Executive summary

This is a summary of the literature and horizon scanning report, produced from 302 abstracts and peer and non-peer reviewed papers in parasitology published in 2013, of relevance to Defra, AHVLA and the wider industry.

Highlights

Gastro-intestinal parasites

- Parasitic diseases in food producing animals, particularly grazing ruminants have a major effect on production. Improved chemical control in the past has allowed vast improvements in food production however the future challenges of drug resistance and climate change have now to be met. Parasite modelling, breeding for resistance, nutraceuticals and vaccination are areas of research currently trying to meet these challenges. It is important that surveillance continues and co-operation with university researchers to enable their outcomes to be used in the field.

- Monepantel resistance on a goat farm in New Zealand emerged less than 2 years after launch, showing that reliance on chemical control of parasites is not sustainable and the importance of sustainable control methods. This is aided by industry led bodies such as SCOPS.

Fasciolosis

- John Dalton wrote a review of potential molecular candidates for vaccines against Fasciola hepatica. This paper contains excellent information on how parasites modulate the immune system to allow their survival in the hostile environments of the host's tissues. Dalton speculates that some of the molecules secreted by the parasite therefore have the potential to treat autoimmune diseases and chronic inflammation. This indicates evaluation of ‘uses’ of parasites other than disease causing organisms.

Vector borne diseases

- Further identification of spotted fever Rickettsia in Dermacentor reticularis and Haemaphysalis punctata ticks in the UK. These two tick species are highly restricted in their distribution in England and Wales, but where they do occur they can be abundant. Research towards clarifying firstly the geographic distribution of rickettsiae in UK ticks, and secondly to assess the prevalence rates in ticks, wild and domesticated animals and humans to identify the drivers for disease transmission and their public health significance should be carried out.
• Links of vector borne disease with climate anomalies such as El Niño and also north Atlantic Oscillations were investigated with possibilities of developing early warning systems in the future. The development of such early warning systems would be potentially useful to all and allow focussed risk assessment for potential new diseases.

• Rift Valley fever (RVF), a vector-borne zoonotic disease caused by a phlebovirus (family Bunyaviridae), is considered to be one of the most important viral zoonoses in Africa. Transmitted by mosquitoes or by direct contact with viraemic products, RVF affects both livestock and humans, causing abortion storms in pregnant ruminants and sudden death in newborns. The disease provokes flu syndrome in most human cases, but also severe encephalitic or haemorrhagic forms and death. Recently the distribution of Rift Valley Fever widened, threatening Europe. The probability of the introduction and a large-scale spread of Rift Valley fever virus (RVFV) in Europe is thought to be low, but localized RVF outbreaks may occur in areas where populations of ruminants and potential vectors are present. A system of modeling is described. This could allow for areas to be identified for targeted surveillance.

• Spread of previously exotic mosquito vectors in Europe was reported. These are known vectors of zoonotic viral diseases. The European Centre for Disease Prevention and Control (ECDC) has launched the production of ‘Guidelines for the surveillance of invasive mosquitoes in Europe’ which should continue and would feed into the above two areas of work.

• Spread of bovine besnoitia continues with in Switzerland after importation of infected animals. Further information on its spread by Stomoxys calitrans also published to further elucidate the transmission of this parasite. The potential for silently infected cattle to be imported into UK remains. Disease was detected in Switzerland by serological testing of imported animals.

• Evidence of a newly detected tick transmitted disease in the UK was published when the first case of Ehrlicia canis in a dog with no previous travel history was reported. This bacterium is transmitted primarily by the brown dog tick. Rhipicephalus sanguineus. Babesia vogeli in a quarantined dog in the UK was also reported. There have been a number of reports of babesiosis in dogs in the UK, including one with no travel history outside of the UK. B. vogeli is endemic in countries across Europe and is intimately associated with its vector, the brown dog tick (Rhipicephalus sanguineus). With harmonisation of companion animal movements within the EU, tick treatment is now recommended but not compulsory. This has led to concern that exotic pathogens and their vectors could be introduced into the UK with increasing frequency. Continued surveillance for both is needed to ensure that the UK can respond adequately to changes in vector ecology and the disease profile of companion animals.

GASTRO-INTESTINAL NEMATODES

Parasitic disease and food security

Global food security will require the production of more food using resources including land more efficiently, and with less waste. This goal must be achieved within the context of climate change and while ensuring minimal adverse environmental impact from both crop and livestock production. Disease, especially infectious disease, is a main constraint of biologically efficient livestock production and both endemic and exotic disease results in mortality and morbidity and hence less food than should ideally be available in current farming systems. A significant proportion of diseases affect the safety of food supplies, in addition to or instead of, their effect on volume and quality of food products. Parasitological diseases including those caused by nematodes, trematodes, protozoa and ectoparasites, have widely differing effects on meat, milk and fibre production and many new technologies have been developed in order to prevent or treat them. Approaches to developing better control of
parasites have included livestock breeding strategies, improved nutrition and management, and the development of new drugs, diagnostic tests and vaccines. Some of the most important examples include both the development of new anthelmintic products, and better means of using existing drugs in order to maximise their effectiveness in the face of rapidly increasing parasite resistance: diagnostic tests which are able to detect low levels of nucleic acids or proteins from infectious agents rapidly; and vaccines derived from either native or recombinant proteins and designed to stimulate the most appropriate protective response from livestock species. Some of the parasitic diseases affect restricted regions around the world, however most affect very large global populations. The development of technologies of suitable and affordable livestock products for use in developing countries where most pressure on increased production for food will occur provides a particular challenge. Most if not all new technologies form part of integrated management schemes on farms and these vary hugely in differing systems and geographical regions of the world. If the benefit of improved technologies for optimal health, welfare and biological efficiency of livestock is to be realised, then the veterinary, farming, commercial animal health and public service communities need to learn lessons from past successes and failures in the delivery of newly developed technologies to the farmer. The combination of technology and rural development in the veterinary parasitological field has played a key role in current food production and is well placed to continue this trend to help in ensuring future food requirements for the world.(Fitzpatrick, 2013)


