Animal &
Plant Health
Agency

APHA Parasitology Group

Annual Review of Literature and Horizon Scanning Report 2018

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The Animal and Plant Health Agency (APHA) is an executive agency of the Department for Environment, Food & Rural Affairs, and also works on behalf of the Scottish Government and Welsh Government.

Contents

Summary

Fasciola hepatica

Research into various vaccination candidates and also why research to date has not produced an effective commercialised vaccine continues. Various studies were also published into many new chemicals (some plant derived), adjuvants and routes of vaccination that appear to have potential.

A study to assist vaccine development showed that a genetic clonal expansion of *F hepatica* occurs within *G. truncatula*, highlighting the potential for amplification of drug resistant genotypes. Interestingly in one experiment, a single miracidium infecting a snail, produced over 3000 metacercariae during the snail's lifetime, showing the potent multiplication step in this intermediate host.

The effect of *F hepatica* on infected hosts' global immune response has continued to be studied. It is modulated toward a nonproliferative type 2 state following natural challenge with *F. hepatica*. This has implications in terms of the timing of the administration of vaccination programs and for host susceptibility to coinfecting pathogens. A paper was published which cautiously suggested a link between *F. hepatica* infection and the shedding of *E. coli* O157 in cattle.

Newer areas of research were also published. In Cuba (and elsewhere but not reported in the UK) the snail *Pseudosuccinea columella* acts as intermediate host of *F. hepatica*, Interestingly in Cuba there are populations of susceptible and naturally-resistant populations to *F. hepatica* within this snail species. Resistance in *P. columella* is characterized by the encapsulation of the parasite by host's immune cells. The effectiveness of *P. columella* resistance to *F. hepatica* does not decline with increasing parasite doses, successive infection or different geographical origins of parasite isolates, Understanding the peculiarities of the *P. columella-F. hepatica* interaction and the extent of the resistant phenotype is crucial for an effective parasite control and for developing alternatives to tackle fasciolosis transmission.

A study was published to determine the effectiveness of *Chaetogaster limnaei* (a freshwater worm) as a natural controller of *Fasciola hepatica* in laboratory conditions. It is naturally symbiotically associated with lymnaeid snails as *Galba truncatula* with this association considered a preventative mechanism against trematode miracidia infection. An effective 70% natural control of *Fasciola hepatica* in *Galba truncatula* was observed, *in vitro*.

Study in dairy herds in Denmark resulted in information pertinent for that country and noted that for diagnosis of infection the serum antibody ELISA was able to detect infection first, whereas both copro-antigen ELISA and faecal egg counts tended to increase in the same animals at a later point. Other diagnostic techmiques such as real time PCR and loop-mediated isothermal amplification (LAMP) have been shown to be able to detect *F hepatica* DNA from as early as one week post infection. Additional work in Poland using serum biochemistry to predict the risk of chronic liver fluke found that the most accurate model was based on glutamate dehydrogenase activity, globulin and urea.

A study in dairy herds and using bulk tank milk ELISA in Cuba, but with perhaps implications for UK, showed reductions in milk production of 18 and 32% were observed in herds with Optical Density Ratios (ODR) of 0.3-0.6 and > 0.6, respectively, when compared to herds with ODR < 0.3. Overall, the longer the milking cows were put out to pasture, the higher the levels of anti-parasite antibodies. Co-grazing with sheep and goats also significantly increased the risk of high ODR.

Work on environmental DNA (eDNA) assays designed to reveal *Galba truncatula*, *Fasciola hepatica* and *Calicophoron daubneyi* presence within water sources on pasture land was published. Further assay development will be required for a field test capable of identifying and quantifying *F. hepatica* and *C. daubneyi* infection risk areas, to support future control strategies. Mapping work both at a farm level and nationally was published from research done in Wales and Ireland.

A study was carried out on faecal samples collected weekly from 10 experimentally-infected Merino lambs. These were subjected to diagnosis via traditional sedimentation, coproELISA and real-time PCR. Samples were first considered positive at 6–8 WPI by coproELISA, real-time PCR and sedimentation, respectively. At 9 WPI 100% of samples were positive by all three methods.The PCR could not be adapted to detect infections sooner.

Rumen fluke (Calicophoron daubneyi)

Molecular studies on *C daubeneyi* have been published and work replicate excystment *in vivo* should aid further research into diagnosis and therapeutic options. There is evidence that at high levels of infection adult rumen fluke are capable of impacting upon rumen fermentation parameters using an *in vitro* gas production study resulting in a significant increase in propionate production. In addition work in Ireland has suggested that rumen fluke is now more common in cattle than liver fluke, doesn't show the same pattern of seasonality and in some areas may be replacing *F. hepatica*.

Parasitic gastro-enteritis (PGE)

Work is continuing into vaccination against the bovine intestinal parasite *Cooperia oncophora.*

The transcriptome of *H. contortus* populations surviving in sheep vaccinated with Barbervax® was compared with worms from control animals. Barbervax® antigens are native integral membrane proteins isolated from the brush border of the intestinal cells of the adult parasite and many of those are proteases. Our findings provide no evidence for changes in expression of genes encoding Barbervax® antigens in the surviving parasite populations. However, surviving parasites from vaccinated animals showed increased expression of other proteases and regulators of lysosome trafficking, and displayed up-regulated lipid storage and defecation abilities that may have circumvented the effect of the vaccine.

Work continues into finding new chemicals as anthelmintics and repurposing existing drugs, often using highly sophisticated screening methods, as well as investigating use of various plant extracts, essential oils and minerals.

Anthelmintic treatments to ewes around lambing time, often with long acting products, have become common practice on UK sheep farms, but these treatments have also been shown to be highly selective for anthelmintic resistance in New Zealand and Australia, with field data supported by modelling results. A study was published which set out to determine (1) the effect of treating or withholding anthelmintic treatments and (2) the effect of treatment of ewes with a persistent or non-persistent anthelmintic, on early infection in lambs in the UK. Faecal egg count data for 10–16 weeks old lambs collected over a three year period (2012–2014) was analysed. The authors conclude that this study supports data generated by other researchers suggesting that the practice of treating ewes at lambing to reduce contamination on pasture and minimise subsequent disease may not in fact always result in lower levels of infection in lambs. The study also demonstrated no significant benefit in early infection in lambs when ewes were treated with long acting compared to short acting anthelmintics. This provides further evidence to support the potential benefits of a more targeted approach to anthelmintic treatment on sheep farms.

A mathematical model to predict the risk arising from the pasture infectivity of four nematode species in Australia has been developed, incorporating weather and management variables and is available for public access (ASKBILL website).

The reduction in pasture infectivity likely to be achieved by the supplementation of grazing sheep with BioWorma®, a product containing the chlamydospores of the nematophagous fungus *Duddingtonia flagrans* strain IAH 1297 in various climatic areas of Australia was published. Given the results of the four trials they conclude that supplementation of pastured sheep with BioWorma was effective in reducing the numbers of parasitic nematode larvae ingested by tracer sheep.

The use of nematophagous fungi incorporated into feed in grazing cattle in Brazil was studied. The pasture that contained the animals that received feed with the fungus showed a reduction of 59% and 52% of larvae recovered at distances of 20 cm and 40 cm from the faecal pats, respectively. The mean number of eggs per gram of faeces each month and animal body weight did not differ ($p > 0.05$) between the treated and control groups.

A study was published which monitored the safety profile of the Barbervax® vaccine in a group of adolescent alpacas in the USA. An increase in rectal temperature for a duration of 2 days (range 2–4 days) was observed post-vaccination. Vaccinated alpacas were lethargic for 2-3 days following vaccination; however, they maintained an appetite and no visible or palpable injection site reactions were observed. Additional studies are required to evaluate the efficacy of the vaccine under field conditions in protecting alpacas against infection with *H. contortus.*

Various tests for diagnosis of Haemonchosis were evaluated. Test sensitivity was: McMaster counting by conventional microscopy < PNA < LAMP < qPCR. The authors conclude, *H. contortus* can be identified by McMaster counting, without major mistakes regarding false positive results. However, molecular methods provide the capacity to diagnose H. contortus eggs with increased accuracy. This is essential when animals are investigated in quarantine or in studies evaluating anthelmintic treatment efficacy. These methods could also be applied to fecal samples from wildlife to investigate nematode transmission between wildlife and livestock.

Anthelmintic resistance

Monepantel resistance was diagnosed on a sheep farm in the UK during routine monitoring of the effectiveness of a farm's roundworm control strategy. Weaned lambs had become ill thrifty and developed diarrhoea, despite the routine use of monepantel. This clinical presentation was caused by trichostrongylosis. The faecal egg count reduction was 76.7% (95% CI: 55.1–82.2%) following treatment with 2.5 mg/kg monepantel. Predominantly *Trichostrongylus vitrinus* along with small proportions of *Oesophagostomum venulosum* were identified by deep amplicon sequencing of pools of larvae recovered from pre and post monepantel treatment coprocultures and on post-mortem examinations. Examination of farm anthelmintic treatment and animal movement records suggested that treatments before movement onto silage aftermaths, putatively with low numbers of susceptible nematodes in refugia, may have placed a high selection pressure on monepantel resistance.

A paper was published on the *in vitro* effects of the newly launched (but not in UK) monepantel abamectin dual active, with evidence that survival of isolates resistant to one or other components could be reduced by the dual active product.

In New Zealand, a replicated field trial was conducted to measure the effect on live weight gain of failing to adequately control anthelmintic resistant populations of *Cooperia oncophora* and to determine whether populations, and hence production losses, increased with time. The data, supported the view that while *C. oncophora* is less pathogenic than other cattle parasite species it can still cause production losses when present in sufficient numbers.

A study looked at practices and factors associated with first lactation heifer exposure to *Ostertagia ostertagi* on pasture that was measured by milk antibody ELISA testing. Heifer individual milk ODR against *O. ostertagi* significantly increased with high stocking rate at first grazing and co-grazing with adult cows before calving. Conversely, heifer individual milk ODR against *O. ostertagi* significantly decreased when heifers had co-grazed with sheep and pasture grass had frequently been mowed.

Cestodes

A further study on the risk based meat inspection of cattle for *Taenia saginata* in UK was published.

Ectoparasites

First evidence of resistance to macrocyclic lactones in *Psoroptes ovis* sheep scab mites in the UK was published, where resistance to moxidectin was demonstrated in mites from commercial farms in an *in vitro* test comparing results from field samples to that obtained from a known susceptible (Moredun) strain.

A study into psoroptic mange in cattle was published. The repeated administration of both IVM formulations at day 0 and 7 accounted for a greater plasma drug availability compared with the single administration. IVM was well distributed from the plasma to the skin without significant differences between both IVM formulations. There was a positive correlation between IVM concentrations in skin and plasma.The repeated administration of IVM increased significantly the IVM concentrations in the skin of areas affected by mange. IVM failed to obtain a parasitological cure in the different groups affected by mange. The failure was observed with both formulations administered at single or repeated doses. Based on the number of animals cured, the range of efficacy was between 0% on day 7 and 60% on day 28 post-treatment. No significant differences in the P. ovis density scores were observed after the IVM treatment at single or repeated doses. They conclude that additional studies are needed to confirm the presence of resistant strains of P.ovis and to establish the appropriate measures to control these parasitic infestations in feedlot cattle.

Vectors and vector-borne diseases

Papers were published this year on the following

- Emerging zoonotic tick borne pathogens in Europe
- Deer as sentinels for tick borne disease surveillance
- Migratory birds as hosts for exotic ticks and vector borne diseases
- Significance of varying wildlife as hosts for zoonotic tick borne infections

ABSTRACTS

Fasciola hepatica

Beesley, N.J., Caminade, C., Charlier, J., Flynn, R.J., Hodgkinson, J.E., Martinez-Moreno, A., Martinez-Valladares, M., Perez, J., Rinaldi, L., Williams, D.J.L.

Fasciola and fasciolosis in ruminants in Europe: Identifying research needs

(2018) Transboundary and Emerging Diseases, 65, pp. 199-216.

Fasciola hepatica is a trematode parasite with a global distribution, which is responsible for considerable disease and production losses in a range of food producing species. It is also identified by WHO as a re-emerging neglected tropical disease associated with endemic and epidemic outbreaks of disease in human populations. In Europe, F. hepatica is mostly associated with disease in sheep, cattle and goats. This study reviews the most recent advances in our understanding of the transmission, diagnosis, epidemiology and the economic impact of fasciolosis. We also focus on the impact of the spread of resistance to anthelmintics used to control F. hepatica and consider how vaccines might be developed and applied in the context of the immune-modulation driven by the parasite. Several major research gaps are identified which, when addressed, will contribute to providing focussed and where possible, bespoke, advice for farmers on how to integrate stock management and diagnosis with vaccination and/or targeted treatment to more effectively control the parasite in the face of increasing the prevalence of infection and spread of anthelmintic resistance that are likely to be exacerbated by climate change.

Hodgkinson, J.E., Cwiklinski, K., Beesley, N., Hartley, C., Allen, K., Williams, D.J.L.

Clonal amplification of Fasciola hepatica in Galba truncatula: Within and between isolate variation of triclabendazole-susceptible and -resistant clones

(2018) *Parasites and Vectors*, 11 (1), art. no. 363.

DOI: 10.1186/s13071-018-2952-z

Background: Fasciola hepatica is of worldwide significance, impacting on the health, welfare and productivity of livestock and regarded by WHO as a re-emerging zoonosis. Triclabendazole (TCBZ), the drug of choice for controlling acute fasciolosis in livestock, is also the drug used to treat human infections. However TCBZ-resistance is now considered a major threat to the effective control of F. hepatica. It has yet to be demonstrated whether F. hepatica undergoes a genetic clonal expansion in the snail intermediate host, Galba truncatula, and to what extent amplification of genotypes within the snail facilitates accumulation of drug resistant parasites. Little is known about genotypic and phenotypic variation within and between F. hepatica isolates. Results: Six clonal isolates of F. hepatica (3× triclabendazole-resistant, TCBZ-R and 3× triclabendazole-susceptible, TCBZ-S) were generated. Snails infected with one miracidium started to shed cercariae 42-56 days post-infection and shed repeatedly up to a maximum of 11 times. A maximum of 884 cercariae were shed by one clonally-infected snail (FhLivS1) at a single time point, with > 3000 clonal metacercariae shed over its lifetime. Following experimental infection all 12 sheep were FEC positive at the time of TCBZ treatment. Sheep infected with one of three putative TCBZ-S clones and treated with TCBZ had no parasites in the liver at post-mortem, whilst sheep each infected with putative TCBZ-R isolates had 35-165 adult fluke at post-mortem, despite TCBZ treatment. All six untreated control animals had between 15-127 parasites. A single multi-locus genotype was reported for every fluke from each of the six clonal isolates. Adult F. hepatica showed considerable variation in weight, ranging from 20-280 mg, with variation in weight evident within and amongst clonal isolates. Conclusions: A genetic clonal expansion occurs within G. truncatula, highlighting the potential for amplification of drug resistant genotypes of F. hepatica. Variation in the weight of parasites within and between clonal isolates and when comparing isolates that are either susceptible or resistant to TCBZ represent inherent variation in liver fluke and cannot be attributed to their resistance or susceptibility traits.

Howell, A.K., Tongue, S.C., Currie, C., Evans, J., Williams, D.J.L., McNeilly, T.N.

Co-infection with Fasciola hepatica may increase the risk of Escherichia coli O157 shedding in British cattle destined for the food chain

(2018) *Preventive Veterinary Medicine*, 150, pp. 70-76.

DOI: 10.1016/j.prevetmed.2017.12.007

Escherichia coli O157 is a zoonotic bacterium that can cause haemorrhagic diarrhoea in humans and is of worldwide public health concern. Cattle are considered to be the main reservoir for human infection. Fasciola hepatica is a globally important parasite of ruminant livestock that is known to modulate its host's immune response and affect susceptibility to bacterial pathogens such as Salmonella Dublin. Shedding of E. coli O157 is triggered by unknown events, but the immune system is thought to play a part. We investigated the hypothesis that shedding of E. coli O157 is associated with F. hepatica infection in cattle. Three hundred and thirty four cattle destined for the food chain, from 14 British farms, were tested between January and October 2015. E. coli O157 was detected by immunomagnetic separation and bacterial load enumerated. F. hepatica infection status was assessed by copro-antigen ELISA. A significant association ($p = 0.01$) was found between the log percent positivity (PP) of the F. hepatica copro-antigen ELISA and E. coli O157 shedding when the fixed effects of day of sampling and the age of the youngest animal in the group, plus the random effect of farm were adjusted for. The results should be interpreted cautiously due to the lower than predicted level of fluke infection in the animals sampled. Nevertheless these results indicate that control of F. hepatica infection may have an impact on the shedding of E. coli O157 in cattle destined for the human food chain.

Cwiklinski, K., Dalton, J.P.

Advances in Fasciola hepatica research using ‘omics’ technologies

(2018) *International Journal for Parasitology*, 48 (5), pp. 321-331.

The liver fluke Fasciola hepatica is an economically important pathogen of livestock worldwide, as well as being an important neglected zoonosis. Parasite control is reliant on the use of drugs, particularly triclabendazole, which is effective against multiple parasite stages. However, the spread of parasites resistant to triclabendazole has intensified the pursuit for novel control strategies. Emerging ‘omics’ technologies are helping advance our understanding of liver fluke biology, specifically the molecules that act at the host-parasite interface and are central to infection, virulence and long-term survival within the definitive host. This review discusses the technological sequencing advances that have facilitated the unbiased analysis of liver fluke biology, resulting in an extensive range of ‘omics’ datasets. In addition, we highlight the ‘omics’ studies of host responses to F. hepatica infection that, when combined with the parasite datasets, provide the opportunity for integrated analyses of host-parasite interactions. These extensive datasets will form the foundation for future in-depth analysis of F. hepatica biology and development, and the search for new drug or vaccine interventions.

Cwiklinski, K., Jewhurst, H., McVeigh, P., Barbour, T., Maule, A.G., Tort, J., O'Neill, S.M., Robinson, M.W., Donnelly, S., Dalton, J.P.

Infection by the helminth parasite fasciola hepatica requires rapid regulation of metabolic, virulence, and invasive factors to adjust to its mammalian host

(2018) *Molecular and Cellular Proteomics*, 17 (4), pp. 792-809. Cited 2 times.

DOI: 10.1074/mcp.RA117.000445

The parasite Fasciola hepatica infects a broad range of mammals with impunity. Following ingestion of parasites (metacercariae) by the host, newly excysted juveniles (NEJ) emerge from their cysts, rapidly penetrate the duodenal wall and migrate to the liver. Successful infection takes just a few hours and involves negotiating hurdles presented by host macromolecules, tissues and micro-environments, as well as the immune system. Here, transcriptome and proteome analysis of ex vivo F. hepatica metacercariae and NEJ reveal the rapidity and multitude of metabolic and developmental alterations that take place in order for the parasite to establish infection. We found that metacercariae despite being encased in a cyst are metabolically active, and primed for infection. Following excystment, NEJ expend vital energy stores and rapidly adjust their metabolic pathways to cope with their new and increasingly anaerobic environment. Temperature increases induce neoblast proliferation and the remarkable up-regulation of genes associated with growth and development. Cysteine proteases synthesized by gastrodermal cells are secreted to facilitate invasion and tissue degradation, and tegumental transporters, such as aquaporins, are varied to deal with osmotic/salinity changes. Major proteins of the total NEJ secretome include proteases, protease inhibitors and anti-oxidants, and an array of immunomodulators that likely disarm host innate immune effector cells. Thus, the challenges of infection by F. hepatica parasites are met by rapid metabolic and physiological adjustments that expedite tissue invasion and immune evasion; these changes facilitate parasite growth, development and maturation. Our molecular analysis of the critical processes involved in host invasion has identified key targets for future drug and vaccine strategies directed at preventing parasite infection.

Graham-Brown, J., Hartley, C., Clough, H., Kadioglu, A., Baylis, M., Williams, D.J.L.

Dairy heifers naturally exposed to Fasciola hepatica develop a type 2 immune response and concomitant suppression of leukocyte proliferation

(2018) *Infection and Immunity*, 86 (1), art. no. e00607-17

DOI: 10.1128/IAI.00607-17

Fasciola hepatica is a parasitic trematode of global importance in livestock. Control strategies reliant on anthelmintics are unsustainable due to the emergence of drug resistance. Vaccines are under development, but efficacies are variable. Evidence from experimental infection suggests that vaccine efficacy may be affected by parasite-induced immunomodulation. Little is known about the immune response to F. hepatica following natural exposure. Hence, we analyzed the immune responses over time in calves naturally exposed to F. hepatica infection. Cohorts of replacement dairy heifer calves (n = 42) with no prior exposure to F. hepatica, on three commercial dairy farms, were sampled over the course of a grazing season. Exposure was determined through an F. hepatica-specific serum antibody enzymelinked immunosorbent assay (ELISA) and fluke egg counts. Concurrent changes in peripheral blood leukocyte subpopulations, lymphocyte proliferation, and cytokine responses were measured. Relationships between fluke infection and immune responses were analyzed by using multivariable linear mixed-effect models. All calves from one farm showed evidence of exposure, while cohorts from the remaining two farms remained negative over the grazing season. A type 2 immune response was associated with exposure, with increased interleukin-4 (IL-4) production, IL-5 transcription, and eosinophilia. Suppression of parasite-specific peripheral blood mononuclear cell (PBMC) proliferation was evident, while decreased mitogen-stimulated gamma interferon (IFN-γ) production suggested immunomodulation, which was not restricted to parasite-specific responses. Our findings show that the global immune response is modulated toward a nonproliferative type 2 state following natural challenge with F. hepatica. This has implications in terms of the timing of the administration of vaccination programs and for host susceptibility to coinfecting pathogens.

Alba, A., Vázquez, A.A., Sánchez, J., Duval, D., Hernández, H.M., Sabourin, E., Vittecoq, M., Hurtrez-Boussés, S., Gourbal, B.

Fasciola hepatica-Pseudosuccinea columella interaction: Effect of increasing parasite doses, successive exposures and geographical origin on the infection outcome of susceptible and naturally-resistant snails from Cuba

(2018) *Parasites and Vectors*, 11 (1), art. no. 559

DOI: 10.1186/s13071-018-3155-3

Background: Pseudosuccinea columella is one of the most widespread vectors of Fasciola hepatica, a globally distributed trematode that affects humans, livestock and wildlife. The exclusive occurrence in Cuba of susceptible and naturally-resistant populations to F. hepatica within this snail species, offers a fascinating model for evolutionary biology, health sciences and vector control strategies. In particular, resistance in P. columella is characterized by the encapsulation of the parasite by host's immune cells and has been experimentally tested using different Cuban F. hepatica isolates with no records of successful infection. Here, we aimed to explore for the first time, the effect of different parasite doses, successive exposures and different parasite origins on the infection outcomes of the two phenotypes of P. columella occurring in Cuba. Methods: To increase the chances for F. hepatica to establish, we challenged Cuban P. columella with increasing single parasite doses of 5, 15 or 30 miracidia and serial exposures (three-times) of 5 miracidia using a sympatric F. hepatica isolate from Cuba, previously characterized by microsatellite markers. Additionally, we exposed the snails to F. hepatica from different geographical origins (i.e. Dominican Republic and France). Parasite prevalence, redial burden and survival of snails were recorded at 25 days post-exposure. Results: No parasite development was noted in snails from the resistant populations independent of the experimental approach. Contrastingly, an overall increase in prevalence and redial burden was observed in susceptible snails when infected with high miracidia doses and after serial exposures. Significant differences in redial burden between single 15 miracidia and serial 3 × 5 miracidia infected snails suggest that immune priming potentially occurs in susceptible P. columella. Compatibility differences of allopatric (Caribbean vs European) F. hepatica with susceptible snails were related to the geographical scale of the combinations. Conclusions: Here, the effectiveness of P. columella resistance to F. hepatica does not decline with increasing parasite doses, successive infection or different geographical origins of parasite isolates, while presenting new evidence for specificity for infection in susceptible P. columella snails. Understanding the peculiarities of the P. columella-F. hepatica interaction and the extent of the resistant phenotype is crucial for an effective parasite control and for developing alternatives to tackle fasciolosis transmission. © 2018 The Author(s). ISSN: 17563305.

Epidemiology

Byrne, A.W., Graham, J., McConville, J., Milne, G., McDowell, S., Hanna, R.E.B., Guelbenzu-Gonzalo, M.

Seasonal variation of Fasciola hepatica antibodies in dairy herds in Northern Ireland measured by bulk tank milk ELISA

(2018) *Parasitology Research*, 117 (9), pp. 2725-2733.

Bovine fasciolosis, caused by the infection of the trematode parasite Fasciola hepatica, remains a problem in dairy herds causing significant production losses. In this study, bulk milk tank samples were utilised to generate a comprehensive survey of the variation in liver fluke exposure over the four seasons of 2016 in Northern Ireland (NI). Samples were tested using an antibody ELISA test; within-herd prevalence levels were categorised relative to sample-to-positive ratio (S/P%). Overall, 1494 herds (~ 50% of all active dairy farms in NI) were sampled. In total, 5750 samples were tested with 91% of herds having a sample result for each season. The proportion of herds with evidence of liver fluke exposure was very high across the year, with 93.03% of all bulk milk samples having some indication of liver fluke antibody presence. A high proportion of samples (2187/5750; 38.03%) fell within the highest infection class (indicating high within-herd prevalence). There was significant seasonal variation in the mean S/P%. A multivariable random effect ordinal logit model suggested that the greatest probability of being in a higher infection class was in winter, whilst the lowest was recorded during summer. There was a significant negative association between increasing herd liver fluke infection class and herd size. Furthermore, there was significant variation in infection levels across regions of Northern Ireland, with higher infection levels in northern administrative areas. This study demonstrates the very high liver fluke exposure in this region of Europe, and that risk is not equally distributed spatially or across seasons in dairy herds.

Takeuchi-Storm, N., Denwood, M., Petersen, H.H., Enemark, H.L., Stensgaard, A.-S., Sengupta, M.E., Beesley, N.J., Hodgkinson, J., Williams, D., Thamsborg, S.M.

Patterns of Fasciola hepatica infection in Danish dairy cattle: Implications for on-farm control of the parasite based on different diagnostic methods

(2018) Parasites and Vectors, 11 (1), art. no. 674

Background: Bovine fasciolosis is an economically important livestock disease in Europe, and represents a particular challenge for organic farms, where cattle are grazed extensively and the use of anthelmintic is limited. A two-year longitudinal study was conducted on two conventional and two organic Danish dairy farms to examine the current temporal trend of F. hepatica infection on-farm, and to gather data of practical relevance for parasite control. Data were collected both at the herd and individual level using currently available diagnostic methods: a commercial serum antibody ELISA, a commercial copro-antigen ELISA, faecal egg counts, and monthly bulk tank milk (BTM) ELISA. The temporal patterns (animal age, farm-level temporal trends and seasonality) in the animal-level test results were analysed by generalised additive mixed models (GAMM). Results: Patterns of infection differed substantially between the farms, due to different grazing management and anthelmintic use. However, animals were first infected at the age of 1.5-2 years (heifers), and most at-risk animals sero-converted in autumn, suggesting that summer infections in snails prevail in Denmark. Our results also suggest that the lifespan of the parasite could be over 2 years, as several cows showed signs of low grade infection even after several years of continuous indoor housing without access to freshly-cut grass. The serum antibody ELISA was able to detect infection first, whereas both copro-antigen ELISA and faecal egg counts tended to increase in the same animals at a later point. Decreasing BTM antibody levels were seen on the two farms that started anthelmintic treatment during the study. Conclusions: While important differences between farms and over time were seen due to varying grazing management, anthelmintic treatment and climatic conditions, the young stock was consistently seen as the main high-risk group and at least one farm also had suspected transmission (re-infection) within the lactating herd. Careful interpretation of test results is necessary for older cows as they can show persistent infections several years after exposure has stopped. Rigorous treatment regimens can reduce BTM ELISA values, but further research is needed to develop a non-medicinal approach for sustainable management of bovine fasciolosis.

Arenal, A., García, Y., Quesada, L., Velázquez, D., Sánchez, D., Peña, M., Suárez, A., Díaz, A., Sánchez, Y., Casaert, S., Van Dijk, J., Vercruysse, J., Charlier, J.

Risk factors for the presence of Fasciola hepatica antibodies in bulk-milk samples and their association with milk production decreases, in Cuban dairy cattle

(2018) BMC Veterinary Research, 14 (1), art. no. 336

Background: Worldwide, Fasciola hepatica infection causes high production losses in the livestock industry. Recently, studies have analyzed the association between measurements of F. hepatica infection intensity and herd management practices. The aim of the present study, the first of its kind in a subtropical region, was to evaluate associations between F. hepatica bulk-tank milk ELISA results with herd management factors and milk yield in dairy herds, in Camagüey, Cuba. The SVANOVIR® F. hepatica-AB ELISA was used to measure F. hepatica antibody levels in a random sample of 516 dairy herds during the period of May-July of 2014. Farm management practice data were collected using a questionnaire. Results: With 82% of the herds testing positive, the results indicate that F. hepatica is very widespread in this area. Reductions in milk production of 18 and 32% were observed in herds with Optical Density Ratios (ODR) of 0.3-0.6 and > 0.6, respectively, when compared to herds with ODR < 0.3. Overall, the longer the milking cows were put out to pasture, the higher the levels of anti-parasite antibodies. Co-grazing with sheep and goats also significantly increased the risk of high ODR. Conclusions: Our data show a widespread occurrence of the parasite as well as a major potential impact of the infection on the Cuban development goal of becoming self-sufficient in milk production. Our risk factor analysis suggests that the prevention of infection around water sources, and the separation of cattle from small ruminants could be useful control measures. This is the first epidemiological survey of F. hepatica abundance, and associated reductions in milk yield, in dairy herds in Cuba.

Lucena, A. N., Corbalán, M. P. M., Martínez-Ibeas, A. M., McGrath, G., Sayers, R., Mulcahy, G. and Zintl, A.

Title: Validation of a spatial liver fluke model under field conditions in Ireland

2018 Geospatial Health, 13 (1) 118-126

Fasciola hepatica is the causative agent of fasciolosis, a global disease of a wide range of mammals, particularly sheep and cattle. Liver fluke infection causes annual losses estimated at around €2.5 billion to livestock and food industries worldwide. Various models have been developed to define risk factors and predict exposure to this liver fluke in ruminants in European countries, most of them based exclusively on data from dairy herds. The aim of this study was to validate a published theoretical baseline risk map of liver fluke exposure and cluster maps in Ireland, by including further explanatory variables and additional herd types that are spatially more widespread. Three approaches were employed: i) comparison of predicted and actual exposure; ii) comparison of cluster distribution of hotspots and coldspots; and iii) development of a new model to compare predicted spatial distribution and risk factors. Based on new survey data, the published baseline predictive map was found to have a sensitivity of 94.7%, a specificity of 5%, a positive predictive value of 60% and a negative predictive value of 38.2%. In agreement with the original model, our validation highlighted temperature and rainfall among the main risk factors. In addition, we identified vegetation indices as important risk factors. Both the previously published and our new model predict that exposure to Fasciola is higher in the western parts of Ireland. However, foci of high probability do not match completely, nor do the location of clusters of hotspots and coldspots.

Non chemical control of *Fasciola hepatica*

Muñiz-Pareja, F., Iturbe-Espinoza, P.

Effectiveness of Chaetogaster limnaei as a controller of Fasciola hepatica in experimental infections of Galba truncatula

(2018) Tropical Parasitology, 8 (2), pp. 88-93.

Background: Chaetogaster limnaei is an annelid symbiotically associated with lymnaeid snails as Galba truncatula. This association is considered a preventive mechanism against trematode miracidia infection, including Fasciola hepatica. The objective of this study was to determine the effectiveness of Chaetogaster limnaei as a natural controller of Fasciola hepatica in laboratory conditions. Procedures: Fasciola hepatica miracidia were inoculated in parallel into snails carrying Chaetogaster limnaei and snails without the annelid. The degree of infection was measured after 40 days of exposure. Furthermore, the number of annelids per snail was quantified, as well as the ability of Chaetogaster limnaei to devour miracidia at different times of exposure. Results: An effective 70% natural control of Fasciola hepatica in Galba truncatula was observed. The carrying capacity of the snail was established to be of 10.6 ± 1 annelids. Chaetogaster limnaei is a predator of Fasciola hepatica devouring an average of 3.79 ± 0.21 miracidia. The results of these experiments have a potential value as a control measure against fascioliasis in the environment.

Vaccination

Azizi, H., Mirzaeei, H., Nasiri, A. A., Bazi, A., Mirzapour, A., Khatami, M., Nahavandi, K. H., Azimi, A. and Yaghoobi, H.

Naltrexone; as an efficient adjuvant in induction of Th1 immunity and protection against Fasciola hepatica infection

(2018) Experimental Parasitology, 189; 66-71

Abstract: Toxic effects of available therapeutics are major drawbacks for conventional management approaches in parasitic infections. Vaccines have provided a promising opportunity to obviate such unwanted complications. In present study, we examined immune augmenting capacities of an emerging adjuvant, Naltrexone, against Fasciola hepatica infection in BALB/c mice. Seventy BALB/c mice were divided into five experimental groups (14 mice per group) including 1- control (received PBS), 2- vaccine (immunized with F. hepatica E/S antigens), 3- Alum-vaccine (immunized with Alum adjuvant and E/S antigens), 4- NLT-vaccine (immunized with NLT adjuvant and E/S antigens), and 5- Alum-NLT-vaccine (immunized with mixed Alum-NLT adjuvant and E/S antigens). Lymphocyte stimulation index was assessed by MTT assay. Production of IFN-γ, IL-4, IgG2a and IgG1 was assessed by ELISA method. Results showed that NLT, either alone or in combination with alum, can induce immune response toward production of IFN-γ and IgG2a as representatives of Th1 immune response. Also, using this adjuvant in immunization experiment was associated with significantly high proliferative response of splenocytes/lymphocytes. Utilization of mixed Alum-NLT adjuvant revealed the highest protection rate (73.8%) in challenge test of mice infected with F. hepatica. These findings suggest the potential role of NLT as an effective adjuvant in induction of protective cellular and Th1 immune responses against fasciolosis.

Dominguez, M.F., González-Miguel, J., Carmona, C., Dalton, J.P., Cwiklinski, K., Tort, J., Siles-Lucas, M.

Low allelic diversity in vaccine candidates genes from different locations sustain hope for Fasciola hepatica immunization

(2018) *Veterinary Parasitology*, 258, pp. 46-52.

Fasciola hepatica is a trematode parasite that causes fasciolosis in animals and humans. Fasciolosis is usually treated with triclabendazole, although drug-resistant parasites have been described in several geographical locations. An alternative to drug treatment would be the use of a vaccine, although vaccination studies that have been performed mainly in ruminants over the last 30 years, show high variability in the achieved protection and are not yet ready for commercialisation. Since F. hepatica exhibits a high degree of genomic polymorphism, variation in vaccine efficacy could be attributed, at least partially, to phenotypic differences in vaccine candidate sequences amongst parasites used in the challenge infections. To begin to address this issue, a collection of F. hepatica isolates from geographically dispersed regions, as well as parasites obtained from vaccination trials performed against a field isolate from Uruguay and the experimentally maintained South Gloucester isolate (Ridgeway Research, UK), were compiled to establish a F. hepatica Biobank. These collected isolates were used for the genetic analysis of several vaccine candidates that are important in host-parasite interactions and are the focus of the H2020 PARAGONE vaccine project (https://www.paragoneh2020.eu/), namely FhCL1, FhCL2, FhPrx, FhLAP and FhHDM. Our results show that F. hepatica exhibits a high level of conservation in the sequences encoding each of these proteins. The consequential low variability in these vaccine candidates amongst parasites from different geographical regions reinforces the idea that they would be suitable immunogens against liver fluke isolates worldwide.

Wesołowska, A., Basałaj, K., Norbury, L.J., Sielicka, A., Wędrychowicz, H., Zawistowska-Deniziak, A.

Vaccination against Fasciola hepatica using cathepsin L3 and B3 proteases delivered alone or in combination

(2018) *Veterinary Parasitology*, 250, pp. 15-21.

No licensed vaccine is currently available for prevention of Fasciola hepatica infections. However, considering the alarming increase in drug resistance, there is an urgent need for a safe and fully effective vaccine against fasciolosis. Here, we tested if cathepsins L (FhCL3-1, FhCL3-2) and B (FhCB3) secreted by juvenile liver flukes are viable vaccine targets when delivered alone or in combination in a rat model. Since control over the early immune response is crucial for parasite's establishment in its host, it was hypothesised that targeting fluke juvenile stages may prove beneficial. Moreover, it was assumed that selected antigens will act in a cumulative manner to interfere with liver fluke migration and thereby will reduce F. hepatica infection. Recombinant FhCL3-1 and FhCL3-2 delivered alone reduced liver fluke burdens by 47 % and 63 %, respectively. A trivalent vaccine containing rFhCL3-1/CL3-2/CB3 did not increase the protective vaccine efficacy compared to the rFhCL3-2 vaccinated group (53 %), although, reductions in liver fluke wet weight (statistically significant) and liver damage score were most pronounced. Further, the highest IgG1 and IgG2a levels were seen in rFhCL3-2 vaccinated rats, the group for which the highest reduction in worm burden was demonstrated. Moreover, IgG1 and IgG2a levels in vaccinated rats were significantly elevated compared to those reported for control groups up to 4 week post-infection. While the mechanism of protection remains unknown, it appears that it depends on vaccine-induced antibodies directed against cathepsins. The obtained results imply that F. hepatica juvenile-specific cathepsins are promising vaccine candidates that induce responses that successfully target early migratory liver fluke stages. Now, the challenge is to evaluate these juvenile-specific cathepsins for use in livestock.

Garza-Cuartero, L., Geurden, T., Mahan, S.M., Hardham, J.M., Dalton, J.P., Mulcahy, G.

Antibody recognition of cathepsin L1-derived peptides in Fasciola hepatica-infected and/or vaccinated cattle and identification of protective linear B-cell epitopes

(2018) *Vaccine*, . Article in Press.

