47 SD and were in the direction of improved resistance for rFH-INF. We estimated favourable dominance effects from same genotypes for rFH-INF and FPR, but dominance effects were antagonistic between rFH-INF and SCS. © 2019, Springer Science+Business Media, LLC, part of Springer Nature.

John, B.C., Davies, D.R., Williams, D.J.L., Hodgkinson, J.E.

A review of our current understanding of parasite survival in silage and stored forages, with a focus on *Fasciola hepatica* metacercariae

(2019) Grass and Forage Science, 74 (2), pp. 211-217.

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85065475444&doi=10.1111%2fgfs.12429&partnerID=40&md5=389cb2fe0347d0d69e29b87b0975b241>

**DOI:** 10.1111/gfs.12429

**Abstract:** *Fasciola hepatica*, the parasitic liver fluke, is a re-emerging zoonotic infection and an important cause of morbidity and mortality in ruminant livestock worldwide. A significant animal welfare concern, fasciolosis also has a detrimental impact on food security, with the effects of sub-clinical infection on growth rate and milk yield estimated to cost the UK cattle industry £40.4 million annually. It is understood that up to 50% of infective *F. hepatica* metacercariae may overwinter on pasture and remain viable to infect grazing livestock the following spring. However, the infection risk posed by feeding grass silage to livestock remains largely unknown as the majority of previous studies are outdated in both experimental design and analysis of ensiled metacercariae viability. Anecdotal reports of fasciolosis outbreaks in housed livestock have reignited interest in *F. hepatica* metacercariae survival during modern ensiling processes and more importantly if they retain viability within stored forages. Consequently, a comprehensive review of the available literature is required in order to identify knowledge gaps and highlight future research opportunities regarding the role of silage in the transmission of *F. hepatica*. © 2019 The Authors. Grass and Forage Science Published by John Wiley & Sons Ltd

Albery, G.F., Becker, D.J., Kenyon, F., Nussey, D.H., Pemberton, J.M.

The Fine-Scale Landscape of Immunity and Parasitism in a Wild Ungulate Population

(2019) Integrative and Comparative Biology, 59 (5), pp. 1165-1175.

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85075440754&doi=10.1093%2ficb%2ficz016&partnerID=40&md5=356db8379c968c10320d73b647558d44>

**DOI:** 10.1093/icb/icz016

**Abstract:** Spatial heterogeneity in susceptibility and exposure to parasites is a common source of confounding variation in disease ecology studies. However, it is not known whether spatial autocorrelation acts on immunity at small scales, within wild animal populations, and whether this predicts spatial patterns in infection. Here we used a well-mixed wild population of individually recognized red deer (*Cervus elaphus*) inhabiting a heterogeneous landscape to investigate fine-scale spatial patterns of immunity and parasitism. We noninvasively collected 842 fecal samples from 141 females with known ranging behavior over 2 years. We quantified total and helminth-specific mucosal antibodies and counted propagules of three gastrointestinal helminth taxa. These data were analyzed with linear mixed models using the Integrated Nested Laplace Approximation, using a Stochastic Partial Differentiation Equation approach to control for and quantify spatial autocorrelation. We also investigated whether spatial patterns of immunity and parasitism changed seasonally. We discovered substantial spatial heterogeneity in general and helminth-specific antibody levels and parasitism with two helminth taxa, all of which exhibited contrasting seasonal variation in their spatial patterns. Notably, *Fasciola hepatica* intensity appeared to be strongly influenced by the presence of wet grazing areas, and antibody hotspots did not correlate with distributions of any parasites. Our results suggest that spatial heterogeneity may be an important factor affecting immunity and parasitism in a wide range of study systems. We discuss these findings with regards to the design of sampling regimes and public health interventions, and suggest that disease ecology studies investigate spatial heterogeneity more regularly to enhance their results, even when examining small geographic areas. © 2019 The Author(s) 2019. Published by Oxford University Press on behalf of the Society for Integrative and Comparative Biology. All rights reserved.