GASTRO-INTESTINAL PARASITES OF SHEEP

The future of parasitic diseases in sheep

Sheep are infected with a range of parasites, for which appropriate and effective control strategies are required. Changing patterns of disease and the inability to control parasitic infections effectively require the implementation of risk-based approaches to parasite control for many of the major parasitic diseases affecting sheep. Developing nematode resistance to anthelmintics necessitates changes to conventionally worming programmes that reduce the risk of further development of anthelmintic resistance through incorporation of new anthelmintic classes and products, along with 'best practice' guidelines for anthelmintic usage. Climatic changes pose a greater risk to sheep flocks through spread of parasites, such as *Haemonchus contortus* and *Fasciola hepatica*, which are parasites inextricably linked to high rainfall and increasing temperatures. Intensification increases the risk from diseases such as coccidiosis and it is now advocated that a risk-based approach to the control of coccidia infection in lambs should be based on strategic, treatments interventions linked to identified periods of risk. Concerns over the risks from the use of ectoparasiticides, have seen the demise of sheep dipping in many countries leading to increasing levels of ectoparasitic infections. At the same time, greater reliance has been placed on the use of injectable macrocyclic lactones, which inadvertently may also increase selection pressure for anthelmintic resistance. As a consequence of all the above factors, risk-based approaches to parasite control need to consider the strategic use of antiparasitics for individual target parasites, as well as needs to integration and rationalisation of all the component strategies.(Taylor, 2013)


Parasitic gastro-enteritis (PGE) diagnosis in sheep

The movement distances of merino sheep were monitored over a 24h period using global positioning system tracking collars and correlated with their faecal egg counts. A linear relationship between the logarithm of faecal egg count and the mean distance moved per time step was found. The results suggest that animal behaviour and productivity outcomes might be influenced even at low faecal egg count levels. The considerable variability of the observations about this linear model suggests that it might be useful for identifying those animals inherently resistant to internal parasites. Such technology could be used as part of a
breeding program aimed at improving stock resistance to internal parasites. (Falzon et al., 2013a)

Parasitic nematodes (roundworms) of small ruminants and other livestock have major economic impacts worldwide. Despite the impact of the diseases caused by these nematodes and the discovery of new therapeutic agents (anthelmintics), there has been relatively limited progress in the development of practical molecular tools to study the epidemiology of these nematodes. Specific diagnosis underpins parasite control, and the detection and monitoring of anthelmintic resistance in livestock parasites, presently a major concern around the world. The purpose of the present article is to provide a concise account of the biology and knowledge of the epidemiology of the gastrointestinal nematodes (order Strongylida), from an Australian perspective, and to emphasize the importance of utilizing advanced molecular tools for the specific diagnosis of nematode infections for refined investigations of parasite epidemiology and drug resistance detection in combination with conventional methods. It also gives a perspective on the possibility of harnessing genetic, genomic and bioinformatic technologies to better understand parasites and control parasitic diseases. (Roeber et al., 2013a)


Teladorsagia circumcincta

Teladorsagia circumcincta is one of the most economically important gastrointestinal nematode parasites of sheep in cool temperate regions, to which sheep show genetically-varying resistance to infection. This is a very common parasite and viable sheep production requires the extensive use of anthelmintic drugs. However, the emergence of drug-resistant parasites has stimulated the search for alternative control strategies to curb production losses. Lambs become infected soon after weaning and begin to control parasite burden within 8-10 weeks of continual infection. This control is an acquired characteristic mediated by the development of parasite-specific antibodies. This paper describes the immunology associated with resistance and susceptibility, focusing on differential T cell activation that regulates the production of specific effector mechanisms. It continues by summarizing the methods used to identify genes that could be exploited as molecular markers of selection for resistance. In particular it focuses on the link between understanding the molecular immunology of infection and the identification of candidate genes for selection. (Venturina et al., 2013)

The FAMACHA (c) system is a practical on-farm method, which can be used to identify small ruminants in need of anthelmintic treatment, depending on the colour of the lower eyelid mucous membrane, which reflects level of anaemia. In the present study, we evaluated the accuracy of the FAMACHA (c) system, for detecting severity of anaemia and trichostrongylid gastrointestinal parasitism in dairy sheep and goats in Greece. The study was carried out in 18 sheep flocks and 12 goat herds, with animals of the dairy breed type, managed in a semi-intensive system. Initially, all female animals in each flock/herd were clinically examined and assigned one of the five scores available in the FAMACHA (c) system eye colour chart (‘1’ to ‘5’). Subsequently, 6 animals from those assigned each of the scores in each flock/herd, i.e., 30 sheep/goats per flock/herd, were selected at random for sample collection. That way, in total, 540 sheep and 360 goats were sampled. From each of the above animals, a blood sample was collected for haematological examination; a faecal sample was collected for faecal egg counting and evaluation of proportion of 3rd stage trichostrongylid larvae. There was a significant positive correlation of haematocrit value of animals and score assignment in the FAMACHA (c) system. Faecal egg counts and proportion of Haemonchus contortus 3rd stage larvae in faecal samples of animals assigned score ‘1’ or ‘5’ were significantly different (P < 0.05) between them. For animals assigned scores ‘2’, ‘3’ and ‘4’, no correlation was evident in score assigned and faecal egg counts or proportion of 3rd stage larvae of H.
contortus in faecal samples. Finally, no significant association was evident between haematocrit values and faecal egg counts. The most frequently identified nematode helminth genus in coprocultures was Teladorsagia spp., which in some farms exceeded 80% of total nematode larvae. It is concluded that in Greece, where prevalence of H. contortus is low, the FAMACHA (c) system may not be useful for identification of animals requiring anthelmintic treatment. In this case, classical parasitological techniques or other selection criteria would be more appropriate. (Papadopoulos et al., 2013)