DOI: 10.1016/j.vaccine.2018.01.020

Fasciola hepatica infection causes important economic losses in livestock and food industries around the world. In the Republic of Ireland F. hepatica infection has a 76% prevalence in cattle. Due to the increase of anti-helminthic resistance, a vaccine-based approach to control of Fasciolosis is urgently needed. A recombinant version of the cysteine protease cathepsin L1 (rmFhCL1) from F. hepatica has been a vaccine candidate for many years. We have found that vaccination of cattle with this immunodominant antigen has provided protection against infection in some experimental trials, but not in others. Differential epitope recognition between animals could be a source of variable levels of vaccine protection. Therefore, we have characterised for first time linear B-cell epitopes recognised within the FhCL1 protein using sera from F. hepatica-infected and/or vaccinated cattle from two independent trials. Results showed that all F. hepatica infected animals recognised the region 19-31 of FhCL1, which is situated in the N-terminal part of the pro-peptide. Vaccinated animals that showed fluke burden reduction elicited antibodies that bound to the regions 120-137, 145-155, 161-171 of FhCL1, which were not recognised by non-protected animals. This data, together with the high production of specific IgG2 in animals showing vaccine efficacy, suggest important targets for vaccine development.

Norbury, L. J., Basałaj, K., Zawistowska-Deniziak, A., Sielicka, A., Wilkowski, P., Wesołowska, A., Smooker, P. M. and Wędrychowicz, H.

Title: Intranasal delivery of a formulation containing stage-specific recombinant proteins of Fasciola hepatica cathepsin L5 and cathepsin B2 triggers an anti-fecundity effect and an adjuvant-mediated reduction in fluke burden in sheep

2018 Veterinary Parasitology, 258: 14-23

DOI: 10.1016/j.vetpar.2018.05.008

Abstract: Fasciola hepatica infection continues to be a major problem in the agriculture sector, particularly in sheep and cattle. Cathepsin L and B proteases are major components of the excretory/secretory material of the parasite, and their roles in several important aspects of parasite invasion and survival has led to their use as targets in rational vaccine design. Previous studies in rats demonstrated that the use of stage-specific antigens, cathepsin B2 and cathepsin L5, as part of a multivalent vaccine, was able to confer significant protection against challenge. In the present study, recombinant versions of cathepsin L5 and cathepsin B2 produced in yeast were used in combination to vaccinate sheep. Intramuscular and intranasal forms of administration were applied, and sheep were subsequently challenged with 150 F. hepatica metacercariae. Intramuscular vaccination was able to induce a strong systemic antibody response against both antigens, but failed to confer significant protection. Conversely, no elevated antibody response was detected against the vaccine antigens following nasal vaccination; however, a reduction in parasite egg viability (>92%) and a statistically significant (p = 0.006), predominantly adjuvant-mediated reduction in worm burdens was observed.

Chemical control of *Fasciola*

Crusco, A., Bordoni, C., Chakroborty, A., Whatley, K.C.L., Whiteland, H., Westwell, A.D., Hoffmann, K.F.

Design, synthesis and anthelmintic activity of 7-keto-sempervirol analogues

(2018) *European Journal of Medicinal Chemistry*, 152, pp. 87-100.

The plant-derived, diterpenoid 7-keto-sempervirol was recently reported to display moderate activity against larval stages of Schistosoma mansoni (IC50 = 19.1 μM) and Fasciola hepatica (IC50 = 17.7 μM), two related parasitic blood and liver flukes responsible for the neglected tropical diseases schistosomiasis and fascioliasis, respectively. Here, we aimed to increase the potency of 7-keto-sempervirol by total synthesis of 30 structural analogues. Subsequent screening of these new diterpenoids against juvenile and adult lifecycle stages of both parasites as well as the human HepG2 liver cell line and the bovine MDBK kidney cell line revealed structure-activity relationship trends. The most active analogue, 7d, displayed improved dual anthelmintic activity over 7-keto-sempervirol (IC50 ≈ 6 μM for larval blood flukes; IC50 ≈ 3 μM for juvenile liver flukes) and moderate selectivity (SI ≈ 4–5 for blood flukes, 8–13 for liver flukes compared to HepG2 and MDBK cells, respectively). Phenotypic studies using scanning electron microscopy revealed substantial tegumental alterations in both helminth species, supporting the hypothesis that the parasite surface is one of the main targets of this family of molecules. Further modifications of 7d could lead to greater potency and selectivity metrics resulting in a new class of broad-spectrum anthelmintic.

Ibarra-Velarde, F., Vera-Montenegro, Y., Flores-Ramos, M., Cantó-Alarcon, G.J., Hernández-Campos, A., Alcala-Canto, Y., Castillo, R.

Assessment of the effective dose of an experimental intramuscular formulation against immature and adult Fasciola hepatica in sheep

(2018) *Veterinary Parasitology*, 260, pp. 38-44.

The effective dose of an injectable prodrug, named compound alpha prodrug, against immature and adult Fasciola hepatica in experimentally infected sheep was determined. In a first experiment, 30 sheep were infected with Fasciola hepatica on day 0 and 50. After microscopic detection of faecal eggs on day 80, groups (n = 6) 1 to 3 were treated with 6, 8 and 10 mg/kg of the experimental water-soluble prodrug compound alpha intramuscularly, respectively. Group 4 was treated with closantel and group 5 remained untreated. Copromicroscopical examinations were made on day 0, 80 and 108. On day 110, trematodes were collected from the bile ducts. Fasciolicide efficacy was assessed as a percentage of fluke-egg and adult-fluke reduction. Fluke length was also recorded. In a second experiment aimed to assess the fasciolicide activity of compound alpha prodrug against four-week-old flukes, 12 sheep were infected on day 0 and allocated into two groups (n = 6). On day 50 post infection, group A was treated with the experimental water-soluble prodrug compound alpha at 6 mg/kg/IM and B remained untreated. Fasciolicide activity was assessed on day 80 after collection, microscopic observation and measurement of flukes present in the parenchyma for immature stages and on day 108 for adults. Egg output decreased 91.2, 96.0, 98.8 and 94.9% for groups 1, 2, 3 and 4, respectively. Compound alpha prodrug cleared 97.6%, 98.51% and 100% of adult stages in a dose-dependent manner. Closantel killed 81.95% flukes. Regarding the second experiment, 81.2% efficacy was achieved. Immature flukes were significantly smaller in the treated group. It is concluded that the intramuscular application of compound alpha prodrug exerted fasciolicide efficacy against adults of Fasciola hepatica.

Kandil, O.M., Hassan, N.M.F., Sedky, D., Ata, E.B., Nassar, S.A., Shalaby, H.A., Nanev, V., Tsocheva-Gaytandzhieva, N., Gabrashanska, M.

Anthelmintic efficacy of Moringa oleifera seed methanolic extract against Fasciola hepatica

(2018) *Journal of Parasitic Diseases*, 42 (3), pp. 391-401.

The current study was carried out to assess in vitro and in vivo effects of Moringa oleifera seed methanolic extract on Fasciola hepatica to develop an alternative source of treatment. The in vitro ovicidal effect of M. oleifera seed extract on immature F. hepatica eggs has provided evidence of inhibitory activity on the vitality and hatchability of F. hepatica eggs. This inhibitory activity was concentration-dependent and also correlated strongly with the exposure time. In the in vivo trial, the oral administration of F. hepatica experimentally infected rabbits with doses of 150 mg/kg BW prepared extract per day for 3 consecutive days on the 63rd day post infection confirmed potent fasciolicide activity of the extract. A gradual decrease in fecal egg count (FEC) was detected from the 1st day post treatment until reaching 100% FEC reduction by the 7th day post treatment. No flukes could be found at post mortem examinations. Significant increments of serum total protein, globulin, the activities of ALT and AST, total cholesterol, triglycerides and urea were recorded during the period of infection, which were improved by treatment. Remarkable histopathological alterations were observed in the infected liver and gallbladder tissues which decreased clearly in the treated rabbits. This study proposes that the used extract has promising and potent fasciolicide activity.

Radio, S., Fontenla, S., Solana, V., Matos Salim, A.C., Araújo, F.M.G., Ortiz, P., Hoban, C., Miranda, E., Gayo, V., Pais, F.S.-M., Solana, H., Oliveira, G., Smircich, P., Tort, J.F.

Pleiotropic alterations in gene expression in Latin American Fasciola hepatica isolates with different susceptibility to drugs

(2018) *Parasites and Vectors*, 11 (1), art. no. 56.

DOI: 10.1186/s13071-017-2553-2

Background: Fasciola hepatica is the main agent of fasciolosis, a zoonotic disease affecting livestock worldwide, and an emerging food-borne disease in humans. Even when effective treatments are available, drugs are costly and can result in tolerance, liver damage and normally they do not prevent reinfection. Drug-resistant strains in livestock have been reported in various countries and, more worryingly, drug resistance in human cases has emerged in South America. The present study aims to characterize the transcriptome of two South American resistant isolates, the Cajamarca isolate from Peru, resistant to both triclabendazole and albendazole (TCBZR/ABZR) and the Rubino isolate from Uruguay, resistant to ABZ (TCBZS/ABZR), and compare them to a sensitive strain (Cenapa, Mexico, TCBZS/ABZS) to reveal putative molecular mechanisms leading to drug resistance. Results: We observed a major reduction in transcription in the Cajamarca TCBZR/ABZR isolate in comparison to the other isolates. While most of the differentially expressed genes are still unannotated, several trends could be detected. Specific reduction in the expression levels of cytoskeleton proteins was consistent with a role of tubulins as putative targets of triclabendazole (TCBZ). A marked reduction of adenylate cyclase might be underlying pleiotropic effects on diverse metabolic pathways of the parasite. Upregulation of GST mu isoforms suggests this detoxifying mechanism as one of the strategies associated with resistance. Conclusions: Our results stress the value of transcriptomic approaches as a means of providing novel insights to advance the understanding of drug mode of action and drug resistance. The results provide evidence for pleiotropic variations in drug-resistant isolates consistent with early observations of TCBZ and ABZ effects and recent proteomic findings.

Whiteland, H.L., Chakroborty, A., Forde-Thomas, J.E., Crusco, A., Cookson, A., Hollinshead, J., Fenn, C.A., Bartholomew, B., Holdsworth, P.A., Fisher, M., Nash, R.J., Hoffmann, K.F.

An Abeis procera-derived tetracyclic triterpene containing a steroid-like nucleus core and a lactone side chain attenuates in vitro survival of both Fasciola hepatica and Schistosoma mansoni

(2018) International Journal for Parasitology: Drugs and Drug Resistance, 8 (3), pp. 465-474.

Two economically and biomedically important platyhelminth species, Fasciola hepatica (liver fluke) and Schistosoma mansoni (blood fluke), are responsible for the neglected tropical diseases (NTDs) fasciolosis and schistosomiasis. Due to the absence of prophylactic vaccines, these NTDs are principally managed by the single class chemotherapies triclabendazole (F. hepatica) and praziquantel (S. mansoni). Unfortunately, liver fluke resistance to triclabendazole has been widely reported and blood fluke insensitivity/resistance to praziquantel has been observed in both laboratory settings as well as in endemic communities. Therefore, the identification of new anthelmintics is necessary for the sustainable control of these NTDs in both animal and human populations. Here, continuing our work with phytochemicals, we isolated ten triterpenoids from the mature bark of Abies species and assessed their anthelmintic activities against F. hepatica and S. mansoni larval and adult lifecycle stages. Full 1H and 13C NMR-mediated structural elucidation of the two most active triterpenoids revealed that a tetracyclic steroid-like nucleus core and a lactone side chain are associated with the observed anthelmintic effects. When compared to representative mammalian cell lines (MDBK and HepG2), the most potent triterpenoid (700015; anthelmintic EC50s range from 0.7 μM–15.6 μM) displayed anthelmintic selectivity (selectivity indices for F. hepatica: 13 for newly excysted juveniles, 46 for immature flukes, 2 for mature flukes; selectivity indices for S. mansoni: 14 for schistosomula, 9 for immature flukes, 4 for adult males and 3 for adult females) and induced severe disruption of surface membranes in both liver and blood flukes. S. mansoni egg production, a process responsible for pathology in schistosomiasis, was also severely inhibited by 700015. Together, our results describe the structural elucidation of a novel broad acting anthelmintic triterpenoid and support further investigations developing this compound into more potent analogues for the control of both fasciolosis and schistosomiasis.

Diagnosis

Kowalczyk, S.J., Czopowicz, M., Weber, C.N., Müller, E., Kaba, J.

Accuracy of a diagnostic model based on serum biochemical parameters in detecting cows at an increased risk of chronic fascioliasis

(2018) *Veterinary Parasitology*, 254, pp. 15-20.

In adult cattle Fasciola hepatica infection usually follows a chronic subclinical course, and reduces both the milk yield and milk quality, resulting in considerable financial losses. Effective control of the disease is based on reliable identification of asymptomatically infected individuals, which now requires special parasitological or serological diagnostic tests. It is also known that F. hepatica infection induces alterations in some serum biochemical parameters. Therefore, the study was conducted to develop a model based on serum biochemical parameters allowing to identify cows at higher risk of chronic fascioliasis. Six hundred sixty eight adult dairy cows from 97 herds located in central and northeastern Poland were clinically examined, and blood and fecal samples from them were collected for a routine monitoring of fascioliasis and metabolic profile. Using the combination of fecal sedimentation test and indirect ELISA based on excretory/secretory products 203 cows, apparently healthy in clinical examination, were definitively classed as affected by (47 cows, 23.2%) or free from (156 cows, 76.8%) chronic fascioliasis. Their serum was screened for the activity of 4 enzymes (γ-glutamyl transpeptidase, glutamate dehydrogenase, aspartate aminotransferase, creatine kinase) and concentration of 18 other components (total bilirubin, total protein, albumin, globulin, urea, glucose, non-esterified fatty acids, β-hydroxybutyrate, sodium, potassium, chloride, calcium, magnesium, copper, zinc, iron, selenium, and haptoglobin). Logistic regression analysis was used to build 4 multivariable models allowing for identification of cows at risk of chronic fascioliasis. Then, the accuracy was compared between the models using the area under the receiver operating characteristic curve (AUROC), and an optimal cut-off value was determined for the most accurate model using Youden J index. The most accurate proved to be the model based on glutamate dehydrogenase activity and globulin, urea (all three positively linked with risk of chronic fascioliasis), and selenium concentration (negatively linked) adjusted by the access to pasture and cow's age. At the optimal cut-off of 0.37 this model had sensitivity of 85.1% (CI 95%:72.3%, 92.6%), specificity of 90.4% (CI 95%:84.7%, 94.1%), positive likelihood ratio of 8.8 (CI 95%:5.4, 14.5), and negative likelihood ratio of 0.16 (CI 95%:0.08, 0.33). This model performed significantly better than model including only γ-glutamyl transpeptidase or model including both γ-glutamyl transpeptidase and aspartate aminotransferase (both also adjusted by the access to pasture and cow's age), and was the only model which performed significantly better than the basic model based solely on the access to pasture and cow's age.

Martínez-Sernández, V., Perteguer, M.J., Hernández-González, A., Mezo, M., González-Warleta, M., Orbegozo-Medina, R.A., Romarís, F., Paniagua, E., Gárate, T., Ubeira, F.M.

Comparison of recombinant cathepsins L1, L2, and L5 as ELISA targets for serodiagnosis of bovine and ovine fascioliasis

(2018) *Parasitology Research*, 117 (5), pp. 1521-1534.

Infections caused by Fasciola hepatica are of great importance in the veterinary field, as they cause important economic losses to livestock producers. Serodiagnostic methods, typically ELISA (with either native or recombinant antigens), are often used for early diagnosis. The use of native antigens, as in the MM3-SERO ELISA (commercialized as BIO K 211, BIO-X Diagnostics), continues to be beneficial in terms of sensitivity and specificity; however, there is interest in developing ELISA tests based on recombinant antigens to avoid the need to culture parasites. Of the antigens secreted by adult flukes, recombinant procathepsin L1 (rFhpCL1) is the most commonly tested in ELISA to date. However, although adult flukes produce three different clades of CLs (FhCL1, FhCL2, and FhCL5), to our knowledge, the diagnostic value of recombinant FhCL2 and FhCL5 has not yet been investigated. In the present study, we developed and tested three indirect ELISAs using rFhpCL1, rFhpCL2, and rFhpCL5 and evaluated their recognition by sera from sheep and cattle naturally infected with F. hepatica. Although the overall antibody response to these three rFhpCLs was similar, some animals displayed preferential recognition for particular rFhpCLs. Moreover, for cattle sera, the highest sensitivity was obtained using rFhpCL2 (97%), being equal for both rFhpCL1 and rFhpCL5 (87.9%), after adjusting cut-offs for maximum specificity. By contrast, for sheep sera, the sensitivity was 100% for the three rFhpCLs. Finally, the presence of truncated and/or partially unfolded molecules in antigen preparations is postulated as a possible source of cross-reactivity.

Jones, R.A., Brophy, P.M., Davis, C.N., Davies, T.E., Emberson, H., Rees Stevens, P., Williams, H.W.

Detection of Galba truncatula, Fasciola hepatica and Calicophoron daubneyi environmental DNA within water sources on pasture land, a future tool for fluke control?

(2018) *Parasites and Vectors*, 11 (1), art. no. 342

DOI: 10.1186/s13071-018-2928-z

Background: Increasing trematode prevalence and disease occurrence in livestock is a major concern. With the global spread of anthelmintic resistant trematodes, future control strategies must incorporate approaches focusing on avoidance of infection. The reliance of trematodes on intermediate snail hosts to successfully complete their life-cycle means livestock infections are linked to the availability of respective snail populations. By identifying intermediate snail host habitats, infection risk models may be strengthened whilst farmers may confidently apply pasture management strategies to disrupt the trematode life-cycle. However, accurately identifying and mapping these risk areas is challenging. Methods: In this study, environmental DNA (eDNA) assays were designed to reveal Galba truncatula, Fasciola hepatica and Calicophoron daubneyi presence within water sources on pasture land. eDNA was captured using a filter-based protocol, with DNA extracted using the DNeasy® PowerSoil® kit and amplified via PCR. In total, 19 potential G. truncatula habitats were analysed on four farms grazed by livestock infected with both F. hepatica and C. daubneyi. Results: Galba truncatula eDNA was identified in 10/10 habitats where the snail was detected by eye. Galba truncatula eDNA was also identified in four further habitats where the snail was not physically detected. Fasciola hepatica and C. daubneyi eDNA was also identified in 5/19 and 8/19 habitats, respectively. Conclusions: This study demonstrated that eDNA assays have the capabilities of detecting G. truncatula, F. hepatica and C. daubneyi DNA in the environment. Further assay development will be required for a field test capable of identifying and quantifying F. hepatica and C. daubneyi infection risk areas, to support future control strategies. An eDNA test would also be a powerful new tool for epidemiological investigations of parasite infections on farms.

Rathinasamy, V., Hosking, C., Tran, L., Kelley, J., Williamson, G., Swan, J., Elliott, T., Rawlin, G., Beddoe, T. and Spithill, T. W.

Development of a multiplex quantitative PCR assay for detection and quantification of DNA from Fasciola hepatica and the intermediate snail host, Austropeplea tomentosa, in water samples

2018 Veterinary Parasitology, 259; 17-24

Liver fluke (Fasciola hepatica) infection is an increasing threat to livestock production resulting in serious economic losses to the beef, dairy and sheep industries in Australia and globally. Triclabendazole (TCBZ) is the main drug used to control liver fluke infections in Australia and the widespread emergence of TCBZ resistance in cattle and sheep threatens liver fluke control. Alternative control measures to lower exposure of livestock to fluke infection would be useful to help preserve the usefulness of current chemical flukicides. Environmental DNA (eDNA) sampling methodology and associated molecular techniques are suited to rapidly assess the presence of pathogens on farms. In the present study, we developed a water sampling method in combination with a multiplex quantitative PCR assay to detect and quantify DNA of F. hepatica and Austropeplea tomentosa (A. tomentosa), a crucial intermediate snail host for liver fluke transmission in

South-east Australia. The multiplex qPCR assay allows for the independent detection of F. hepatica and A. tomentosa DNA using specific primers and a probe targeting the ITS-2 region of the liver fluke or snail. The method allows the highly specific and sensitive (minimal DNA detection levels to 14–50 fg) detection of F. hepatica or A. tomentosa. The method allows the detection of both liver fluke and snail eDNA in water samples. The effective quantification of liver fluke and snail eDNA in water samples using this assay could potentially allow researchers to both identify and monitor F. hepatica transmission zones on farming properties in South-east Australia which will better inform control strategies, with potential application of the assay worldwide.

Calvani, N.E.D., George, S.D., Windsor, P.A., Bush, R.D., Šlapeta, J.

Comparison of early detection of Fasciola hepatica in experimentally infected Merino sheep by real-time PCR, coproantigen ELISA and sedimentation

(2018) *Veterinary Parasitology*, 251, pp. 85-89.

Fasciolosis due to infection with Fasciola hepatica, Fasciola gigantica or their hybrids is a significant global cause of livestock production loss. Infection is commonly diagnosed by a labour-intensive sedimentation and faecal egg count (FEC), which has limited throughput and is only applicable after completion of the 8–12 week pre-patent period (PPP). A commercially-available ELISA for the detection of coprological antigen (coproELISA) enables detection prior to the completion of the PPP and is suitable for diagnosis of larger sample sizes, although the sensitivity reported under experimental infection settings can be difficult to replicate in the field, particularly in cattle. A recently-published real-time PCR workflow for the sensitive detection of Fasciola spp. DNA in faecal samples provides increased sample throughput, although the point at which this technique is first able to diagnose infection remains unknown. Other tools for the molecular diagnosis of fasciolosis, such as conventional PCR and loop-mediated isothermal amplification (LAMP), have been shown to detect F. hepatica DNA as early as 1 week post infection (WPI). In this study, faecal samples were collected weekly from 10 experimentally-infected Merino lambs and subjected to diagnosis via traditional sedimentation, coproELISA and real-time PCR. Samples were first considered positive at 6–8 WPI by coproELISA, real-time PCR and sedimentation, respectively. At 9 WPI 100% of samples were positive by all three methods. To evaluate the capacity of the real-time PCR approach to detect infection prior to completion of the PPP, two methods of sample preparation were compared at 2 WPI: (i) 150 mg raw faecal samples and (ii) 3 g faecal starting volume prior to sedimentation and pelleting. Neither method of sample preparation yielded positive results at 2 WPI suggesting that DNA amplification by real-time PCR is associated with faecal egg load.

Calvani, N.E.D., Cheng, T., Green, C., Hughes, P., Kwan, E., Maher, E., Bush, R.D., Šlapeta, J.

A quick and simple benchtop vortex egg-disruption approach for the molecular diagnosis of Fasciola hepatica from ruminant faecal samples

(2018) *Parasitology Research*, 117 (8), pp.2685-2688.

Commonly employed diagnostic methods for Fasciola spp., such as a traditional sedimentation and faecal egg count, or a commercially available coprological ELISA, have limitations in their sensitivity or ability to differentiate species. A reliable DNA isolation method coupled with real-time PCR addresses these issues by providing highly sensitive and quantitative molecular diagnosis from faecal samples. The current study evaluated a standard benchtop vortex for F. hepatica egg disruption in sheep and cattle faecal samples and determined the minimum faecal egg load required for a positive result from un-concentrated (raw) faecal samples. The minimum faecal egg load for a positive real-time PCR result from 150 mg raw faecal sample was 10 and 20 eggs per gram for sheep and cattle, respectively. No significant difference ($P = 0.4467$) between disruptions on a benchtop vortex for 5 or 10 min was observed when compared to 40 s of disruption at 6.0 m/s in a benchtop homogeniser.

Mokhtarian, K., Meamar, A.R., Khoshmirsafa, M., Razmjou, E., Masoori, L., Khanmohammadi, M., Akhlaghi, L., Falak, R.

Comparative assessment of recombinant and native immunogenic forms of Fasciola hepatica proteins for serodiagnosis of sheep fasciolosis

(2018) *Parasitology Research*, 117 (1), pp. 225-232.

Laboratory diagnosis of sheep fasciolosis is commonly performed by coprological examinations; however, this method may lead to false negative results during the acute phase of the infection. Furthermore, the poor sensitivity of coprological methods is considered to be a paradox in the chronic phase of the infection. In this study, we compared the immunoreactivity of native and recombinant forms of Fasciola hepatica excretory/secretory antigens and determined their capabilities for the development of F. hepatica-specific immunoassays. Immunoreactivity and specificity of recombinant and native forms of F. hepatica antigens, including fatty acid binding protein (FABP), glutathione-S-transferase (GST), and cathepsin L-1 (CL1), in parallel with native forms of FABP and GST, were studied for serodiagnosis of the chronic form of sheep fasciolosis, individually or in combination with each other by enzyme-linked immunosorbent assays (ELISA). The correlation of the findings was assessed by receiver-operator characteristic (ROC); furthermore, the specificity and sensitivity were assessed by Youden’s J. Serologic cross-reactivity was evaluated using samples from healthy sheep ($n = 40$), Fasciola-infected sheep ($n = 30$), and sheep with other parasitic infections ($n = 43$). The FABPs were determined to be greater than 95% sensitive for F. hepatica serodiagnosis. The most desirable diagnostic recombinant antigen was rCL1, which showed 100% sensitivity and 97% specificity in ELISA and was capable of discriminating the positive and negative samples by maximum Youden’s J results. We conclude that rCL1 can be used for routine serodiagnosis of chronic fasciolosis. Thus, it could be advantageous in development of immunoassays for screening of ovine herds in fasciolosis-endemic areas and as a reliable agent for detection of fasciolosis in non-endemic regions.

Rumen fluke (Calicophoron daubneyi)

Huson, K.M., Morphew, R.M., Allen, N.R., Hegarty, M.J., Worgan, H.J., Girdwood, S.E., Jones, E.L., Phillips, H.C., Vickers, M., Swain, M., Smith, D., Kingston-Smith, A.H., Brophy, P.M.

Polyomic tools for an emerging livestock parasite, the rumen fluke Calicophoron daubneyi; Identifying shifts in rumen functionality

(2018) Parasites and Vectors, 11 (1), art. no. 617

Background: Diseases caused by parasitic flatworms of rumen tissues (paramphistomosis) are a significant threat to global food security as a cause of morbidity and mortality in ruminant livestock in subtropical and tropical climates. Calicophoron daubneyi is currently the only paramphistome species commonly infecting ruminant livestock in temperate European climates. However, recorded incidences of C. daubneyi infection in European livestock have been increasing over the last decade. Whilst clinical paramphistomosis caused by adult worms has not been confirmed in Europe, fatalities have been attributed to severe haemorrhagic enteritis of the small intestine resulting from the migration of immature paramphistomes. Large numbers of mature adults can reside in the rumen, yet to date, the impact on rumen fermentation, and consequently on productivity and economic management of infected livestock, have not been resolved. Limited publicly available nucleotide and protein sequences for C. daubneyi underpin this lack of biological and economic understanding. Here we present for the first time a de novo assembled transcriptome, with functional annotations, for adult C. daubneyi, which provides a reference database for protein and nucleotide sequence identification to facilitate fundamental biology, anthelmintic, vaccine and diagnostics discoveries. Results: This dataset identifies a number of genes potentially unique to C. daubneyi and, by comparison to an existing transcriptome for the related Paramphistomum cervi, identifies novel genes which may be unique to the paramphistome group of platyhelminthes. Additionally, we present the first coverage of the excretory/secretory and soluble somatic proteome profiles for adult C. daubneyi and identify the release of extracellular vesicles from adult C. daubneyi parasites during in vitro, ex-host culture. Finally, we have performed the first analysis of rumen fluke impacting upon rumen fermentation parameters using an in vitro gas production study resulting in a significant increase in propionate production. Conclusions: The resulting data provide a discovery platform (transcriptome, proteomes, EV isolation pipeline and in vitro fermentation system) to further study C. daubneyi-host interaction. In addition, the acetate: propionate ratio has been demonstrated to decrease with rumen fluke infection suggesting that acidotic conditions in the rumen may occur.

O'Shaughnessy, J., Garcia-Campos, A., McAloon, C. G., Fagan, S., De Waal, T., McElroy, M., Casey, M., Good, B., Mulcahy, G., Fagan, J., Murphy, D. and Zintl, A.

Epidemiological investigation of a severe rumen fluke outbreak on an Irish dairy farm

(2018) Parasitology , 145 (7) 948-952

Although the rumen fluke, Calicophoron daubneyi is now very common and widespread throughout Western Europe, reports of clinical cases are still rare. This study explores the epidemiological background to a severe rumen fluke outbreak in 6-month-old heifers on a dairy farm in Ireland. Sequence analysis of the cytochrome oxidase subunit 1 (Cox1) gene of the rumen fluke metacercariae on pasture failed to identify predominant, possibly pathogenic subtypes. However, estimates of metacercarial load indicated that the animals were exposed to a daily dose of about 5334 C. daubneyi metacercariae for a period of 3 weeks resulting in the build-up of very large numbers of immature worms in the small intestine. It is hypothesized that specific environmental conditions may favour this parasite over its competitor, the liver fluke, Fasciola hepatica, possibly by allowing it to emerge earlier. The possibility that C. daubneyi may be better adapted to the Irish climate than F. hepatica together with the fact that selective treatment against F. hepatica effectively frees the niche for C. daubneyi, may result in the gradual replacement of F. hepatica by C. daubneyi.

Naranjo-Lucena, A., Munita Corbalán, M. P., Martínez-Ibeas, A. M., McGrath, G., Murray, G., Casey, M., Good, B., Sayers, R., Mulcahy, G. and Zintl, A.

Spatial patterns of Fasciola hepatica and Calicophoron daubneyi infections in ruminants in Ireland and modelling of C. daubneyi infection

2018 Parasites and Vectors, 11 (1)

Background: Fasciola hepatica has always represented a threat to Irish livestock because the Irish climate is highly suitable for the main local intermediate host of the parasite, the snail Galba truncatula. The recent clinical emergence of infections due to Calicophoron daubneyi has raised the question of whether the two parasites, which share a niche during part of their life-cycles, interact in some way. Here, we used geographical information systems (GIS) to analyse the distribution of both parasites in cattle and sheep. We also developed the first predictive model of paramphistomosis in Ireland. Results: Our results indicated that, in cattle, liver fluke infection is less common than rumen fluke infection and does not exhibit the same seasonal fluctuations. Overall, we found that cattle had a higher likelihood of being infected with rumen fluke than sheep (OR = 3.134, $P < 0.01$). In addition, infection with one parasite increased the odds of infection with the other in both host species. Rumen fluke in cattle showed the highest spatial density of infection. Environmental variables such as soil drainage, land cover and habitat appeared to be the most important risk factors for C. daubneyi infection, followed by rainfall and vegetation. Overall the risk of infection with this parasite was predicted to be higher in the west of the country. Conclusions: This study shows differences between the infection rates and spatial patterns of bovine and ovine infections with F. hepatica and C. daubneyi in Ireland. Whether the reasons for this are due to susceptibility, exposure and/or management factors is yet to be determined. Furthermore, the rumen fluke model indicates distinct risk factors and predicted distribution to those of F. hepatica, suggesting potential biological differences between both parasite species.

Huson, K. M., Wild, C., Fenn, C. and Robinson, M. W.

2018

Optimized conditions for the in vitro excystment of Calicophoron daubneyi metacercariae

Parasitology 145(8), 1015-1019

Paramphistomosis, caused by Calicophoron daubneyi, is an emerging infection of ruminants throughout Western Europe. Despite its prevalence, many questions remain regarding the basic biology of this parasite and how it interacts with its host. Consequently, there is a need to develop methods to study C. daubneyi in vitro to improve our understanding of rumen fluke biology. Towards this, we aimed to identify a suitable protocol for in vitro excystment of C. daubneyi metacercariae. Six methods that have been used to excyst metacercariae from a number of trematode species were tested with C. daubneyi metacercariae. Three of these achieved an average of >50% excystment whilst one method, which included an acid-pepsin treatment, incubation in reducing conditions and an alkaline/bile salt solution to activate the larvae, consistently gave >80% excystment. The latter protocol also showed no detrimental effect on the motility of newly excysted juvenile (NEJ) parasites when observed for up to 24 h in RPMI 1640 medium post-excystment. The successful production of C. daubneyi NEJs in vitro is a significant step forward, and will enable the discovery of infective stage-specific parasite antigens and facilitate drug screening trials, to aid the development of much needed diagnostic and therapeutic options for paramphistomosis.

Parasitic gastro-enteritis (PGE)

Waghorn, T.S., Bouchet, C.L.G., Bekelaar, K., Leathwick, D.M.

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

(2018) *New Zealand Veterinary Journal*, Article in Press.

DOI: 10.1080/00480169.2018.1532849

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 Tararua 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.

Diagnosis of PGE

Elmahalawy, S.T., Halvarsson, P., Skarin, M., Höglund, J.

Droplet digital polymerase chain reaction (ddPCR) as a novel method for absolute quantification of major gastrointestinal nematodes in sheep

(2018) *Veterinary Parasitology*, 261, pp. 1-8.

In this paper, we present for the first time a new tool, based on Droplet Digital™ Polymerase Chain Reaction (ddPCR), for absolute quantification of key genera of gastrointestinal (GI) nematode parasites of grazing livestock. Four combinations of primers/probe sets targeting the internal transcribed spacer region 2 (ITS2) of the ribosomal RNA gene array were designed using the Primer3 software, following in silico analysis of nucleotide sequences from nematodes of interest downloaded from common databases. The amplified regions include both a universal region for detection of any strongylid gastrointestinal parasite and three different genus specific regions, making it possible to differentiate between the most important GI nematodes of sheep in Sweden: Haemonchus, Teladorsagia and Trichostrongylus. Analysis of samples containing serial dilutions and different mixtures of genomic DNA extracted from different species of adult worms proved useful in assessment of different threshold settings with the QuantaSoft software. Analysis of template DNA from these worms indicated that ddPCR is a viable choice for detection and absolute quantification of the different genera and also in samples with multiple species. Interpretation of the ddPCR results was straightforward and choice of analytical approach had little influence on the final results. Thus, the results obtained in the different analytical approaches seemed to be robust and the concentrations determined were uniform. Furthermore, the linear range of the Haemonchus ddPCR assay was similar to that of real-time PCR (qPCR). Taken together, our data confirm the suitability of ddPCR for detection and absolute quantification of three major sheep pathogens when tested on larval cultures from pooled ovine faeces. The results also indicate that ddPCR can be a useful complement to applications based on conventional egg counting methods such as the faecal egg reduction test (FECRT).

Vaccination

González-Hernández, A., Borloo, J., Peelaers, I., Casaert, S., Leclercq, G., Claerebout, E., Geldhof, P.

Comparative analysis of the immune responses induced by native versus recombinant versions of the ASP-based vaccine against the bovine intestinal parasite Cooperia oncophora

(2018) *International Journal for Parasitology*, 48 (1), pp. 41-49.

The protective capacities of a native double-domain activation-associated secreted protein (ndd-ASP)-based vaccine against the cattle intestinal nematode Cooperia oncophora has previously been demonstrated. However, protection analysis upon vaccination with a recombinantly produced antigen has never been performed. Therefore, the aim of the current study was to test the protective potential of a Pichia-produced double-domain ASP (pdd-ASP)-based vaccine against C. oncophora. Additionally, we aimed to compare the cellular and humoral mechanisms underlying the vaccine-induced responses by the native (ndd-ASP) and recombinant vaccines. Immunisation of cattle with the native C. oncophora vaccine conferred significant levels of protection after an experimental challenge infection, whereas the recombinant vaccine did not. Moreover, vaccination with ndd-ASP resulted in a higher proliferation of CD4-T cells both systemically and in the small intestinal mucosa when compared with animals vaccinated with the recombinant antigen. In terms of humoral response, although both native and recombinant vaccines induced similar levels of antibodies, animals vaccinated with the native vaccine were able to raise antibodies with greater specificity towards ndd-ASP in comparison with antibodies raised by vaccination with the recombinant vaccine, suggesting a differential immune recognition towards the ndd-ASP and pdd-ASP. Finally, the observation that animals displaying antibodies with higher percentages of recognition towards ndd-ASP also exhibited the lowest egg counts suggests a potential relationship between antibody specificity and protection.

Sallé, G., Laing, R., Cotton, J.A., Maitland, K., Martinelli, A., Holroyd, N., Tracey, A., Berriman, M., Smith, W.D., Newlands, G.F.J., Hanks, E., Devaney, E., Britton, C.

Transcriptomic profiling of nematode parasites surviving vaccine exposure

(2018) *International Journal for Parasitology*, Article in Press.

DOI: 10.1016/j.ijpara.2018.01.004

Some nematode species are economically important parasites of livestock, while others are important human pathogens causing some of the most important neglected tropical diseases. In both humans and animals, anthelmintic drug administration is the main control strategy, but the emergence of drug-resistant worms has stimulated the development of alternative control approaches. Among these, vaccination is considered to be a sustainable and cost effective strategy. Currently, Barbervax® for the ruminant strongylid Haemonchus contortus is the only registered subunit vaccine for a nematode parasite, although a vaccine for the human hookworm Necator americanus is undergoing clinical trials (HOOKVAC consortium). As both these vaccines comprise a limited number of proteins, there is potential for selection of nematodes with altered sequences or expression of the vaccine antigens. Here we compared the transcriptome of H. contortus populations from sheep vaccinated with Barbervax® with worms from control animals. Barbervax® antigens are native integral membrane proteins isolated from the brush border of the intestinal cells of the adult parasite and many of those are proteases. Our findings provide no evidence for changes in expression of genes encoding Barbervax® antigens in the surviving parasite populations. However, surviving parasites from vaccinated animals showed increased expression of other proteases and regulators of lysosome trafficking, and displayed up-regulated lipid storage and defecation abilities that may have circumvented the effect of the vaccine. Implications for other potential vaccines for human and veterinary nematodes are discussed.

PGE Control

Charlier, J., Barkema, H.W.

DISCONTOOLS supplement: Current research gaps for advancing control of infectious diseases in production animals

(2018) *Transboundary and Emerging Diseases*, 65, pp. 5-8.

DISCONTOOLS (DISease CONtrol TOOLS) is an open-access database to assist public and private funders of animal health research in identifying research gaps and planning future research. This database is supported by a range of national funders of animal health research in Europe, with industry providing secretariat support. Information in the database is generated by disease-specific expert groups. In this DISCONTOOLS Supplement, contributing experts expanded their gap analyses into review papers for 15 diseases, covering zoonotic, production and epizootic diseases. Across this diverse array of diseases, it is clear that fundamental research on host-pathogen relationships and immune responses remains critical for evidence-based development of novel vaccines, pharmaceuticals and diagnostics to improve animal health. Furthermore, it is also obvious that there is need to better utilize economics and knowledge regarding “human factors” to optimise uptake and use of a broad range of tools and insights.