French, A.S., Zadoks, R.N., Skuce, P.J., Mitchell, G., Gordon-Gibbs, D.K., Taggart, M.A.

Habitat and host factors associated with liver fluke (*Fasciola hepatica*) diagnoses in wild red deer (*Cervus elaphus*) in the Scottish Highlands

(2019) Parasites and Vectors, 12 (1), art. no. 535, .

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85074922787&doi=10.1186%2fs13071-019-3782-3&partnerID=40&md5=76e10a3a233730c6bf789b9c9d11b397>

**DOI:** 10.1186/s13071-019-3782-3

**Abstract:** Background: Red deer (*Cervus elaphus*) are a common wild definitive host for liver fluke (*Fasciola hepatica*) that have been the subject of limited diagnostic surveillance.

This study aimed to explore the extent to which coprological diagnoses for *F. hepatica* in red deer in the Scottish Highlands, Scotland, are associated with variability among hosts and habitats. Methods: Our analyses were based on coproantigen ELISA diagnoses derived from faecal samples that were collected from carcasses of culled deer on nine hunting estates during two sampling seasons. Sampling locations were used as centroids about which circular home ranges were quantified. Data were stratified by season, and associations between host, hydrological, land cover and meteorological variables and binary diagnoses during 2013-2014 (n = 390) were explored by mixed effect logistic regression. The ability of our model to predict diagnoses relative to that which would be expected by chance was quantified, and data collected during 2012-2013 (n = 289) were used to assess model transferability. Results: During 2013-2014, habitat and host characteristics explained 28% of variation in diagnoses, whereby half of the explained variation was attributed to differences among estates. The probability of a positive diagnosis was positively associated with the length of streams in the immediate surroundings of each sampling location, but no non-zero relationships were found for land cover or lifetime average weather variables. Regardless of habitat, the probability of a positive diagnosis remained greatest for males, although males were always sampled earlier in the year than females. A slight decrease in prediction efficacy occurred when our model was used to predict diagnoses for out-of-sample data. Conclusions: We are cautious to extrapolate our findings geographically, owing to a large proportion of variation attributable to overarching differences among estates. Nevertheless, the temporal transferability of our model is encouraging. While we did not identify any non-zero relationship between meteorological variables and probability of diagnosis, we attribute this (in part) to limitations of interpolated meteorological data. Further study into non-independent diagnoses within estates and differences among estates in terms of deer management, would improve our understanding of *F. hepatica* prevalence in wild deer. © 2019 The Author(s).

Mollinedo, S., Gutierrez, P., Azurduy, R., Valle, F., Salas, A., Mollinedo, Z., Soto, P., Villarroel, C.F., Ransom, J., Lawrence, R., Berman, J., Soto, J.

Mass drug administration of triclabendazole for fasciola hepatica in Bolivia

(2019) American Journal of Tropical Medicine and Hygiene, 100 (6), pp. 1494-1497.

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85067375206&doi=10.4269%2fajtmh.19-0060&partnerID=40&md5=e983dbec7ed83f9dd519ad18277645d6>

**DOI:** 10.4269/ajtmh.19-0060

**Abstract:** Human infection with *Fasciola hepatica* leads to obstruction of the common bile duct by adult worms and disease characterized by biliary colic, epigastric pain, and nausea. Recommended treatment is a single dose of triclabendazole (TCBZ) (10 mg/kg). Because in the 1990s the Bolivian Altiplano bordering Lake Titicaca was thought to have the highest prevalence of human fascioliasis worldwide, the Bolivian Ministry of Health instituted TCBZ mass drug administration (MDA). From 2008 to 2016 (excepting 2015),

one dose of 250 mg was administered, usually in September/ October, to each resident of highly endemic regions willing to participate. This is apparently the first reported use of MDA for Fasciola. The proportion of persons in key regions receiving TCBZ MDA was 87% in 2016. In 2017, we resurveyed key regions, and found that the MDA program had been dramatically successful. Whereas Fasciola prevalence was reported as 26.9% in Huacullani/Tiahuanaco and 12.6% in Batallas in 1999, there was 0.7% prevalence in Huacullani/Tiahuanaco and 1% in Batallas in 2017. However, lessons from schistosomiasis control efforts suggest that for sustained control of Fasciola infection, Fasciola MDA needs to be maintained and coupled with measures to control infection in the intermediary snail and in the animal hosts of *F. hepatica*. © 2019 by The American Society of Tropical Medicine and Hygiene.