**Trichostrongyulus spp**

Three climate-controlled chamber experiments were conducted to determine the effect of 32 mm simulated rainfall applied prior to (days -4 to -1) or after (days 0-7) faecal deposition and as a single (32 mm) or split (2 x 16 mm) application on faecal moisture (FM) and development of H. contortus and T. colubriformis to third stage infective larvae (L3). The timing of simulated rainfall regulated extra-pellet L3 recovery for H. contortus (P < 0.05) but not T. colubriformis. Recovery of L3 was highest (P < 0.05) when simulated rainfall was applied on the day of deposition followed by days -1, 1 and 2, which resulted in similar but lower development success rates. Recovery of intra-pellet T. colubriformis L3 was two-fold greater (P = 0.008) than for H. contortus and was higher (P = 0.007) following simulated rainfall on days 0 and 1 than on other days. There was a positive association between FM and total L3 recovery indicating the importance of FM in the period 48-72 h (H. contortus) and 72-96 h (T. colubriformis) after deposition. Simulated rainfall on the day prior to deposition was as effective in supporting total L3 recovery as application on days 1 or 2 and this effect could be predicted through FM. This highlights the importance of soil in transferring moisture to the faecal pellet. The importance of precedent rainfall and soil moisture in determining the development success of H. contortus and T. colubriformis, in addition to the general effects of the timing of simulated rainfall, need to be accommodated in grazing management programs to combat these species. (Khadijah et al., 2013a)

Gastrointestinal (GI) strongyle infection remains one of the main constraints to goat production worldwide. Samples of small intestine from 15 Syrian goats naturally infected with Trichostrongyulus colubriformis were examined by routine histology, histochemistry and immunohistochemistry to describe the histological changes and the phenotypes of inflammatory cellular components of the mucosa. Results indicated that the immune response to infection by T. colubriformis was characterized by an increased rate of the severity of the histologic lesions, an increase rate of T cell lymphocytes recruitment to the intestinal mucosa and quantitative and qualitative changes in the histochemical composition of mucin in goblet cells. (Trapani et al., 2013)


**Haemonchus spp**

A cross-over experiment was conducted to compare six different phenotypic measures of resilience to gastro-intestinal nematodes (predominantly Haemonchus contortus) in Merino...
sheep and their association with resistance and production levels. On each of six farms, 120 ewes born in 2006 and 120 older mixed age ewes were selected at shearing in 2007. Of these, 60 in each mob were serially treated with long-acting anthelmintics to suppress worm populations. The other 60 ewes were managed according to management practices employed on the farm (infected, INF). At shearing in 2008, the experimental sheep had their anthelmintic treatments switched. The experiment concluded at shearing in 2009. Measures of resilience were greasy fleece weight (GFW), live weight gain (LWG) and haematocrit (HCT) when infected and the difference in these variables between infected and suppressed. Resistance was determined from multiple faecal egg count (WEC) when infected. Measures of resilience based on GFW, LWG and HCT were moderately correlated with each other (r=0.25-0.50) suggesting that they represent different traits. Correlations between a measure in infected animals, and the difference in the same measurement between infected and uninfected animals were higher (r=0.37 to -0.82), indicating that measurement during infection is an adequate measure of resilience. WEC was negatively correlated with LWG and HCT during infection but not GFW. Correlations with resilience measures based on difference between infected and uninfected were positive. Surviving infected sheep were found to have higher haematocrit (HCT), and lower WEC in summer and autumn than sheep that died following the measurement. These results show that measurement of performance traits while infected is a reasonable approximation of measurement of resilience based on the difference in performance between infected and non-infected. They also show that resilience to worm infection is not a single trait, but rather a suite of moderately correlated traits. (Kelly et al., 2013)

The structural changes induced in adult Haemonchus contortus after in vitro and in vivo contact with a tannin-rich (TR) plant, either tizalam (Lysiloma latisiliquum) or sainfoin (Onobrychis vicifolia), were assessed using scanning electron microscopy (SEM). All the worms used in the study were adult females. The Haemonchus adult worms were obtained from the abomasum of infected donor goats. Adult H. contortus were kept in contact with the extracts of TR plants for 24 h for the in vitro assays and were compared to worms maintained in PBS (control). For the in vivo studies, the adult H. contortus parasites were obtained from artificially infected goats. All the goats were fed a tannin-free diet until D27 post-infection when infection was patent. On D28 some goats were fed fodder of one of the TR plants for seven consecutive days. Thus, their H. contortus were in contact with TR fodder within the gastrointestinal tract. The control worms were obtained from goats fed only a tannin-free diet. In the in vitro assays and in vivo studies, the SEM observations revealed structural alterations in the worms after contact with TR plants when compared to the control worms (i.e.: longitudinal and transversal folds and thicker cuticular ridges, material aggregates around the buccal capsule and/or vulva or anus). The main changes concerned the cuticle and the buccal area. The structural changes found in the worms exposed to TR plants might affect their motility and nutrition with possible consequences on their reproduction, as suggested by previous in vivo trials. (Martinez-Ortiz-de-Montellano et al., 2013)

Recent experiments on the effects of rainfall and/or soil moisture (SM) on development of sheep gastro-intestinal nematodes to infective L3 stage have used soil of relatively low moisture content in small experimental samples that dry out faster than field soil. To determine whether higher and more sustained SM content modulates the effects of rainfall amount and timing on faecal moisture (FM) and development of H. contortus and T. colubriformis to infective third stage larvae (L3), a climate-controlled chamber experiment was conducted. It was designed to test the effects of rainfall amount (0, 12 and 24 mm), rainfall timing (days -1, 0 and 3 relative to faecal deposition) and soil moisture maintained at 10, 20 and 30% on these variables. Total recovery of L3 14 days after faecal deposition was significantly affected by SM, rainfall timing and their interaction (P < 0.01), but not by rainfall amount or species or other two-way interactions. Recovery of L3 was maximal (28%) with a SM treatment of 30% and simulated rainfall on day 3. Faecal moisture was significantly affected by collection day, SM treatment, rainfall amount and rainfall timing with significant interaction between many of these effects (P < 0.05). A positive linear association between FM and total L3 recovery was strongest on day 4 after faecal deposition (R² = 0.64, P < 0.001) for H. contortus and day 6 (R² = 0.78, P < 0.001) for T. colubriformis. Overall the results show that SM is able to modulate the effects of rainfall timing and amount with increased SM acting to broaden the window of opportunity for the free-living stages to respond to post deposition rainfall to complete development to L3. If SM is maintained in the
A chapter summarizes progress made in vaccine development against *Haemonchus contortus* infection, including discussions on lead vaccine antigens (i.e., dipeptidyl peptidases, aminopeptidases, aspartyl and metalloproteases, cysteine proteases). Current challenges and possible solutions are also discussed. (Knox, 2013)