Charlier, J., Thamsborg, S.M., Bartley, D.J., Skuce, P.J., Kenyon, F., Geurden, T., Hoste, H., Williams, A.R., Sotiraki, S., Höglund, J., Chartier, C., Geldhof, P., van Dijk, J., Rinaldi, L., Morgan, E.R., von Samson-Himmelstjerna, G., Vercruysse, J., Claerebout, E.

Mind the gaps in research on the control of gastrointestinal nematodes of farmed ruminants and pigs

(2018) *Transboundary and Emerging Diseases*, 65, pp. 217-234.

Summary: Gastrointestinal (GI) nematode control has an important role to play in increasing livestock production from a limited natural resource base and to improve animal health and welfare. In this synthetic review, we identify key research priorities for GI nematode control in farmed ruminants and pigs, to support the development of roadmaps and strategic research agendas by governments, industry and policymakers. These priorities were derived from the DISCONTOOLS gap analysis for nematodes and follow-up discussions within the recently formed Livestock Helminth Research Alliance (LiHRA). In the face of ongoing spread of anthelmintic resistance (AR), we are increasingly faced with a failure of existing control methods against GI nematodes. Effective vaccines against GI nematodes are generally not available, and anthelmintic treatment will therefore remain a cornerstone for their effective control. At the same time, consumers and producers are increasingly concerned with environmental issues associated with chemical parasite control. To address current challenges in GI nematode control, it is crucial to deepen our insights into diverse aspects of epidemiology, AR, host immune mechanisms and the socio-psychological aspects of nematode control. This will enhance the development, and subsequent uptake, of the new diagnostics, vaccines, pharma-/nutraceuticals, control methods and decision support tools required to respond to the spread of AR and the shifting epidemiology of GI nematodes in response to climatic, land-use and farm husbandry changes. More emphasis needs to be placed on the upfront evaluation of the economic value of these innovations as well as the socio-psychological aspects to prioritize research and facilitate uptake of innovations in practice. Finally, targeted regulatory guidance is needed to create an innovation-supportive environment for industries and to accelerate the access to market of new control tools.

Laurenson, Y.C.S.M., Kahn, L.P.

A mathematical model to predict the risk arising from the pasture infectivity of four nematode species in Australia

(2018) *Animal Production Science*, 58 (8), pp. 1504-1514.

Gastrointestinal parasites cost the Australian sheep industry AU436 million annually. Early warning of impending worm risk may reduce this cost by providing producers with sufficient time to implement control strategies. A biophysical model was developed to simulate the on-pasture lifecycle stages of the four predominant nematode species in Australia (Haemonchus contortus, Teladorsagia circumcincta, Trichostrongylus colubriformis and Trichostrongylus vitrinus). The influence of climatic variables (temperature and water availability) on the survival, development and migration of each lifecycle stage was incorporated and parameterised to available point estimates (H. contortus: R 2 = 0.88, n = 1409- T. circumcincta: R 2 = 0.56, n = 243- T. colubriformis: R 2 = 0.61, n = 355- T. vitrinus: R 2 = 0.66, n = 147). Constant fecundities (eggs/worm.day) provided the daily quantity of eggs deposited per sheep (H. contortus = 3275- T. circumcincta = 140- T. colubriformis = 300- T. vitrinus = 300). Farm management practices were considered via the specification of stocking rates (sheep/ha), and the administration of anthelmintic treatments (reducing egg deposition by a defined efficacy and duration for each nematode species). Pasture infectivity per nematode species was calculated as the quotient of larvae on herbage and herbage availability (t/ha). Risk was calculated as the product of pasture infectivity and the potential productive impact of each nematode species (H. contortus = 3.9%- T. circumcincta = 9.22%- T. colubriformis = 9.31% T. vitrinus = 9.31%), and then summed across nematode species. This predictive model has been incorporated into the Sheep CRC's 'ASKBILL' application (www.askbill.com.au, verified 13 April 2018), which uses 90-day weather forecast data (5-km grid resolution) provided by the Australian Bureau of Meteorology. © 2018 CSIRO.

de Castro Oliveira, I., Vieira, Í.S., de Carvalho, L.M., Campos, A.K., Freitas, S.G., de Araujo, J.M., Braga, F.R., de Araújo, J.V.

Reduction of bovine strongilides in naturally contaminated pastures in the southeast region of Brazil. (2018) Experimental Parasitology, 194, pp. 9-15.

Biological control through the use of nematophagous fungi is a sustainable alternative for combatting helminthes in domestic animals and allows a reduction in the use of anthelmintics. The objective of this research was to evaluate the efficacy of the Arthrobotrys cladodes var macroides fungus in a pelleted formulation, based on sodium alginate and administered twice a week orally, as an alternative for the biological control of nematodes in field-grown young cattle. The experiment was conducted in a farm located in the municipality of Viçosa, MG, where 12 cattle, seven to nine months old, were allocated in two groups (treated group and control group) and distributed in pickets of Brachiaria decumbens, naturally infested with nematode larvae. The animals in the treated group received 1g of sodium alginate matrix pellets for every 10 kg of animal live weight, containing the nematophagous fungus Arthrobotrys cladodes var macroides and administered twice a week in conjunction with commercial feed. In the control group, each animal received 1 g of pellets for every 10 kg of animal live weight, without fungal mycelium added to the feed. Samples of feces and pastures were collected fortnightly for 12 months. The results showed that the most prevalent nematode genera in the coprocultures were Haemonchus sp., Cooperia sp. and Oesophagostomum sp., reflecting the results found in forage. The pasture that contained the animals that received feed with the fungus presented a reduction of 59% and 52% of larvae recovered at distances of 20 cm and 40 cm from the fecal pats, respectively. The mean number of eggs per gram of feces each month and animal body weight did not differ ($p > 0.05$) between the treated and control groups. Stool and soil samples from both groups were colonized by A. cladodes fungus and other fungi. Administration of Arthrobotrys cladodes var macroides mycelium by means of a sodium alginate matrix twice weekly reduced larval infestation of the surrounding pasture, indicating that this fungus may be a promising biological control of infecting forms of nematodes present in the environment.

Mendes, J.B., Cintra, M.C.R., Nascimento, L.V., De Jesus, R.M.M., Maia, D., Ostrensky, A., Teixeira, V.N., Sotomaior, C.S.

Effects of protein supplementation on resistance and resilience of lambs naturally infected with gastrointestinal parasites

(2018) *Semina:Ciencias Agrarias*, 39 (2), pp. 643-655.

In this study, the objective was to evaluate the ejficacy of protein supplementation on gastrointestinal nematode control in lambs naturally infected. Sixty weaned Ile de France and Texel crossbred lambs, with 60 days of age, were divided into three groups (n=20) according to the level of protein in the concentrate: low protein (LP; 8.5%), moderate protein (MP; 15%), and high protein (HP; 25%). The daily amount of concentrate offered was based on 3% of live weight, which was continued throughout the experiment of 98 days. Evaluations were conducted biweekly from days 0 (D0) to 98 (D98). There were no significant differences (p > 0.05) in the average weight among the LP, MP and HP groups, which was 22.0, 21.4, and 21.1 kg, respectively, on D0 and 46.4, 48.3, and 48.2 kg, respectively, on D98. The daily weight gain was not significantly different among the groups (LP, 250 g; MP and HP, 276 g; p > 0.05). The average egg count per gram of feces (epg) of the LP group on D98 (6, 765; an increase of 5, 690 epg from D0) was higher than that of the MP and HP groups (1, 617.5, p < 0.05 and 3, 435, p > 0.05, respectively), which remained constant throughout the study. The hematocrit values decreased (p < 0.05) from D0 to D98 only in the LP group. Only in the HP group (p < 0.05), the mean serum total protein concentration increased from D0 (5.3 g dL-1) to D98 (5.8 g dL-1). The average albumin level on D98 (2.10 g dL-1) was lower than that on D0 (2.52 g dL-1) in the LP group (p < 0.05). During the study, 10 lambs overall were treated with anthelmintic because the epg values exceeded 10, 000; six treated lambs were from the LP group, 3 from the HP group, and only 1 from the MP group. These results indicate that supplementation of diet with 15% protein increases the resistance and resilience of lambs to gastrointestinal parasites.

Lello, J., McClure, S.J., Tyrrell, K., Viney, M.E.

Predicting the effects of parasite co-infection across species boundaries

(2018) *Proceedings of the Royal Society B: Biological Sciences*, 285 (1874), art. no. 20172610.

DOI: 10.1098/rspb.2017.2610

It is normal for hosts to be co-infected by parasites. Interactions among co-infecting species can have profound consequences, including changing parasite transmission dynamics, altering disease severity and confounding attempts at parasite control. Despite the importance of co-infection, there is currently no way to predict how different parasite species may interact with one another, nor the consequences of those interactions. Here, we demonstrate a method that enables such prediction by identifying two nematode parasite groups based on taxonomy and characteristics of the parasitological niche. From an understanding of the interactions between the two defined groups in one host system (wild rabbits), we predict how two different nematode species, from the same defined groups, will interact in co-infections in a different host system (sheep), and then we test this experimentally. We show that, as predicted, in co-infections, the blood-feeding nematode Haemonchus contortus suppresses aspects of the sheep immune response, thereby facilitating the establishment and/or survival of the nematode Trichostrongylus colubriformis; and that the T. colubriformis-induced immune response negatively affects H.

contortus. This work is, to our knowledge, the first to use empirical data from one host system to successfully predict the specific outcome of a different co-infection in a second host species. The study therefore takes the first step in defining a practical framework for predicting interspecific parasite interactions in other animal systems.

Learmount, J., Callaby, R., Taylor, M.

An observational study of ewe treatments at lambing on early infection in lambs on UK sheep farms

(2018) *Veterinary Parasitology*, 253, pp. 55-59.

Anthelmintic treatments to ewes around lambing time, often with long acting products, have become common practice on UK sheep farms, but these treatments have also been shown to be highly selective for anthelmintic resistance in New Zealand and Australia, with field data supported by modelling results. The purpose of this study was to determine (1) the effect of treating or withholding anthelmintic treatments and (2) the effect of treatment of ewes with a persistent or non-persistent anthelmintic, on early infection in lambs in the UK. Faecal egg count data for 10–16 weeks old lambs collected over a three year period (2012–2014) was analysed. Samples were grouped according to whether the ewes on the farm had or had not been treated with an anthelmintic at lambing. For both analyses, data for early infection were analysed by generalised linear mixed model. There was no effect of withholding or treating ewes on subsequent early infection in lambs. In addition, there was no effect of region, farm type or management type on the faecal egg counts. There was, however an effect of year, with lambs having lower counts in 2014 than in 2012 and an interaction between year and ewe treatment, with data suggesting lower infection levels over time for those farms withholding anthelmintic treatments altogether. There was no effect of drug type on early infection in lambs nor region, farm type or treatment on the lamb faecal egg counts. However, there was an effect of year and an interaction between year and drug type with lower egg count over time with the short acting drugs. Our study supports data generated by other researchers suggesting that the practice of treating ewes at lambing to reduce contamination on pasture and minimise subsequent disease may not in fact always result in lower levels of infection in lambs. The study also demonstrated no significant benefit in early infection in lambs when ewes were treated with long acting compared to short acting anthelmintics. This provides further evidence to support the potential benefits of a more targeted approach to anthelmintic treatment on sheep farms.

Parasite immunity

Greer, A.W., McKenzie, J.L., McAnulty, R.W., Huntley, J.F., McNeilly, T.N.

Immune development and performance characteristics of Romney sheep selected for either resistance or resilience to gastrointestinal nematodes

(2018) *Veterinary Parasitology*, 250, pp. 60-67.

Immunological and performance characteristics were explored in Romney sheep from lines selected for either resistance or resilience to parasite infection. At a mean 78 days-of-age, twin lambs from a line selected for resistance (RT) and lambs from a line selected for resilience (RL) were infected with the intestinal nematode Trichostrongylus colubriformis for 100 days (I) while their twin remained as an uninfected control (C). Compared with RL, RT animals had lower levels of circulating CD4+ T-cells (P = 0.003) but a greater proportion of these were activated (CD4+CD25+) in response to infection (P = 0.007). Differences between the lines in humoral immune responses to nematode infection varied with higher levels of T. colubriformis specific immunoglobulin (Ig) E in RT-I than RL-I (P = 0.002) but similar levels of both IgG (P = 0.926) and IgA (P = 0.321) responses. Temporal differences in the immune response also existed between the lines with RT-I animals displaying an earlier peak and more rapid reduction in FEC and an earlier peak in T. colubriformis specific IgA. In addition, compared with their RT-C and RL-C counterparts, infection caused a 22% reduction in feed intake from day 56 (P = 0.001) with total feed intake reduced by 15% and 9% for RT-I and RL-I, respectively. Cumulative liveweight gain was greatest for RL animals (P = 0.026) and relative to RT-C and RL-C was reduced by 5.8 kg and 4.9 kg for RT-I and RL-I, respectively. Overall, the selection lines appear to have differences in immunological characteristics that are both dependent on, and independent of parasite infection. Further, the difference in growth in the uninfected animals coupled with the similar cost of infection suggests the lower liveweight gain of RT-I compared with RL-I may be due to inherent differences between the lines in their growth potential, rather than a greater cost of infection in animals selected for resistance.

Arif, R., Satrija, F., Winarto, A., Boediono, A., Manalu, W.

Production of lambs’ resilience to Haemonchus contortus

(2018) *Small Ruminant Research*, 167, pp. 110-116.

Haemonchus (H.) contortus infection in sheep is a major health problem in tropical and sub-tropical regions that cause great economic losses. Our previous studies have shown that improved uterine environments during pregnancy can improve postnatal growth and health performance of the offspring, indicated by lower mortality and morbidity. In the present experiment, we evaluated the resistance and resilience to H. contortus of lambs born to ewes injected with pregnant mare's serum gonadotropin (PMSG) prior to mating. Improvement of the uterine environment was conducted by increasing endogenous secretion of estrogen and progesterone as pregnancy hormones during pregnancy by injecting the ewes with PMSG prior to mating. A total of 16 lambs, regardless of sex, at the age of 5 months were assigned into a 2 × 2 factorial experiment with 4 replications. The first factor was PMSG injection, consisting of two levels, i.e., lambs born to ewes without PMSG injection (Non-PMSG lambs) and those born to PMSG-injected ewes (PMSG lambs). The second factor was the infection of lambs with H. contortus at the age of 5 months, consisting of two levels, i.e., lambs without infection (Non-infected lambs) and lambs infected with H. contortus (Infected lambs). Non-infected lambs were administered with distilled water in a capsule without infective larvae. Infected lambs were individually infected with a single dose containing 1200 L3 of H. contortus. Compared to non-PMSG lambs, PMSG lambs tended to have better prenatal growth indicated by greater birth weights (P = 0.06). The improved prenatal growth during pregnancy improved the postnatal growth and health performance of the lambs. Three months after infection of H. contortus, non-PMSG lambs and PMSG lambs had similar worm counts. However, the PMSG lambs showed significantly higher resilience to H. contortus as indicated by the lower fecal egg counts 6–10 weeks after infection. The higher resilience of the PMSG lambs was shown by the positive growth rate during infection, while non-PMSG lambs had a negative growth rate after infection. Prior to infection, PMSG lambs showed a higher segmented neutrophil percentage with lower lymphocyte numbers. Three months after infection, PMSG lambs had significantly higher lymphocyte and thrombocyte numbers as well as mean corpuscular hemoglobin concentration (MCHC) with lower neutrophil and monocyte numbers. The conclusion of this study is that the improvement of the uterine environment during pregnancy could be used to produce superior offspring with high resilience to the infection of H. contortus.

Haemonchus contortus

Doyle, S.R., Laing, R., Bartley, D.J., Britton, C., Chaudhry, U., Gilleard, J.S., Holroyd, N., Mable, B.K., Maitland, K., Morrison, A.A., Tait, A., Tracey, A., Berriman, M., Devaney, E., Cotton, J.A., Sargison, N.D.

A Genome Resequencing-Based Genetic Map Reveals the Recombination Landscape of an Outbred Parasitic Nematode in the Presence of Polyploidy and Polyandry

(2018) *Genome Biology and Evolution*, 10 (2), pp. 396-409.

The parasitic nematode Haemonchus contortus is an economically and clinically important pathogen of small ruminants, and a model system for understanding the mechanisms and evolution of traits such as anthelmintic resistance. Anthelmintic resistance is widespread and is a major threat to the sustainability of livestock agriculture globally; however, little is known about the genome architecture and parameters such as recombination that will ultimately influence the rate at which resistance may evolve and spread. Here, we performed a genetic cross between two divergent strains of H. contortus, and subsequently used whole-genome resequencing of a female worm and her brood to identify the distribution of genome-wide variation that characterizes these strains. Using a novel bioinformatic approach to identify variants that segregate as expected in a pseudotestcross, we characterized linkage groups and estimated genetic distances between markers to generate a chromosome-scale F 1 genetic map. We exploited this map to reveal the recombination landscape, the first for any helminth species, demonstrating extensive variation in recombination rate within and between chromosomes. Analyses of these data also revealed the extent of polyandry, whereby at least eight males were found to have contributed to the genetic variation of the progeny analyzed. Triploid offspring were also identified, which we hypothesize are the result of nondisjunction during female meiosis or polyspermy. These results expand our knowledge of the genetics of parasitic helminths and the unusual life-history of H. contortus, and enhance ongoing efforts to understand the genetic basis of resistance to the drugs used to control these worms and for related species that infect livestock and humans throughout the world. This study also demonstrates the feasibility of using whole-genome resequencing data to directly construct a genetic map in a single generation cross from a noninbred nonmodel organism with a complex lifecycle.

Baptistiolli, L., Narciso, L.G., De Almeida, B.F.M., Bosco, A.M., De Souza, J.C., Torrecilha, R.B.P., Pereira, P.P., Figueiredo, R.N., Garcia, J.F., Kaneto, C.N., Ciarlini, P.C.

Systemic oxidative stress in Suffolk and Santa Ines sheep experimentally infected with Haemonchus contortus

(2018) *Acta Parasitologica*, 63 (3), pp. 504-514.

The mechanisms responsible for the imbalance between oxidants and antioxidants in sheep infected with Haemonchus contortus are not well established. This study aimed to prove the hypothesis that oxidative stress occurring during infection by H. contortus varies according to breed, and that the parasite burden correlates with hypoalbuminaemia and anaemia. Thus, after deworming and confirming the absence of infection, two different sheep breeds, Suffolk (n = 15) and Santa Ines (n = 22), were orally inoculated with a single dose of 5,000 L3 of H. contortus. The egg counts per gram of faeces (EPG), packed cell volume (PCV) and concentrations of several plasma markers of oxidative stress (lipid peroxidation, albumin, uric acid, total bilirubin, total antioxidant capacity [TAC], total oxidant concentration [TOC] and the oxidative stress index [OSI]) were quantified before (control group) and during the experimental infection (28, 34 and 42 days post-inoculation). In both breeds, TOC increased at 28 days and TAC increased at 42 days. In Suffolk sheep, there was a positive correlation of EPG with oxidant components (28 days) and a negative correlation of EPG with PCV (42 days). In Santa Ines sheep, there was a positive correlation of EPG with bilirubin (r = 0.492; p = 0.020). H. contortus infection caused oxidative stress, which varied according to the breed. Parasite burden was not associated with hypoalbuminaemia, whereas there was a negative correlation with PCV. This research provides the first evidence that the antioxidant status contributes more to the resilience to H. contortus in Santa Ines sheep compared to Suffolk sheep.

Sinnathamby, G., Henderson, G., Umair, S., Janssen, P., Bland, R., Simpson, H.

The bacterial community associated with the sheep gastrointestinal nematode parasite Haemonchus contortus

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DOI: 10.1371/journal.pone.0192164

Culture-independent methods were used to study the microbiota of adult worms, third-stage larvae and eggs, both in faeces and laid in vitro, of Haemonchus contortus, a nematode parasite of the abomasa of ruminants which is a major cause of production losses and ill-health. Bacteria were identified in eggs, the female reproductive tract and the gut of adult and third-stage larvae (L3). PCR amplification of 16S rRNA sequences, denaturing gradient gel electrophoresis (DGGE) and clone libraries were used to compare the composition of the microbial communities of the different life-cycle stages of the parasites, as well as parasites and their natural environments. The microbiomes of adult worms and L3 were different from those in the abomasum or faeces respectively. The H. contortus microbiota was mainly comprised of members of the phyla Proteobacteria, Firmicutes and Bacteroidetes. Bacteria were localised in the gut, inside eggs and within the uterus of adult female worms using the universal FISH Eub338 probe, which targets most bacteria, and were also seen in these tissues by light and transmission electron microscopy. Streptococcus/Lactococcus sp. were identified within the distal uterus with the probe Strc493. Sequences from the genera Weissella and Leuconostoc were found in all life-cycle stages, except eggs collected from faeces, in which most sequences belonged to Clostridium sp. Bacteria affiliated with Weissella/Leuconostoc were identified in both PCR-DGGE short sequences and clone libraries of nearly full length 16S rRNA bacterial sequences in all life-cycle stages and subsequently visualised in eggs by fluorescent in situ hybridisation (FISH) with group-specific probes. This strongly suggests they are vertically transmitted endosymbionts. As this study was carried out on a parasite strain which has been maintained in the laboratory, other field isolates will need to be examined to establish whether these bacteria are more widely dispersed and have potential as targets to control H. contortus infections.

Vaccination

González-Sánchez, M.E., Cuquerella, M., Alunda, J.M.

Vaccination of lambs against Haemonchus contortus with the recombinant rHc23. Effect of adjuvant and antigen dose

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DOI: 10.1371/journal.pone.0193118

Haemonchus contortus is the most pathogenic gastrointestinal helminth of small ruminants. Natural or experimental repeated infections and several native antigens confer a partially protective immune response but vaccination with subunit antigens has been elusive. Promising results have been obtained with a recombinant form of a somatic antigen (rHc23). In this paper we present the results obtained in vaccination trials in lambs using two dosages of rHc23 and standard adjuvants. Six-months old Manchego females lambs were vaccinated with rHc23 (50 or 200 µg/dose) adjuvanted with 1mL aluminum hydroxide on days -42, -28 and -14 or with 200 µg/dose rHc23 and 5 mg Quil A on days -49, -28 and -7. Control lambs were kept receiving only the adjuvants the same days or no treatment. Moreover one group did not receive any treatment or infection. On day 0 vaccinated lambs, untreated animals and those receiving the adjuvant were infected per os with a monospecific single infection of 4000 L3 of H. contortus. Infection was kept for 58 days and follow-up included the determination of serum specific antibody respons (ELISA, WB), hematological parameters (eosinophil counts, hematocrit) and fecal egg counts (epg). Absence of hematocrit alterations, reduction of helminth's eggs output and abomasal parasite burden at the end of the experiment were the efficacy criteria of vaccination with the recombinant. Immunization with both adjuvants and antigen dosages elicited strong antibody responses particularly with Quil A. Vaccinated groups showed significant reduction of fecal egg excretion and abomasal helminth burdens. Highest protection of lambs against challenge was achieved with aluminum hydroxide and 200 µg/dose rHc23 with a reduction of over 70% of the abomasal burden and over 80% of fecal egg output. Results suggest that rHc23 could be a valuable recombinant candidate for vaccination against haemonchosis. No clear relationship was found between antibody levels and protection this pointing towards involvement of both humoral and cellular components in the protective response elicited by rHc23.

VanHoy, G., Carman, M., Habing, G., Lakritz, J., Hinds, C.A., Niehaus, A., Kaplan, R.M., Marsh, A.E.

Safety and serologic response to a Haemonchus contortus vaccine in alpacas

(2018) *Veterinary Parasitology*, 252, pp. 180-186.

Haemonchosis in camelids remains a challenging disease to treat, and prevention has become increasingly problematic due to widespread anthelmintic resistance. Barbervax® is an adjuvanted vaccine containing natural H-11, H-gal-GP antigens obtained from Haemonchus contortus adults via a proprietary process and solubilized in Quil A. This vaccine is approved for use in Australia, after demonstrating its safety and efficacy in sheep and goats. There are no published studies evaluating Barbervax in other ruminants/pseudoruminants such as camelids which can be parasitized with H. contortus. The vaccine utilizes a mixture of the parasite gut mucosal membrane enzymes including H-gal-GP and H11, involved in digesting a blood meal from the host. This study monitored the safety profile of the Barbervax® vaccine in a group of adolescent alpacas. Although designed into the original study of vaccine efficacy, the experimental infection with viable H. contortus third stage larvae could not be completed due to lack of detectable significant variation of infection following experimental challenge. Twelve alpacas (158 + 15 days) were randomized to vaccination with Barbervax® or no treatment. Three doses of Barbervax® were administered at 3 week intervals and investigators involved in animal monitoring and sample collection were blinded to the groupings. Clinical pathologic parameters were evaluated 7 days before vaccination, and 1 and 2 months post-vaccination. Daily clinical observations were made and specific observations regarding the injection site and rectal temperatures were monitored in each alpaca twice daily for 1 week following vaccination. Fecal egg counts, packed cell volume, and total protein were monitored following challenge with 1500 H. contortus larvae on days 42, 46, and 50. An increase in rectal temperature for a duration of 2 days (range 2–4 days) was observed post-vaccination. Vaccinated alpacas were lethargic for 2-3 days following vaccination; however, they maintained an appetite and no visible or palpable injection site reactions were observed. Following the first vaccination, all animals maintained normal clinical pathologic parameters throughout the study period. The vaccinated animals did develop titers to the H. contortus antigen as measured by ELISA. In conclusion, the Barbervax®

vaccine demonstrated safety in this small group of young, healthy alpacas, but additional studies are required to evaluate the efficacy of the vaccine under field conditions in protecting alpacas against infection with H. contortus.

Immunity

Garza, J.J., Greiner, S.P., Bowdridge, S.A.

Ovine vital neutrophil extracellular traps bind and impair Haemonchus contortus L3 in a breed-dependent manner

(2018) *Parasite Immunology*, 40 (9), art. no. e12572.

DOI: 10.1111/pim.12572

This study aimed to characterize neutrophil response to Haemonchus contortus (Hc) in vitro using cells from parasite-resistant St. Croix (STC) and parasite-susceptible Suffolk (SUF) sheep. Neutrophils from Hc-primed and naive STC and SUF sheep were incubated with Hc larval antigen (HcLA), Hc worm antigen (HcWA) or complete media (CM). After HcLA exposure, neutrophils from STC and SUF formed extracellular traps composed of DNA. Stimulation with HcLA induced a 35-fold increase in extracellular DNA compared to CM controls. However, extracellular DNA was not found when neutrophils were cultured with HcWA. The formation of neutrophil extracellular traps (NET) in response to HcLA yields a low percentage of necrotic cells indicating a form of vital NETosis. Neutrophils from primed and naïve STC bound Hc L3 greater (93% and 68%) than SUF (78% and 45%; $P < 0.001$). Furthermore, STC neutrophils significantly reduced larval ATP levels compared to SUF neutrophils (0.05 µmol/L vs 0.1 µmol/L ATP, $P < 0.001$). These data indicate that ovine neutrophils bind, form vital NET and reduce ATP to Hc L3 in a breed and infection status–dependent manner.

El-Ashram, S., Li, C., Abouhajer, F., Mehmood, R., Al Nasr, I., Zhang, Y., Lu, T., Yili, D., Suo, X., Haoji, Z., Li, Z., Huang, S.

An ex vivo abomasal ovine model to study the immediate immune response in the context of Haemonchus contortus larval-stage

(2018) *Veterinary Parasitology*, 254, pp. 105-113.

We have set up an ex vivo ovine abomasal model, which can mimic the multicellular process to explore the early steps in haemonchine nematode infection using RNA-seq technology. Ovine abomasal explants were collected for histological and transcriptional analysis and supernatants collected to quantitate lactate dehydrogenase (LDH) enzymes. Atotal of 233 were substantially induced genes between L4-inoculated and uninoculated-control tissues, respectively. However, a total of 14 were considerably down-regulated genes between the 51 aforementioned tissues. Fifteen pathways were annotated by Kyoto Encyclopedia of Genes, and Genomes pathway analysis accounted for the significant percentage in immediate response to larval-stage of H. contortus. Key genes upregulated in response to the addition of L4-inoculum of H. contortus were IL-6, IL-8, C1q, Atypical chemokine receptor-3, chemokine ligand-2, manganese superoxide dismutase, integrin alpha-7, -8, -9, integrin subunit beta-1, integrin subunit beta 6, intercellular adhesion molecule-1 and actin alpha-1. This study shows for the first time that galectin-1 is up-regulated in an ex vivo abomasal segment model exposed to L4-inoculum of H. contortus following 6 h of incubation. The abomasal segment model has been shown to be a suitable tool to study the haemonchine larval-stage effects on the ovine abomasal tissues prior to in vivo assessment.

Molecular studies

Habibi, S.A., Callanan, M., Forrester, S.G.

Molecular and pharmacological characterization of an acetylcholine-gated chloride channel (ACC-2) from the parasitic nematode Haemonchus contortus

(2018) *International Journal for Parasitology: Drugs and Drug Resistance*. Article in Press.

DOI:10.1016/j.ijpddr.2018.09.004

Nematode cys-loop ligand-gated ion channels (LGICs) have been shown to be attractive targets for the development of novel anti-parasitic drugs. The ACC-1 family of receptors are a unique group of acetylcholine-gated chloride channels present only in invertebrates, and sequence analysis suggests that they contain a novel binding site for acetylcholine. We have isolated a novel member of this family, Hco-ACC-2, from the parasitic nematode Haemonchus contortus and using site-directed mutagenesis, electrophysiology and molecular modelling examined how two aromatic amino acids in the binding site contributed to agonist recognition. It was found that instead of a tryptophan residue in binding loop B, which essential for ligand binding in mammalian nAChRs, there is a phenylalanine (F200) in Hco-ACC-2. Amino acid changes at F200 to either a tyrosine or tryptophan were fairly well tolerated, where a F200Y mutation resulted in a channel hypersensitive to ACh and nicotine as well as other cholinergic agonists such as carbachol and methacholine. In addition, both pyrantel and levamisole were partial agonists at the wild-type receptor and like the other agonists showed an increase in sensitivity at F200Y. On the other hand, in Hco-ACC-2 there is a tryptophan residue at position 248 in loop C that appears to be essential for receptor function, as mutations to either phenylalanine or tyrosine resulted in a marked decrease in agonist sensitivity. Moreover, mutations that swapped the residues F200 and W248 (ie. F200W/W248F) produced non-functional receptors. Overall, Hco-ACC-2 appears to have a novel cholinergic binding site that could have implications for the design of specific anthelmintics that target this family of receptors in parasitic nematodes.

Callanan, M.K., Habibi, S.A., Law, W.J., Nazareth, K., Komuniecki, R.L., Forrester, S.G.

Investigating the function and possible biological role of an acetylcholine-gated chloride channel subunit (ACC-1) from the parasitic nematode Haemonchus contortus

(2018) *International Journal for Parasitology: Drugs and Drug Resistance*. Article in Press.

DOI: 10.1016/j.ijpddr.2018.10.010

The cys-loop superfamily of ligand-gated ion channels are well recognized as important drug targets for many invertebrate specific compounds. With the rise in resistance seen worldwide to existing anthelmintics, novel drug targets must be identified so new treatments can be developed. The acetylcholine-gated chloride channel (ACC) family is a unique family of cholinergic receptors that have been shown, using Caenorhabditis elegans as a model, to have potential as anti-parasitic drug targets. However, there is little known about the function of these receptors in parasitic nematodes. Here, we have identified an acc gene (hco-acc-1) from the sheep parasitic nematode Haemonchus contortus. While similar in sequence to the previously characterized C. elegans ACC-1 receptor, Hco-ACC-1 does not form a functional homomeric channel in Xenopus oocytes. Instead, co-expression of Hco-ACC-1 with a previously characterized subunit Hco-ACC-2 produced a functional heteromeric channel which was 3x more sensitive to acetylcholine compared to the Hco-ACC-2 homomeric channel. We have also found that Hco-ACC-1 can be functionally expressed in C. elegans. Overexpression of both cel-acc-1 and hco-acc-1 in both C. elegans N2 and acc-1 null mutants decreased the time for worms to initiate reversal avoidance to octanol. Moreover, antibodies were generated against the Hco-ACC-1 protein for use in immunolocalization studies. Hco-ACC-1 consistently localized to the anterior half of the pharynx, specifically in pharyngeal muscle tissue in H. contortus. On the other hand, expression of Hco-ACC-1 in C. elegans was restricted to neuronal tissue. Overall, this research has provided new insight into the potential role of ACC receptors in parasitic nematodes.

Matoušková, P., Lecová, L., Laing, R., Dimunová, D., Vogel, H., Raisová Stuchlíková, L., Nguyen, L.T., Kellerová, P., Vokřál, I., Lamka, J., Szotáková, B., Várady, M., Skálová, L.

UDP-glycosyltransferase family in Haemonchus contortus: Phylogenetic analysis, constitutive expression, sex-differences and resistance-related differences.

(2018) International Journal for Parasitology: Drugs and Drug Resistance, 8 (3), pp. 420-429.

UDP-glycosyltransferases (UGT), catalysing conjugation of UDP-activated sugar donors to small lipophilic chemicals, are widespread in living organisms from bacteria to fungi, plant, or animals. The progress of genome sequencing has enabled an assessment of the UGT multigene family in Haemonchus contortus (family Trichostrongylidae, Nematoda), a hematophagous gastrointestinal parasite of small ruminants. Here we report 32 putative UGT genes divided into 15 UGT families. Phylogenetic analysis in comparison with UGTs from Caenorhabditis elegans, a free-living model nematode, revealed several single member homologues, a lack of the dramatic gene expansion seen in C. elegans, but also several families (UGT365, UGT366, UGT368) expanded in H. contortus only. The assessment of constitutive UGT mRNA expression in H. contortus adults identified significant differences between females and males. In addition, we compared the expression of selected UGTs in the drug-sensitive ISE strain to two benzimidazole-resistant strains, IRE and WR, with different genetic backgrounds. Constitutive expression of UGT368B2 was significantly higher in both resistant strains than in the sensitive strain. As resistant strains were able to deactivate benzimidazole anthelmintics via glycosylation more effectively then the sensitive strain, UGT368B2 enhanced constitutive expression might contribute to drug resistance in H. contortus.

Diagnosis

Ljungström, S., Melville, L., Skuce, P.J., Höglund, J.

Comparison of four diagnostic methods for detection and relative quantification of Haemonchus contortus eggs in feces samples

(2018) *Frontiers in Veterinary Science*, 4 (JAN), art. no. 239

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We compared four methods for identification of Haemonchus contortus eggs. With increased trade in animals within and between countries and continents, it has become important to correctly identify H. contortus eggs in fecal samples. To validate the outcome of diagnostic tests, sheep feces (n = 38) were collected from naturally infected flocks in Sweden. Subsamples were analyzed with (a) McMaster egg counting; (b) differential counting of eggs after staining with peanut agglutinin (PNA); (c) detection of DNA following amplification by real-time quantitative polymerase chain reaction (qPCR); and (d) loop-mediated isothermal amplification (LAMP). Differences between similar tests (microscopic and molecular) and SD (±SD) were analyzed with Bland-Altman plots and Spearman rank correlation. Strongylid egg counts ranged from 200 to 12,100 eggs per gram (epg) (mean epg ± SD = 1,278 ± 2,049). Microscopy showed presence of H. contortus eggs in 27 (73%) unstained samples and in 28 (76%) samples stained with PNA, whereas 29 samples (78%) tested positive in LAMP and 34 (91%) in qPCR analysis. The cycle threshold (Ct) values with LAMP ranged between 13 and 38 (mean ± SD = 21 ± 7), and those in qPCR between 25 and 49 (mean ± SD = 33 ± 6). In the LAMP and qPCR analyses, seven (19%) and three (8%) samples, respectively, had a cycle threshold (Ct) > 35, whereas no reactions were observed in eight (22%) and three (8%) samples, respectively. There was good agreement between the diagnostic tests based on microscopic examination and DNA detection, although the molecular tests were more sensitive. The bias between the microscopy methods (-4.2 ± 11) was smaller than for the molecular tests (-9.8 ± 10). The observed ranking in terms of test sensitivity was: McMaster counting by conventional microscopy < PNA < LAMP < qPCR. In conclusion, H. contortus can be identified by McMaster counting, without major mistakes regarding false positive results. However, molecular methods provide the capacity to diagnose H. contortus eggs with increased accuracy. This is essential when animals are investigated in quarantine or in studies evaluating anthelmintic treatment efficacy. These methods could also be applied to fecal samples from wildlife to investigate nematode transmission between wildlife and livestock.

Anthelmintics

Abongwa, M., Marjanovic, D.S., Tipton, J.G., Zheng, F., Martin, R.J., Trailovic, S.M., Robertson, A.P.

Monepantel is a non-competitive antagonist of nicotinic acetylcholine receptors from Ascaris suum and Oesophagostomum dentatum.

(2018) International Journal for Parasitology: Drugs and Drug Resistance, 8 (1), pp. 36-42.

Zolvix® is a recently introduced anthelmintic drench containing monepantel as the active ingredient. Monepantel is a positive allosteric modulator of DEG-3/DES-2 type nicotinic acetylcholine receptors (nAChRs) in several nematode species. The drug has been reported to produce hypercontraction of Caenorhabditis elegans and Haemonchus contortus somatic muscle. We investigated the effects of monepantel on nAChRs from Ascaris suum and Oesophagostomum dentatum heterologously expressed in Xenopus laevis oocytes. Using two-electrode voltage-clamp electrophysiology, we studied the effects of monepantel on a nicotine preferring homomeric nAChR subtype from A. suum comprising of ACR-16; a pyrantel/tribendimidine preferring heteromeric subtype from O. dentatum comprising UNC-29, UNC-38 and UNC-63 subunits; and a levamisole preferring subtype (O. dentatum) comprising UNC-29, UNC-38, UNC-63 and ACR-8 subunits. For each subtype tested, monepantel applied in isolation produced no measurable currents thereby ruling out an agonist action. When monepantel was continuously applied, it reduced the amplitude of acetylcholine induced currents in a concentration-dependent manner. In all three subtypes, monepantel acted as a non-competitive antagonist on the expressed receptors. ACR-16 from A. suum was particularly sensitive to monepantel inhibition (IC50 values: 1.6 ± 3.1 nM and 0.2 ± 2.3 μM). We also investigated the effects of monepantel on muscle flaps isolated from adult A. suum. The drug did not significantly increase baseline tension when applied on its own. As with acetylcholine induced currents in the heterologously expressed receptors, contractions induced by acetylcholine were antagonized by monepantel. Further investigation revealed that the inhibition was a mixture of competitive and non-competitive antagonism. Our findings suggest that monepantel is active on multiple nAChR subtypes.