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A high throughput deep amplicon sequencing method to show the emergence and spread of *Calicophoron daubneyi* rumen fluke infection in United Kingdom cattle herds

(2019) *Veterinary Parasitology*, 268, pp. 9-15.

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85062720687&doi=10.1016%2fj.vetpar.2019.02.007&partnerID=40&md5=bfc2a35d94fb9c6559d03c590e747c2e>

**DOI:** 10.1016/j.vetpar.2019.02.007

**Abstract:** The prevalence of *C. daubneyi* infection in the United Kingdom has increased, but despite the potential for rumen flukes to cause production loss in ruminant livestock, understanding of their emergence and spread is poor. Here we describe the development of a method to explore the multiplicity of *C. daubneyi* infection and patterns of the parasite's emergence and spread, based on Illumina MiSeq deep sequencing of meta barcoded amplicons of a fragment of the cytochrome c oxidase subunit I (mt-COX-1) locus. Our results show high levels of genetic diversity in 32 *C. daubneyi* populations derived from finished prime cattle consigned to slaughter from northern United Kingdom. The results are consistent with a single introduction of *C. daubneyi* infection to some of the farms where the cattle had been grazed during their lifetime and multiple introductions to most. The results illustrate the impact of high levels of animal movements in the United Kingdom, whereby multiple common mt-COX-1 haplotypes were identified in 26 populations in the absence of geographical clustering of clades. This has implications for the adaptability of environmental and intermediate host stages of the parasite to changing climatic and animal management conditions, or of parasitic stages to exposure to anthelmintic drugs; potentially allowing for greater pathogenicity, or the development of anthelmintic resistance, respectively. © 2019 Elsevier B.V.

## *Dicrocoelium dendriticum*

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Spatiotemporal patterns of infection for emerging larval liver fluke (*Dicrocoelium dendriticum*) in three species of land snail in Southern Alberta, Canada

(2019) *Journal of Parasitology*, 105 (1), pp. 155-161.

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85062278857&doi=10.1645%2f18-124&partnerID=40&md5=b543808e58fe56f917a361107b00415b>

**DOI:** 10.1645/18-124

**Abstract:** The control of emerging parasites requires a fundamental knowledge of where and when rates of transmission are high. Data on spatiotemporal patterns of infection are challenging to obtain, particularly for complex life cycle parasites that involve transmission into multiple obligate hosts. The lancet liver fluke, *Dicrocoelium dendriticum*, has a long history of colonization outside its native host and geographical range in continental Europe. Infection patterns involving adult and metacercarial stages have been characterized for this trematode in a region of emergence in western Canada within co-grazing herbivores and ants, but infection patterns in snail intermediate hosts in this region are unknown. We combined spatiotemporal prevalence surveys with sequence analyses of the cytochrome c oxidase subunit 1 (COI) barcoding gene from samples of sporocyst tissue in infected snails to confirm that *D. dendriticum* utilizes 3 sympatric species of Oreohelid land snail (*Oreohelix subrudis*, *Oreohelix* sp., and *Oreohelix cooperi*) as first intermediate host. Mean prevalence within a total sample of 900 adult snails collected over 1 field season from 6 sites was  $9.9 \pm 2.4\%$ . For each species of snail, prevalence ranged between 5-30% within monthly samples, with peaks in midsummer followed by declines in fall. Between-site variation in prevalence was low and nonsignificant, implying that rates of transmission of *D. dendriticum* miracidia from domestic stock and wildlife into snails are similar within localized sites, despite high variation in local habitat characteristics and in the structure of the definitive host community. © © American Society of Parasitologists 2019.