The small ruminant parasite *Haemonchus contortus* is the most widely used parasitic nematode in drug discovery, vaccine development and anthelmintic resistance research. Its remarkable propensity to develop resistance threatens the viability of the sheep industry in many regions of the world and provides a cautionary example of the effect of mass drug administration to control parasitic nematodes. Its phylogenetic position makes it particularly well placed for comparison with the free-living nematode *Caenorhabditis elegans* and the most economically important parasites of livestock and humans. Here we report the detailed analysis of a draft genome assembly and extensive transcriptomic dataset for *H. contortus*. This represents the first genome to be published for a strongylid nematode and the most extensive transcriptomic dataset for any parasitic nematode reported to date. We show a general pattern of conservation of genome structure and gene content between *H. contortus* and *C. elegans*, but also a dramatic expansion of important parasite gene families. We identify genes involved in parasite-specific pathways such as blood feeding, neurological function, and drug metabolism. In particular, we describe complete gene repertoires for known drug target families, providing the most comprehensive understanding yet of the action of several important anthelmintics. Also, we identify a set of genes enriched in the parasitic stages of the lifecycle and the parasite gut that provide a rich source of vaccine and drug target candidates. The *H. contortus* genome and transcriptome provides an essential platform for postgenomic research in this and other important strongylid parasites. (Laing et al., 2013)

This work aimed to study the possible relationships among the magnitude of abomasal worm burden and the proliferation of globular leucocytes and mucosal mast cells in the abomasal mucosa, and the white blood cell count. Eighteen Suffolk x Greyface lambs were infected with *Haemonchus-contortus*, and 1-2 were kept free of nematodes. Blood samples were collected on days 0, 30, and 57 post-infection (p.i.) for leucogram determination. At day 62, all animals were euthanized to count the total number of nematodes recovered in the abomasum and to count the number of mucosal mast cells and globular leucocytes. On day 57, higher levels of parasitism corresponded to lower leucocyte counts. The infected groups had lower lymphocyte counts throughout the experimental period. Animals with higher numbers of parasites had lower neutrophil and eosinophil counts on day 57. The lower the worm burden, the greater the number of mucosal mast cells (r = -0.85; p < 0.01) and globular leucocytes (r = -0.87, p < 0.01) observed. The sheep most resistant to haemochosis had greater peripheral and tissue cellular immune responses (Ortolani et al., 2013)

The barber's pole worm, *Haemonchus contortus*, is one of the most economically important parasites of small ruminants worldwide. Although this parasite can be controlled using anthelmintic drugs, resistance against most drugs in common use has become a widespread problem. We provide a draft of the genome and the transcriptomes of all key developmental stages of *H. contortus* to support biological and biotechnological research areas of this and related parasites. The draft genome of *H. contortus* is 320 Mb in size and encodes 23,610 protein-coding genes. On a fundamental level, we elucidate transcriptional alterations taking place throughout the life cycle, characterize the parasite's gene silencing machinery, and explore molecules involved in development, reproduction, host-parasite interactions, immunity and disease. The secretome of *H. contortus* is particularly rich in peptidases linked to blood feeding activity and interactions with host tissues, and a diverse array of molecules is involved in complex immune responses. On an applied level, we predict drug targets and identify vaccine molecules. Conclusions: The draft genome and developmental transcriptome of *H. contortus* provide a major resource to the scientific community for a wide range of genomic, genetic, proteomic, metabolomic, evolutionary, biological, ecological and epidemiological investigations, and a solid foundation for biotechnological outcomes, including new anthelmintics, vaccines and diagnostic tests. This first draft genome of any strongylid nematode paves the way for a rapid acceleration in our understanding of a wide range of
Haemonchus contortus is one of main blood-sucking gastrointestinal nematodes that infects in sheep and goats, which induces anaemia and decreases production performance of hosts and results in economic lose to producers. Chemical anthelmintic drugs were used to control infections of gastrointestinal nematodes in animals. However, the problems of drug resistance and the safety of animal products urge us to choose alternative strategies. To probe mechanisms of immune responses of H. contortus and improve immunity and resilience of hosts through nutritional regulations have been reported to be effective. Theories of nutritional manipulation are based on the physiological and pathological responses of hosts to H. contortus infection and strategies of improving host resistance and resilience to H. contortus. This review focused on the life cycle of H. contortus, immune mechanisms, and nutritional manipulation strategies of controlling H. contortus infection, such as manipulate dietary protein level, mineral level, and bioactive compounds. (Zhong and Zhou, 2013)