Partridge, F.A., Brown, A.E., Buckingham, S.D., Willis, N.J., Wynne, G.M., Forman, R., Else, K.J., Morrison, A.A., Matthews, J.B., Russell, A.J., Lomas, D.A., Sattelle, D.B.

An automated high-throughput system for phenotypic screening of chemical libraries on C. elegans and parasitic nematodes

(2018) *International Journal for Parasitology: Drugs and Drug Resistance*, 8 (1), pp. 8-21.

Parasitic nematodes infect hundreds of millions of people and farmed livestock. Further, plant parasitic nematodes result in major crop damage. The pipeline of therapeutic compounds is limited and parasite resistance to the existing anthelmintic compounds is a global threat. We have developed an INVertebrate Automated Phenotyping Platform (INVAPP) for high-throughput, plate-based chemical screening, and an algorithm

(Paragon) which allows screening for compounds that have an effect on motility and development of parasitic worms. We have validated its utility by determining the efficacy of a panel of known anthelmintics against model and parasitic nematodes: Caenorhabditis elegans, Haemonchus contortus, Teladorsagia circumcincta, and Trichuris muris. We then applied the system to screen the Pathogen Box chemical library in a blinded fashion and identified compounds already known to have anthelmintic or anti-parasitic activity, including tolfenpyrad, auranofin, and mebendazole; and 14 compounds previously undescribed as anthelmintics, including benzoxaborole and isoxazole chemotypes. This system offers an effective, high-throughput system for the discovery of novel anthelmintics.

Hamel, D., Visser, M., Mayr, S., Rauh, R., Wang, H., Fankhauser, R., Rehbein, S.

Eprinomectin pour-on: Prevention of gastrointestinal and pulmonary nematode infections in sheep.

(2018) Veterinary Parasitology, 264, pp. 42-46.

The present study was conducted to further characterize the anthelmintic activity of the 0.5% w/v topical formulation of eprinomectin (EPRINEX® Pour-on, Merial) when administered at 1 mg/kg body weight to sheep in preventing the establishment of induced gastrointestinal and pulmonary nematode infections. Thirty-six female Merino sheep (~4 months of age, weighing 27.0–36.0 kg) were blocked by pre-treatment body weight to form blocks of four animals. Within blocks, the animals were randomly allocated to either remain untreated (control) or be treated once with EPRINEX® either on Day 0, Day 7 or Day 14. Starting on Day 15, the sheep were given trickle infections with infective larvae of seven species of gastrointestinal nematodes and Dictyocaulus filaria lungworms daily for seven consecutive days. Five weeks after completion of the daily challenge (Day 56), the animals were necropsied for parasite recovery and count. Treatment with EPRINEX® prevented the establishment (>90%, $p \leq 0.027$) of D. filaria, Teladorsagia circumcincta (pinnata/trifurcata), Cooperia curticei, Nematodirus battus, Trichostrongylus colubriformis and Oesophagostomum venulosum for at least 21 days, and of Haemonchus contortus and Trichostrongylus axei for at least 14 days. Sheep in the groups treated with EPRINEX® at Days 7 and 14 had significantly ($p \leq 0.018$) higher Day -1 to Day 56 wt gains than the untreated controls. No treatment-related health problems or any other health problems were observed throughout the study.

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., Jabbar, A., Wells, T.N.C., Palmer, M.J., Gasser, R.B., Baell, J.B.

Optimization of Novel 1-Methyl-1 H-Pyrazole-5-carboxamides Leads to High Potency Larval Development Inhibitors of the Barber's Pole Worm.

(2018) Journal of Medicinal Chemistry, 61 (23), pp. 10875-10894.

A phenotypic screen of a diverse library of small molecules for inhibition of the development of larvae of the parasitic nematode Haemonchus contortus led to the identification of a 1-methyl-1H-pyrazole-5-carboxamide derivative with an IC 50 of 0.29 µM. Medicinal chemistry optimization targeted modifications on the left-hand side (LHS), middle section, and right-hand side (RHS) of the scaffold in order to elucidate the structure-activity relationship (SAR). Strong SAR allowed for the iterative and directed assembly of a focus set of 64 analogues, from which compound 60 was identified as the most potent compound, inhibiting the development of the fourth larval (L4) stage with an IC 50 of 0.01 µM. In contrast, only 18% inhibition of the mammary epithelial cell line MCF10A viability was observed, even at concentrations as high as 50 µM.

Dilrukshi Herath, H.M.P., Song, H., Preston, S., Jabbar, A., Wang, T., McGee, S.L., Hofmann, A., Garcia-Bustos, J., Chang, B.C.H., Koehler, A.V., Liu, Y., Ma, Q., Zhang, P., Zhao, Q., Wang, Q., Gasser, R.B.

Arylpyrrole and fipronil analogues that inhibit the motility and/or development of Haemonchus contortus in vitro

(2018) International Journal for Parasitology: Drugs and Drug Resistance, 8 (3), pp. 379-385.

Due to widespread drug resistance in parasitic nematodes, there is a need to develop new anthelmintics. Given the cost and time involved in developing a new drug, the repurposing of known chemicals can be a promising, alternative approach. In this context, we tested a library (n = 600) of natural product-inspired pesticide analogues against exsheathed third stage-larvae (xL3s) of Haemonchus contortus (barber's pole worm) using a whole-organism, phenotypic screening technique that measures the inhibition of motility and development in treated larvae. In the primary screen, we identified 32 active analogues derived from chemical scaffolds of arylpyrrole or fipronil. The seven most promising compounds, selected based on their anthelmintic activity and/or limited cytotoxicity, are arylpyrroles that reduced the motility of fourth-stage larvae (L4s) with significant potency (IC50 values ranged from 0.04 ± 0.01 µM to 4.25 ± 0.82 µM, and selectivity indices ranged from 10.6 to 412.5). Since the parent structures of the active compounds are uncouplers of oxidative phosphorylation, we tested the effect of selected analogues on oxygen consumption in xL3s using the Seahorse XF24 flux analyser. Larvae treated with the test compounds showed a significant increase in oxygen consumption compared with the untreated control, demonstrating their uncoupling activity. Overall, the results of the present study have identified natural product-derived molecules that are worth considering for chemical optimisation as anthelmintic drug leads.

Sugawara, A., Kubo, M., Hirose, T., Yahagi, K., Tsunoda, N., Noguchi, Y., Nakashima, T., Takahashi, Y., Welz, C., Mueller, D., Mertens, C., Koebberling, J., Ōmura, S., Sunazuka, T.

Jietacins, azoxy antibiotics with potent nematocidal activity: Design, synthesis, and biological evaluation against parasitic nematodes

(2018) *European Journal of Medicinal Chemistry*, 145, pp. 524-538.

Jietacins, an azoxy antibiotic class of chemicals, were isolated from the culture broth of Streptomyces sp. KP-197. They have a unique structural motif, including a vinyl azoxy group and a long acyclic aliphatic chain, which is usually branched but non-branched in the case of jietacin C. During a drug discovery program, we found that jietacins display potent anthelmintic activity against parasitic nematodes and that jietacin A has a moderate or low acute toxicity (LD50 > 300 mg/kg) and no mutagenic potential in a mini Ames screen. This suggests that jietacins have potential for drug discovery research. In order to create a novel anthelmintic agent, we performed design, synthesis, and biological evaluation of jietacin derivatives against parasitic nematodes. Of these derivatives, we found that a fully synthesized simplified derivative exhibited better anthelmintic activity against three parasitic nematodes than natural jietacins. In addition, it had a better efficacy in vivo through oral administration against a mouse nematode. This indicated that the azoxy motif could prove useful as a template for anthelmintic discovery, possibly creating a class of anthelmintic with novel skeletons, a potential new mode of action, and providing further insight for rational drug design.

Anthelmintic resistance

Charvet, C.L., Guégnard, F., Courtot, E., Cortet, J., Neveu, C.

Nicotine-sensitive acetylcholine receptors are relevant pharmacological targets for the control of multidrug resistant parasitic nematodes

(2018) International Journal for Parasitology: Drugs and Drug Resistance, 8 (3), pp. 540-549.

The control of parasitic nematodes impacting animal health relies on the use of broad spectrum anthelmintics. However, intensive use of these drugs has led to the selection of resistant parasites in livestock industry. In that respect, there is currently an urgent need for novel compounds able to control resistant parasites. Nicotine has also historically been used as a de-wormer but was removed from the market when modern anthelmintics became available. The pharmacological target of nicotine has been identified in nematodes as acetylcholine-gated ion channels. Nicotinic-sensitive acetylcholine receptors (N-AChRs) therefore represent validated pharmacological targets that remain largely under-exploited. In the present study, using an automated larval migration assay (ALMA), we report that nicotinic derivatives efficiently paralyzed a multiple (benzimidazoles/levamisole/pyrantel/ivermectin) resistant field isolate of H. contortus. Using C. elegans as a model we confirmed that N-AChRs are preferential targets for nornicotine and anabasine. Functional expression of the homomeric N-AChR from C. elegans and the distantly related horse parasite Parascaris equorum in Xenopus oocytes highlighted some striking differences in their respective pharmacological properties towards nicotine derivative sensitivity. This work validates the exploitation of the nicotine receptors of parasitic nematodes as targets for the development of resistance-breaking compounds.

Cazajous, T., Prevot, F., Kerbiriou, A., Milhes, M., Grisez, C., Tropee, A., Godart, C., Aragon, A., Jacquiet, P.

Multiple-resistance to ivermectin and benzimidazole of a Haemonchus contortus population in a sheep flock from mainland France, first report.

(2018) Veterinary Parasitology: Regional Studies and Reports, 14, pp. 103-105.

Anthelmintic resistance was explored by fecal egg count reduction test in a sheep flock from the French Pyrenees at the request of the veterinary practitioner after a poor response to anthelmintics was noted. The FECRT confirmed the suspicion with a mean percentage of reduction in egg excretions of 45% (CI 95%: - 40 to 78.5) and 0% (CI95%: -162 to 49) within the ivermectin and the benzimidazole groups respectively. Haemonchus contortus was shown to be the IVM and BZ resistant species after morphological and molecular characterizations whereas Teladorsagia circumcincta was probably resistant to BZ only. The H. contortus population was still susceptible to moxidectin, closantel and levamisole. As this sheep flock is a transhumant flock, the spread of this multiple-resistant Haemonchus contortus population to the other sheep flocks sharing the same pastures in Pyrenean Mountains is highly likely. From the knowledge of the authors, this is the first report of multi-resistance to ivermectin and benzimidazole of a Haemonchus contortus population in mainland France.

Maté, L., Ballent, M., Cantón, C., Ceballos, L., Lifschitz, A., Lanusse, C., Alvarez, L., Liron, J.P.

Assessment of P-glycoprotein gene expression in adult stage of Haemonchus contortus in vivo exposed to ivermectin

(2018) Veterinary Parasitology, 264, pp. 1-7.

The efflux transporter P-glycoprotein (P-gp) has been implicated in multidrug resistance of different nematode parasites affecting livestock species. Increased expression of P-gp in nematodes after their in vitro as well as in vivo exposure to anthelmintics suggests a role of P-gp in drug resistance. The current study evaluated the P-gp gene expression in a highly-resistant isolate of the sheep nematode Haemonchus contortus, selected after exposure to ivermectin (IVM) treatments at 10-fold the therapeutic dose. Four lambs were artificially infected with L 3 (7000 L 3 /animal) of a previously selected IVM highly resistant H. contortus isolate. Forty five (45) days after infection, adult worms were collected at 0 (untreated), 6, 12 and 24 h post-oral IVM (2 mg/kg) administration. The relative transcription levels of different H. contortus P-gp genes were studied by quantitative real-time PCR (qPCR) and confirmed by RNA-seq. P-gp1 and P-gp11 gene expressions did not change throughout the experimental sampling period. P-gp3 and P-gp9.1 transcripts decreased significantly at both 12 and 24 h post IVM exposure. P-gp2 expression was progressively increased in a time-dependent manner at 1.81 (6 h), 2.08 (12 h) and 2.49 (24 h)-fold compared to adult worms not exposed (control 0 h) to IVM, although without reaching statistically significant differences (P > 0.05). P-gp12 was neither detected by qPCR nor by RNA-seq analysis. These relatively modest changes in the P-gp gene expression could not be enough to explain the high level of IVM resistance displayed by the H. contortus isolate under assessment. Overexpression of membrane drug transporters including P-gp has been associated with IVM resistance in different nematode parasites. However, some evidences suggest that resistance to IVM and other macrocyclic lactones may develop by multiple mechanisms. Further studies are needed to improve the understanding of resistance mechanisms in adult stages of H. contortus.

Cintra, M.C.R., Teixeira, V.N., Nascimento, L.V., Ollhoff, R.D., Sotomaior, C.S.

Monepantel resistant Trichostrongylus colubriformis in goats in Brazil

(2018) *Veterinary Parasitology: Regional Studies and Reports*, 11, pp. 12-14.

In a farm where Trichostrongylus colubriformis in sheep had been found resistant to monepantel (MNP), we aimed to evaluate the efficacy against gastrointestinal nematodes in a controlled efficacy test (CT) and a fecal egg count reduction test (FECRT) in goats. We used 15 (CT) and 30 goats (FECRT), naturally infected, and equally divided into control and treated groups. The efficacy of MNP in the CT against Haemonchus contortus, Trichostrongylus axei and Cooperia curticei was 100%. For T. colubriformis, the efficacy was 90.5% in goats treated with 2.5 mg/kg, and 88.1% in goats treated with 3.5 mg/kg. In the FECRT, the overall reduction was 91% (2.5 mg/kg) and 90% (3.75 mg/kg). In the fecal cultures, Trichostrongylus spp. larvae represented 25% (pre-treatment) and 15% (post-treatment) of the larvae in the control group, but they were 75% (2.5 mg/kg) and 98% (3.75 mg/kg) of the recovered larvae after MNP treatment. In accordance with published standards, and based on FECRT and CT, MNP was considered ineffective against T. colubriformis in the studied flock.

Pyziel, A.M., Björck, S., Wiklund, R., Skarin, M., Demiaszkiewicz, A.W., Höglund, J.

Gastrointestinal parasites of captive European bison Bison bonasus (L.) with a sign of reduced efficacy of Haemonchus contortus to fenbendazole

(2018) *Parasitology Research*, 117 (1), pp.295-302.

The history of European bison Bison bonasus Linnaeus, 1758 has been stormy since its extinction in the wild after the First World War. Due to the fact that the species was restored from just 12 founders, further expansion has suffered from low genetic variability, rendering the bison vulnerable to various pathogens due to inbreeding depression. Parasites are recognised as a key biological threat to bison population. Thus, parasitological examination including monitoring of the level of anthelmintic resistance in a herd should be a routine procedure involved in management and protection of European bison. This study was conducted in a group of 27 bison kept in a European bison breeding centre in Sweden. In April 2015, a faecal egg count reduction test (FECRT) was performed in animals with ≥ 100 gastrointestinal nematode (GIN) eggs per gram faeces, to determine effectiveness of fenbendazole (FBZ) treatment. Additionally, the third stage larvae were cultured for molecular examination by a conventional PCR as well as by real-time quantitative PCR (q-PCR) for detection of the blood-sucking nematode Haemonchus contortus. Faecal sampling was conducted 1 day before and 8 days after deworming each animal. Anthelmintic treatment turned to be entirely efficient toward intestinal nematodes of genera Nematodirus and Trichuris, whereas shedding of strongylid eggs from the subfamily Ostertagiinae was reduced from 81 to 30%. Polymerase chain reaction (PCR) on cultured third-stage larvae (L3) before treatment was positive for H. contortus, Ostertagia ostertagi and Cooperia oncophora, whereas post-treatment examination revealed exclusively the DNA of H. contortus. Thus, only H. contortus was involved in post-treatment faecal egg count (FEC). FECRT showed that the reduction in strongylid FEC to FBZ in the examined bison herd was 87% (95%-confidence intervals [95% CI] = 76–93), suggesting reduced efficacy of FBZ to strongylid GIN including mainly H. contortus.

Page, A.P.

The sensory amphidial structures of Caenorhabditis elegans are involved in macrocyclic lactone uptake and anthelmintic resistance

(2018) *International Journal for Parasitology*, 48 (13), pp. 1035-1042.

Parasitic nematodes represent formidable pathogens of humans, livestock and crop plants. Control of these parasites is almost exclusively dependent on a small group of anthelmintic drugs, the most important of which belong to the macrocyclic lactone class. The extensive use of these drugs to control the ubiquitous trichostrongylid parasites of grazing livestock has resulted in the emergence of both single and multi-drug resistance. The expectation is that this resistance will eventually occur in the human parasites such as the common and debilitating soil transmitted nematodes and vector-borne filarial nematodes. While the modes of action of anthelmintics such as ivermectin, have been elucidated, notably in the model nematode Caenorhabditis elegans, the molecular nature of this resistance remains to be fully determined. Here we show that the anterior amphids play a key role in ivermectin uptake and mutations in these sensory structures result in ivermectin resistance in C. elegans. Random genetic mutant screens, detailed analysis of existing amphid mutants and lipophilic dye uptake indicate that the non-motile ciliated amphid neurons are a major route of ivermectin ingress; the majority of the mutants characterised in this study are predicted to be involved in intraflagellar transport. In addition to a role in ivermectin resistance, a subset of the amphid mutants are resistant to the non-related benzimidazole class of anthelmintics, raising the potential link to a multi-drug resistance mechanism. The amphid structures are present in all nematodes and are clearly defined in a drug-sensitive strain of Haemonchus contortus. It is predicted that amphidial drug uptake and intraflagellar transport may prove to be significant in the development of single and multi-drug resistance in the nematode pathogens of veterinary and human importance.

Kotze, A.C., Ruffell, A., Lamb, J., Elliott, T.P.

Response of drug-susceptible and -resistant Haemonchus contortus larvae to monepantel and abamectin alone or in combination in vitro

(2018) *Veterinary Parasitology*, 249, pp. 57-62.

There is an increasing interest in the use of combination anthelmintic products for the control of intestinal nematode parasites of livestock. These products are seen as attractive options for parasite control in the face of increasing levels of resistance to the different anthelmintic drug classes, as well as a means to slow the rate at which resistance develops to the individual components of the combination. With the recent introduction of an anthelmintic combination product containing abamectin and monepantel (at 1:12.5), we were interested in measuring the response of drug-susceptible and drug-resistant isolates of Haemonchus contortus to these two drugs alone and in combination, using larval development assays. The GWBII isolate showed resistance to abamectin (12-fold) alongside susceptibility to monepantel. The resistance ratio was reduced from 12- to 3.2-fold when the two drugs were combined. The MPL-R isolate was resistant to both drugs, with resistance factors of 6-fold towards abamectin, and 10.6- and 1008-fold towards monepantel in two sub-populations present in the isolate. This isolate showed 6.4-fold resistance to the drug combination. Hence, for both GWBII and MPL-R, the level of resistance towards the combination was reduced compared to the resistance towards abamectin or monepantel alone, respectively, but was not abolished. However, for GWBII, this in vitro resistance to the drug combination would be expected to have no impact on the in vivo efficacy of the combination drench product as the isolate is resistant to only the abamectin component of the drench, with monepantel remaining effective. On the other hand, the observed in vitro resistance to the combination shown by the MPL-R isolate is derived from significant levels of resistance towards both components separately, and hence may impact on in vivo efficacy of the combination. Isobologram analysis did not find any evidence for a synergistic interaction between the two drugs in larval development assays. We examined the predicted effects of varying the abamectin:monepantel ratio in drug combinations, assuming that the two drugs acted in an additive fashion. For GWBII, resistance to the drug combination was reduced to almost zero as the abamectin:monepantel ratio increased from 1:12.5 to 1:100, reflecting its resistance to only the abamectin component of the combination. For MPL-R, on the other hand, the resistance increased as the relative proportion of monepantel in the combination was increased, reflecting the extreme level of in vitro resistance shown by this isolate to monepantel.

Pacheco, P.A., Rodrigues, L.N.C., Ferreira, J.F.S., Gomes, A.C.P., Veríssimo, C.J., Louvandini, H., Costa, R.L.D., Katiki, L.M.

Inclusion complex and nanoclusters of cyclodextrin to increase the solubility and efficacy of albendazole

(2018) *Parasitology Research*, 117 (3), pp. 705-712.

Albendazole (ABZ), a benzimidazole widely used to control gastrointestinal parasites, is poorly soluble in water, resulting in variable and incomplete bioavailability. This has favored the appearance ABZ-resistant nematodes and, consequently, an increase in its clinical ineffectiveness. Among the pharmaceutical techniques developed to increase drug efficacy, cyclodextrins (CDs) and other polymers have been extensively used with water-insoluble pharmaceutical drugs to increase their solubility and availability. Our objective was to prepare ABZ formulations, including β-cyclodextrin (βCD) or hydroxypropyl-β-cyclodextrin (HPβCD), associated or not to the water-soluble polymer polyvinylpyrrolidone (PVP). These formulations had their solubility and anthelmintic effect both evaluated in vitro. Also, their anthelmintic efficacy was evaluated in lambs naturally infected with gastrointestinal nematodes (GIN) through the fecal egg count (FEC) reduction test. In vitro, the complex ABZ/HPβCD had higher solubility than ABZ/βCD. The addition of PVP to the complexes increased solubility and dissolution rates more effectively for ABZ/HPβCD than for ABZ/βCD. In vivo, 48 lambs naturally infected with GIN were divided into six experimental groups: control, ABZ, ABZ/βCD, ABZ/βCD-PVP, ABZ/HPβCD, and ABZ/HPβCD-PVP. Each treated animal received 10 mg/kg of body weight (based on the ABZ dose) for three consecutive days. After 10 days of the last administered dose, treatment efficacy was calculated. The efficacy values were as follows: ABZ (70.33%), ABZ/βCD (85.33%), ABZ/βCD-PVP (82.86%), ABZ/HPβCD (78.37%), and ABZ/HPβCD-PVP (43.79%). In vitro, ABZ/HPβCD and ABZ/HPβCD-PVP had high solubility and dissolution rates. In vivo, although the efficacies of ABZ/βCD, ABZ/βCD-PVP, and ABZ/HPβCD increased slightly when compared to pure ABZ, this increase was not significant (P CloseSPigtSPi 0.05).

David, M., Lebrun, C., Duguet, T., Talmont, F., Beech, R., Orlowski, S., André, F., Prichard, R.K., Lespine, A.

Structural model, functional modulation by ivermectin and tissue localization of Haemonchus contortus P-glycoprotein-13

(2018) *International Journal for Parasitology: Drugs and Drug Resistance*, 8 (1), pp. 145-157.

Haemonchus contortus, one of the most economically important parasites of small ruminants, has become resistant to the anthelmintic ivermectin. Deciphering the role of P-glycoproteins in ivermectin resistance is desirable for understanding and overcoming this resistance. In the model nematode, Caenorhabditis elegans, P-glycoprotein-13 is expressed in the amphids, important neuronal structures for ivermectin activity. We have focused on its ortholog in the parasite, Hco-Pgp-13. A 3D model of Hco-Pgp-13, presenting an open inward-facing conformation, has been constructed by homology with the Cel-Pgp-1 crystal structure. In silico docking calculations predicted high affinity binding of ivermectin and actinomycin D to the inner chamber of the protein. Following in vitro expression, we showed that ivermectin and actinomycin D modulated Hco-Pgp-13 ATPase activity with high affinity. Finally, we found in vivo Hco-Pgp-13 localization in epithelial, pharyngeal and neuronal tissues. Taken together, these data suggest a role for Hco-Pgp-13 in ivermectin transport, which could contribute to anthelmintic resistance.

Turnbull, F., Jonsson, N.N., Kenyon, F., Skuce, P.J., Bisset, S.A.

P-glycoprotein-9 and macrocyclic lactone resistance status in selected strains of the ovine gastrointestinal nematode, Teladorsagia circumcincta

(2018) *International Journal for Parasitology: Drugs and Drug Resistance*, 8 (1), pp. 70-80.

The Teladorsagia circumcincta P-glycoprotein-9 (Tci-pgp-9) gene has previously been implicated in multiple-anthelmintic resistance in this parasite. Here we further characterise genetic diversity in Tci-pgp-9 and its possible role in ivermectin (IVM) and multi-drug resistance using two UK field isolates of T. circumcincta, one susceptible to anthelmintics (MTci2) and the other resistant to most available anthelmintics including IVM (MTci5). A comparison of full-length Tci-pgp-9 cDNA transcripts from the MTci2 and MTci5 isolates (~3.8 kb in both cases) indicated that they shared 95.6% and 99.5% identity at the nucleotide and amino acid levels, respectively. Nine non-synonymous SNPs were found in the MTci5 sequences relative to their MTci2 counterparts. Twelve genomic sequence variants of the first internucleotide binding domain of Tci-pgp-9 were identified and up to 10 of these were present in some individual worms, strongly supporting previous evidence that amplification of this gene has occurred in T. circumcincta. On average, fewer distinct sequence variants of Tci-pgp-9 were present in individual worms of the MTci5 isolate than in those of the MTci2 isolate. A further reduction in the number of sequence variants was observed in individuals derived from an IVM-treated sub-population of MTci5. These findings suggest that Tci-pgp-9 was under purifying selection in the face of IVM treatment in T. circumcincta, with some sequence variants being selected against.

Stuchlíková, L.R., Matoušková, P., Vokřál, I., Lamka, J., Szotáková, B., Sečkařová, A., Dimunová, D., Nguyen, L.T., Várady, M., Skálová, L.

Metabolism of albendazole, ricobendazole and flubendazole in Haemonchus contortus adults: Sex differences, resistance-related differences and the identification of new metabolites

(2018) *International Journal for Parasitology: Drugs and Drug Resistance*, 8 (1), pp. 50-58.

Haemonchus contortus (family Trichostrongylidae, Nematoda), a hematophagous gastrointestinal parasite found in small ruminants, has a great ability to develop resistance to anthelmintic drugs. We studied the biotransformation of the three benzimidazole anthelmintics: albendazole (ABZ), ricobendazole (albendazole S-oxide; RCB) and flubendazole (FLU) in females and males of H. contortus in both a susceptible ISE strain and resistant IRE strain. The ex vivo cultivation of living nematodes in culture medium with or without the anthelmintics was used. Ultrasensitive UHPLC/MS/MS analysis revealed 9, 7 and 12 metabolites of ABZ, RCB and FLU, respectively, with most of these metabolites now described in the present study for the first time in H. contortus. The structure of certain metabolites shows the presence of biotransformation reactions not previously reported in nematodes. There were significant qualitative and semi-quantitative differences in the metabolites formed by male and female worms. In most cases, females metabolized drugs more extensively than males. Adults of the IRE strain were able to form many more metabolites of all the drugs than adults of the ISE strain. Some metabolites were even found only in adults of the IRE strain. These findings suggest that increased drug metabolism may play a role in resistance to benzimidazole drugs in H. contortus.

Muchiut, S.M., Fernández, A.S., Steffan, P.E., Riva, E., Fiel, C.A.

Anthelmintic resistance: Management of parasite refugia for Haemonchus contortus through the replacement of resistant with susceptible populations

(2018) *Veterinary Parasitology*, 254, pp. 43-48.

Sheep production in tropical and temperate regions is hampered by the presence of Haemonchus contortus, the blood-sucking nematode that is the major cause of economic losses in small ruminant enterprises. The most limiting factor in the control of this parasitic disease is the steady progress of anthelmintic resistance worldwide. The search for control strategies that minimise the use of anthelmintics is therefore central to various efforts worldwide. One strategy is the introduction of susceptible parasites in refugia when these refugia are at low levels. This strategy could lead to a renewed possibility anthelmintics being effective. At farm level, this management practice could recover the use of anthelmintics in flocks with high levels of resistance. This review explores the possibility of replacing resistant H. contortus populations with susceptible ones through refugia management and. highlights the experiences of on-farm research attempts carried out in different geographical areas, reaching various degrees of success.

Babják, M., Königová, A., Urda Dolinská, M., Vadlejch, J., Várady, M.

Anthelmintic resistance in goat herds—In vivo versus in vitro detection methods

(2018) *Veterinary Parasitology*, 254, pp. 10-14.

Anthelmintic resistance (AR) is a serious threat to animal health and has a major economic impact worldwide due to production and financial losses. The aim of this study was to determine the occurrence of AR on 30 goat farms in Slovakia during the pasturing seasons and to compare three widely used in vitro and in vivo methods for detecting AR in field conditions. A three-year survey was conducted during the pasturing seasons of 2014–2016. Goats on each farm were split into treated and control groups and were treated by recommended (5 mg/kg body weight) and double doses (10 mg/kg b.w.) of albendazole. Comparisons between percent reduction in a faecal egg count reduction test (FECRT) and an egg hatch test (EHT) and the presence of L3 larvae in a larval development test (LDT) using resistant concentrations of benzimidazole (BZ) were monitored after treatment. The FECRT indicated percent reductions of 69.2–86.2% for the single dose and of 36.3–45.4% for the double dose. The EHT indicated that all farms had BZ-resistant nematodes. Low (<15% hatching) and high (>15% hatching) levels of resistance were detected on 13 and 17 farms, respectively. The LDT failed to detect resistant larvae on seven farms but detected low and high levels of resistance on seven and 14 farms, respectively. The data indicate a moderate correlation between in vitro and in vivo tests for detecting BZ resistance among the 30 goat farms. The hatching detected by the EHT and the presence of L3 larvae by the LDT at resistant BZ concentrations provided reasonable identification of low levels of resistance in the parasite populations, but the use of a double dose for a treatment may underestimate the real occurrence of low levels of resistant parasites on goat farms.

Hamer, K., Bartley, D., Jennings, A., Morrison, A., Sargison, N.

Lack of efficacy of monepantel against trichostrongyle nematodes in a UK sheep flock

(2018) *Veterinary Parasitology*, 257, pp. 48-53.

Monepantel resistance was diagnosed during routine monitoring of the effectiveness of a farm's roundworm control strategy. Weaned lambs had become ill thrifty and developed diarrhoea, despite the routine use of monepantel. This clinical presentation was caused by trichostrongylosis. The faecal egg count reduction was 76.7% (95% CI: 55.1–82.2%) following treatment with 2.5 mg/kg monepantel. Predominantly Trichostrongylus vitrinus along with small proportions of Oesophagostomum venulosum and Trichostrongylus vitrinus were identified by deep amplicon sequencing of pools of larvae recovered from pre and post monepantel treatment coprocultures and on postmortem examinations. The undifferentiated FECRT showed resistance to monepantel, but not to levamisole, ivermectin, or moxidectin. Examination of farm anthelmintic treatment and animal movement records suggested that treatments before movement onto silage aftermaths, putatively with low numbers of susceptible nematodes in refugia, may have placed a high selection pressure on monepantel resistance. Effective control of parasitic gastroenteritis using anthelmintic drugs is a prerequisite for sustainable sheep production. This case reiterates the need for care when combining anthelmintic treatments with movements to safe grazing, and the value of monitoring of anthelmintic efficacy as part of iterative planned animal health management.

George, M.M., Lopez-Soberal, L., Storey, B.E., Howell, S.B., Kaplan, R.M.

Motility in the L3 stage is a poor phenotype for detecting and measuring resistance to avermectin/milbemycin drugs in gastrointestinal nematodes of livestock

(2018) *International Journal for Parasitology: Drugs and Drug Resistance*, 8 (1), pp.22-30.

Motility is a commonly used in vitro phenotype for assessing anthelmintic activity of candidate compounds, and for detecting anthelmintic resistance in nematodes. Third-stage larvae (L3) of parasitic nematodes are commonly used in motility-based assays because L3 are simple to obtain and can remain viable in storage for extended periods. To improve the measurement of motility of microscopic stages of nematodes, our laboratory developed the Worminator, which quantitatively measures motility of parasites. Using the Worminator, we compared the dose-response characteristics of several avermectin/milbemycin (AM) compounds using L3 from both AM-susceptible and AM-resistant Cooperia spp. (abamectin, doramectin, eprinomectin, ivermectin, moxidectin) and Haemonchus contortus (eprinomectin, ivermectin, moxidectin). Concentrations tested with the Worminator ranged from 0.156 to 40 μM. Differences in EC50 between AM-susceptible and AM-resistant isolates of Cooperia spp. and Haemonchus contortus were small, with resistance ratios ranging from 1.00 to 1.34 for Cooperia spp., 0.99 to 1.65 for Haemonchus contortus. Larval migration inhibition assays were conducted using the same isolates and were equally ineffective for detection of resistance with resistance ratios less than 2.0. These results contrast with those of the Larval Development Assay where we obtained a resistance ratio of 16.48 using the same isolates of Haemonchus contortus. Moreover, even at the highest concentration tested (40 μM), 100% inhibition of motility was never achieved and EC50 for Worminator assays were more than 100× higher than peak plasma levels achieved in vivo following treatment. These data demonstrate that dose-response characteristics for inhibition of motility in L3 of gastrointestinal nematodes of livestock do not significantly differ for AM-susceptible and AM-resistant isolates. These data challenge the suitability of motility as a phenotype for detecting and measuring resistance to AM drugs in gastrointestinal nematodes of livestock.

Fontaine, P., Choe, K.

The transcription factor SKN-1 and detoxification gene ugt-22 alter albendazole efficacy in Caenorhabditis elegans

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Parasitic nematodes infect over 1/4 th of the human population and are a major burden on livestock and crop production. Benzimidazole class anthelmintics are widely used to treat infections, but resistance is a widespread problem. Mutation of genes encoding the benzimidazole target β-tubulin is a well-established mechanism of resistance, but recent evidence suggests that metabolism of the drugs may also occur. Our objective was to investigate contributions of the detoxification-response transcription factor SKN-1 to anthelmintic drug resistance using C. elegans. We find that skn-1 mutations alter EC50 of the common benzimidazole albendazole in motility assays by 1.5–1.7 fold. We also identify ugt-22 as a detoxification gene associated with SKN-1 that influences albendazole efficacy. Mutation and overexpression of ugt-22 alter albendazole EC50 by 2.3–2.5-fold. The influence of a nematode UGT on albendazole efficacy is consistent with recent studies demonstrating glucose conjugation of benzimidazoles.

Elmahalawy, S.T., Halvarsson, P., Skarin, M., Höglund, J.

Genetic variants in dyf-7 validated by droplet digital PCR are not drivers for ivermectin resistance in Haemonchus contortus

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Resistance to ivermectin (IVM) in the nematode Haemonchus contortus in small ruminants is an increasing problem throughout the world. Access to molecular diagnostics will allow early detection of IVM resistance, which in turn can limit the spread of resistant isolates. One candidate gene which has recently been suggested as a marker for IVM resistance is that for dye-filling protein (dyf-7). In this study, we critically investigated the suitability of A141G and G153T single nucleotide polymorphisms (SNP) of dyf-7 as a marker in larval cultures collected from sheep farms in Sweden, involving several isolates for which resistance status had been characterised by the faecal egg count reduction test (FECRT). Initially, we designed dyf-7 primers from a worldwide collection of adult Haemonchus contortus DNA. With the sequence data, we created a haplotype network. We then optimised and used the same sets of primers and probes in a droplet digital PCR (ddPCR) assay for precise quantification of dyf-7 allele frequencies in pre- and post-anthelmintic treatment faecal larval cultures. The fractional abundance (FA) of the mutant SNP was within the range 7.8 and 31%. However, the FA was generally stable in samples collected from the same farms, even though they were obtained on different occasions up to 25 months apart. There was also no indication that the level of IVM resistance as measured by the faecal egg count reduction test was higher on farms with high FA. Furthermore, by comparing FA in samples from the same farms pre- and post-IVM treatment, we found no evidence of a correlation between dyf-7 and level of IVM resistance. Based on these results, dyf-7 is not a suitable marker for field testing of IVM resistance in H. contortus.

Cazajous, T., Prevot, F., Kerbiriou, A., Milhes, M., Grisez, C., Tropee, A., Godart, C., Aragon, A., Jacquiet, P.

Multiple-resistance to ivermectin and benzimidazole of a Haemonchus contortus population in a sheep flock from mainland France, first report

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Anthelmintic resistance was explored by fecal egg count reduction test in a sheep flock from the French Pyrenees at the request of the veterinary practitioner after a poor response to anthelmintics was noted. The FECRT confirmed the suspicion with a mean percentage of reduction in egg excretions of 45% (CI 95%: - 40 to 78.5) and 0% (CI95%: -162 to 49) within the ivermectin and the benzimidazole groups respectively. Haemonchus contortus was shown to be the IVM and BZ resistant species after morphological and molecular characterizations whereas Teladorsagia circumcincta was probably resistant to BZ only. The H. contortus population was still susceptible to moxidectin, closantel and levamisole. As this sheep flock is a transhumant flock, the spread of this multiple-resistant Haemonchus contortus population to the other sheep flocks sharing the same pastures in Pyrenean Mountains is highly likely. From the knowledge of the authors, this is the first report of multi-resistance to ivermectin and benzimidazole of a Haemonchus contortus population in mainland France.

Candy, P.M., Waghorn, T.S., Miller, C.M., Ganesh, S., Leathwick, D.M.

The effect on liveweight gain of using anthelmintics with incomplete efficacy against resistant Cooperia oncophora in cattle

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A replicated field trial was conducted to measure the effect on liveweight gain of failing to adequately control anthelmintic resistant populations of Cooperia oncophora and to determine whether populations, and hence production losses, increased with time. Eight mobs of 10 Friesian-Hereford calves were run on independent farmlets from January to December, over each of two years. All mobs were routinely treated with a pour-on formulation of eprinomectin every six weeks, which controlled parasites other than

Cooperia. Four mobs also received six weekly treatments with an oral levamisole plus albendazole combination anthelmintic to control Cooperia. Liveweights, condition scores, faecal egg counts and larval numbers on pasture were measured throughout. In the first year animals treated with eprinomectin alone were 12.9 kg lighter in November than those treated with eprinomectin plus albendazole and levamisole, however, in the second year there was no difference between the treatment groups. The data, therefore, support the view that while C. oncophora is less pathogenic than other cattle parasite species it can still cause production losses when present in sufficient numbers. In the first year of the study, parasite load, as measured by faecal nematode egg count and larval numbers on herbage, tended to be higher and calf growth rates lower than in the second year. In both years, counts of infective larvae on herbage declined over winter–spring to be at low levels before mid-summer. This suggests that the carry-over of infection from one crop of calves to the next was relatively small and hence that the level of challenge to the young calves at the start of each year was largely due to the effectiveness of the quarantine treatments administered when the animals arrived on the trial site. Low survival of larvae on pasture between grazing seasons, resulting in small larval populations on pasture when drenching programmes start each summer, might help to explain the widespread development of anthelmintic resistance in this parasite under New Zealand grazing systems.