Haemonchus contortus infections have been increasingly reported in ungulates from cold climates even though past studies have shown that the free-living juveniles from this species survive poorly under freezing conditions. Overwintering strategies of H. contortus have not been documented in the United States Northern Great Plains. A PCR survey identified H. contortus as vastly predominant trichostrongyle species present (in addition to occasional detections of Teladorsagia sp.) in a closed farm flock of sheep from Brookings County, SD. Benzimidazole (BZ) and avermectin (AV) anthelmintics had been used intensely for many years on this flock. During the autumn season, three fecal egg count reduction tests (doramectin, albendazole, and moxidectin) were performed over a 4-year span to assess drug effectiveness within the flock. Significant drug resistance was found in Haemonchus adults with doramectin (69% efficacy), marginal resistance was found with albendazole (90% efficacy) and no resistance was found in moxidectin (100% efficacy). The following spring, pre-lambing and post-lambing fecals were obtained from albendazole and moxidectin treatment years to assess the resistance of the tissue-dwelling fourth-stage juveniles (J4s) at those times. Albendazole treated pre-lambing fecals averaged only 4 EPG and treated post-lambing fecals increased to 454 EPG, indicating that many of the J4s were not killed during the autumn treatment. Moxidectin pre-lambing fecals averaged only 1 EPG, and post-lambing fecals only increased to 6 EPG in the treated moxidectin population and 1422 EPG in the untreated moxidectin population. In addition to evaluating the ability of H. contortus to overwinter as drug-resistant tissue-dwelling J4s, this study also evaluated the overwintering ability of pasture-dwelling, free-living third-stage juveniles at this farm. In the summers of 2010 and 2011, naive tracer lambs were placed on a H. contortus contaminated pasture for 3 weeks to assess J3 winter survival. In 2010, tracer lambs only averaged 7 EPG whereas drylotted control lambs averaged 2 EPG; in 2011, tracer lambs averaged 2 EPG while the control lambs averaged 1 EPG. These results suggest that at this northern plains farm, yearly transmission of H. contortus is predominately through drug-resistant J4s. This is consistent with other cold-climate, overwintering studies involving H. contortus from Europe. (Grosz et al., 2013)


Khadijah, S., Kahn, L.P., Walkden-Brown, S.W., Bailey, J.N., Bowers, S.F., 2013b. Soil moisture modulates the effects of the timing and amount of rainfall on faecal moisture and development of Haemonchus contortus and Trichostrongylus colubriformis to infective third stage larvae. Veterinary Parasitology 196, 347-357.****


Trichuris spp

A recurrent problem in the control of whipworm (Trichuris spp.) infections in many animal species and man is the relatively low efficacy of treatment with a single application of benzimidazoles (BZs). The presence of single nucleotide polymorphisms (SNPs) in codons 167, 198 and 200 in the beta-tubulin gene has been associated with BZ anthelmintic resistance in intestinal nematodes of veterinary importance. We hypothesized that the low susceptibility to BZ could be related to a natural tolerance or induced resistance caused by BZ-resistant associated SNPs. The aim of the present study was therefore to investigate the presence of these SNPs in the beta-tubulin gene of Trichuris spp. obtained from a range of animals. DNA was extracted from a total of 121 Trichuris spp. adult whipworm specimens obtained from 6 different host species. The number of worms from each host was pig: 31, deer: 21, sheep: 18, mouse: 17, dog: 19 and Arabian camels: 14. A pooled sample of Trichuris eggs from 3 moose was also used. In order to amplify the beta-tubulin fragments which covered codons 167, 198 and 200 of the gene, degenerate primers were designed. The sequences obtained were used to design specific primers and used to amplify a similar to 476 bp fragment of the beta-tubulin gene. The PCR products were sequenced, analysed and evaluated. We did not identify SNPs in codons 167, 198 or 200 that led to amino acid substitutions in any of the studied Trichuris spp., but genetic variation expected to be related to species differences was observed. The cluster analysis showed close evolutionary relationship between Trichuris spp. from ruminants and between mouse and dog whereas the pig-derived worms, T. suis, clustered with T. trichiura obtained from Genbank.(Hansen et al., 2013)


PARASITE CONTROL

An article was published on parasite control at grass (Doherty, 2013)

Parasitic helminths present one of the most pervasive challenges to grazing herbivores. Many macro-parasite transmission models focus on host physiological defence strategies, omitting more complex interactions between hosts and their environments. This work represents the first model that integrates both the behavioural and physiological elements of gastro-intestinal nematode transmission dynamics in a managed grazing system. A spatially explicit, individual-based, stochastic model is developed, that incorporates both the hosts' immunological responses to parasitism, and key grazing behaviours including faecal avoidance. The results demonstrate that grazing behaviour affects both the timing and
intensity of parasite outbreaks, through generating spatial heterogeneity in parasite risk and nutritional resources, and changing the timing of exposure to the parasites’ free-living stages. The influence of grazing behaviour varies with the host-parasite combination, dependent on the development times of different parasite species and variations in host immune response. Our outputs include the counterintuitive finding that under certain conditions perceived parasite avoidance behaviours (faecal avoidance) can increase parasite risk, for certain host-parasite combinations. Through incorporating the two-way interaction between infection dynamics and grazing behaviour, the potential benefits of parasite-induced anorexia are also demonstrated. Hosts with phenotypic plasticity in grazing behaviour, that make grazing decisions dependent on current parasite burden can reduce infection with minimal loss of intake over the grazing season. This paper explores how both host behaviours and immunity influence macro-parasite transmission in a spatially and temporally heterogeneous environment. The magnitude and timing of parasite outbreaks is influenced by host immunity and behaviour, and the interactions between them; the incorporation of both regulatory processes is required to fully understand transmission dynamics. Understanding of both physiological and behavioural defence strategies will aid the development of novel approaches for control. (Fox et al., 2013)

Modern livestock breeding practices provide new opportunities for producing animals that are adapted to their production environment and are free of disease. Using current knowledge of biology and by seeking ‘the desired outcome’ animal selection strategies can be designed that deliver more precisely defined results so maximising genetic gain and minimising risk. This review briefly describes the evolution of genetic selection in livestock and considers some of the positive and negative aspects of selection practices over time. The selection of sheep to withstand gastro-intestinal nematode parasitism is used as an example to explain how developments in selection strategy have improved genetic progress for complex traits. Re-evaluation of the understanding of the outcomes of selection for parasite resistance is used here to examine whether a more sophisticated approach is desirable, and to propose a number of additional phenotype measurement strategies that could complement and improve the quality of information used for animal selection. Finally some ideas are presented for creating a situation where a designed, highly defined breeding objective might be used to increase precision and reduce risk. This may become possible via research to adapt or develop tools for more sophisticated phenotypic evaluation, to discover biological processes integral to desired breed changes, and to define desired animal types which match economic and societal expectations. (Hunt et al., 2013)