Blanchard, A., Guégnard, F., Charvet, C.L., Crisford, A., Courtot, E., Sauvé, C., Harmache, A., Duguet, T., O'Connor, V., Castagnone-Sereno, P., Reaves, B., Wolstenholme, A.J., Beech, R.N., Holden-Dye, L., Neveu, C.

Deciphering the molecular determinants of cholinergic anthelmintic sensitivity in nematodes: When novel functional validation approaches highlight major differences between the model Caenorhabditis elegans and parasitic species

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Cholinergic agonists such as levamisole and pyrantel are widely used as anthelmintics to treat parasitic nematode infestations. These drugs elicit spastic paralysis by activating acetylcholine receptors (AChRs) expressed in nematode body wall muscles. In the model nematode Caenorhabditis elegans, genetic screens led to the identification of five genes encoding levamisole-sensitive-AChR (L-AChR) subunits: unc-38, unc-63, unc-29, lev-1 and lev-8. These subunits form a functional L-AChR when heterologously expressed in Xenopus laevis oocytes. Here we show that the majority of parasitic species that are sensitive to levamisole lack a gene orthologous to C. elegans lev-8. This raises important questions concerning the properties of the native receptor that constitutes the target for cholinergic anthelmintics. We demonstrate that the closely related ACR-8 subunit from phylogenetically distant animal and plant parasitic nematode species functionally substitutes for LEV-8 in the C. elegans L-AChR when expressed in Xenopus oocytes. The importance of ACR-8 in parasitic nematode sensitivity to cholinergic anthelmintics is reinforced by a 'model hopping' approach in which we demonstrate the ability of ACR-8 from the hematophagous parasitic nematode Haemonchus contortus to fully restore levamisole sensitivity, and to confer high sensitivity to pyrantel, when expressed in the body wall muscle of C. elegans lev-8 null mutants. The critical role of acr-8 to in vivo drug sensitivity is substantiated by the successful demonstration of RNAi gene silencing for Hco-acr-8 which reduced the sensitivity of H. contortus larvae to levamisole. Intriguingly, the pyrantel sensitivity remained unchanged thus providing new evidence for distinct modes of action of these important anthelmintics in parasitic species versus C. elegans. More broadly, this highlights the limits of C. elegans as a predictive model to decipher cholinergic agonist targets from parasitic nematode species and provides key molecular insight to inform the discovery of next generation anthelmintic compounds.

Baltrušis, P., Halvarsson, P., Höglund, J.

Exploring benzimidazole resistance in Haemonchus contortus by next generation sequencing and droplet digital PCR

(2018) International Journal for Parasitology: Drugs and Drug Resistance, 8 (3), pp. 411-419.

Anthelmintic resistance in gastrointestinal nematode (GIN) parasites of grazing ruminants is on the rise in countries across the world. Haemonchus contortus is one of most frequently encountered drug-resistant GINs in small ruminants. This blood-sucking abomasal nematode contributes to massive treatment costs and poses a serious threat to farm animal health. To prevent the establishment of resistant strains of this parasite, up-to-date molecular techniques need to be proposed which would allow for quick, cheap and accurate identification of individuals infected with resistant worms. The effort has been made in the previous decade, with the development of the pyrosequencing method to detect resistance-predicting alleles. Here we propose a novel droplet digital PCR (ddPCR) assay for rapid and precise identification of H. contortus strains as being resistant or susceptible to benzimidazole drugs based on the presence or absence of the most common resistance-conferring mutation F200Y (TAC) in the β tubulin isotype 1 gene. The newly developed ddPCR assay was first optimized and validated utilizing DNA templates from single-worm samples, which were previously sequenced using the next generation PacBio RSII Sequencing (NGS) platform. Subsequent NGS results for faecal larval cultures were then used as a reference to compare the obtained values for fractional abundances of the resistance-determining mutant allele between ddPCR and NGS techniques in each sample. Both methods managed to produce highly similar results and ddPCR proved to be a reliable tool which, when utilized at full capacity, can be used to create a powerful mutation detection and quantification assay.

Baiak, B.H.B., Lehnen, C.R., da Rocha, R.A.

Anthelmintic resistance in cattle: A systematic review and meta-analysis

(2018) Livestock Science, 217, pp. 127-135.

A systematic review and meta-analysis was conducted to identify, evaluate, and synthesize primary literature reporting the efficacy of anthelmintic drugs in cattle. Information on the bibliographic data, anthelmintic drugs, animals, reduction method, days after application, parasite genera, type of application, and dosage were collected. The final data base was composed of 70 articles published between 1986 and 2016 with a total of 8,976 animals. The mode of application interfered with the efficacy of anthelmintic drugs in cattle ($P < 0.05$); oral application was superior to injectable and pour-on drugs. The combined use of drugs was superior to single macrocyclic lactones including ivermectin ($P < 0.05$), in this way the combined use was more effective than the single use of drugs for the control of gastro-intestinal nematodes in cattle ($P < 0.05$). Nematode genera in the abomasum (Ostertagia spp., Haemonchus spp.) and intestine (Cooperia spp.) were evaluated. Levamisole had a greater efficacy for Cooperia spp. than Ostertagia spp. ($P < 0.05$); ivermectin had a greater efficacy for Ostertagia spp. than Haemonchus spp. ($P < 0.05$); doramectin had a greater efficacy for Ostertagia spp. than Cooperia spp. ($P < 0.05$). Dosing in larger quantities and according to the manufacturer's recommendation was most efficient, and the dose limiting species differed between substance classes. The test based on the count of eggs per gram of faeces indicated a reduced efficacy of the drug as days passed following treatment. The forest plot did not show a difference ($P > 0.05$) between interventions (resistance and efficacy). The results of this systematic review and meta-analysis suggest that anthelmintic resistance in cattle is present on several continents. Therefore, there is a need to replace the schemes based on the exclusive use of drugs in order to decrease the selection pressure.

Ali, Q., Rashid, I., Shabbir, M.Z., Shahzad, K., Ashraf, K., Sargison, N.D., Chaudhry, U.

Population genetics of benzimidazole-resistant Haemonchus contortus and Haemonchus placei from buffalo and cattle: implications for the emergence and spread of resistance mutations

(2018) *Parasitology Research*, 117 (11), pp. 3575-3583.

The population genetics of nematode parasites are poorly understood with practical reference to the selection and spread of anthelmintic resistance mutations. Haemonchus species are important to study the nematode population genetics due to their clinical importance in ruminant livestock, and the availability of genomic resources. In the present study, it has been examined that Haemonchus contortus and Haemonchus placei populations from three buffalo and nine cattle hosts. Seventy-three individual adult worms of H. contortus and 148 of H. placei were analysed using a panel of seven microsatellite markers. The number of alleles per locus in H. contortus and H. placei indicated that all populations were polymorphic for the microsatellites used in the present study. Genetic diversity parameters included high levels of allelic richness and heterozygosity, indicating effective population sizes, high mutation rates and high transmission frequencies in the area. Genetic structure parameters revealed low genetic differentiation between and high levels of genetic variation within H. contortus and H. placei populations. Population dynamic analyses showed an absence of heterozygosity excess in both species, suggesting that there was no deviation from genetic drift equilibrium. Our results provide a proof of concept for better understanding of the consequences of specific control strategies, climatic change or management strategies on the population genetics of anthelmintic resistance alleles in Haemonchus spp. infecting co-managed buffalo and cattle.

Parasites of cattle

Craig, T.M.

Gastrointestinal Nematodes, Diagnosis and Control

(2018) *Veterinary Clinics of North America - Food Animal Practice*, 34 (1), pp. 185-199.

Disease caused by nematodes in the gastrointestinal tract of cattle is primarily economic and the effect of treatment is cattle grow larger faster because of increased feed intake. The disease, control measures, and drugs used must be focused on different ages and environments. Different drugs should be used against the parasites most likely affected by the specific drug and administered how and when it will accomplish the best sustainable control. Management needs to ensure that at-risk animals are exposed to sufficient worms to stimulate their immunologic response but not overwhelm it.

Sauermann, C.W., Ganesh, S., Scott, I., Pomroy, W.E., Leathwick, D.M.

Establishment of Cooperia oncophora in calves

(2018) Veterinary Parasitology, 264, pp. 64-68.

The establishment rate of Cooperia oncophora related to host age and previous infection was investigated in young calves. Calves of similar age were kept on a feed pad and allocated into multiple groups, based on their age and weight. Two groups (each n = 16) received trickle infections with an ivermectin-susceptible C. oncophora isolate of 2000 or 10,000 infective stage larvae per week while another group (n = 16) was kept as an uninfected control. At intervals over a period of 11 months, two animals from each group were challenged with 15,000 infective stage larvae of an ivermectin-resistant isolate, 25 days later orally treated with ivermectin and 5 days after that slaughtered for worm counts. On three occasions additional calves (n = 2), subjected to the high trickle infection rate, received an ivermectin treatment to remove the existing worm burden, prior to challenge as above. Further calves (n = 4) of similar age were introduced at the beginning and the end of the experiment to determine the effect of larval age on establishment rate. The establishment in the two trickle infection groups declined to <10% within the first three months, which was significantly different from the control group. In the animals receiving the high trickle infection, but an anthelmintic treatment before challenge the establishment rate was not significantly different from the controls. Over the duration of the experiment establishment in the control group declined from 53% to <20%, which was similar to the decrease recorded at the beginning and the end of the experiment in the animals to determine the effect of larval age. The findings indicate that an existing C. oncophora burden had a strong effect on the establishment of incoming larvae in the trickle infected groups, but this was not observed if the existing burden was removed before the final challenge. The decline in establishment rate in the control group was attributed to the age of the larvae and not the age of the calves per se.

Bellet, C., Green, M.J., Bradley, A.J., Kaler, J.

A longitudinal study of gastrointestinal parasites in English dairy farms. Practices and factors associated with first lactation heifer exposure to Ostertagia ostertagi on pasture

(2018) *Journal of Dairy Science*, 101 (1), pp. 537-546.

The gastrointestinal nematode Ostertagia ostertagi is an important cause of lost production, health, and welfare in cattle. Detailed records were obtained over a 5-yr period (2010–2015) by questionnaires and qualitative interviews to investigate the practices adopted by dairy farmers to control cattle helminth infections and the factors associated with heifer exposure to O. ostertagi on pasture. In total, 1,454 heifers' individual milk samples were collected over a 1-yr period (2014–2015) in 43 dairy farms in England and tested for O. ostertagi antibody by ELISA. Multilevel linear regression models were used to investigate the association between individual milk optical density ratio (ODR) against O. ostertagi and heifer management from birth to time of sampling. Farm and heifer median ODR against O. ostertagi were 0.98 (interquartile range = 0.76–1.02) and 0.64 (interquartile range = 0.42–0.84), respectively. The majority of heifers (88%) received an anthelmintic treatment before sampling in this study. After controlling for the effect of anthelmintic treatments, heifer individual milk ODR against O. ostertagi significantly increased with high stocking rate at first grazing and co-grazing with adult cows before calving. Conversely, heifer individual milk ODR against O. ostertagi significantly decreased when heifers had co-grazed with sheep and pasture grass had frequently been mowed. Overall, these results provide evidence to support targeting grazing management toward limiting the use of anthelmintics in dairy young stock to enable sustainable control of cattle helminth infections in England. However, to be accepted and adopted by farmers, these best practices would need to take into account farmers' perspectives and contextual challenges.

Höglund, J., Hessle, A., Zaralis, K., Arvidsson-Segerkvist, K., Athanasiadou, S.

Weight gain and resistance to gastrointestinal nematode infections in two genetically diverse groups of cattle

(2018) *Veterinary Parasitology*, 249, pp. 88-91.

Body weight gain (BWG) and gastrointestinal nematode challenge (GIN) were investigated in two genetically diverse groups of cattle. Thirty-two dairy calves (D = Swedish Red/Holstein) and 31 dairy × beef crosses (C = Swedish Red/Holstein × Charolais) pairwise matched by dam breed and birth dates, were monitored for ≈20 weeks on a pasture grazed by cattle in the previous year. At turn-out, animals (between 6 and 12 months age) from each genotype were either infected with 5000 third stage (L3) Ostertagia ostertagi (50%) and Cooperia oncophora (50%) larvae (H, high-exposure); or treated monthly with 0.5 mg ivermectin (Noromectin®, Pour-on) per kg bodyweight to remove worms ingested (L, low-exposure). Animals were weighed every fortnight and individual BWG was calculated. Faecal and blood samples were collected every four weeks throughout the experiment for nematode faecal egg counts (FEC) and larvae cultures and serum pepsinogen concentrations (SPC), respectively. Nematode eggs were observed 29 days post turn-out in both H groups. FEC peaked to around 200 eggs per gram (epg) on days 58 and 85 respectively in both H groups. FEC were also observed in the L groups at the same time, but mean epg remained very low (<20 epg) and constituted exclusively of C. oncophora. Although, there was no significant difference in SPC values in animals of the different genotypes, ten animals of CH showed a SPC >3.5 IU tyrosine whereas only six DH animals reached similar pepsinogen levels. The level of infection (H and L) significantly affected BWG in both genotypes. Even though there was no statistically significant genotype (C or D) × treatment (H or L) interaction, there was a larger difference in body weight of H and L in C (37 kg) compared to D (17 kg) genotypes at the end of the experiment. Our data collectively support the view crossbred (C) animals experience the impact of gastrointestinal parasitism more severely compared to pure dairy (D) first season grazers. The mechanisms that underpin this remains speculative.

Frey, C.F., Eicher, R., Raue, K., Strube, C., Bodmer, M., Hentrich, B., Gottstein, B., Marreros, N.

Apparent prevalence of and risk factors for infection with Ostertagia ostertagi, Fasciola hepatica and Dictyocaulus viviparus in Swiss dairy herds

(2018) *Veterinary Parasitology*, 250, pp. 52-59.

Infections with helminth parasites can negatively affect performance of dairy cows. Knowledge on infection intensity, spatial distributions and risk factors are key to develop targeted treatment strategies. Canada and most EU countries have conducted large investigations, but respective data for Switzerland were missing. We now performed a bulk tank milk serosurvey for Ostertagia ostertagi, Fasciola hepatica, and Dictyocaulus viviparus on a total of 1036 voluntarily participating dairy herds that were sampled at confinement periods, i.e. in winter 2014/15 or 2015/16, respectively. All samples were analyzed with commercial ELISAs for antibodies (AB) against O. ostertagi and F. hepatica, and those of the first sampling period additionally with an in-house ELISA for AB against D. viviparus. Testing for the latter parasite was not done in the second year of the study, as the sampling period might have missed infections due to the short lived nature of specific antibodies. The possible influence of geographic, climatic, and farm management variables on AB levels were assessed for each parasite using scanning cluster and multiple regression analysis. Overall seroprevalence for O. ostertagi was 95.5% (95% C.I.: 94.0–96.6), with a mean optical density ratio (ODR) of 0.83, for F. hepatica 41.3% (95% C.I.: 38.3–44.4), and for D. viviparus 2.9% (95% C.I.: 1.6–4.7). There were no significant differences between the two sampling periods. For all parasites, significant geographic clusters of higher AB levels could be established. Furthermore, AB levels against all three parasites were positively correlated with each other, indicating either cross-reactions or co-infections. For O. ostertagi, herd size and percentage of pasture in the ration were positively correlated with AB levels. For F. hepatica, altitude above sea level (a.s.l.)

positively, and milk production per cow and year was negatively correlated with AB levels. This work provides baseline data for further studies performing in-depth risk factor analysis and investigating management as well as targeted treatment options to control the parasites.

Twomey, A.J., Carroll, R.I., Doherty, M.L., Byrne, N., Graham, D.A., Sayers, R.G., Blom, A., Berry, D.P.

Genetic correlations between endo-parasite phenotypes and economically important traits in dairy and beef cattle

(2018) *Journal of Animal Science*, 96 (2), pp. 407-421.

Parasitic diseases have economic consequences in cattle production systems. Although breeding for parasite resistance can complement current control practices to reduce the prevalence globally, there is little knowledge of the implications of such a strategy on other performance traits. Records on individual animal antibody responses to Fasciola hepatica, Ostertagia ostertagi, and Neospora caninum were available from cows in 68 dairy herds (study herds); national abattoir data on F. hepatica–damaged livers were also available from dairy and beef cattle. After data edits, 9,271 dairy cows remained in the study herd dataset, whereas 19,542 dairy cows and 68,048 young dairy and beef animals had a record for the presence or absence of F. hepatica–damaged liver in the national dataset. Milk, reproductive, and carcass phenotypes were also available for a proportion of these animals as well as their contemporaries. Linear mixed models were used to estimate variance components of antibody responses to the three parasites; covariance components were estimated between the parasite phenotypes and economically important traits. Heritability of antibody responses to the different parasites, when treated as a continuous trait, ranged from 0.07 (O. ostertagi) to 0.13 (F. hepatica), whereas the coefficient of genetic variation ranged from 4% (O. ostertagi) to 20% (F. hepatica). The antibody response to N. caninum was genetically correlated with the antibody response to both F. hepatica (−0.29) and O. ostertagi (−0.67); a moderately positive genetic correlation existed between the antibody response to F. hepatica and O. ostertagi (0.66). Genetic correlations between the parasite phenotypes and the milk production traits were all close to zero (−0.14 to 0.10), as were the genetic correlations between F. hepatica–damaged livers and the carcass traits of carcass weight, conformation, and fat score evaluated in cows and young animals (0.00 to 0.16). The genetic correlation between F. hepatica–damaged livers in cows and milk somatic cell score was 0.32 (SE = 0.20). Antibody responses to F. hepatica and O. ostertagi had favorable genetic correlations with fertility traits, but conversely, antibody response to N. caninum and F. hepatica–damaged livers were unfavorably genetically correlated with fertility. This study provides the necessary information to undertake national multitrait genetic evaluations for parasite phenotypes.

Parasitism and dairy sheep

Aguerre, S., Jacquiet, P., Brodier, H., Bournazel, J.P., Grisez, C., Prévot, F., Michot, L., Fidelle, F., Astruc, J.M., Moreno, C.R.

Resistance to gastrointestinal nematodes in dairy sheep: Genetic variability and relevance of artificial infection of nucleus rams to select for resistant ewes on farms

(2018) *Veterinary Parasitology*, 256, pp. 16-23.

Breeding sheep for enhanced resistance to gastrointestinal parasites is a promising strategy to limit the use of anthelmintics due to the now widespread resistance of parasites to these molecules. This paper reports the genetic parameters estimated for parasite resistance and resilience traits in the Blond-faced Manech dairy sheep breed and the putative impacts of the selection for resistance to gastrointestinal nematodes (GIN) on farms. Two datasets were used. First, the rams of the selection scheme were artificially infected twice with L3 Haemonchus contortus larvae. Faecal egg counts (FEC) and packed cell volume (PCV) loss were measured 30 days after each infection. Secondly, the FEC, PCV and body condition score (BCS) (1–6 measures per ewe) of naturally infected ewes on farms were measured in the spring, summer and autumn over a two-year period. Genetic parameters were estimated for each dataset independently but also globally based on the pedigree connections between the two datasets. For the experimentally infected sires, the FEC following the second infection was moderately heritable (heritability: 0.35) and strongly correlated with FEC after the first infection (genetic correlation: 0.92). For the naturally infected ewes, FEC was also heritable (0.18). Using the two datasets together, a genetic correlation of 0.56-0.71 was estimated between the FEC values of the experimentally infected rams and naturally infected ewes. Consequently, the genetic variability of parasite resistance is similar whatever the physiological status (males or milking/pregnant ewes) and the infection conditions (experimental infection with one parasite or natural infection with several parasites). In practice, when the sire population is divided into two groups based on their genetic value, the FEC of the ewes born to the 50% most resistant sires is half that of the ewes born to the 50% most susceptible sires. Our study shows the feasibility and efficiency of genetic selection for parasitism resistance based on the sires' FEC records to improve parasite resistance in naturally grazing ewes. For breed improvement, and to increase the selection pressure on parasite resistance, it seems more appropriate to measure FEC values on rams after experimental infection rather than on ewes in natural infection conditions because this limits the number and standardizes the conditions of FEC measurements.

Parasitism of dairy goats

Fthenakis, G.C., Papadopoulos, E.

Impact of parasitism in goat production

(2018) *Small Ruminant Research*, 163, pp. 21-23.

Goats can be infected by protozoan or metazoan parasites, in internal infections or external infestations, in certain predilection organs or found at various sites. Parasitism leads to depletion of resources of affected animals, as well as, in many cases, in decreased feed intake, resulting in reduced production. These production constraints are particularly important in many tropical or subtropical areas, where goats play an essential role in the agricultural economy. The present paper summarises the potential impact of parasitism in the production of goats and discusses specific examples of adverse effects in the various facets of goat production. Animals with highest milk yield have particularly significantly decrease in milk production in cases of parasitic infections, but no changes in milk composition. In relation to effects of gastrointestinal parasitism in goat-kids, it has been found that growth rate of kids increased and carcasses produced were of better quality after administration of effective anthelmintic drug. Various parasitic infections adversely affect reproductive efficiency in herds; for example, genital myiosis would cause lesions impeding mating, scrotal sarcoptic mange can lead to reduction of testicular mass and besnoitosis may affect the testes, whilst protozoa have a foetopathic effect, causing abortions and/or stillbirths, and long-term reduction of reproductive output in affected does, as well as infecting young kids, causing increased neonatal mortality. Finally, the various ectoparasites affecting goats (e.g., Mallophaga lice or Przhevalskiana silenus) cause severe damage on the skin of affected animals, which would render their hair unsuitable for processing or would reduce its value.

Alternative control of parasites

Plant based products

Hernández-Bolio, G.I., García-Sosa, K., Escalante-Erosa, F., Castañeda-Ramírez, G.S., Sauri-Duch, E., Torres-Acosta, J.F.D.J., Peña-Rodríguez, L.M.

Effects of polyphenol removal methods on the in vitro exsheathment inhibitory activity of Lysiloma latisiliquum extracts against Haemonchus contortus larvae

(2018) *Natural Product Research*, 32 (5), pp. 508-513.

It has been suggested that the anthelmintic (AH) activity of tannin-rich forages is related to their tannin content. However, reports on tropical fodders such as Lysiloma latisiliquum describe the same AH activity after the addition of tannin-blocking agents, suggesting that the activity either depends on the method of tannin blocking/removal or is due to other type of secondary metabolites. This study compared both the effect of the drying process of the plant material and the effect of different polyphenol removal methods on the AH activity of L. latisiliquum acetone-water extracts when tested against Haemonchus contortus. The results showed that the extraction of oven-dried (OD) leaves of L. latisiliquum yielded a CT-free extract with high AH activity. However, polyphenol-free fractions showed similar or lower AH activity levels as of those original OD extract. HPLC analysis confirmed that common polyphenolic metabolites are not responsible for the AH activity of L. latisiliquum.

Castañeda-Ramírez, G.S., Rodríguez-Labastida, M., Ortiz-Ocampo, G.I., González-Pech, P.G., Ventura-Cordero, J., Borges-Argáez, R., Torres-Acosta, J.F.J., Sandoval-Castro, C.A., Mathieu, C.

An in vitro approach to evaluate the nutraceutical value of plant foliage against Haemonchus contortus

(2018) *Parasitology Research*, 117 (12), pp. 3979-3991.

Nutraceutical plants provide nutrients for the animal as well as secondary compounds that can affect the biology and survival of gastrointestinal nematodes (GIN). Current screening of nutraceutical plants is based on in vitro evidence of anthelmintic (AH) activity against different life stages of GIN, but nutritional information is omitted or scarce. This study proposes an integral in vitro screening protocol to identify the nutraceutical value of the foliage from plant species consumed by small ruminants, using Haemonchus contortus as a biological model. The leaves from Acacia collinsii, A. pennatula, Bunchosia swartziana, Gymnopodium floribundum, Havardia albicans, Leucaena leucocephala, Lysiloma latisiliquum, Mimosa bahamensis, Piscidia piscipula, and Senegalia gaumeri were evaluated for their chemical composition and in vitro digestibility. Acetone:water extracts (70:30) from leaves of each plant were evaluated using the egg hatch assay and larval exsheathment inhibition assay. Respective effective concentrations 50% (EC50) were determined for each assay. The ten plant species showed good nutritional value for ruminants, including crude protein (> 10%), metabolizable energy (> 2.9 MJ/kg DM), and varied CT content (from 1.0 to 37.6%). The best AH activity against H. contortus eggs (EC50 = 401.8 µg/mL) and L3 (EC50 = 83.1 µg/mL) was observed for S. gaumeri extract. Although all the plant species showed in vitro nutraceutical potential, the leaves of S. gaumeri had the best values. The proposed in vitro protocol showed to be useful for the integral assessment of the nutraceutical potential of different plant species as it included the nutritional value and the AH activity against eggs and L3 in the selected plant species.

Váradyová, Z., Mravčáková, D., Babják, M., Bryszak, M., Grešáková, L., Čobanová, K., Kišidayová, S., Plachá, I., Königová, A., Cieslak, A., Slusarczyk, S., Pecio, L., Kowalczyk, M., Várady, M.

Effects of herbal nutraceuticals and/or zinc against Haemonchus contortus in lambs experimentally infected

(2018) *BMC Veterinary Research*, 14 (1), art. no. 78.

Background: The gastrointestinal parasitic nematode Haemonchus contortus is a pathogenic organism resistant to several anthelmintics. This study assessed the efficacy of a medicinal herbal mixture (Herbmix) and organic zinc, as an essential trace element for the proper functioning of both unspecific and specific immune defensive mechanisms, against experimental infections with H. contortus in lambs. All lambs were infected orally with approximately 5000 third-stage larvae of a strain of H. contortus susceptible to anthelmintics (MHco1). Twenty-four female lambs 3-4 months of age were divided into four groups: unsupplemented animals (control), animals supplemented with Herbmix (Hmix), animals supplemented with organic zinc (Zn) and animals supplemented with Herbmix and organic zinc (Hmix+Zn). Eggs per gram (EPG) of faeces were quantified 20, 28, 35, 42, 49, 56, 62 and 70 d post-infection and mean abomasal worm counts were assessed 70 d post-infection. Samples of blood were collected from each animal 7, 35, 49 and 70 d post-infection. Results: Quantitative analyses of the bioactive compounds in Herbmix identified three main groups: flavonoids (9964.7 μg/g), diterpenes (4886.1 μg/g) and phenolic acids (3549.2 μg/g). Egg counts in the lambs treated with Hmix, Zn and Hmix+Zn decreased after 49 d. The EPGs in the Zn and Hmix+Zn groups were significantly lower on day 56 ($P < 0.05$ and $P < 0.01$, respectively), and the EPGs and mean worm counts were significantly lower on day 70 in all supplemented groups ($P < 0.05$ and $P < 0.01$). Hemograms of complete red blood cells of each animal identified clinical signs of haemonchosis after day 35. Serum calprotectin concentrations and IgA levels were significantly affected by treatment. The treatment influenced serum malondialdehyde concentrations ($P < 0.05$) and sulfhydryl groups ($P < 0.01$) of antioxidant status. The mineral status was unaltered in all lambs. Conclusion: A direct anthelmintic impact on the viability of nematodes was not fully demonstrated, but the treatments with herbal nutraceuticals and zinc likely indirectly contributed to the increase in the resistance of the lambs to nematode infection.

Quijada, J., Drake, C., Gaudin, E., El-Korso, R., Hoste, H., Mueller-Harvey, I.

Condensed Tannin Changes along the Digestive Tract in Lambs Fed with Sainfoin Pellets or Hazelnut Skins

(2018) *Journal of Agricultural and Food Chemistry*, 66 (9), pp. 2136-2142.

The variable anthelmintic efficacy of condensed tannins (CT) against gastrointestinal nematodes may depend on CT concentration, composition, or fate along the digestive tract. We analyzed CT concentration and composition by acetone-HCl-butanol and thiolysis coupled to HPLC-MS in digesta and feces of lambs. Lambs had been infected with Haemonchus contortus and Trichostrongylus colubriformis and received sainfoin pellets and hazelnut skins of contrasting prodelphinidin/procyanidin ratios. The digesta and feces had lower CT concentrations than the original feeds but similar concentration patterns across the digestive compartments. The changes in assayable CT concentrations between rumen, abomasum, and small intestine may be due to complex formation between CT and other dietary components. However, the large CT disappearance (61-85%) from feed to feces could also indicate that CT may have been structurally modified, degraded, or absorbed during digestion. Interestingly, there were no changes in the structural features of assayable CT in the digesta.

Brito, D.R.B., Costa-Júnior, L.M., Garcia, J.L., Torres-Acosta, J.F.J., Louvandini, H., Cutrim-Júnior, J.A.A., Araújo, J.F.M., Soares, E.D.S.

Supplementation with dry Mimosa caesalpiniifolia leaves can reduce the Haemonchus contortus worm burden of goats

(2018) *Veterinary Parasitology*, 252, pp. 47-51.

Gastrointestinal nematodes (GINs) cause considerable economic losses in grazing goat herds. At present, GIN control cannot rely on conventional anthelmintic (AH) drugs because parasites have developed resistance against such drugs. Thus, alternative control methods are being sought to reduce the dependence on AH. Many tannin-rich plants exhibit AH activity and may be used as alternatives for GIN control. Mimosa caesalpiniifolia is a tannin-rich shrub consumed by small ruminants in Brazil. This study evaluated the in vivo AH effect of M. caesalpiniifolia leaf powder supplementation on GIN egg fecal excretion and worm burden in goats. Plant leaves were harvested, dried and ground to obtain a powder. Twenty-four castrated male goats, aged six to eight months, with a mean body weight of 15.0 ± 2.5 kg were used in the experiment. Animals were infected orally with 16,000 larvae comprising 50% Haemonchus spp., 41% Trichostrongylus spp. and 9% Oesophagostomum spp. Once the infection was patent, the goats were distributed into four groups of six animals. The control group received concentrate without condensed tannins (CTs) and did not receive any drench against GINs. The monepantel group received concentrate without CTs and were drenched once with monepantel. The other two groups received the M. caesalpiniifolia leaf powder in two periods of seven consecutive days (days 1–7 and 14–21), with one of the groups also receiving 10 g of polyethyleneglycol (PEG)/day. The animals were weighed weekly, and individual fecal eggs counts (FECs) were performed daily. After 28 days, the animals were humanly slaughtered, and the worm burden was estimated. Although live weight gain and FECs did not differ among the groups ($P > 0.05$), post-mortem worm counts showed a reduction in Haemonchus contortus adult worm burden (57.7%) in goats of the CT group compared to control goats ($P < 0.05$). The addition of PEG did not diminish AH activity in the CT + PEG group (66.9% reduction compared to the control). No AH effect against other GIN species was found. The result for the addition of PEG suggested that the observed AH activity was associated with plant secondary compounds, as opposed to CTs. As expected, no AH effect against Oesophagostomum columbianum was found for the monepantel group showed. Thus, feeding dry leaves of M. caesalpiniifolia represent a promising alternative for the control of GIN infections in goats.

Barone, C.D., Zajac, A.M., Manzi-Smith, L.A., Howell, A.B., Reed, J.D., Krueger, C.G., Petersson, K.H.

Anthelmintic efficacy of cranberry vine extracts on ovine Haemonchus contortus

(2018) *Veterinary Parasitology*, 253, pp. 122-129.

The discovery that plant secondary compounds, including proanthocyanidins (PAC), suppress gastrointestinal nematode (GIN) infection has provided promise for alternative methods of GIN control in small ruminants. This investigation is the first to examine the anthelmintic potential of cranberry vine (CV) against the GIN Haemonchus contortus. The purpose of this study was to explore the anti-parasitic activity of CV in the form of a specific organic proanthocyanidin extract (CV-PAC) and an aqueous extract (CV-AqE) containing PAC and other compounds. In vitro egg hatching, first (L1) and third (L3) stage larval and adult worm motility and L3 exsheathment were evaluated after a 24-h incubation with CV products. In addition, CV treated worms were observed via scanning electron microscopy, and a preliminary investigation of the efficacy of CV powder against an experimental infection of H. contortus was conducted. The in vivo effect on an experimental infection was determined by administering 21.1 g CV powder to lambs (n = 9 per group) for three consecutive days, and collecting fecal egg count data for four weeks post-treatment. The effect of CV-PAC on egg hatching, L3 motility and exsheathment was limited. However, a substantial effect was observed on motility of post-hatch L1 (EC50 0.3 mg PAC/mL) and adults (EC50 0.2 mg PAC/mL). The CV-AqE showed more effect on egg hatching (EC50 5.3 mg/mL containing 0.6 mg PAC/mL) as well as impacting motility of L1 (EC50 1.5 mg/mL with 0.2 mg PAC/mL) and adults (EC50 3.4 mg/mL with 0.4 mg PAC/mL), but like CV-PAC, did not substantially effect L3 motility or exsheathment. Scanning electron microscopy revealed an accumulation of aggregate on the cuticle around the buccal area of adult worms incubated in CV-AqE and CV-PAC. In the preliminary in vivo study, there was a significant effect of treatment over time (p =.04), although differences in individual weeks were not significant. In summary, both extracts inhibited motility of L1 and adult worms. The higher efficacy of CV-AqE than CV-PAC at levels that contained the same concentrations of PAC tested alone, suggest that other secondary compounds in the CV-AqE contributed to the observed effects on the parasites. This first study of the in vitro and in vivo effects of CV suggest that this readily available plant product may have utility in integrated control of H. contortus and support the need for additional testing to provide further information.

González-Cortazar, M., Zamilpa, A., López-Arellano, M.E., Aguilar-Marcelino, L., Reyes-Guerrero, D.E., Olazarán-Jenkins, S., Ramírez-Vargas, G., Olmedo-Juárez, A., Mendoza-de-Gives, P.

Lysiloma acapulcensis leaves contain anthelmintic metabolites that reduce the gastrointestinal nematode egg population in sheep faeces

(2018) *Comparative Clinical Pathology*, 27 (1), pp. 189-197.

One metabolite against lamb gastrointestinal nematodes (GIN) was identified from extracts of Lysiloma acapulcensis leaves. Firstly, an aqueous extract of L. acapulcensis leaves and two fractions, organic and aqueous, were evaluated at different concentrations against Haemonchus contortus eggs and histotrophic larvae. In other experiment, 35 Pelibuey GIN-infected lambs were randomly divided into five groups, as follows: (1) ivermectin (0.2 mg/kg BW); (2) control (untreated); (3) rutin (10 mg/kg BW, as a flavonoid comparison control); (4) L. acapulcensis leaves ethyl acetate fraction (EtAc-F, 25 mg/kg BW); and (5) L. acapulcensis leaves (dried and grinded, 5 g/kg BW). The EtAc-F fraction caused 94.8% egg-hatching inhibition ($P < 0.05$, 6.25 mg/ml). Likewise, at 50 mg/ml, this fraction resulted in 100% larval mortality. In the second experiment, the highest eggs per gram (EPG) of faeces reduction caused by ivermectin was 80.2% ($\bar{y}$ = 61.8%); meanwhile, rutin showed a highest EPG reduction = 66.2% ($\bar{y}$ = 37.5%). Similarly, EtAc-F fraction showed 62.9% EPG highest reduction ($\bar{y}$ = 32.5%). On the other hand, lambs fed with L. acapulcensis leaves showed a maximum EPG reduction = 50.1% ($\bar{y}$ = 29.4%). Chromatography analysis (HPLC; NRM) of EtAc-F fraction revealed the presence of myricitrin as a major compound. Lysiloma acapulcensis leaves EtAc-F exhibited the highest in vitro activity against H.

contortus eggs and larvae. The low efficacy of ivermectin suggests the presence of anthelmintic resistance. Lysiloma acapulcensis leaves and fractions can be considered in future experiments searching for a sustainable alternative of GIN control.

Giovanelli, F., Mattellini, M., Fichi, G., Flamini, G., Perrucci, S.

In vitro anthelmintic activity of four plant-derived compounds against sheep gastrointestinal nematodes

(2018) *Veterinary Sciences*, 5 (3), art. no. 78.

DOI: 10.3390/vetsci5030078

By using the egg hatch test (EHT), the larval development test (LDT) and the larval mortality/paralysis test (LMT), the in vitro anthelmintic activity on sheep gastrointestinal strongyles (GIS) of four plant-derived pure compounds, mangiferin (at 0.25%, 0.125% and 0.0625%), rutin (at 1%, 0.75%, 0.5%), quercetin (at 1%), and β-sitosterol (at 1%, 0.75%, 0.5%), was investigated. For comparison, untreated and treated (0.1% thiabendazole, 0.1% TBZ) controls were used. Six repetitions were made throughout the experiment. Data were statistically elaborated using the χ2 test. The concentration able to inhibit the development of the 50% of L1s to L3s and causing the mortality of the 50% of L3s (EC50) was also calculated. L3s recovered from untreated Petri dishes were identified at the genus level. In EHT, all tested compounds at all concentrations significantly (p < 0.01) inhibited the hatch of the eggs when compared to the untreated controls, but none of them was as effective as 0.1% TBZ. In LDT, rutin (at 1%, 0.75% and 0.5%), mangiferin (at 0.25% and 0.125%), β-sitosterol (at 1%) and 0.1% TBZ completely prevented the larval development from L1 to L3 in respect to the untreated controls (p < 0.01). In LMT, all tested compounds significantly (p < 0.01) increased the death of L3s compared to the untreated controls, except for β-sitosterol at 0.5%. However, only rutin at all concentrations and 0.25% and 0.125% mangiferin were as effective as 0.1% TBZ. Haemonchus, Trichostrongylus, Chabertia and Teladorsagia/Ostertagia GIS genera, were identified.

Ferreira, L.E., Benincasa, B.I., Fachin, A.L., Contini, S.H.T., França, S.C., Chagas, A.C.S., Beleboni, R.O.

Essential oils of Citrus aurantifolia, Anthemis nobile and Lavandula officinalis: in vitro anthelmintic activities against Haemonchus contortus

(2018) *Parasites & vectors*, 11 (1), p. 269.