Genetic selection for enhanced levels of protective antibody to specific nematode antigens may be a more user-friendly means of selecting animals for resistance to gastrointestinal nematodes than obtaining faecal samples and selecting on the basis of faecal egg counts. Saliva IgA antibody levels to the L3-specific surface glycan known as carbohydrate larval antigen were measured on six occasions over a 5 month period in approximately 350 lambs. The carbohydrate larval antigen IgA response increased markedly with time as the lambs grazed on pasture naturally contaminated with nematode parasite larvae. The monthly log(e) transformed carbohydrate larval antigen IgA levels were moderately heritable at all samplings, with a combined value of 0.28 +/- 0.10 and a repeatability of 0.35 +/- 0.03. The genetic correlations between all samplings were high (0.86), suggesting that testing for a carbohydrate larval antigen IgA response could be carried out at any time in the 5 months post-weaning. The transformed carbohydrate larval antigen IgA levels were genetically and phenotypically correlated negatively with log-transformed (faecal egg count + 50), averaging -0.57 +/- 0.20 and -0.12 +/- 0.03 (P < 0.05), respectively. The correlations between carbohydrate larval antigen IgA and breech-soiling (dag score) never reached significance. However, genetic correlations between carbohydrate larval antigen IgA and live weight were always positive and significantly so, especially at the beginning and end of the trial, indicating that carbohydrate larval antigen IgA production may be an important genetic determinant of growth rate for lambs experiencing a larval challenge. The data suggest that the ideal time to sample for a carbohydrate larval antigen IgA response and maximise selection for lowered faecal egg count and increased live-weight would be in the first 2 months after weaning. (Shaw et al., 2013)
Despite the fact that there are many beneficial worm species, veterinarians, physicians and parasitologists have multiple reasons to combat parasitic worms. The pros-and-cons of various approaches for the discovery of new control methods are discussed, including novel anthelmintics, vaccines and genetic approaches to identify novel drug and vaccine targets. Currently, the mainstay of worm control remains chemotherapy and prophylaxis. The importance of knowledgeable and wise use of the available anthelmintics is highlighted. (Kaminsky et al., 2013)

Seven 3-month-old, female, helminth-free lambs were immunized intranasally with three doses (1 mg total) of a recombinant part of the catalytic region of the serine/threonine phosphatase 2A (PP2Ar) (group 1 [G1]). In addition, four lambs were used as an adjuvant control group (G2), four as unimmunized, infected controls (G3), and four as unimmunized, uninfected controls (G4). Fifteen days after the last immunization, lambs from G1, G2, and G3 were challenged with 10,000 larval stage 3 (L3) organisms in a plurispecific nematode infection composed of ca. 40% Trichostrongylus colubriformis, 40% Haemonchus contortus, and 20% Teladorsagia circumcincta. All the lambs were clinically monitored throughout the experiment. Parasitological (fecal egg output and immunological response), biopathological (packed-cell volume and leukocyte and eosinophil counts), and zootecanical (live-weight gain) analyses were conducted. On day 105 of the experiment, all the animals were slaughtered and the adult worm population in their abomasum examined. Intranasal administration of PP2Ar with bacterial walls as an adjuvant elicited a strong immune response in the immunized lambs, as evidenced by their humoral immune response. Immunized animals and animals receiving the adjuvant shed significantly (P < 0.001) fewer numbers of parasites' eggs in their feces. The immunization significantly reduced the helminth burden in the abomasum by the end of the experiment (>68%), protection being provided against both Haemonchus and Teladorsagia. Live-weight gain in the immunized lambs was similar to that in the uninfected controls versus the infected or adjuvanted animal groups. Our results suggest that heterologous immunization of ruminants by intranasal administration may be efficacious in the struggle to control gastrointestinal helminths in these livestock. (Mohamed Fawzi et al., 2013)

Managing infections of sheep with gastrointestinal nematode parasites (worms) and problems of resistance to anthelmintic treatments continue to be major challenges for graziers on the Northern Tablelands of New South Wales, Australia. The whole-farmlet study of grazing enterprises undertaken by the Cicerone Project tested the broad hypotheses that compared with typical management (farmlet B), internal parasites can be more effectively managed with improved nutrition (farmlet A) or by intensive rotational grazing (farmlet C). Further aims were to identify the major sources of variation in faecal worm egg count (WEC) over the 6-year period and to examine the efficacy of the various anthelmintic treatments used during the experiment. This paper describes the management of sheep worms at the whole-farmlet level during the experiment, and analyses data from the routine WEC monitoring (5644 records) and larval differentiation tests (322 records) carried out on behalf of the Cicerone Management Board and by a doctoral candidate. It complements more detailed investigations published elsewhere. Over the period from July 2000 to December 2006, worm infections in ewes, lambs, hoggets and wethers were, with some exceptions, successfully controlled on the farmlets through a combination of regular monitoring of WEC, treatment with a wide array of anthelmintics and grazing management. Farmlet C had lower mean WEC (444 epg) and annual anthelmintic treatment frequency (3.1 treatments/year) over the whole experimental period than farmlets B (1122 epg, 4.3 treatments/year) or A (1374 epg, 4.7 treatments/year). The main factors influencing WEC were the time since the last anthelmintic treatment, and the anthelmintic used at that treatment. The magnitude of these effects dwarfed those of climatic and management factors that might be expected to influence the epidemiology of gastrointestinal nematode infections via environmental or host-mediated mechanisms. Nevertheless management factors associated with stocking rate and grazed proportion (proportion of each farmlet grazed at any one time), and climatic indicators of both temperature and moisture availability had significant effects on WEC. The results show that, in a region with Haemonchus contortus as the major sheep nematode, improved host nutrition in a higher input system (farmlet A) did not provide more effective control of gastrointestinal nematodes than typical management (farmlet B); however, it was observed that gastrointestinal nematode control was no worse on farmlet A than on farmlet B in spite of farmlet A supporting a 48% higher stocking rate by later in the trial period (2005). The study
provided strong support for the proposition that intensive rotational grazing (farmlet C) provided more effective control of gastrointestinal nematodes than typical management (farmlet B) as evidenced by significantly lower WEC counts and anthelmintic treatment frequency. Tactical worm control based on routine monitoring of WEC provided adequate control of worms on all three farmlets for much of the experimental period but failed to prevent significant spikes in WEC to values associated with significant production loss on multiple occasions, and significant ewe mortality on farmlets A and B on one occasion. (Walkden-Brown et al., 2013)