Infections of sheep with gastrointestinal parasites, especially Haemonchus contortus, have caused serious losses in livestock production, particularly after the emergence of resistance to conventional anthelmintics. The search for new anthelmintic agents, especially those of botanical origin, has grown substantially due to the perspective of less contamination of meat and milk, as well as other advantages related to their cost and accessibility in less developed countries. The aim of this study was to evaluate the in vitro anthelmintic activity of essential oils of the plant species Citrus aurantifolia, Anthemis nobile and Lavandula officinalis against the main developmental stages of the parasite H. contortus.

RESULTS: Plant species were selected based on substantial ethnopharmacological information. Analysis of the composition of each oil by gas chromatography coupled to mass spectrometry (GC-MS) demonstrated the presence of limonene (56.37%), isobutyl angelate (29.26%) and linalool acetate (35.97%) as the major constituents in C. aurantifolia, A. nobile and L. officinalis, respectively. Different concentrations of each oil were tested in vitro for their capacity to inhibit egg hatching (EHT), larval development (LDT) and adult worm motility (AWMT) using a multidrug-resistant strain of H. contortus (Embrapa 2010). The IC50 values obtained for the oils of C. aurantifolia, A. nobile and L. officinalis were 0.694, 0.842 and 0.316 mg/ml in the EHT and 0.044, 0.117 and 0.280 mg/ml in the LDT, respectively. The three oils were able to inhibit adult worm motility completely within the first 8-12 h of observation in the AWMT.

CONCLUSIONS: The present results demonstrate significant anthelmintic activity of the three oils against the different developmental stages of H. contortus. Furthermore, this study is of ethnopharmacological importance by validating the anthelmintic activity of the oils studied. Although new experiments are necessary, these data contribute to the development of pharmaceutical-veterinary products for sheep farming by opening up new therapeutic possibilities against gastrointestinal infections caused by H. contortus.

Abidi, A., Sebai, E., Dhibi, M., Alimi, D., Rekik, M., B'chir, F., Maizels, R.M., Akkari, H.

Chemical analyses and anthelmintic effects of Artemisia campestris essential oil

(2018) *Veterinary Parasitology*, 263, pp. 59-65.

The present study aimed at analyzing the chemical composition and evaluating the in vitro and in vivo anthelmintic activity of Artemisia campestris essential oil aerial parts. The chemical composition was analysed by gaz chromatography/mass chromatography (GC/MS). Fifty compounds were identified representing 99.98% of the total oil. A. campestris essential oil was dominated by beta-pinene (36.40%) and 2-undecanone (14.7%). The in vitro anthelmintic activity tests of A. campestris essential oil were performed on Haemonchus contortus using egg hatch assay (EHA) and adult worm's motility assay (AWMA) compared with a reference drug albendazole. In the EHA 100% inhibition was observed at 2 mg/ml after 48 h incubation (IC50 = 0.93 mg/ml). In the AWMA, essential oil induced 66.6% inhibition at 0.5 mg/ml after 8 h post exposure. The nematicidal effect of essential oil was evaluated on Heligmosomoides polygyrus. It was monitored through faecal egg count reduction (FECR) and total worm count reduction (TWCR). Three doses (2000, 4000 and 5000 mg/kg) were studied using a bioassay. The dose of 5000 mg/kg showed a high nematicidal activity (72.1% FECR and 72% TWCR), 7 days post-treatment. The results of the present study suggest that A. campestris essential oil has a potential anthelmintic activity and further studies are required in order to establish its mechanisms of action.

Fungi

Healey, K., Lawlor, C., Knox, M.R., Chambers, M., Lamb, J.

Field evaluation of Duddingtonia flagrans IAH 1297 for the reduction of worm burden in grazing animals: Tracer studies in sheep

(2018) *Veterinary Parasitology*, 253, pp. 48-54.

The aim of these studies was to determine the reduction in pasture infectivity likely to be achieved by the supplementation of grazing sheep with BioWorma®, a product containing the chlamydospores of the nematophagous fungus Duddingtonia flagrans strain IAH 1297. Four placebo-controlled trials were conducted between 2009 and 2013 in sheep in different climatic regions of New South Wales and Queensland, Australia and across several seasons. The effectiveness of BioWorma was assessed by total worm counts in tracer sheep placed in paddocks grazed by parasitised sheep which were fed a daily supplement with and without BioWorma under group-feeding conditions. Further proof of concept was obtained by assessing the worm burdens and weight gains of the parasitised sheep, as well as the number of anthelmintic (“salvage”) treatments required when faecal egg counts exceeded a threshold level. Significant reductions ranging from 57 to 84% (P < 0.05) in worm burdens of the tracer sheep placed in the paddock grazed by BioWorma treated sheep were obtained in all four trials, compared to the Control group. In two of the studies the treatment effect was greater at the end of the trial, indicating that pasture infectivity in the Control paddocks had risen considerably. The main nematodes encountered were Haemonchus spp., Trichostrongylus spp., and Teladorsagia spp. (including multi-resistant strains) and significant reductions were demonstrated for each of these species. Given the results of the four trials it can be concluded that supplementation of pastured sheep with BioWorma was effective in reducing the numbers of parasitic nematode larvae ingested by tracer sheep. It is considered that these levels of reduced pasture larvae would result in productivity increases in grazing sheep and reduce the requirement for intervention with anthelmintic chemicals. Therefore, use of BioWorma will provide an alternative means for control of gastrointestinal nematode (GIN) parasites on pasture.

Rodríguez-Martínez, R., Mendoza-de-Gives, P., Aguilar-Marcelino, L., López-Arellano, M.E., Gamboa-Angulo, M., Hanako Rosas-Saito, G., Reyes-Estébanez, M., Guadalupe García-Rubio, V.

In Vitro Lethal Activity of the Nematophagous Fungus Clonostachys rosea (Ascomycota: Hypocreales) against Nematodes of Five Different Taxa

(2018) *BioMed research international*, 2018, p. 3501827.

This study was aimed to evaluate the in vitro lethal activity of the nematophagous fungi Clonostachys rosea against 5 nematodes species belonging to different taxa. Two groups of 35 Petri dishes (PD) each were divided into 5 series of 7 (PD). Group 1 (series 1, 2, 3, 4, and 5) contained only water agar; meanwhile group 2 plates (series 6, 7, 8, 9, and 10) contained C. rosea cultures growth on water agar. Every plate from the two groups was added with 500 nematodes corresponding to the following genera/specie: Haemonchus contortus, Caenorhabditis elegans, Rhabditis sp., Panagrellus redivivus, and Butlerius sp. After 5-day incubation at room temperature, free (nontrapped) larvae were recovered from plates using the Baermann funnel technique. Recovered nematodes were counted and compared with their proper controls. Results shown an important reduction percentage of the nematode population attributed to the fungal lethal activity as follows: H. contortus (L3) 87.7%; C. elegans 94.7%; Rhabditis sp. 71.9%; P. redivivus 92.7%; and Butlerius sp. 100% ($p \leq 0.05$). The activity showed by C. rosea against the H. contortus can be crucial for further studies focused to the biological control of sheep haemonchosis, although the environmental impact against beneficial nematodes should be evaluated.

Miscellaneous products

Benelli, G.

Gold nanoparticles – against parasites and insect vectors

(2018) *Acta Tropica*, 178, pp. 73-80.

Nanomaterials are currently considered for many biological, biomedical and environmental purposes, due to their outstanding physical and chemical properties. The synthesis of gold nanoparticles (Au NPs) is of high interest for research in parasitology and entomology, since these nanomaterials showed promising applications, ranging from detection techniques to drug development, against a rather wide range of parasites of public health relevance, as well as on insect vectors. Here, I reviewed current knowledge about the bioactivity of Au NPs on selected insect species of public health relevance, including major mosquito vectors, such as Aedes aegypti, Anopheles stephensi and Culex quinquefasciatus. The toxicity of Au NPs against helminths was reviewed, covering Schistosoma mansoni trematodes as well as Raillietina cestodes. Furthermore, I summarized the information available on the antiparasitic role of Au NPs in the fight against malaria, leishmaniosis, toxoplasmosis, trypanosomiasis, cryptosporidiosis, and microsporidian parasites affecting human and animals health. Besides, I examined the employ of Au NPs as biomarkers, tools for diagnostics and adjuvants for the induction of transmission blocking immunity in malaria vaccine research. In the final section, major challenges and future outlooks for further research are discussed, with special reference to the pressing need of further knowledge about the effect of Au NPs on other arthropod vectors, such as ticks, tsetse flies, tabanids, sandflies and blackflies, and related ecotoxicology assays.

Alimi, D., Rekik, M., Akkari, H.

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

(2018) *Journal of Helminthology*, pp. 1-7. Article in Press.

DOI: 10.1017/S0022149X18000378

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.

Silva, N.C.S., Lima, A.S., Silva, C.R., Brito, D.R.B., Cutrim-Junior, J.A.A., Milhomem, M.N., Costa-Junior, L.M.

In vitro and in vivo activity of hydrolyzed Saccharomyces cerevisiae against goat nematodes

(2018) *Veterinary Parasitology*, 254, pp.6-9.

Haemonchus contortus is the most prevalent nematode in tropical and subtropical regions and is responsible for significant losses in small ruminant production systems. Control of this parasite is based on the use of synthetic anthelmintics; although effective, prolonged and indiscriminate use can select for populations of resistant nematodes. Hydrolyzed yeast can help prevent populations of nematodes and is low in cost. The present study aimed to evaluate the preventive effect of hydrolyzed yeast on H. contortus through in vitro and in vivo assays. In in vitro experiments, the yeast preparation was tested at different stages of the H. contortus life cycle via tests of egg hatchability, larval development, larval exsheathment and larval migration. Both eggs and L3 larvae were obtained from sheep artificially infected with a monospecific strain of H. contortus. In in vivo experiments, animals were treated with hydrolyzed yeast (53.4 mg/kg/BW), and fecal egg counts (FECs), feces culture and Famacha were performed. The yeast preparation inhibited larval exsheathment in a dose-dependent manner (IC95 of 0.46 mg/mL), though no activity at the other stages of the H. contortus life cycle was observed. The hydrolyzed yeast preparation was able to reduce FECs in treated animals from D40 to D54 (p < 0.05), showing an average efficiency of 60%. The yeast exhibited preventive properties, and the IC95 value suggests that H. contortus is highly susceptible. Our results indicate that administration of hydrolyzed yeast, which is already used commercially in ruminant nutrition, is an alternative method for the control of nematodes, mainly H. contortus.

Dictyocaulus spp.

2 A Forbes Livestock papers

Lurier T; Delignette-Muller ML; Rannou B; Strube C; Arcangioli MA; Bourgoin G (2018)

Diagnosis of bovine dictyocaulosis by bronchoalveolar lavage technique: A comparative study using a Bayesian approach.

Preventive Veterinary Medicine 154 124-131

Bovine dictyocaulosis is a pulmonary parasitic disease present in temperate countries, with potential important clinical and economic impacts. The Baermann technique is routinely used despite its low sensitivity in adult cows. Recently developed serological tests seem to offer better sensitivity, but validations of these tests in field conditions are few. We aimed to study two non-previously evaluated diagnosis methods of dictyocaulosis based on bronchoalveolar lavage sampling (BAL), which allows finding lungworm stages in the lungs as well as determination of eosinophilia. We compared them to the Baermann technique and serological tests. As no gold standard was available, we performed a Bayesian analysis by the simultaneous use of latent class and mixture models. The study was carried out during the 2015 pasture season on 60 adult cows originating from 11 herds with clinical signs of dictyocaulosis, and 10 apparently healthy cows originating from the teaching herd of VetAgro Sup, in France. Prevalence of infection was highly variable among herds with clinical signs (10–90%). Despite a maximal specificity (100%), the sensitivity of parasitological methods was low (7.4% for the Baermann sedimentation and 24.7% for the examination of BAL fluids). Better results were observed with serology (Se = 74.9%, Sp = 85.5%) with an optimal cut-off value estimated at 0.397 for the optical density ratio. Even better results were obtained with the count of eosinophil in BAL (Se = 89.4%, Sp = 85.2%) with an optimal cut-off value estimated at 4.77% for the eosinophil proportion. The BAL is a relevant diagnostic method of dictyocaulosis for practitioners due to the opportunity to perform two analyses (direct parasitic research and the eosinophil count) and to its good sensitivity and specificity.

May K; Brügemann K; König S; Strube C. (2018)

The effect of patent Dictyocaulus viviparus (re)infections on individual milk yield and milk quality in pastured dairy cows and correlation with clinical signs

Parasites and Vectors 11(1)

Infections with the bovine lungworm Dictyocaulus viviparus might lead to reduced milk production and detrimental impacts on milk quality resulting in considerable economic losses in dairy farming. Methods: In the presented field study, 1988 faecal samples were collected from 1166 Black and White dairy cows allocated in 17 small and medium-sized German grassland farms. Faecal samples were collected in summer and autumn 2015 to assess D. viviparus larvae excretion. Test-day records were used to estimate the association between patent D. viviparus infections in individual cows and the milk production parameters milk yield, milk protein and milk fat content by using linear mixed models. Bulk tank milk (BTM) samples from each farm and individual milk samples from those cows which were excreting larvae in summer were collected in autumn. In addition, occurrence of the clinical symptom "coughing" was noted in individual cows during autumn sampling to determine its association with patent lungworm infections. Results: Patent D. viviparus infections were found on 23.5% (4/17) of farms with a prevalence at the individual cow level of 0.9% (9/960) in summer and 3.4% (35/1028) in autumn. No BTM sample exceeded the BTM ELISA cut-off value of 0.410 optical density ratio (ODR), the mean value was 0.168 ODR. Only one individual milk sample exceeded the individual milk ELISA cut-off value of 0.573 ODR (mean value of 0.302 ODR). A patent D. viviparus infection status was associated with a lower average daily milk yield of 1.62 kg/cow/day (P = 0.0406). No significant association was found with milk protein or fat content representing milk quality parameters. Coughing was observed in 5.9% (61/1028) of cows. Of the coughing cows, only 4.9% (3/61) had a patent lungworm infection. Fisher's exact test showed no significant difference between infected and non-infected coughing cows. Conclusions: Farmers and veterinarians should be aware that patent lungworm (re)infections in dairy cows reduce milk yield, despite the absence of clinical signs. Furthermore, if dairy cows present with coughing, other differential diagnoses need to be considered in addition to dictyocaulosis.

Pyziel AM; Dolka I; Werszko J; Laskowski Z; Steiner-Bogdaszewska Ż; Wiśniewski J; Demiaszkiewicz AW; Anusz K (2018)

Pathological lesions in the lungs of red deer Cervus elaphus (L.) induced by a newly-described Dictyocaulus cervi (Nematoda: Trichostrongyloidea)

Veterinary Parasitology 261 22-26

The large lungworms of the genus Dictyocaulus are causative agents of parasitic bronchitis in various ungulate hosts, including red deer. Recently, the red deer-derived lungworm D. cervi was described and separated from D. eckerti. Little is known of the transmission patterns, epidemiology, geographical distribution and pathogenicity of D. cervi. Histological examinations were performed on 22 formalin-fixed lung tissue samples of hunted red deer. Exclusively, D. cervi adults were derived from 15 red deer and confirmed molecularly (GenBank accession: MH183394). Dictyocaulus cervi infection was associated with various degrees of lung pathology, including interstitial pneumonia, bronchitis and bronchiolitis with an influx of eosinophils, lymphocytes, plasma cells and macrophages; massive hyperplasia of lymphoid follicles within bronchiolar tissue, and hyperplasia of the bronchial and bronchiolar epithelium. Furthermore, emphysema, atelectasis and lung tissue congestion were noted. Interestingly, interstitial and subpleural fibrosis was seen in adult Dictyocaulus-negative samples, suggesting either a prepatent phase of Dictyocaulus infection or infection/coinfection with protostrongylid nematodes. © 2018 Elsevier B.V.

Pyziel AM; Laskowski Z; Höglund J (2018)

An assessment of the use of cox1 and cox3 mitochondrial genetic markers for the identification of Dictyocaulus spp. (Nematoda: Trichostrongyloidea) in wild ruminants

Parasitology Research 117(7) 2341-2345

Lungworms of the genus Dictyocaulus Railliet and Henry, 1907 (Nematoda: Trichostrongyloidea) are the causative agents of parasitic bronchitis (dictyocaulosis, husk) of various ungulate hosts, including domestic and wild ruminants. Correct diagnosis of lungworm species and a better understanding of the transmission patterns of Dictyocaulus spp. are crucial in minimising the risk of its cross transmission between wildlife and livestock, and for the control of dictyocaulosis. The study was conducted on large lungworms collected from European bison, roe deer and red deer. The study resulted in 14 sequences of the partial cox1 region of Dictyocaulus spp. and 10 novel DNA sequences of partial cox3 region, including the first available mt cox3 sequence, of the roe deer lungworm (D. capreolus). The European bison was infected with bison genotype of D. viviparus, whereas red deer and roe deer were infected with D. cervi and D. capreolus respectively. The current study revealed that the cox3 nucleotide sequences of D. capreolus and D. viviparus were 100% homologous to each other. Our findings indicate that the mt cox3 gene does not serve as an efficient mt marker for systematic, population genetic or molecular epidemiological studies of Dictyocaulus lungworms.

Cestodes and other zoonotic parasites

Lucy J. Robertson,1,* Paul R. Torgerson,2 and Joke van der Giessen3

Foodborne Parasitic Diseases in Europe: Social Cost-Benefit Analyses of Interventions

Trends in parasitolofy. Volume 34, Issue 11, November 2018, Pages 919-923

Social cost-benefit analysis (SCBA) can be used to evaluate the benefit to society as a whole of a particular intervention. Describing preliminary steps of an SCBA for two food-borne parasitic diseases, echinococcosis and cryptosporidi- osis, indicates where data are needed in order to identify those interventions of greatest benefit. SCBAs depend on the availability of high- quality data. CONCLUSION For all three parasites con- sidered here, the actual incidence of human cases is probably underesti- mated, and empirical data on the food-attributable proportion of cases are lack- ing. Nevertheless, for cryptosporidiosis, a relatively acute disease and for which foodborne outbreaks have been identi- fied and documented, a preliminary SCBA seems possible, although this would be improved with better data. DALYs are an important input, and there- fore improved human surveillance in European countries is needed. Although both CE and AE are chronic diseases, and prevalence data are probably under- estimated, in areas of high endemicity it should be feasible to conduct an SCBA for CE. Improved human and slaughter- house surveillance would improve the available data (e.g., the European Regis- ter of Cystic Echinococcosis; [17]). For AE in Europe, for which the associated parasite life cycle is largely in wildlife, data are currently insufficient for an SCBA. However, based on the potential for spread of this parasite, improved human surveillance could provide useful data for a future SCBA.

Yaoyu Feng,1 Una M. Ryan,2 and Lihua Xiao1

Genetic Diversity and Population Structure of Cryptosporidium

Trends in parasitology Volume 34, Issue 11, November 2018, Pages 997-1011

Cryptosporidium species and subtypes differ in host range and public health significance

Host-adapted subtype families exist in the human-pathogenic species *C. parvum* and *C. hominis*.

Genetic recombination is common within the zoonotic *C. parvum*, especially its hypertransmissible IIaA15G2R1 subtype.

Genetic recombination in *C. hominis* is mostly restricted to its virulent subtypes, especially IbA10G2.

Host and geographical segregation, genetic recombination, and selective pressure shape the population structure of *C. parvum*and *C. hominis*, leading to the emergence of host-adapted, virulent, and hypertransmissible subtypes with public health significance.

Chengat Prakashbabu, B., Marshall, L.R., Crotta, M., Gilbert, W., Johnson, J.C., Alban, L., Guitian, J.

Risk-based inspection as a cost-effective strategy to reduce human exposure to cysticerci of Taenia saginata in low-prevalence settings

(2018) *Parasites and Vectors*, 11 (1), art. no. 257.

Background: Taenia saginata cysticercus is the larval stage of the zoonotic parasite Taenia saginata, with a life-cycle involving both cattle and humans. The public health impact is considered low. The current surveillance system, based on post-mortem inspection of carcasses has low sensitivity and leads to considerable economic burden. Therefore, in the interests of public health and food production efficiency, this study aims to explore the potential of risk-based and cost-effective meat inspection activities for the detection and control of T. saginata cysticercus in low prevalence settings. Methods: Building on the findings of a study on risk factors for T. saginata cysticercus infection in cattle in Great Britain, we simulated scenarios using a stochastic scenario tree model, where animals are allocated to different risk categories based on their age, sex and movement history. These animals underwent different types of meat inspection (alternative or current) depending on their risk category. Expert elicitation was conducted to assess feasibility of scenarios and provide data for economic analysis. The cost-effectiveness of these scenarios was calculated as an incremental cost-effectiveness ratio, using the number of infected carcasses detected as the technical outcome. Results: Targeting the high-risk population with more incisions into the heart while abandoning incisions into the masseter muscles was found to reduce the total number of inspections and cost, while simultaneously increasing the number of infected carcasses found. Conclusions: The results suggest that, under reasonable assumptions regarding potential improvements to current inspection methods, a more efficient and sensitive meat inspection system could be used on animals categorised according to their risk of harbouring T. saginata cysticercus at slaughter. Such a system could reduce associated cost to the beef industry and lower microbial contamination of beef products, improving public health outcomes.

Jansen, F., Dorny, P., Berkvens, D., Gabriël, S.

Bovine cysticercosis and taeniosis: The effect of an alternative post-mortem detection method on prevalence and economic impact

(2018) Preventive Veterinary Medicine, 161, pp. 1-8.

In Europe, bovine cysticercosis (BCC) is detected by routine meat inspection (MI) at the slaughterhouse. The prevalence of BCC in Belgium based on MI is estimated at 0.23%. MI has a known low sensitivity for animals with localised infections and alternative detection techniques should be considered. A mathematical scenario-analysis model was built to determine the current prevalence of BCC in Belgium based on MI results combined with results of dissection of the predilection sites (PS) and the B158/B60 Ag-ELISA on serum of 614 carcasses found negative on MI. Additionally, the impact of the introduction of the B158/B60 Ag-ELISA at slaughter on the prevalence of BCC and taeniosis and on the economic impact related to Taenia saginata was estimated for Belgium. Data for estimating the economic impact were gathered from multiple sources within the meat and human health sectors and included value loss for infected carcasses, inspection costs, carcass destruction costs, cattle insurance costs and costs related to taeniosis (consultation physician, drugs and laboratory test). The model estimated the current prevalence of BCC to be 42.5% (95% CI: 32.4–60.7%) and the sensitivity of the MI for viable and degenerated cysticerci at only 0.54% (95% CI: 0.37–0.71%). A total of 213,344 viable cysticerci (95% CI: 122,962–386.249) were estimated to be present in the infected carcasses in one year and only 408 (95% CI: 356–464) of these were present in the carcasses detected at current MI. The annual number of human taeniosis cases is estimated at 11,000 by using the sale numbers of niclosamide in Belgium. Implementation of the Ag-ELISA at slaughter (Se = 36.37%; Sp = 99.36%) would greatly reduce the prevalence of BCC to 0.6% and the number of taeniosis cases to 89 in year 10. Unfortunately, the accompanying resulting increase in costs for the animal owners, slaughterhouses and the insurance company, would be extremely high in the first years. Cattle owners would suffer losses of up to €21 million in the first year after implementation of the Ag-ELISA (compared to an annual loss of €3.5 million in the current situation), slaughterhouses of €10 million (compared to €200,000 currently) and the insurance company of almost €6 million (compared to €2.3 million profit currently). Therefore, implementation of the Ag-ELISA might not be feasible and other options for controlling T. saginata should be investigated.

Lett WS, Boufana B, Lahmar S, Bradshaw H, Walters TMH, Brouwer A, Fraser AR, Maskell D, Craig PS (2018).

Canine echinococcosis screening in foxhound hunts in England and Wales using coproantigen ELISA and coproPCR.

Parasitology Open 4, e17, 1–6. https://doi.org/10.1017/pao.2018.14

A total of eight foxhound packs in England and Wales were screened for Echinococcus species using a genus-specific coproantigen ELISA and for Echinococcus granulosus sensu lato and Echinococcus equinus by coproPCR. Main screening (n = 364 hounds) occurred during 2010–2011 wherein a quarter (25.6%) of the foxhound fecal samples tested were Echinococcus coproantigen-positive (93/364). In total, five of eight (62.5%) hunts screened had coproantigen-positive hounds; coproantigen prevalence for individual foxhound packs ranged from 0 to 61.2% and was shown to be >30% in three hunts (in counties of Powys, Wales and Northumberland, England). Foxhound fecal samples from six of the eight tested hunts (four Welsh and two English hunts) were positive by coproPCR for E. granulosus s.l (including one sequence confirmation of E. granulosus sensu stricto) and E. equinus DNA.

Analysis of hunt questionnaire data suggested that there was an association between poor foxhound husbandry, especially feeding practices and Echinococcus coproantigen prevalence.

Clearer guidelines regarding the risk of canine echinococcosis are required for safe management of foxhound hunts in England and Wales.

Wu, M., Yan, M., Xu, J., Liang, Y., Gu, X., Xie, Y., Jing, B., Lai, W., Peng, X., Yang, G.

Expression, tissue localization and serodiagnostic potential of Echinococcus granulosus leucine aminopeptidase

(2018) *International Journal of Molecular Sciences*, 19 (4), art. no. 1063.

DOI: 10.3390/ijms19041063

Echinococcus granulosus is the causative agent of cystic echinococcosis (CE), a widespread parasitic zoonosis. Leucine aminopeptidases (LAPs) of the M17 peptidase family have important functions in regulating the balance of catabolism and anabolism, cell maintenance, growth and defense. In this study, we presented a bioinformatic characterization and experimentally determined the tissue distribution characteristics of E. granulosus LAP (Eg-LAP), and explored its potential value for diagnosis of CE in sheep based on indirect ELISA. Through fluorescence immunohistochemistry, we found that Eg-LAP was present in the tegument and hooks of PSCs, the whole germinal layer and adult worm parenchymatous tissue. Western blotting results revealed that the recombinant protein could be identified using E. granulosus-infected sheep serum. The diagnostic value of this recombinant protein was assessed by indirect ELISA, and compared with indirect ELISA based on hydatid fluid antigen. The sensitivity and specificity rEgLAP-ELISA were 95.8% (23/24) and 79.09% (87/110), respectively, while using hydatid fluid as antigen showed the values 41.7% (10/24) and 65.45% (72/110). This is the first report concerning leucine aminopeptidase from E. granulosus, and the results showed that Eg-LAP belong to M17 peptidase families, and that it is involved in important biological function of E. granulosus. Furthermore, rEg-LAP is appropriate for diagnosing and monitoring CE in sheep in field. Development of a rapid test using rEg-LAP to diagnose sheep CE deserves further study.

Dán, Á., Rónai, Z., Széll, Z., Sréter, T.

Prevalence and genetic characterization of Echinococcus spp. in cattle, sheep, and swine in Hungary

(2018) *Parasitology Research*, 117 (9), pp. 3019-3022.

DOI: 10.1007/s00436-018-5977-5

A study was conducted to investigate genetic diversity of Echinococcus isolates collected in Hungarian slaughterhouses between 2015 and 2018. Organs of 219 animals with suspected hydatidosis were collected during routine meat inspection and sent to our laboratory. Echinococcus infection was confirmed in 65 animals. These results indicate that prevalence data based on reporting of slaughterhouses are not reliable without the appropriate training of the people responsible for meat inspection. The genetic diversity was evaluated by the DNA sequence analysis of the cytochrome c oxydase subunit 1 (cox1) mitochondrial gene. Echinococcus intermedius (n = 31), Echinococcus granulosus s.s. (n = 2), and Echinococcus multilocularis (n = 3) was identified in swine. In cattle, only E. granulosus s.s. (n = 20) was detected. E. granulosus s.s. (n = 7) was the dominant species in sheep; nevertheless, E. intermedius was also identified in two animals. E. granulosus s.s. (n = 29) and E. intermedius (n = 33) were classified in 13 and three haplotypes, respectively. The genetic diversity and haplotype network of E. granulosus s.s. were similar to that observed in some other countries of Eastern Europe. The genetic diversity of E. intermedius was low with a single dominant haplotype. Cysts were fertile in nine sheep (100%), 22 swine (61%), and three cattle (15%) indicating that all three species play a role in some extent in the epidemiology of cystic echinococcosis in Hungary. Based on the number of animals killed in the slaughterhouses involved in the present study, the rate of infection was 0.013% in sheep, 0.007% in cattle, and 0.001% in swine. As animals with hydatidosis originated from family farms, control programs should mainly focus on these facilities.

Siles-Lucas, M., Casulli, A., Cirilli, R., Carmena, D.

Progress in the pharmacological treatment of human cystic and alveolar echinococcosis: Compounds and therapeutic targets

(2018) *PLoS Neglected Tropical Diseases*, 12 (4), art. no. e0006422.

Human cystic and alveolar echinococcosis are helmintic zoonotic diseases caused by infections with the larval stages of the cestode parasites Echinococcus granulosus and E. multilocularis, respectively. Both diseases are progressive and chronic, and often fatal if left unattended for E. multilocularis. As a treatment approach, chemotherapy against these orphan and neglected diseases has been available for more than 40 years. However, drug options were limited to the benzimidazoles albendazole and mebendazole, the only chemical compounds currently licensed for treatment in humans. To compensate this therapeutic shortfall, new treatment alternatives are urgently needed, including the identification, development, and assessment of novel compound classes and drug targets. Here is presented a thorough overview of the range of compounds that have been tested against E. granulosus and E. multilocularis in recent years, including in vitro and in vivo data on their mode of action, dosage, administration regimen, therapeutic outcomes, and associated clinical symptoms. Drugs covered included albendazole, mebendazole, and other members of the benzimidazole family and their derivatives, including improved formulations and combined therapies with other biocidal agents. Chemically synthetized molecules previously known to be effective against other infectious and non-infectious conditions such as anti-virals, antibiotics, anti-parasites, anti-mycotics, and anti-neoplastics are addressed. In view of their increasing relevance, natural occurring compounds derived from plant and fungal extracts are also discussed. Special attention has been paid to the recent application of genomic science on drug discovery and clinical medicine, particularly through the identification of small inhibitor molecules tackling key metabolic enzymes or signalling pathways.

Gopinath, H., Aishwarya, M., Karthikeyan, K.

Tackling scabies: novel agents for a neglected disease

(2018) International Journal of Dermatology, 57 (11), pp. 1293-1298.

The scabies mite, Sarcoptes scabiei var hominis, is an obligate ectoparasite of humans. It has been a source of distress for humanity since antiquity. The troublesome mite is emerging triumphant over current acaricidal agents with reports of emerging resistance and treatment failures. Scabies in endemic areas and crusted scabies offer additional management challenges. Exploration of indigenous plants and better understanding of mite biology and pathogenesis provide opportunities for the development of novel agents for this common pest. We review the recent diverse approaches to scabies, including the use of novel plant products with a better safety profile, translating the use of moxidectin from veterinary practice to human scabies, vaccination, immunotherapy, and development of drugs that directly target mite molecules.

Rašović, M.B.

Helminthes that are transmitted through food to humans

(2018) *Journal of Hygienic Engineering and Design*, 22, pp. 11-17.

Helminthes which contaminate food cause different diseases in humans and animals, many of which are zoonoses. The largest number of species of parasites infect humans and animals entering through the contaminated food and water, but it is possible infection through skin - by arthropods. Helminthes are mostly parasites of the small intestine and people from the body eliminate eggs or larvae of helminths through faeces. In order these eliminated forms of the parasites to become infectious for susceptible animals, they have to perform part of its development freely in the external environment or in one or two transitional host. Helminths are divided into two phylums: Plathelmintes (tapeworms - flukes and cestodes) and Nemathelmintes (roundworms). The most common helminths that can be transmitted through food to humans are flukes (Fasciola hepatica, Dicrocoelium lanceolatum, Opistorchis felineus, Paragonimus spp., Fasciolopsis buski), cestodes (Tae-nia solium, Taenia saginata, Diphyllobotrium latum, Echinococcus granulosus, Dipilidium caninum, Taenia multiceps, Spirometra spp., Hymenolepis nana), nematodes (Anisakis simplex, Ascaris lumbricoides, Enterobius vermicularis, Trichuris trichiura, Trichinella spiralis, Gnathostoma spp., Toxocara canis, Ancylostoma du-odenale, Necator americanus). Some of them are described in this paper. In order to prevent the diseases, it is necessary to know well all possibilities of contamination of food by these parasites, their life cycle and resistance in the environment. Significant measures to prevent the occurrence hel-minthoses transmitted through food and water are: adequate implementation of hygiene measures during production, processing, preparation and consumption of food, appropriate thermal treatment of food, personal hygiene measures, hygiene of environment, bio-thermally treatment of manure and others.

Perandin, F., Pomari, E., Bonizzi, C., Mistretta, M., Formenti, F., Bisoffi, Z.

Assessment of real-time polymerase chain reaction for the detection of trichostrongylus spp. DNA from human fecal samples

(2018) *American Journal of Tropical Medicine and Hygiene*, 98 (3), pp. 768-771.

DOI: 10.4269/ajtmh.17-0733

Sporadic cases of Tricostrongylosis are reported in humans. Diagnosis of enteric Trichostrongylus relies primarily on coproscopic analysis but morphological identification is difficult because of similarity among nematode species. The method is time consuming and requires some expertise. To overcome these limitations, we developed a molecular approach by real-time polymerase chain reaction (PCR) to provide a rapid, specific, and sensitive tool to detect Trichostrongylus spp. in human feces. We designed primers and probe specific for Trichostrongylus rDNA region 5.8S and internal transcribed spacer 2. Three Italian family clusters were analyzed and DNA sequencing was performed to confirm real-time PCR results comparing with known GenBank sequence data. Sequence analysis showed3 99% identity to Trichostrongylus colubriformis and Trichostrongylus axei. This study provides a molecular methodology suitable for fast and specific detection of Trichostrongylus in fecal specimens and to distinguish the zoonotic species.

Robertson, L.J.

Parasites in Food: From a Neglected Position to an Emerging Issue

(2018) *Advances in Food and Nutrition Research*, 86, pp. 71-113.

Foodborne parasites have long been a neglected group of pathogens, as they often have insidious, chronic effects, rather than being acute diseases, and they are often associated with impoverished or marginalized populations. In addition, due to the long incubation period for most foodborne parasites, source attribution is often difficult, if not impossible. However, global trends have enabled foodborne parasites to emerge in different populations in new locations, transmitted through different food types, and sometimes with unexpected symptoms. This emergence of foodborne parasites has brought them into focus. In this chapter, six foodborne parasites are used as examples on emergence: Echinococcus multilocularis is spreading to new locations; Cryptosporidium spp. are beginning to be associated not only with water, but also with salads; Trypanosoma cruzi is being manifest with acute disease due to foodborne transmission, particularly transmitted with juices; Trichinella spp. have become less of a burden regarding transmission via pork in many countries, but now game animals are becoming a concern; anisakiasis is becoming a global problem as the world develops a taste for sushi, and similarly for opisthorchiasis, which is increasingly being associated with cholangiocarcinoma. However, the emergence of these foodborne parasites provides an incentive for increased efforts being made toward control. In this chapter, having described how the parasites are emerging from their neglected position, the focus turns toward control. In addition to considering control measures that may be applied to the specific parasites, an overview is provided of some of the organized collaborations, projects, and consortia, as well as some of their outputs, that have in focus the control of these emerging and important pathogens.

Ectoparasites

Lifschitz, A., Fiel, C., Steffan, P., Cantón, C., Muchiut, S., Dominguez, P., Lanusse, C., Alvarez, L.

Failure of ivermectin efficacy against Psoroptes ovis infestation in cattle: Integrated pharmacokinetic-pharmacodynamic evaluation of two commercial formulations

(2018) Veterinary Parasitology, 263, pp. 18-22.

Psoroptic mange is an important parasitic disease that mainly affects beef cattle producing marked economic losses. Ivermectin (IVM) is considered one of the most effective treatments against psoroptic mange and is used worldwide to control both endo and ectoparasites in different species. The current work assessed the relationship between pharmacokinetic behavior of IVM and its efficacy against Psoroptes ovis after the subcutaneous administration of two commercial formulations in a cattle feedlot. Aberdeen Angus and Hereford steers were selected based on the presence of active mite infestations. Animals were allocated into 4 experimental groups and treated with a single (day 0) or repeated subcutaneous injection (days 0 and 7) of one of two commercial formulations of IVM (1%) at 0.2 mg/kg. Blood and skin samples were taken from 8 randomly selected animals of each experimental group to measure IVM concentrations by HPLC. Skin scrapings were also collected from six different sites in each animal, mites were counted and ranked based on a density score. Equivalent plasma concentrations of IVM were measured after the administration of IVM formulations under study. The repeated administration of both IVM formulations at day 0 and 7 accounted for a greater plasma drug availability compared with the single administration ($P < 0.05$). IVM was well distributed from the plasma to the skin without significant differences between both IVM formulations. There was a positive correlation between IVM concentrations in skin and plasma (r: 0.73 P < 0.0001). The mean ratios between IVM availabililty (measured as AUC) in the skin and in plasma were between 1.2 and 2.1. The repeated administration of IVM increased significantly the IVM concentrations in the skin of areas affected by mange. IVM failed to obtain a parasitological cure in the different groups affected by mange. The failure was observed with both formulations administeredat single or repeated doses. Based on the number of animals cured, the range of efficacy was between 0% on day 7 and 60% on day 28 post-treatment. No significant differences in the P. ovis density scores were observed after the IVM treatment at single or repeated doses. Additional studies are needed to confirm the presence of resistant strains of P.ovis and to establish the appropriate measures to control these parasitic infestations in feedlot cattle.

Khater, H.F., Geden, C.J.

Potential of essential oils to prevent fly strike and their effects on the longevity of adult Lucilia sericata

(2018) Journal of Vector Ecology, 43 (2), pp. 261-270.

Lucilia sericata is a facultative ectoparasite causing fly strike or myiasis in warm-blooded vertebrates. It is controlled by traps or insecticides, but both have drawbacks and alternative ways of control are urgently needed. Essential oils (EOs) of vetiver (Chrysopogon zizanioides), cinnamon (Cinnamomum zeylanicum), and lavender (Lavandula angustifolia) and their blends (OBs); OB1 (2 ml of each EO plus 4 ml of sunflower oil as a carrier) and OB2 (2 ml of each EO) were tested. Oils were tested at 5% for deterrence assays, and a dose response assay 0.01-0.6%, was conducted to determine forced-contact toxicity. We evaluated the efficacy of oils as oviposition deterrents, repellents/attractants, and their effects on mortality and longevity of adult L. sericata. Our data indicated that 0.2% EOs killed all flies by 5 min post-treatment and that vetiver oil greatly deterred flies from the oviposition medium and reduced adult longevity. Sunflower oil repelled all flies from ovipositing and greatly reduced the lifespan of treated adults. The blend of the four oils (OB1) had the greatest repellent effect on the flies. EOs have insecticidal, repellent, and oviposition-deterrent activities against L. sericata that could be used for suppression of blow fly populations.