ANTHELMINTIC RESISTANCE

Northern Ireland

A questionnaire to obtain information on nematode control practices and sheep management was sent to over 1000 farmers in Northern Ireland. Replies were received from 305 flock owners, and data from 252 of them were analysed. Farms were divided into lowland and upland areas. Sizes of pasture and stocking rates on lowland and upland farms were 59.5 hectares, 6.99 sheep/hectare and 62.9 hectares and 10.01 sheep/hectare, respectively. Mean drenching rates for lambs and adults were 2.33 and 2.44, respectively, in lowland flocks and 2.73 and 2.71, respectively, in upland flocks. Between 2008 and 2011, the most frequently identified compounds in use were benzimidazoles and moxidectin in lowland flocks, and benzimidazoles and avermectins in upland flocks. Over the same period the most frequently identified commercial formulations were Tramazole (R), Panacur (R) and Allverm (R) (white drench), Levacide (R) (yellow drench), Oramec (R) (clear drench; avermectin), Cydectin (R) (clear drench; moxidectin) and Monepantel (R) (orange drench). Most respondents (56.35%) treated their lambs at weaning and the most common time to treat ewes was identified to be pre-mating (67.86% of respondents). The results of the questionnaire survey revealed that lowland annual drench frequency was 233 and 2.44 in lambs and ewes, respectively, although drench frequencies were higher in upland flocks: 2.73 and 2.71 for lambs and ewes, respectively. Annual drench rotation was practised by 43.96% of flock owners, but whether this was true rotation or pseudo-rotation (i.e., substitution of one anthelmintic product by another product belonging to the same chemical group of anthelmintics) could not be explicitly determined. (McMahon et al., 2013a)

The prevalence of anthelmintic resistance in Northern Ireland sheep flocks was evaluated between July and October 2011. Sampling kits were sent to 172 flock owners and returns were received from 91. Within this survey population, 27 flock owners used benzimidazole products, 10 used levamisole products, 15 used avermectin products, 26 used milbemycin
products and 4 flock owners used the amino acetonitrile derivative, Monepantel. The remaining 9 flock owners used combination drenches (broad spectrum wormer plus fasciocide). However, 15 sets of samples were ineligible for faecal egg count reduction testing due to either too low an egg count or insufficient faecal volume. Treatment efficacy below 95%, indicating significant resistance, was detected in 81% (n=24) of flocks tested for benzimidazole resistance; in 14% (n=1) of flocks tested for levamisole resistance; and in 50% (n=7) and 62% (n=13) of flocks tested for avermectin and milbemycin resistance, respectively. Monepantel resistance was absent in all (n=3) flocks tested. Combination products (broad spectrum nematocide plus fluikide) containing levamisole were entirely effective, while treatment efficacy below 95% was detected in 60% (n=3) of flocks where the nematocide in the combination product was a benzimidazole. Where parasite identification based on coproculture was completed, Trichostrongyulus was the dominant genus detected in all cases post-treatment, indicating the occurrence of anthelmintic-resistant Trichostrongyulus spp. populations. Benzimidazole efficacy was highest in treating Trichostrongyulus spp. (51%) and lowest when treating Teladorsagia spp. Levamisole was 100% effective in treating Cooperia, but ineffective (0%) in treating Trichostrongyulus spp. Avermectin efficacy was highest when treating Haemonchus contortus (100%) and Teladorsagia spp. (73%), with a marginally lower efficacy against Trichostrongyulus spp. (71%). Moxidectin efficacy was 33% against Trichostrongyulus spp., 68% against Teladorsagia spp., 97% against Cooperia spp. and 100% against Haemonchus contortus infections.(McMahon et al., 2013b)

Canada

Gastrointestinal nematodes (GIN) are a significant constraint to pasture-based sheep production worldwide. Anthelmintic resistance (AR) has been reported in most sheep-raising areas in the world, yet little is known about the AR status in Canada. This study was conducted to determine the frequency of AR in GIN in sheep flocks in Ontario, Canada. Forty-seven sheep flocks were enrolled in the study, and their level of parasitism was monitored monthly throughout a grazing season by analyzing owner-acquired fecal samples from 15 grazing lambs per flock. When the mean GIN fecal egg count (FEC) reached a threshold of 200 eggs per gram (epg), oral ivermectin was supplied to producers to check ivermectin efficacy; the reduction in mean FEC 14 days after ivermectin treatment was calculated.

'Drench failure' was defined as a reduction in mean FEC of <95%. In those flocks with apparent drench failure, researchers performed a Fecal Egg Count Reduction Test (FECRT), dividing sheep into 4 treatment groups (n=10-15): control (i.e. untreated), ivermectin, and, if sufficient numbers of animals - fenbendazole and levamisole. AR was defined as a reduction in mean FEC <95% and a lower 95% confidence interval <90%. Larval cultures were performed on pooled post-treatment FECRT samples. Larval Development Assays (LDAs) to detect the presence of resistance to thiabendazole and levamisole were performed prior to the ivermectin drench check on pooled owner-acquired fecal samples that reached the 200 epg threshold. Approximately 89% (42/47) of the farms reached the FEC threshold of 200 epg; 93% (39/42) of these farms performed an ivermectin drench check, and 88% (34/39) of these farms had drench failure. The FECRT was performed on 29 of the 34 farms. Resistance to ivermectin, fenbendazole and levamisole was demonstrated on 97% (28/29), 95% (19/20) and 6% (1/17) of the farms tested, respectively, with considerable variability in resistance levels among farms. Haemonchus sp. was the most commonly cultured parasite from post-treatment fecal samples. The FECRT was performed on 21 farms were available; of these, 14% (3/21) and 62% (13/21) had low and high levels of thiabendazole resistance, respectively, while none of the farms exhibited resistance to levamisole. Amongst these tested farms, resistance to both ivermectin and benzimidazoles was very common. These findings strongly suggest that AR, particularly in Haemonchus sp., is a serious problem in these sheep flocks. Thus, marked changes in GIN management need to be instituted immediately to mitigate a worsening situation.(Falzon et al., 2013b)