Fraser, T.A., Carver, S., Martin, A.M., Mounsey, K., Polkinghorne, A., Jelocnik, M.

A Sarcoptes scabiei specific isothermal amplification assay for detection of this important ectoparasite of wombats and other animals

(2018) *PeerJ*, 2018 (7), art. no. e5291.

Background. The globally distributed epidermal ectoparasite, Sarcoptes scabiei, is a serious health and welfare burden to at-risk human and animal populations. Rapid and sensitive detection of S. scabiei infestation is critical for intervention strategies. While direct microscopy of skin scrapings is a widely utilised diagnostic method, it has low sensitivity. PCR, alternatively, has been shown to readily detect mite DNA even in microscopy-negative skin scrapings. However, a limitation to the latter method is the requirements for specialised equipment and reagents. Such resources may not be readily available in regional or remote clinical settings and are an important consideration in diagnosis of this parasitic disease. Methodology. A Loop Mediated Isothermal Amplification (LAMP) assay targeting the ITS-2 gene for S. scabiei was developed and evaluated on clinical samples from various hosts, previously screened with conventional S. scabies-specific PCR. Species specificity of the newly developed LAMP assay was tested against a range of DNA samples from other arthropods. The LAMP assays were performed on a real-time fluorometer as well as thermal cycler to evaluate an end-point of detection. Using skin scrapings, a rapid sample processing method was assessed to eliminate extensive processing times involved with DNA extractions prior to diagnostic assays, including LAMP. Results. The S. scabiei LAMP assay was demonstrated to be species-specific and able to detect DNA extracted from a single mite within a skin scraping in under 30 minutes. Application of this assay to DNA extracts from skin scrapings taken from a range of hosts revealed 92.3% congruence (with 92.50% specificity and 100% sensitivity) to the conventional PCR detection of S. scabiei. Preliminary results have indicated that diagnostic outcome from rapidly processed dry skin scrapings using our newly developed LAMP is possible in approximately 40 minutes. Discussion. We have developed a novel, rapid and robust molecular assay for detecting S. scabiei infesting humans and animals. Based on these findings, we anticipate that this assay will serve an important role as an ancillary diagnostic tool at the point-of-care, complementing existing diagnostic protocols for S. scabiei.

Filazi, A., Yurdakok-Dikmen, B.

Amitraz

(2018) *Veterinary Toxicology: Basic and Clinical Principles: Third Edition*, pp. 525-531.

Amitraz is a formamidine derivative pesticide that is widely used in the preservation of agricultural products and the control of ectoparasites in animals. Due to its lipophilic property, amitraz is rapidly absorbed from the applied area, and may cause poisonings in horses and humans, and especially in cats and dogs. Therefore, its use is not recommended in these animals (especially for small dog breeds such as Chihuahua, Pomeranian) and diabetic ones. It has a long half-life and is primarily excreted in urine. The toxic effects are mainly related to the stimulation of α2-adrenergic receptors, and the inhibition of the monoamine oxidase enzyme along with prostaglandins. The most common signs of toxicity are central nervous system depression, respiratory depression, bradycardia, hypotension, mydriasis, hypothermia, vomiting, diarrhea, and coma. Chronic poisoning cause reproductive and developmental toxicity through endocrine disrupting effects. The diagnosis is based on anamnesis, clinical signs, and the detection of amitraz and its metabolites in biological fluids. For the treatment of amitraz poisoning, α2-adrenergic receptor antagonists (yohimbine or atipamezole) along with symptomatic and supportive substances are suggested.

Doherty, E., Burgess, S., Mitchell, S., Wall, R.

First evidence of resistance to macrocyclic lactones in Psoroptes ovis sheep scab mites in the UK

(2018) *Veterinary Record*, 182 (4), p. 106.

Ovine psoroptic mange (sheep scab) is an infection of substantial economic and animal welfare concern in the UK. Its prevalence has increased rapidly over the last 20 years and management is dependent on a small number of acaricidal compounds, many of which are also used to control a range of other endoparasites and ectoparasites. Here, the effects of the macrocyclic lactone (ML) moxidectin was considered using in vitro assays against mites from four farm populations where persistent treatment failure had been reported: two in West Wales, one from the England/Wales border and one in Herefordshire. The data demonstrate resistance in mites from all four farms. This is the first quantitative evidence of ML resistance in Psoroptes mites in the UK. Given the similarities in their mode of action it is highly likely that cross-resistance across the range of this class of compound will be found. The development of resistance to moxidectin is of considerable concern given the already high prevalence of scab infection in some regions; major difficulties in scab management should be anticipated if ML resistance becomes widely established in the UK.

Bosco, A., Morgoglione, M.E., Amadesi, A., Masiello, I., Antenucci, P., Cringoli, G., Rinaldi, L.

Efficacy of deltamethrin pour-on (BUTOX® 7.5 Pour-On) against Haematopinus tuberculatus in Italian Mediterranean buffalo (Bubalus bubalis) [Efficacia della deltametrina pour-on (BUTOX® 7,5 Pour-On) per il controllo di Haematopinus tuberculatus nel bufalo di razza Mediterranea Italiana (Bubalus bubalis)]

(2018) *Large Animal Review*, 24 (2), pp. 73-79.

Introduction: The sucking louse Haematopinus tuberculatus is a harmful ectoparasite found on water buffalo (Bubalus bubalis). Louse infestation often leads to skin irritation, anemia, anorexia, restlessness and reduced productivity. Specific treatments for lice control are uncommon throughout the world; however, deltamethrin is used to control ectoparasites in farm animals and is considered the most effective among synthetic pyrethroids. Aims: The present paper reports the results of a field trial conducted in two buffalo farms in southern Italy to assess the efficacy of deltamethrin pour-on against the sucking louse H. tuberculatus and the influence of treatment on milk yield. Materials and methods: The trial was conducted between March and September 2016. The parasitological investigation was performed on 45 lactating buffaloes, 15 belonging to the Control Group, 15 belonging to the DELTA 1 Group (treated with deltamethrin pour-on at the dosage of 75 mg/head) and 15 belonging to DELTA 2 Group (treated with deltamethrin pour-on at the dosage of 150 mg/head). The groups were homogeneous for number of lice, age and milk production. Louse counts were performed on days -1, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77 and 84 at thirteen predilection sites on the skin of each buffalo.

Statistical analysis was performed using ANOVA one way test, by Graphpad Prism 5. Results and discussion: In terms of percent lice reduction, deltamethrin pour-on was highly effective in DELTA 1 Group (100% in farm no. 1 and 99.1% in farm no. 2) and 100% in DELTA 2 Group (in both farms). A significantly ($P<0.05$) higher milk yield was recorded in both treated groups (DELTA 1 and DELTA 2) compared to the control group. No differences were observed between the two groups. During the trial, deltamethrin was well tolerated by all animals, demonstrating an optimal tolerability of the drug in the buffalo species. Conclusions: The availability of deltamethrin in a pour-on formulation for buffaloes during the lactating stage would be highly beneficial to buffalo farmers who derive an important part of their income from buffalo milk.

Bovine besnoitiosis

Treatment developments

Vet Parasitol. 2018 Sep 15;261:77-85. doi: 10.1016/j.vetpar.2018.08.015. Epub 2018 Aug 31.

Repurposing of commercially available anti-coccidials identifies diclazuril and decoquinate as potential therapeutic candidates against Besnoitia besnoiti infection

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Repurposing of currently marketed compounds with proven efficacy against apicomplexan parasites was used as an approach to define novel candidate therapeutics for bovine besnoitiosis. Besnoitia besnoiti tachyzoites grown in MARC-145 cells were exposed to different concentrations of toltrazuril, diclazuril, imidocarb, decoquinate, sulfadiazine and trimethoprim alone or in combination with sulfadiazine. Drugs were added either just prior to infection of MARC-145 cells (0 h post infection, hpi) or at 6 hpi. A primary evaluation of drug effects was done by direct immunofluorescence staining and counting. Potential effects on the host cells were assessed using a XTT kit for cell proliferation. Compounds displaying promising efficacy were selected for IC_{50} and IC_{99} determination by qPCR. In addition, the impact of drugs on the tachyzoite ultrastructure was assessed by TEM and long-term treatment assays were performed. Cytotoxicity assays confirmed that none of the compounds affected the host cells. Decoquinate and diclazuril displayed invasion inhibition rates of 90 and 83% at 0 h pi and 73 and 72% at 6 h pi, respectively. The remaining drugs showed lower efficacy and were not further studied. Decoquinate and diclazuril exhibited IC_{99} values of 100 nM and 29.9 µM, respectively. TEM showed that decoquinate primarily affected the parasite mitochondrium, whilst diclazuril interfered in cytokinesis of daughter zoites. The present study demonstrates the efficacy of diclazuril and decoquinate against B. besnoiti in vitro and further assessments of safety and efficacy of both drugs should be performed in the target species.

Baron, S., Barrero, R.A., Black, M., Bellgard, M.I., van Dalen, E., Maritz-Olivier, C.

Differentially expressed genes in response to amitraz treatment suggests a proposed model of resistance to amitraz in R. decoloratus ticks

(2018) International Journal for Parasitology: Drugs and Drug Resistance, 8 (3), pp. 361-371.

The widespread geographical distribution of Rhipicephalus decoloratus in southern Africa and its ability to transmit the pathogens causing redwater, gallsickness and spirochaetosis in cattle makes this hematophagous ectoparasite of economic importance. In South Africa, the most commonly used chemical acaricides to control tick populations are pyrethroids and amitraz. The current amitraz resistance mechanism described in R. microplus, from South Africa and Australia, involves mutations in the octopamine receptor, but it is unlikely that this will be the only contributing factor to mediate resistance. Therefore, in this study we aimed to gain insight into the more complex mechanism(s) underlying amitraz resistance in R. decoloratus using RNA-sequencing. Differentially expressed genes (DEGs) were identified when comparing amitraz susceptible and resistant ticks in the presence of amitraz while fed on bovine hosts. The most significant DEGs were further analysed using several annotation tools. The predicted annotations from these genes, as well as KEGG pathways potentially point towards a relationship between the α-adrenergic-like octopamine receptor and ionotropic glutamate receptors in establishing amitraz resistance. All genes with KEGG pathway annotations were further validated using RT-qPCR across all life stages of the tick. In susceptible ticks, the proposed model is that in the presence of amitraz, there is inhibition of Ca 2+ entry into cells and subsequent membrane hyperpolarization which prevents the release of neurotransmitters. In resistant ticks, we hypothesize that this is overcome by ionotropic glutamate receptors (NMDA and AMPA) to enhance synaptic transmission and plasticity in the presence of neurosteroids. Activation of NMDA receptors initiates long term potentiation (LTP) which may allow the ticks to respond more rapidly and with less stimulus when exposed to amitraz in future. Overactivation of the NMDA receptor and excitotoxicity is attenuated by the estrone, NAD + and ATP hydrolysing enzymes. This proposed pathway paves the way to future studies on understanding amitraz resistance and should be validated using in vivo activity assays (through the use of inhibitors or antagonists) in combination with metabolome analyses.

Epidemiology

Int J Parasitol Parasites Wildl. 2018 Aug 17;7(3):317-321. doi: 10.1016/j.ijppaw.2018.08.002. eCollection 2018 Dec.

Exposure to *Neospora* spp. and *Besnoitia* spp. in wildlife from Israel.

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A wide panel of 871 sera from two wild carnivores and nine wild ruminant species were tested. *Neospora* antibodies were present in six out of the 11 species investigated, whereas *Besnoitia* antibodies were undetected. Golden jackal, red fox, addax, Arabian oryx, Persian fallow deer, mouflon, mountain gazelle, Nubian ibex, scimitar horned oryx and water buffalo were seropositive against *N. caninum* infection by IFAT and/or MAT. Moreover, the presence of *Neospora* spp.-specific antibodies was confirmed by Western blot in golden jackal (6/189; 3.2%), red fox (1/75; 1.3%), Persian fallow deer (13/232; 5.6%), mouflon (1/15; 16.7%), Nubian ibex (22/55; 40%) and water buffalo (12/18; 66.7%). Addax (1/49) and water buffalo (1/18) were MAT-seropositive against *B. besnoiti* but were seronegative by Western blot. Hence, *Neospora* sylvatic cycle is present in Israel and may cross over to a domestic life cycle. In contrast, wildlife species investigated are unlikely to present a risk of transmitting *Besnoitia* to livestock in Israel.

Transbound Emerg Dis. 2018 Dec;65(6):1979-1990. doi: 10.1111/tbed.12980. Epub 2018 Aug 12.

Effect of parasite dose and host age on the infection with Besnoitia besnoiti tachyzoites in cattle

Diezma-Díaz C[1], Jiménez-Meléndez A[1], Re MT[1,2], Ferre I[1], Ferreras MDC[3], Gutiérrez-Expósito D[1,3], Rojo-Montejo S[1,4], Román-Trufero A[4], Benavides-Silván J[3], García-Lunar P[1], Calleja-Bueno L[2], Blanco-Murcia J[1,2], Osoro K[4], Ortega-Mora LM[1], Álvarez-García G[1]. (Spain)

Bovine besnoitiosis is continuing to spread in Europe. Therefore, the development of ruminant animal models of infection is urgently needed to evaluate therapeutic and prophylactic tools. Herein, we studied the effect of parasite dose and host age on the infection dynamics with Besnoitia besnoiti tachyzoites in cattle in two independent experimental infections. In experiment A, twelve 3-month-old male calves were inoculated intravenously with either three different doses of tachyzoites (G1: 10^8 ; G2: 10^7 ; G3: 10^6) or with PBS (G4). In experiment B, six 14-month-old bulls were inoculated with 10^6 tachyzoites based on results obtained in experiment A. In both trials, clinical signs compatible with acute and chronic besnoitiosis were monitored daily; blood and skin samples were collected regularly for 70-115 days post-infection (pi). Finally, animals were killed, and tissues were collected for lesion and parasite detections. Infected animals developed mild-moderate signs compatible with acute besnoitiosis. Lymphadenopathy and fever were observed in both calves (from 12 hr until 7 days pi) and bulls (from 6 days until 9 days pi). Seroconversion was detected at 16-19 days pi, and antibody levels remained high. Infected animals did not developed characteristic clinical signs and macroscopic lesions of chronic besnoitiosis. However, successfully, parasite-DNA was detected in a reduced number of target tissues: conjunctiva, ocular sclera, epididymis, skin of the scrotum and carpus in calves (n = 10, 6 of which belonged to G3), and pampiniform plexus and testicular parenchyma in bulls. Remarkably, one tissue cyst and mild microscopic lesions were also detected. In summary, inoculated animals developed the acute besnoitiosis and chronic infection was evidenced by microscopic findings. However, our results suggest that tachyzoite dose and host age are not key variables for inducing clinical signs and macroscopic lesions characteristic of chronic besnoitiosis. Thus, a further refinement of this model should evaluate other parasite- and host-dependent variables.

Neospora caninum

Vet Res. 2018 Oct 17;49(1):106. doi: 10.1186/s13567-018-0601-3.

Endogenous transplacental transmission of Neospora caninum during successive pregnancies across three generations of naturally infected sheep

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Endogenous transplacental transmission, which occurs during pregnancy as the result of reactivation of a latent infection in the dam, is the main mechanism of propagation of Neospora caninum within cattle herds. However, the importance of this propagation mechanism has not yet been evaluated in relation to ovine neosporosis. In this study, involving three generations of ewes naturally infected by N. caninum, we demonstrated that endogenous transplacental transmission may also be highly efficient in the ovine host since transmission of infection occurred in 96.6% of gestations and the congenital infection rate ranged between 66.7 and 93%. Nevertheless, parasite burdens decreased gradually in consecutive generations. Reactivation of latent infections had a strong impact on the pregnancy outcome, with high mortality rates recorded in the offspring of the two first generations of ewes (21.4-46.1%). Histological examination of the brain revealed that all aborted foetuses had characteristic lesions of neosporosis (necrotic glial foci) and a few parasite cysts, whereas most stillborn and newborn lambs that died shortly after birth had non-specific lesions (mild glial foci without necrosis) and parasite cysts were more frequent. Microsatellite analysis revealed scarce genetic variability in the N. caninum population, in accordance with a scenario in which infections were of a single origin and were exclusively maintained by clonal propagation through endogenous transplacental transmission.

Vet Res. 2018 May 8;49(1):42. doi: 10.1186/s13567-018-0539-5.

Influence of dose and route of administration on the outcome of infection with the virulent Neospora caninum isolate Nc-Spain7 in pregnant sheep at mid-gestation

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Experimental infections in pregnant sheep have been focused on studying the effect of the time of challenge on the outcome of N. caninum infection, whereas the impact of the dose and route of challenge has not been studied in depth. Therefore, clinical outcome, immune responses, parasite detection and burden, and lesion severity in placental tissues and foetal brains were investigated in 90-day-pregnant sheep inoculated intravenously with 10^5 (G1), 10^4 (G2), 10^3 (G3), or 10^2 (G4) tachyzoites or subcutaneously with 10^4 (G5) tachyzoites of the virulent Nc-Spain7 isolate and an uninfected group (G6). Comparing challenge doses, G1 was the only group that had 100% abortion. Likewise, IFNγ levels in G1 increased earlier than those in other intravenously infected groups, and IgG levels on day 21 post-infection (pi) were higher in G1 than those in other intravenously infected groups. Concerning vertical transmission, G1 shows a higher parasite burden in the foetal brain than did G2 and G3. Comparing routes of administration, no differences in foetal survival rate or parasite load in the foetal brain were found. Although G2 had higher IFNγ levels than G5 on day 10 pi, no differences were found in humoral immune responses. Because the outcome after intravenous infection with 10^5 tachyzoites was similar to that observed after intravenous infection with 10^6 tachyzoites used in a previous work (100% abortion and vertical transmission), we conclude that it may be reasonable to use 10^5 tachyzoites administered by the intravenous route in further experiments when assessing drugs or vaccine candidates.

Parasitol Int. 2018 Aug;67(4):397-402. doi: 10.1016/j.parint.2018.03.009. Epub 2018 Mar 31.

Neospora caninum in birds: A review

de Barros LD[1], Miura AC[2], Minutti AF[2], Vidotto O[2], Garcia JL[2].(Brazil)

Neospora caninum is an obligate intracellular protozoan parasite that infects domestic and wild animals. Canids are considered to be definitive hosts since they may shed oocysts into the environment through their feces. The disease is recognized as one of the major causes of bovine abortion worldwide, leading to important economic losses in the dairy and beef cattle industries. Previous studies have reported N. caninum infection in different species of birds; infection in birds has been associated with increased seroprevalence and reproductive problems in dairy cattle. Although the role of birds in the epidemiological cycle of neosporosis is unknown, birds are exposed to infection because they feed on the ground and could thus contribute to parasite dissemination. This review is focused on the current state of knowledge of neosporosis in birds.

Vector borne diseases

Developing controls

Saudi J Biol Sci. 2019 Jul;26(5):921-929. doi: 10.1016/j.sjbs.2018.06.007. Epub 2018 Jun 28.

Managing mosquitoes and ticks in a rapidly changing world - Facts and trends

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Vector-borne diseases transmitted by mosquitoes and ticks are on the rise. The effective and sustainable control of these arthropod vectors is a puzzling challenge for public health worldwide. In the present review, I attempted to provide a concise and updated overview of the current mosquito and tick research scenario. The wide array of control tools recently developed has been considered, with special reference to those approved by the World Health Organization Vector Control Advisory Group (WHO VCAG), as well as novel ones with an extremely promising potential to be exploited in vector control programs. Concerning mosquitoes, a major focus has been given on genetically modified vectors, eave tubes, attractive toxic sugar baits (ATSB) and biocontrol agents. Regarding ticks, the recent development of highly effective repellents and acaricides (including nanoformulated ones) as well as behavior-based control tools, has been highlighted. In the second part of the review, key research questions about biology and control of mosquitoes and ticks have been critically formulated. A timely research agenda outlining hot issues to be addressed in mosquito and tick research is provided. Overall, it is expected that the present review will contribute to boost research and applications on successful mosquito and tick control strategies, along with an improved knowledge of their biology and ecology.

Epidemiology

Ticks Tick Borne Dis. 2018 Mar;9(3):605-614. doi: 10.1016/j.ttbdis.2018.01.011. Epub 2018 Feb 3.

Surveillance of British ticks: An overview of species records, host associations, and new records of Ixodes ricinus distribution

Cull B[1], Pietzsch ME[2], Hansford KM[2], Gillingham EL[2], Medlock JM[3]. (UK)

Public Health England's passive Tick Surveillance Scheme (TSS) records the distribution, seasonality and host associations of ticks submitted from across the United Kingdom (UK), and helps to inform the UK government on emerging tick-borne disease risks. Here we summarise data collected through surveillance during 2010-2016, and compare with previous TSS data from 2005 to 2009, particularly in relation to the primary Lyme borreliosis vector Ixodes ricinus. 4173 records were submitted, constituting >14,000 ticks; 97% were endemic tick records (13,833 ticks of 11 species), with an additional 97 records of imported ticks (438 ticks of 17 species). Tick submissions were mainly from veterinary professionals (n = 1954; 46.8%) and members of the public and amateur entomologists (n = 1600; 38.3%), as well as from academic institutions (n = 249; 6.0%), wildlife groups (n = 239; 5.7%) and health professionals (n = 131; 3.1%). The most commonly reported hosts of endemic ticks were dogs (n = 1593; 39.1% of all records), humans (n = 835; 20.5%) and cats (n = 569; 14%). New host associations were recorded for a number of tick species. Ixodes ricinus was the most frequently recorded endemic tick species (n = 2413; 59.2% of all records), followed by I. hexagonus (n = 1355; 33.2%), I. canisuga (n = 132; 3.2%) and I. frontalis (n = 56; 1.4%), with other species each making up <1% total records. 81% of I. ricinus recorded from humans were nymphs, whereas 93.4% of I. ricinus from companion animals were adults. Recent TSS records of I. ricinus in the UK add a considerable amount of new presence data for this species, particularly in the southern regions of England, and confirm that this species is widespread across the UK. The scheme remains a valuable method of collecting continuous national distribution data on ticks from a variety of host species.

Parasit Vectors. 2018 Jan 8;11(1):20. doi: 10.1186/s13071-017-2570-1.

Epidemiology, genetic variants and clinical course of natural infections with Anaplasma phagocytophilum in a dairy cattle herd

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(Germany)

Natural infections with A. phagocytophilum in a dairy cattle herd in Germany were investigated over one pasture season by using serology, haematology, blood chemistry and polymerase chain reaction (PCR). Sequence analysis of partial 16S rRNA, groEL, msp2 and msp4 genes of A. phagocytophilum was carried out in order to trace possible genetic variants and their relations between cattle, roe deer (Capreolus capreolus) and ticks (Ixodes ricinus) in this area. In total 533 samples from 58 cattle, 310 ticks, three roe deer and one wild boar were examined. Our results show (i) typical clinical symptoms of TBF in first-time infected heifers, such as high fever, reduced milk yield, lower limb oedema and typical haematological and biochemical findings such as severe leukopenia, erythropenia, neutropenia, lymphocytopenia, monocytopenia, a significant increase in creatinine and bilirubin and a significant decrease in serum albumin, γ-GT, GLDH, magnesium and calcium; (ii) a high overall prevalence of A. phagocytophilum infections in this herd as 78.9% (15/19) of the naïve heifers were real-time PCR-positive and 75.9% (44/58) of the entire herd seroconverted; and (iii) a high level of sequence variation in the analysed genes with five variants of the 16S rRNA gene, two variants of the groEL gene, three variants of the msp2 gene and four variants in the msp4 gene with certain combinations of these variants. In cattle particular combinations of the genetic variants of A. phagocytophilum occurred, whereas three roe deer showed different variants altogether. This is indicative for a sympatric circulation of variants in this small geographical region (< 1 km^2). Both re- and superinfections with A. phagocytophilum were observed in five cattle showing that infection does not result in sterile immunity. For prevention of clinical cases we suggest pasturing of young, not pregnant heifers to reduce economical losses.

Ann Parasitol. 2018;64(4):265-284. doi: 10.17420/ap6404.162.

The role of particular ticks developmental stages in the circulation of tick-borne pathogens in Central Europe. 6. Babesia

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(Poland)

In the Central European conditions, three species of Babesia have epidemiological significance as human pathogens – Babesia divergens, B. microti and B. venatorum. Tick Ixodes ricinus is considered as their main vector, wild mammals as the animal reservoir. The zoonotic cycles of small and large Babesia differ in details. Due to the lack of transovarial mode transmission in small species B. microti, the circulation goes mainly between immature ticks and vertebrate hosts; pathogen circulates primarily in the cycle: infected rodent → the tick larva → the nymph → the mammal reservoir →the larva of the tick. The tick stages able to effectively infect human are nymphs and adult females, males do not participate in the follow transmission. For large Babesia – B. divergens and B.

venatorum, the transovarial and transstadial transmission enable the presence of the agent in adult ticks, moreover, that larvae and nymphs feed on not-susceptible hosts. The tick stages able to effectively infect cattle and other ruminants are adult females. Resuming, pathogen circulates primarily in the cycle the ruminant host – adult female tick – the larva – the nymph – adult female of the next generation – the ruminant. Due to the compound developmental transmission has place after the outflow of a tick began feeding.

Ticks Tick Borne Dis. 2019 Apr;10(3):505-512. doi: 10.1016/j.ttbdis.2018.12.009. Epub 2018 Dec 28.

Unravelling the phenology of Ixodes frontalis, a common but understudied tick species in Europe

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Tick-borne diseases have a complex epidemiology that depends on different ecological communities, associating several species of vertebrate hosts, vectors and pathogens. While most studies in Europe are focused on Ixodes ricinus, the common sheep tick, other Ixodes species may also be involved in the transmission or maintenance of different pathogens. In this study, we describe for the first time the activity pattern of I. frontalis, an understudied but widespread tick species associated with several common bird species in Europe. Questing ixodid tick stages (larvae, nymphs and adults) of both I. frontalis and I. ricinus were monitored by the drag sampling method over three years at the same locations in Western France. Differential activities were observed depending on I. frontalis life stages: nymphs and adults were present sporadically on the ground throughout the year, while larvae exhibited a marked peak of activity around October-November with tens or even hundreds of individuals per m^2, followed by a slow decrease in winter. Larvae were completely absent in summer, which contrasts with the high numbers of I. ricinus larvae at this time of the year. The vegetation and the litter where the two tick species were found also exhibited marked differences, with I. frontalis mostly collected under bamboo bushes.

Ticks Tick Borne Dis. 2019 Jan;10(1):191-206. doi: 10.1016/j.ttbdis.2018.10.003. Epub 2018 Oct 18.

Dermacentor reticulatus in Berlin/Brandenburg (Germany): Activity patterns and associated pathogens

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Dermacentor reticulatus is one of the most important European tick species. However, its spatial distribution, seasonality and regional vector role are not well known. This study aimed to gather information about abundance patterns of questing ticks and associated pathogens in unfed female adult D. reticulatus in the Berlin/Brandenburg area. Using the flagging method, questing ticks were collected at four sites in 2010-2012 and 2000 D. reticulatus were analysed regarding infection with Rickettsia, Babesia, Borrelia and

Anaplasmataceae by conventional or real-time PCR. Dermacentor reticulatus showed a bimodal activity pattern: highest numbers of adult ticks were recorded between March and end of May (mean 50 ticks/h) and from mid-August until end of November (mean 102 ticks/h). During summer, almost complete inactivity was observed (mean 0.4 ticks/h). Sporadic samplings from December to February revealed tick activity also during winter (mean 47 ticks/h), which was characterised by large fluctuations. Using negative binomial regression analysis, significant influences of the variables sampling site, season and temperature on the abundance of questing D. reticulatus were determined. The parameters relative humidity and year were not of significant importance. PCR analyses showed an average prevalence of 64% for Rickettsia sp. Large differences in pathogen frequencies were observed between sampling sites (31.4-78.3%). Regression analysis demonstrated a significant influence of the sampling site but not of season and year. Examinations regarding other pathogen groups indicated prevalences of 0.25% (Borrelia sp.) and 0.05% (Anaplasmataceae) but absence of Babesia sp. Sequencing of positive samples revealed infections with Rickettsia raoultii, Borrelia miyamotoi, Borrelia afzelii and Anaplasma phagocytophilum. The study shows stable populations of D. reticulatus in Berlin/Brandenburg. People should be aware of ticks throughout the year since Ixodes ricinus is co-endemic and active in spring, summer and autumn while adult D. reticulatus are active throughout the year and even in winter during periods of frost as long as it is warming up during the day. Prevalence of R. raoultii in the present study is among the highest described for D. reticulatus. Borrelia miyamotoi was detected for the first time in D. reticulatus, illustrating the importance of screening studies to evaluate the pathogen structure in D. reticulatus populations.

Med Vet Entomol. 2018 Dec;32(4):473-480. doi: 10.1111/mve.12335. Epub 2018 Sep 8.

Has the red sheep tick, Haemaphysalis punctata, recently expanded its range in England?

Medlock JM[1,2,3], Hansford KM[1,3], Vaux AGC[1], Cull B[1], Pietzsch ME[1], Gillingham EL[1,3], Johnson N[4], Phipps LP[4]. (UK)

The red sheep tick, Haemaphysalis punctata (Ixodida: Ixodidae), has been reported as present in the U.K. for more than a century; however, very little has been written about its distribution. In recent years, numbers of detections of this tick species reported to the Public Health England (PHE) Tick Surveillance Scheme have increased. This rise in the number of records may be attributable to increased tick surveillance activities or to the increased distribution of this species of tick in parts of England. This paper reviews published reports of H. punctata and all data held by the Biological Records Centre and PHE, and summarizes a number of field studies conducted by PHE and the Animal and Plant Health Agency over the past 8 years. It would appear from the evidence presented here that H. punctata may be expanding its range across the eastern part of the South Downs National Park, where there have also been reports of this tick species biting humans. It is possible that the movement of sheep between grassland sites is facilitating this spread. Further studies that better elucidate the ecology of this tick and its possible role as a vector of human and veterinary diseases are now warranted.

Ticks Tick Borne Dis. 2018 Sep;9(6):1555-1564. doi: 10.1016/j.ttbdis.2018.07.010. Epub 2018 Jul 26.

Detection of Anaplasma phagocytophilum, Candidatus Neoehrlichia sp., Coxiella burnetii and Rickettsia spp. in questing ticks from a recreational park, Portugal.

Santos AS[1], de Bruin A[2], Veloso AR[3], Marques C[3], Pereira da Fonseca I[3], de Sousa R[4], Sprong H[2], Santos-Silva MM[4]. (Portugal, Netherlands)

Tick-borne agents with medical relevance have been recorded in Portugal but little is known about their occurrence in urban outdoor leisure areas. This study aimed to investigate ticks and tick-borne agents in three public parks of Lisbon's metropolitan area. A total of 234 questing ticks belonging to eight species were found in Parque Florestal de Monsanto (PFM). Ixodes ventalloi represented 40% of collections. Mitochondrial genes confirmed Ixodes morphological identification, evidencing the intraspecific variability of I. ricinus and particularly I. frontalis populations. Regarding tick-borne agents, Rickettsia massiliae DNA were found in 21 (9.0%) ticks, Coxiella burnetii in 15 (6.4%), Anaplasma phagocytophilum in five (2.1%), an agent closely related to Candidatus Neoehrlichia mikurensis in two (0.9%), Rickettsia sibirica mongolitimonae and Rickettsia monacensis each in one (0.4%). Active enzootic cycles were suggested for these agents by the detection of positives in different time periods. Five tick species were founded with C. burnetii, including I. ventalloi which seems to be a new association record. This tick was also the only species found positive for A. phagocytophilum and the Candidatus Neoehrlichia mikurensis-like agent. Two A. phagocytophilum variants were detected in PFM, one of them representing a potentially new ecotype already found in I. ventalloi from another Portuguese area. To the authors´ knowledge, this is also the first report of such a Candidatus Neoehrlichia mikurensis-like microorganism. These data show an interesting diversity of ticks and tick-borne agents with potential public health relevance in PFM, an urban recreational area commonly frequented by humans and their pets.

New Microbes New Infect. 2018 Jan 6;22:30-36. doi: 10.1016/j.nmni.2017.12.011. eCollection 2018 Mar.

'*Candidatus* Neoehrlichia mikurensis' in Europe

Portillo A[1], Santibáñez P[1], Palomar AM[1], Santibáñez S[1], Oteo JA[1]. (Spain)

'*Candidatus* Neoehrlichia mikurensis' is an uncultured emerging bacterium that is provisionally included in the family *Anaplasmataceae.* In Europe, it is transmitted by *Ixodes ricinus* ticks. Rodents are the reservoirs. It is widely distributed in mammals (both wild and domestic) and birds. It causes an inflammatory disease in humans with underlying diseases, but the microorganism also affects immunocompetent individuals in which asymptomatic infection has been recognized. A high degree of suspicion and the use of molecular tools are needed for the correct diagnosis. Efforts to cultivate it and to investigate its pathogenesis should be a priority.

Parasit Vectors. 2018 Mar 20;11(1):130. doi: 10.1186/s13071-018-2713-z.

First detection of Borrelia miyamotoi in Ixodes ricinus ticks from northern Italy

Ravagnan S[1], Tomassone L[2], Montarsi F[1], Krawczyk AI[3], Mastrorilli E[1], Sprong H[3], Milani A[1], Rossi L[2], Capelli G[4]. (Italy, Netherlands)

Borrelia miyamotoi is a spirochete transmitted by several ixodid tick species. It causes a relapsing fever in humans and is currently considered as an emerging pathogen. In Europe, B. miyamotoi seems to occur at low prevalence in Ixodes ricinus ticks but has a wide distribution. Here we report the first detection of B. miyamotoi in Ixodes ricinus ticks collected in two independent studies conducted in 2016 in the north-eastern and north-western Alps, Italy. Three out of 405 nymphs (0.74%) tested positive for Borrelia miyamotoi. In particular, B. miyamotoi was found in 2/365 nymphs in the western and in 1/40 nymphs in the eastern alpine area. These are the first findings of B. miyamotoi in Italy. Exposure to B. miyamotoi and risk of human infection may occur through tick bites in northern Italy. Relapsing fever caused by Borrelia miyamotoi has not yet been reported in Italy, but misdiagnoses with tick-borne encephalitis, human granulocytic anaplasmosis or other relapsing fever can occur. Our findings suggest that B. miyamotoi should be considered in the differential diagnosis of febrile patients originating from Lyme borreliosis endemic regions. The distribution of this pathogen and its relevance to public health need further investigation.Study in Netherlands (2017) of animal hosts - Both rodents (9%) and birds (8%) were found positive for B. miyamotoi by PCR, whereas the roe deer samples were negative.

Exp Appl Acarol. 2018 Feb;74(2):191-199. doi: 10.1007/s10493-018-0220-8. Epub 2018 Jan 30.

First report of Borrelia miyamotoi in an Ixodes ricinus tick in Augsburg, Germany.

Page S, Daschkin C, Anniko S, Krey V, Nicolaus C, Maxeiner HG.

Tick borne infections and wild and domestic hosts

Ticks Tick Borne Dis. 2018 Feb;9(2):164-170. doi: 10.1016/j.ttbdis.2017.08.012. Epub 2017 Sep 1.

Tick-borne pathogens and their reservoir hosts in northern Italy

Baráková I[1], Derdáková M[2], Selyemová D[2], Chvostáč M[2], Špitalská E[3], Rosso F[4], Collini M[4], Rosà R[4], Tagliapietra V[4], Girardi M[4], Ramponi C[5], Hauffe HC[4], Rizzoli A[6]. (Slovakia, Italy)

During 2011-2012, 848 feeding Ixodeds sp. ticks at all life stages (adults, nymphs and larvae) from various hosts (wild ungulates, birds and rodents; domestic sheep, dogs and humans) were collected. The highest prevalences of A. phagocytophilum and Rickettsia spp. were detected in adult and nymphal tick stages feeding on wild ungulates (11.4% prevalence for both pathogens), while the Babesia spp. prevailed in nymphal and larval ticks feeding on wild birds (7.7%). A wide spectrum of tick-borne agents was present in larval ticks: those detached from wild ungulates were positive for A. phagocytophilum, B. venatorum, R. helvetica, R. monacensis and R. raoultii, while those removed from wild rodents were positive for B. venatorum, R. helvetica, R. monacensis and Candidatus Neoehrlichia mikurensis, and ticks from wild birds carried A. phagocytophilum, B. venatorum, B. capreoli and R. helvetica. This study provides evidence of circulation of five tick-borne pathogens not reported in this region before, specifically R. raoultii, R. monacensis, B. venatorum, B. capreoli and B. microti. Furthermore, it discusses the epidemiological role of the animal species from which the ticks were collected highlighting the needs for more experimental studies especially for those pathogens where transovarial transmission in ticks has been demonstrated.

Parasit Vectors. 2018 Sep 3;11(1):495. doi: 10.1186/s13071-018-3068-1

Diverse tick-borne microorganisms identified in free-living ungulates in Slovakia

Kazimírová M[1], Hamšíková Z[2], Špitalská E[3], Minichová L[3], Mahríková L[2], Caban R[4], Sprong H[5], Fonville M[5], Schnittger L[6,7], Kocianová E[3]. (Slovakia, Netherlands, Argentina)

Free-living ungulates are hosts of ixodid ticks and reservoirs of tick-borne microorganisms in central Europe and many regions around the world. Tissue samples and engorged ticks were obtained from roe deer, red deer, fallow deer, mouflon, and wild boar hunted in deciduous forests of south-western Slovakia. DNA isolated from these samples was screened for the presence of tick-borne microorganisms by PCR-based methods.Ticks were found to infest all examined ungulate species. The principal infesting tick was Ixodes ricinus, identified on 90.4% of wildlife, and included all developmental stages. Larvae and nymphs of Haemaphysalis concinna were feeding on 9.6% of wildlife. Two specimens of Dermacentor reticulatus were also identified. Ungulates were positive for A. phagocytophilum and Theileria spp. Anaplasma phagocytophilum was found to infect 96.1% of cervids, 88.9% of mouflon, and 28.2% of wild boar, whereas Theileria spp. was detected only in cervids (94.6%). Importantly, a high rate of cervids (89%) showed mixed infections with both these microorganisms. In addition to A. phagocytophilum and Theileria spp., Rickettsia helvetica, R. monacensis, unidentified Rickettsia sp., Coxiella burnetii, "Candidatus Neoehrlichia mikurensis", Borrelia burgdorferi (s.l.) and Babesia venatorum were identified in engorged I. ricinus. Furthermore, A. phagocytophilum, Babesia spp. and Theileria spp. were detected in engorged H. concinna. Analysis of 16S rRNA and groEL gene sequences revealed the presence of five and two A. phagocytophilum variants, respectively, among which sequences identified in wild boar showed identity to the sequence of the causative agent of human granulocytic anaplasmosis (HGA). Phylogenetic analysis of Theileria 18S rRNA gene sequences amplified from cervids and engorged I. ricinus ticks segregated jointly with sequences of T. capreoli isolates into a moderately supported monophyletic clade.The findings indicate that free-living ungulates are reservoirs for A. phagocytophilum and Theileria spp. and engorged ixodid ticks attached to ungulates are good sentinels for the presence of agents of public and veterinary concern. Further analyses of the A. phagocytophilum genetic variants and Theileria species and their associations with vector ticks and free-living ungulates are required.

Pol J Vet Sci. 2018 Jun;21(2):415-417. doi: 10.24425/122607.

Investigation of the tick-borne pathogens Rickettsia helvetica and Anaplasma phagocytophilum in the blood of the domestic goat (Capra hircus)

Rymaszewska A[1]. (Poland)

The bacterial species Anaplasma phagocytophilum and Rickettsia helvetica are pathogenic for humans and domestic animals and are transmitted by ticks, e.g., of the Ixodes genus. Most of the vertebrate species constituting reservoirs for anaplasmas are known, but the potential reservoirs of rickettsiae are still under discussion. This study presents an analysis of the DNA of tick-borne pathogens isolated from the whole blood of goats grazing on meadows in West Pomerania, Poland. No DNA of A. phagocytophilum was found in the blood of the goats, while the DNA of R. helvetica was detected in 5.5% of the animals. The potential role of ruminants in the circulation of R. helvetica remains unknown.

Ticks Tick Borne Dis. 2019 Feb;10(2):377-385. doi: 10.1016/j.ttbdis.2018.11.020. Epub 2018 Nov 27.

Coxiella burnetii in ticks and wild birds

Tokarevich NK[1], Panferova YA[2], Freylikhman OA[2], Blinova OV[2], Medvedev SG[3], Mironov SV[3], Grigoryeva LA[3], Tretyakov KA[3], Dimova T[4], Zaharieva MM[5], Nikolov B[6], Zehtindjiev P[6], Najdenski H[5]. (Russia, Bulgaria)

The study objective was to get more information on C. burnetii prevalence in wild birds and ticks feeding on them, and the potentialities of the pathogen dissemination over Europe by both. Blood, blood sera, feces of wild birds and ticks removed from those birds or from vegetation were studied at two sites in Russia: the Curonian Spit (site KK), and the vicinity of St. Petersburg (site SPb), and at two sites in Bulgaria: the Atanasovsko Lake (site AL), and the vicinity of Sofia (site SR). C. burnetii DNA was detected in blood, feces, and ticks by PCR (polymerase chain reaction). All positive results were confirmed by Sanger's sequencing of 16SrRNA gene target fragments. The antibodies to C. burnetii in sera were detected by CFR (complement fixation reaction). Eleven of 55 bird species captured at KK site hosted Ixodes ricinus. C. burnetii DNA was detected in three I. ricinus nymphs removed from one bird (Erithacus rubecula), and in adult ticks flagged from vegetation: 0.7% I. persulcatus (site SPb), 0.9% I. ricinus (site KK), 1.0% D. reticulatus (AL site). C. burnetii DNA was also detected in 1.4% of bird blood samples at SPb site, and in 0.5% of those at AL site. Antibodies to C. burnetii were found in 8.1% of bird sera (site SPb). C. burnetii DNA was revealed in feces of birds: 0.6% at AL site, and 13.7% at SR site. Both molecular-genetic and immunological methods were applied to confirm the role of birds as a natural reservoir of C. burnetii. The places of wild bird stopover in Russia (Baltic region) and in Bulgaria (Atanasovsko Lake and Sofia region) proved to be natural foci of C. burnetii infection. Migratory birds are likely to act as efficient "vehicles" in dispersal of C. burnetii -infested ixodid ticks.

Folia Parasitol (Praha). 2018 Jun 15;65. pii: 2018.008. doi: 10.14411/fp.2018.008.

Molecular screening for bacterial pathogens in ticks (Ixodes ricinus) collected on migratory birds captured in northern Italy

Pajoro M[1], Pistone D[2,3], Varotto Boccazzi I[3], Mereghetti V[4], Bandi C[1,3], Fabbi M[5], Scattorin F[3], Sassera D[6], Montagna M[4]. (Italy)

Migratory birds have an important role in transporting ticks and associated tick-borne pathogens over long distances. In this study, 2,793 migratory birds were captured by nets in a ringing station, located in northern Italy, and checked for the presence of ticks. Two-hundred and fifty-one ticks were identified as nymphs and larvae of Ixodes ricinus (Linnaeus, 1758) and they were PCR-screened for the presence of bacteria belonging to Borrelia burgdorferi sensu lato, Rickettsia spp., Francisella tularensis and Coxiella burnetii. Four species of Borrelia (B. garinii, B. afzelii, B. valaisiana and B. lusitaniae) and three species of Rickettsia (R. monacensis, R. helvetica and Candidatus Rickettsia mendelii) were detected in 74 (30%) and 25 (10%) respectively out of 251 ticks examined. Co-infection with Borrelia spp. and Rickettsia spp. in the same tick sample was encountered in 7 (7%) out of the 99 infected ticks. We report for the first time the presence of Candidatus Rickettsia mendelii in I. ricinus collected on birds in Italy. This study, besides confirming the role of birds in dispersal of I. ricinus, highlights an important route by which tick-borne pathogens might spread across different countries and from natural environments towards urbanised areas.

Vet Microbiol. 2018 Nov;226:15-22. doi: 10.1016/j.vetmic.2018.10.008. Epub 2018 Oct 11.

Differential identification of Anaplasma in cattle and potential of cattle to serve as reservoirs of Anaplasma capra, an emerging tick-borne zoonotic pathogen

Seo MG[1], Ouh IO[2], Lee H[3], Geraldino PJL[4], Rhee MH[3], Kwon OD[3], Kwak D[5]. (South Korea)

Bovine anaplasmosis is a tick-borne, infectious, non-contagious disease caused by Anaplasma marginale, A. centrale, A. bovis, and zoonotic A. phagocytophilum. Recently, Anaplasma capra detected in goats was identified as a novel zoonotic pathogen. To determine whether A. capra can infect bovines, we used PCR to differentially diagnose Anaplasma spp. in 1219 South Korean cattle by performing multilocus gene typing and restriction fragment length polymorphism (RFLP). Nucleotide sequencing and phylogenetic analysis detected the 16S rRNA gene of A. bovis and four genes from A. capra in 12 (1.0%) and five (0.4%) cattle, respectively. Supplementary discrimination between A. bovis and A. capra was accomplished by RFLP. The 16S rRNA, msp4, groEL, and gltA genes of A. capra identified in this study had much lower degrees of identity to those in A. centrale and other Anaplasma spp. A. phagocytophilum was not detected in any of the tested cattle. Although the prevalence was low, this study suggests the potential of cattle to serve as reservoirs of A. capra. Thus, further studies are needed to clarify the pathogenesis of A. capra in cattle and its possible involvement in transmission to humans.

Ann Parasitol. 2018;64(3):151-171. doi: 10.17420/ap6403.147.

The role of particular ticks developmental stages in the circulation of tick-borne pathogens in Central Europe. 5. Borreliaceae

Karbowiak G[1], Biernat B[2], Stańczak J[2], Werszko, J[1], Szewczyk T[1], Sytykiewicz H[3]. (Poland)

The developmental cycles of all B. burgdorferi s.l. genospecies present typical, main pattern described in the 90thies. The simple scheme might be modified according to the biology of species and hosts preference. Central European genospecies of B. burgdorferi s.l. can be associated with four groups of hosts playing the role of animal reservoirs. The group 1 contains genospecies associated with rodents as primary animal reservoir – B. afzelii, B. garinii, B. burgdorferi sensu stricto, strains B. bavariensis (B. garinii OspA serotype 4). The group 2 involves B. valaisiana and most of B. garinii strains, associated with birds. The group 3 involves B. spielmanii, the reservoir hosts are Gliridae, and hedgehogs. The group 4 includes B. lusitaniae, the hosts are lizards. B. miyamotoi enzootic cycle seems to be similar to B. burgdorferi complex, however, differs by the transovarial transmission possibility. The divisions are not extreme; in the hosts group, infected with appropriate Borrelia genospecies, very often are found the specimens infected with other genospecies.

Vet Parasitol. 2018 Feb 15;251:17-26. doi: 10.1016/j.vetpar.2017.12.018. Epub 2017 Dec 25.

Neglected vector-borne zoonoses in Europe: Into the wild

Tomassone L[1], Berriatua E[2], De Sousa R[3], Duscher GG[4], Mihalca AD[5], Silaghi C[6], Sprong H[7], Zintl A[8]. (Multinational)

Wild vertebrates are involved in the transmission cycles of numerous pathogens. Additionally, they can affect the abundance of arthropod vectors. Urbanization, landscape and climate changes, and the adaptation of vectors and wildlife to human habitats represent complex and evolving scenarios, which affect the interface of vector, wildlife and human populations, frequently with a consequent increase in zoonotic risk. While considerable attention has focused on these interrelations with regard to certain major vector-borne pathogens such as Borrelia burgdorferi s.l. and tick-borne encephalitis virus, information regarding many other zoonotic pathogens is more dispersed. In this review, we discuss the possible role of wildlife in the maintenance and spread of some of these neglected zoonoses in Europe. We present case studies on the role of rodents in the cycles of Bartonella spp., of wild ungulates in the cycle of Babesia spp., and of various wildlife species in the life cycle of Leishmania infantum, Anaplasma phagocytophilum and Rickettsia spp. These examples highlight the usefulness of surveillance strategies focused on neglected zoonotic agents in wildlife as a source of valuable information for health professionals, nature managers and (local) decision-makers. These benefits could be further enhanced by increased collaboration between researchers and stakeholders across Europe and a more harmonised and coordinated approach for data collection.

Sci Rep. 2018 Jan 30;8(1):1865. doi: 10.1038/s41598-018-20138-1.

Detection of tick-borne bacteria and babesia with zoonotic potential in Argas (Carios) vespertilionis (Latreille, 1802) ticks from British bats

Lv J[1,2], Fernández de Marco MDM[2], Goharriz H[2], Phipps LP[2], McElhinney LM[2,3], Hernández-Triana LM[2], Wu S[1], Lin X[1], Fooks AR[2,3], Johnson N[4,5]. (China, UK)

Ticks host a wide range of zoonotic pathogens and are a significant source of diseases that affect humans and livestock. However, little is known about the pathogens associated with bat ticks. We have collected ectoparasites from bat carcasses over a seven year period. Nucleic acids (DNA and RNA) were extracted from 296 ticks removed from bats and the species designation was confirmed in all ticks as Argas (Carios) vespertilionis. A subset of these samples (n = 120) were tested for the presence of zoonotic pathogens by molecular methods. Babesia species, Rickettsia spp., within the spotted fever group (SFG), and Ehrlichia spp. were detected in ticks removed from 26 bats submitted from 14 counties across England. The prevalence of Rickettsia spp. was found to be highest in Pipistrellus pipistrellus from southern England. This study suggests that the tick species that host B. venatorum may include the genus Argas in addition to the genus Ixodes. As A. vespertilionis has been reported to feed on humans, detection of B. venatorum and SFG Rickettsia spp. could present a risk of disease transmission in England. No evidence for the presence of flaviviruses or Issyk-Kul virus (nairovirus) was found in these tick samples.

Transbound Emerg Dis. 2018 Jun;65(3):765-772. doi: 10.1111/tbed.12801. Epub 2018 Jan 11.

Exposure to West Nile virus and tick-borne encephalitis virus in dogs in Spain.

García-Bocanegra I, Jurado-Tarifa E, Cano-Terriza D, Martínez R, Pérez-Marín JE, Lecollinet S.

Serological screening dogs can show presence of these viruses in certain areas.

Zoonoses

Zoonoses Public Health. 2019 Mar;66(2):216-222. doi: 10.1111/zph.12554. Epub 2018 Dec 28.

Tick-borne encephalitis virus in cows and unpasteurized cow milk from Norway

Paulsen KM[1,2], Stuen S[3], das Neves CG[4], Suhel F[4], Gurung D[1], Soleng A[5], Stiasny K[6], Vikse R[1], Andreassen ÅK[1], Granquist EG[2]. (Norway)

Tick-borne encephalitis virus (TBEV) is recognized as the most important zoonotic tick-transmitted virus in Europe. TBEV is mainly transmitted to humans through bites from TBEV-infected ticks (Ixodes ricinus and Ixodes persulcatus). However, alimentary infection after consumption of unpasteurized milk and cheese from domestic ruminants has been reported. There is little information about TBEV in ruminants in Norway. The objectives of this study were to analyse unpasteurized cow milk for TBEV RNA and to study the presence of IgG antibodies to TBEV in the same animals. A total of 112 milk and blood samples were collected from cows from five different farms spread from southern to northern Norway. The milk samples were analysed by an in-house reverse transcription (RT) real-time polymerase chain reaction and confirmed by pyrosequencing. Serum samples were screened by a commercial enzyme-linked immunosorbent assay and verified by a TBEV-specific serum neutralization test. We found TBEV RNA in unpasteurized milk collected from farms in the municipalities of Mandal, Skedsmo and Brønnøy in 5.4% of the tested animals. Specific antibodies to TBEV were only detected in Arendal, where 88.2% of the tested animals were positive. Further studies on milk containing TBEV RNA should be performed to conclude if TBEV found in unpasteurized milk in Norway is infectious, which could be of great importance in a One Health perspective.

Other abstracts were on emerging/ potential threat zoonoses

- Thrombocytopenia syndrome virus (China) – animal hosts demonstrated
- Tick-borne phleboviruses (Japan, Albania, Balkan peninsula)
- Vector borne bacterial and arbovirus infections Mediterranean and new threats to Europe
- Crimean-Congo haemorrhagic fever Bulgaria – evidence of animal exposure
- Alkhurma hemorrhagic fever virus in ticks on migrating birds in Europe and Asia minor

Int J Environ Res Public Health. 2018 Sep 29;15(10). pii: E2145. doi: 10.3390/ijerph15102145.

Assessment of the Public Health Threats Posed by Vector-Borne Disease in the United Kingdom (UK)

Medlock JM[1,2,3], Hansford KM[4,5], Vaux AGC[6], Cull B[7], Gillingham E[8,9], Leach S[10,11]. (UK)

In recent years, the known distribution of vector-borne diseases in Europe has changed, with much new information also available now on the status of vectors in the United Kingdom (UK). For example, in 2016, the UK reported their first detection of the non-native mosquito *Aedes albopictus*, which is a known vector for dengue and chikungunya virus. In 2010, *Culex modestus*, a principal mosquito vector for West Nile virus was detected in large numbers in the Thames estuary. For tick-borne diseases, data on the changing distribution of the Lyme borreliosis tick vector, *Ixodes ricinus*, has recently been published, at a time when there has been an increase in the numbers of reported human cases of Lyme disease. This paper brings together the latest surveillance data and pertinent research on vector-borne disease in the UK, and its relevance to public health. It highlights the need for continued vector surveillance systems to monitor our native mosquito and tick fauna, as well as the need to expand surveillance for invasive species. It illustrates the importance of maintaining surveillance capacity that is sufficient to ensure accurate and timely disease risk assessment to help mitigate the UK's changing emerging infectious disease risks, especially in a time of climatic and environmental change and increasing global connectivity.

Vet Rec. 2018 May 26;182(21):609. doi: 10.1136/vr.k2264. No abstract available.

Tick bites and tick-transmitted diseases.

Rocchi M, Dagleish M, McInnes C.

This was wriiten in response to the research comment ‘What is the risk of tickborne disease to UK pets?’ (*VR*, 5 May 2018, vol 182, pp 511-513). Tulloch, commenting on an original research article printed in the same issue – Be tick aware: when and where to check cats and dogs for ticks (p 514) – emphasises the requirement for further research into the prevalence of tick-borne diseases in domestic pets within the UK. We agree, but while Tulloch indicated only two tick-borne infections (*Babesia canis* and *Borrelia burgdoferi*) that can be acquired in the UK by pets and two that may be encountered through travel on the European continent (tickborne encephalitis [TBE] and Crimean-Congo haemorrhagic fever [CCHF]), we wish to highlight a further two infections, louping-ill, caused by louping-ill virus and tick-borne fever (TBF), caused by *Anaplasma phagocytophilum*. These are endemic to the UK and can also be transmitted to pets from the same tick that transmits the other infections – *Ixodes ricinus*.

Int J Infect Dis. 2019 Feb;79:77-84. doi: 10.1016/j.ijid.2018.11.017. Epub 2018 Nov 27.

Animals as amplification hosts in the spread of severe fever with thrombocytopenia syndrome virus: A systematic review and meta-analysis

Chen C[1], Li P[2], Li KF[2], Wang HL[2], Dai YX[2], Cheng X[1], Yan JB[3]. (China)

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging tick-borne infectious disease caused by severe fever with thrombocytopenia syndrome virus (SFTSV). The seroprevalence of anti-SFTSV antibodies specific to SFTSV (IgG or IgM) has been investigated in different animal hosts in many epidemiological studies, but no systematic estimation of seroprevalence has yet been performed. Hence, this meta-analysis was conducted in order to obtain a more comprehensive result to clarify the prevalence of SFTSV in animals. A search for all relevant articles was conducted in the major national and international electronic databases up to August 2018. Data on seroprevalence of SFTSV antibodies (IgM and IgG) were extracted as the primary outcome. The pooled seroprevalence rates and 95% confidence intervals (95% CI) were determined. Overall, anti-SFTSV antibodies (IgG or IgM) were detected in 15 animal species. The pooled seroprevalence of anti-SFTSV antibodies was 45.70% in goats and sheep, 36.70% in cattle, 29.50% in dogs, 9.60% in chickens, 3.20% in rodents, and 3.20% in pigs. The seroprevalence of SFTSV in animals that had a confined range was significantly lower than that in free-range animals. SFTSV RNA was detected in 11 animal species, with a carriage rate varying from 0.23% to 26.31%.SFTSV has a wide spectrum of animal hosts, including domestic and wild animals. The prevalence of SFTSV is high among specific animal species.

Ticks Tick Borne Dis. 2019 Feb;10(2):328-335. doi: 10.1016/j.ttbdis.2018.11.012. Epub 2018 Nov 16.

Infection of newly identified phleboviruses in ticks and wild animals in Hokkaido, Japan indicating tick-borne life cycles

Torii S[1], Matsuno K[2], Qiu Y[3], Mori-Kajihara A[4], Kajihara M[4], Nakao R[5], Nao N[4], Okazaki K[6], Sashika M[7], Hiono T[8], Okamatsu M[8], Sakoda Y[9], Ebihara H[10], Takada A[11], Sawa H[12]. (Japan)

Recent discoveries of tick-borne pathogens have raised public health concerns on tick-borne infectious diseases and emphasize the need to assess potential risks of unrecognized tick-borne pathogens. First, to determine the existence of tick-borne phleboviruses (TBPVs), genetic surveillance of phleboviruses in ticks was conducted mainly in Hokkaido, the northernmost island in Japan from 2013 to 2015. Genes of two TBPVs, previously reported as Mukawa virus (MKWV) and a newly identified relative of MKWV, Kuriyama virus (KURV), were detected and the viruses were isolated from Ixodes persulcatus collected in Hokkaido, but not in I. persulcatus collected from other areas of Japan. These viruses were phylogenetically and antigenically similar to each other. Next, to investigate the infection of MKWV in mammals, serum samples from wildlife captured in Hokkaido from 2007 to 2011 were used for serological screening. Neutralizing antibodies against MKWV were detected in both Yezo-deer (Cervus nippon yesoensis) (2/50) and raccoons (Procyon lotor) (16/64). However, no infectious MKWV was recovered from laboratory mice in experimental infections, though viral RNAs were detected in their tissues. Thus, MKWV and KURV may maintain tick-mammalian life cycles in Hokkaido, suggesting their potential as causative agents of tick-borne.

Pathog Dis. 2018 Nov 1;76(8). doi: 10.1093/femspd/fty083.

Prevalence of tick-borne viruses in Ixodes ricinus assessed by high-throughput real-time PCR

Gondard M[1], Michelet L[1], Nisavanh A[1], Devillers E[1], Delannoy S[2], Fach P[2], Aspan A[3], Ullman K[3], Chirico J[3], Hoffmann B[4], van der Wal FJ[5], de Koeijer A[5], van Solt-Smits C[5], Jahfari S[6], Sprong H[6], Mansfield KL[7], Fooks AR[7], Klitgaard K[8], Bødker R[8], Moutailler S[1]. (Multi-national)

Ticks are one of the principal arthropod vectors of human and animal infectious diseases. Whereas the prevalence of tick-borne encephalitis virus in ticks in Europe is well studied, there is less information available on the prevalence of the other tick-borne viruses (TBVs) existing worldwide. The aim of this study was to improve the epidemiological survey tools of TBVs by the development of an efficient high-throughput test to screen a wide range of viruses in ticks.In this study, we developed a new high-throughput virus-detection assay based on parallel real-time PCRs on a microfluidic system, and used it to perform a large scale epidemiological survey screening for the presence of 21 TBVs in 18 135 nymphs of Ixodes ricinus collected from five European countries. This extensive investigation has (i) evaluated the prevalence of four viruses present in the collected ticks, (ii) allowed the identification of viruses in regions where they were previously undetected.In conclusion, we have demonstrated the capabilities of this new screening method that allows the detection of numerous TBVs in a large number of ticks. This tool represents a powerful and rapid system for TBVs surveillance in Europe and could be easily customized to assess viral emergence.

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Detection of a Novel Phlebovirus (Drin Virus) from Sand Flies in Albania

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Phlebotomine sand flies are generalist vectors with significant implications for public health. They are able to transmit phleboviruses that cause sand fly fever, headaches, or meningitis in humans. Albania is a country in Southeast Europe with a typical Mediterranean climate which provides convenient conditions for the presence of sand flies. Hence, the circulation of phleboviruses, such as the Toscana and Balkan viruses, has been recently described in the country. We followed a virus discovery approach on sand fly samples collected in 2015 and 2016 in seven regions of Albania, with the aim to investigate and characterize potentially circulating phleboviruses in phlebotomine sand flies. A presumed novel phlebovirus was detected in a pool consisting of 24 *Phlebotomus neglectus* males. The virus was provisionally named the *Drin virus* after a river near the locality of Kukës, where the infected sand flies were trapped. Genetic and phylogenetic analysis revealed that the Drin virus is closely related to the Corfou (CFUV) virus, isolated in the 1980s from *Phlebotomus major* sand flies on the eponymous island of Greece, and may also be involved in human infections because of its similarity to the sand fly fever Sicilian virus. The latter justifies further studies to specifically address this concern. Together with recent findings, this study confirms that Albania and the Balkan peninsula are hot spots for phleboviruses.

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Neglected vector-borne bacterial diseases and arboviruses in the Mediterranean area

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Arthropod vectors can transmit pathogenic microorganisms from one vertebrate to another during their blood meal. Although some vector-borne diseases have been eradicated in the Mediterranean area, such as malaria and dengue, recent endemic microorganisms (Toscana virus, *Rickettsia* spp.) remain neglected even though they cause many more cases. New diagnostic tools and innovative tools for the identification and characterization of vector species and microorganisms have been developed at IHU Méditerranée Infection, either internally or through collaborative and integrated projects. We have detected *Rickettsia slovaca* as a human pathogen and have described the disease; we have shown that *Rickettsia felis* can be transmitted by *Anopheles* mosquitoes; we have emphasized the increasing importance of bedbug (*Cimex lectularius*) as a potential vector of *Bartonella quintana*; and we have described the Toscana virus, a major agent of meningitis and meningoencephalitis which was disseminated in North Africa and Central and Eastern Europe, where it frequently cocirculates with a large number of newly described phleboviruses transmitted by sand flies.

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Ongoing and emerging arbovirus threats in Europe

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During the last decades, arboviruses that are endemic in Europe have expanded their geographic range and caused an increasing number of human outbreaks. These viruses include West Nile virus, which is expanding its area of circulation in central and southern Europe; Usutu virus, with increasing evidence of a role in human disease; tick-borne encephalitis virus, which is being detected in northern areas and at higher altitudes as a consequence of climate warming; Crimean-Congo hemorrhagic fever virus, which is endemic in Eastern Europe and the Middle East, but has been recently detected in Spain; other viruses, such as California encephalitis virus antigenic group, which circulate in northern and central Europe but whose relevance for human disease in largely unknown. In addition, the rise in global travel and trade has posed Europe to an increased risk of introduction and expansion of exotic arthropod vectors and autochthonous transmission of arboviruses, like dengue and chikungunya viruses, following new introductions from endemic areas. Implementation of integrated arbovirus surveillance programs has been crucial to adopt proper control measures. The identification of emerging outbreaks is however challenging and requires a high degree of awareness and laboratory capacity.

Euro Surveill. 2018 Nov;23(45). doi: 10.2807/1560-7917.ES.2018.23.45.1800201.

Tick-borne encephalitis in Europe, 2012 to 2016

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Since 2012, tick-borne encephalitis (TBE) is a notifiable in the European Union. The European Centre for Disease Prevention and Control annually collects data from 28 countries plus Iceland and Norway, based on the EU case definition. Between 2012 and 2016, 23 countries reported 12,500 TBE cases (Ireland and Spain reported none), of which 11,623 (93.0%) were confirmed cases and 878 (7.0%) probable cases. Two countries (Czech Republic and Lithuania) accounted for 38.6% of all reported cases, although their combined population represented only 2.7% of the population under surveillance.

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High seroprevalence for Crimean-Congo haemorrhagic fever virus in ruminants in the absence of reported human cases in many regions of Bulgaria

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Crimean-Congo haemorrhagic fever (CCHF) is a severe tick-borne zoonotic disease in humans caused by CCHF virus. It has been observed in Bulgaria since 1952 and over the years more than 1600 cases have been reported in the country. Close contact with viraemic livestock was shown as one of the main causes of the infection. Detection of CCHF virus specific antibodies in livestock can be used as an indicator for virus circulation and risk assessment. CCHF seroprevalence was investigated in 843 cattle, 88 goats and 130 sheep, originating from all 28 districts of Bulgaria. CCHF virus-specific IgG antibodies were observed in 165 cattle (19.6, 95% CI 17.0-22.4%), in 20 goats (22.7, 95% CI 15.2-32.5%) and in 10 sheep (7.7, 95% CI 4.2-13.6%). The highest seroprevalence was detected in the district of Kardzhali, South Bulgaria (86.7, 95% CI 73.8-93.7%), a well-known focus of CCHF in humans. The other two districts with human CCHF cases, Blagoevgrad and Burgas, located in southwest and southeast Bulgaria, showed overall seroprevalences in livestock of 41.9% (95% CI 28.4-56.7%) and 31.3% (95% CI 22.2-42.1%), respectively. Seroprevalences in districts with no history of human CCHF cases varied between 55% (95% CI 39.8-69.3%) and 22.5% (95% CI 12.3-37.5%). These results suggest frequent CCHF virus infections even in regions without human CCHF cases and an enhanced risk of infection for humans in close contact with the infected livestock.

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Alkhurma Hemorrhagic Fever Virus RNA in Hyalomma rufipes Ticks Infesting Migratory Birds, Europe and Asia Minor

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Alkhurma hemorrhagic fever virus RNA was detected in immature Hyalomma rufipes ticks infesting northward migratory birds caught in the North Mediterranean Basin. This finding suggests a role for birds in the ecology of the Alkhurma hemorrhagic fever virus and a potential mechanism for dissemination to novel regions. Increased surveillance is warranted.

Ticks Tick Borne Dis. 2018 Mar;9(3):500-505. doi: 10.1016/j.ttbdis.2018.01.009. Epub 2018 Feb 3.

High prevalence of Rickettsia helvetica in wild small mammal populations in Germany

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Since the beginning of the 21st century, spotted fever rickettsioses are known as emerging diseases worldwide. Rickettsiae are obligately intracellular bacteria transmitted by arthropod vectors. The ecology of Rickettsia species has not been investigated in detail, but small mammals are considered to play a role as reservoirs. Aim of this study was to monitor rickettsiae in wild small mammals over a period of five years in four federal states of Germany. Initial screening of ear pinna tissues of 3939 animals by Pan-Rick real-time PCR targeting the citrate synthase (gltA) gene revealed 296 rodents of seven species and 19 shrews of two species positive for rickettsial DNA. Outer membrane protein gene (ompB, ompAIV) PCRs based typing resulted in the identification of three species: Rickettsia helvetica (90.9%) was found as the dominantly occurring species in the four investigated federal states, but Rickettsia felis (7.8%) and Rickettsia raoultii (1.3%) were also detected. The prevalence of Rickettsia spp. in rodents of the genus Apodemus was found to be higher (approximately 14%) than in all other rodent and shrew species at all investigated sites. General linear mixed model analyses indicated that heavier (older) individuals of yellow-necked mice and male common voles seem to contain more often rickettsial DNA than younger ones. Furthermore, rodents generally collected in forests in summer and autumn more often carried rickettsial DNA. In conclusion, this study indicated a high prevalence of R. helvetica in small mammal populations and suggests an age-dependent increase of the DNA prevalence in some of the species and in animals originating from forest habitats. The finding of R. helvetica and R. felis DNA in multiple small mammal species may indicate frequent trans-species transmission by feeding of vectors on different species. Further investigations should target the reason for the discrepancy between the high rickettsial DNA prevalence in rodents and the so far almost absence of clinical apparent human infections.

Poultry mites

Tomley, F.M., Sparagano, O.

Spotlight on avian pathology: red mite, a serious emergent problem in layer hens

(2018) Avian Pathology, 47 (6), pp. 533-535.

Dermanyssus gallinae, the poultry red mite, is currently the most important ectoparasite of the egg laying industry worldwide with an expanding global prevalence. As a blood-feeder, it causes anaemia and severe welfare issues to the hens and it is a major cause of economic losses. It is also a vector for Salmonella species, avian influenza and potentially for other vector-borne pathogens. Paradoxically, there is a notable lack of funding for research into poultry red mite and an urgent need for effective and safe control strategies, sustainable therapies, prophylactics and integrated pest management.

Camarda, A., Pugliese, N., Bevilacqua, A., Circella, E., Gradoni, L., George, D., Sparagano, O., Giangaspero, A.

Efficacy of a novel neem oil formulation (RP03™) to control the poultry red mite Dermanyssus gallinae

(2018) *Medical and Veterinary Entomology*, 32 (3), pp. 290-297.

Dermanyssus gallinae (Mesostigmata: Dermanyssidae) is the most harmful ectoparasite of laying hens, represents an occupational hazard for poultry workers, and a growing threat to medical science per se. There is increasing demand for alternative products, including plant-derived acaricides, with which to control the mite. The present study investigated the efficacy of neem oil against D. gallinae on a heavily infested commercial laying hen farm. A novel formulation of 20% neem oil, diluted from a 2400-p.p.m. azadirachtin-concentrated stock (RP03™), was administered by nebulization three times in 1 week. Using corrugated cardboard traps, mite density was monitored before, during and after treatment and results were statistically analysed. Mite populations in the treated block showed 94.65%, 99.64% and 99.80% reductions after the first, second and third product administrations, respectively. The rate of reduction of the mite population was significantly higher in the treated block ($P < 0.001$) compared with the control and buffer blocks. The results suggest the strong bioactivity of neem, and specifically of the patented neem-based formulation RP03™, against D. gallinae. The treatment was most effective in the 10 days following the first application and its effects persisted for over 2 months. Further studies will aim to overcome observed side effects of treatment represented by an oily layer on equipment and eggs.

Abdelfattah, E.M., Vezzoli, G., Buczkowski, G., Makagon, M.M.

Essential oils: effects of application rate and modality on potential for combating northern fowl mite infestations

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The northern fowl mite (NFM), Ornithonyssus sylviarum (Mesostigmata: Macronyssidae), is the primary blood-feeding ectoparasite found on poultry in the U.S.A. Three experiments were conducted in vitro to test the acaricidal properties of cade, garlic, lavender, lemongrass, pine and thyme essential oils against NFM, and to evaluate whether these effects are altered by adjusting oil application rates and application modality (direct vs. vapour contact). Applied at the rate of 0.21 mg/cm2, the essential oils of cade, thyme, lemongrass and garlic resulted in higher NFM mortality at 24 h post-application than lavender and pine oils, and the untreated and ethanol-treated controls. Cade and thyme were the most consistent and fast-acting of the essential oils in terms of toxicity to NFM. Cade applied at 0.21 mg/cm2 and 0.11 mg/cm2 and thyme applied at 0.21 mg/cm2 were effective in eliminating NFM within 2 h through direct contact. The modality of application did not affect the efficacy of cade and thyme essential oils. The results suggest that essential oils may be utilized as alternatives to chemical pesticides and could be used as fumigants for the control of NFM.

Wildlife

Sparks, A.M., Watt, K., Sinclair, R., Pilkington, J.G., Pemberton, J.M., Johnston, S.E., McNeilly, T.N., Nussey, D.H.

Natural selection on antihelminth antibodies in a wild mammal population

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An effective immune response is expected to confer fitness benefits through improved resistance to parasites but also incur energetic costs that negatively impact fitness-related traits, such as reproduction. The fitness costs and benefits of an immune response are likely to depend on host age, sex, and levels of parasite exposure. Few studies have examined the full extent to which patterns of natural selection on immune phenotypes vary across demographic groups and environments in the wild. Here, we assessed natural selection on plasma levels of three functionally distinct isotypes (IgA, IgE, and IgG) of antibodies against a prevalent nematode parasite measured in a wild Soay sheep population over 26 years. We found little support for environment-dependent selection or reproductive costs. However, antibody levels were negatively associated with parasite egg counts and positively associated with subsequent survival, albeit in a highly age-and isotype-dependent manner. Raised levels of antiparasite IgA best predicted reduced egg counts, but this did not predict survival in lambs. In adults increased antiparasite IgG predicted reduced egg counts, and in adult females IgG levels also positively predicted overwinter survival. Our results highlight the potential importance of age-and sex-dependent selection on immune phenotypes in nature and show that patterns of selection can vary even among functionally related immune markers.

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Causes of mortality and morbidity in free-ranging mustelids in Switzerland: Necropsy data from over 50 years of general health surveillance

(2018) *BMC Veterinary Research*, 14 (1), art. no. 195.

Background: Although mustelids occur worldwide and include a wide range of species, little is known about the diseases affecting them. Mustelids have regularly been submitted for post mortem investigation in the framework of the program for general wildlife health surveillance in Switzerland, which has been in place for nearly 60 years. We performed a retrospective analysis of the necropsy reports on mustelids submitted to the diagnostic service of the University of Bern. The aims of this study were to present an overview of the causes of mortality and morbidity observed in these carnivores, to assess differences among species, to assess changes in disease detection over the study period, and to describe the pathology of selected diseases. Results: Five hundred and sixty-six reports from 1958 to 2015 were analyzed. Most animals were stone martens (Martes foina, 46%) and badgers (Meles meles, 44%); the remaining species were polecats (Mustela putorius, 4.7%), pine martens (Martes martes, 2%), stoats (Mustela erminea, 1.4%), weasels (Mustela nivalis, 0.8%) and otters (Lutra lutra, 0.3%). Infectious diseases (n = 262) were frequent and were mostly bacterial or viral; non-infectious conditions (n = 169) were less common and were mostly traumatic or due to metabolic disorders. The most frequent diagnoses included distemper (75% were badgers), amyloidosis (96% were martens), bacterial respiratory infections (all mustelids), biting lice (badgers only) and pulmonary and gastro-intestinal helminths (all species). Less frequent diseases included histoplasmosis (badgers only), aspergillosis, toxoplasmosis, hepatozoonosis, and sarcoptic mange. Lesions due to infection with distemper virus were primarily appreciated in the respiratory tract and central nervous system; they presented species-specific characteristics such as necrosis in the ependyma in badgers and absence of syncytia in stone martens. Amyloidosis in martens was multisystemic in most cases and included both AA and AL amyloidosis; the main macroscopic change was severe splenomegaly. Conclusion: Infectious diseases were the most frequent causes of morbidity and mortality of mustelids, with marked species-specific differences. Lung and skin were the most commonly affected organs. Contagious diseases such as canine distemper, sarcoptic mange and rabies in mustelids showed a similar temporal pattern as in red foxes (Vulpes vulpes), suggesting pathogen spillovers from foxes to mustelids.

Companion animals

Otranto, D.

Arthropod-borne pathogens of dogs and cats: From pathways and times of transmission to disease control.

(2018) *Veterinary Parasitology*, 251, pp. 68-77.

Vector-borne pathogens have developed a close relationship with blood feeding arthropod ectoparasites (e.g., mosquitoes, ticks, phlebotomine sand flies, black flies, fleas, kissing bugs, lice) and exploited a huge variety of vector transmission routes. Therefore, the life cycles of these pathogens result in a long evolved balance with the respective arthropod biology, ecology and blood feeding habits, instrumentally to the infection of several animal species, including humans. Amongst the many parasite transmission modes, such as ingestion of the arthropod, with its faeces or secretions, blood feeding represents the main focus for this article, as it is a central event to the life of almost all arthropod vectors. The time frame in which pathogens are transmitted to any animal host is governed by a large number of biological variables related to the vector, the pathogen, the host and environmental factors. Scientific data available on transmission times for each pathogen are discussed relative to their impact for the success of vector-borne disease control strategies. Blocking pathogen transmission, and thus preventing the infection of dogs and cats, may be achievable by the use of chemical compounds if they are characterised by a fast onset of killing activity or repellence against arthropods. The fast speed of kill exerted by systemic isoxazoline, as well as the repellent effect of pyrethroids have renewed the interest of the scientific community and pharmaceutical companies towards reducing the burden of vector-borne diseases under field conditions. However, endosymbionts and vaccines targeting arthropods or pathogen antigens should be further investigated as alternative strategies towards the goal of achieving an effective integrated control of vector-borne diseases.