In 2011, a field study was conducted to assess drug resistance of gastro-intestinal nematodes in sheep flocks in Ontario, Canada. Benzimidazole resistance in Haemonchus contortus was assessed by genetic analysis of eggs; measurement of resistant allele percentages at codons 167,198 and 200 in the beta-tubulin gene was determined on pools of H. contortus eggs using pyrosequencing. Susceptibility to benzimidazoles in gastro-intestinal nematodes was also determined using a Faecal Egg Count Reduction Test (FECRT) and a Larval Development
In total, 16 farms were assessed with the genetic test. Based on resistant allele frequencies, all of the farms (16/16) tested had benzimidazole resistance in *H. contortus*; the overall percentage of benzimidazole-resistant *H. contortus* (estimated prior to treatment using the Hardy-Weinberg formula) was 68.5%. The FECRT and LDA were performed on 11 and 13 farms, respectively. Resistance to fenbendazole was detected on 100% (11/11) of the farms where the FECRT was performed. The LDA revealed the presence of thiabendazole resistance in *H. contortus* in 92% (12/13) of the farms. Estimated percentages of resistant parasites in *H. contortus* populations obtained with the two biological tests and the genetic test were compared. The results of the genetic test were in agreement with the biological tests and confirmed that benzimidazole resistance in *H. contortus* is present in Ontario sheep flocks. Differences between the different methods of drug resistance detection are discussed in terms of cost, time and sampling. (Barrere et al., 2013a)

**South Africa**

Controlled-release albendazole capsules (CRCs) are currently registered for use in Australia and New Zealand as anthelmintic treatment in sheep. However, reports on the efficacy of such products on resistant parasite populations are sometimes controversial. This is the first study to report on the efficacy of such products under South African field conditions in sheep harbouring a population of *Haemonchus contortus* with known multiple anthelmintic resistance, including to albendazole. Treatment groups were comprised of CRC-treated and single dose albendazole-treated sheep, as well as negative controls. Groups were compared by using faecal egg count reduction tests, FAMACHA® anaemia scoring, conception rates and comparative weight gains over three and a half months. Based on a comparison of faecal egg counts, no advantage could be found using CRCs. Moreover, the use of the product actually decreased weight gain when compared with the control group animals. (Fisher and van Sittert, 2013)

**Resistance to monepantel**

After reports of the apparent failure of monepantel to reduce the egg counts of goats on a farm in the lower North Island of New Zealand, faecal egg count reduction tests were conducted in goats and lambs resident on the property, and a confirmatory, slaughter study was conducted using 12 sheep, sourced elsewhere, that were grazed on the farm for approximately 5 weeks. In the egg count reduction test in goats, 8 animals were given monepantel at 3.9 mg/kg (just over 1.5x the sheep dose rate of 2.5 mg/kg), whilst four received 7.7 mg/kg (just over 3 x the sheep dose). In the egg count reduction test in sheep, 15 lambs were treated with 3.0 mg/kg of monepantel. For the confirmatory study, the sheep were housed indoors for 2 weeks before half were treated with 2.9 mg/kg monepantel and the animals were killed for worm counts 9 days later. There was no evidence of efficacy in either egg count reduction test, or in the goats, the two dose rates used appeared equally ineffective. Likewise, there were no significant reductions in egg counts or worm burdens in the slaughter study. Monepantel was ineffective against at least two gastrointestinal nematode species, *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*. These findings represent the first report from the field of resistance having developed to the anthelmintic monepantel with severe resistance developing in more than one species after being administered on 17 separate occasions to different stock classes and in less than 2 years of the product first being used on the farm in question. (Scott et al., 2013)

**Testing for anthelmintic resistance**

Evaluation of resistance in selected field strain of *Haemonchus contortus* to ivermectin and moxidectin using the Larval Migration on Agar Test. *Haemonchus contortus* is one of the most common and economically significant causes of disease in small ruminants worldwide, and the control programs of parasitic nematodes - including *H. contortus* - rely mostly on the use of anthelmintic drugs. The consequence of the use of this, as the sole sanitary strategy to avoid parasite infections, was the reduction of the efficacy of all chemotherapeutic products with a heavy selection for resistance. The widespread of anthelmintic resistance and the difficulty of its early diagnosis has been a major concern for the sustainable parasite management on farms. The objective of this research was to determine and compare the
Ivermectin (IVM) and moxidectin (MOX) effect in a selected field strain of *H. contortus* with a known resistance status, using the *in vitro* larval migration on agar test (LMAT). Third stage larvae of the selected isolate were obtained from faecal cultures of experimentally infected sheep and incubated in eleven increasing diluted concentrations of IVM and MOX (6, 12, 24, 48, 96, 192, 384, 768, 1536, 3072 and 6144 μg/mL). The dose-response sigmoidal curves were obtained using the R-2 value of >0.90 and the lethal concentration (LC50) dose for the tested anthelmintic drugs using a four-parameter logistic model. The LC50 value for MOX was significantly lower than IVM (1.253 μg/mL and 91.06 μg/mL), identifying the *H. contortus* isolate as considerably less susceptible to IVM compared to MOX. Furthermore, the LMAT showed a high consistency (p<0.0001) and provided to be a useful diagnostic tool for monitoring the resistance status of IVM and MOX in *H. contortus* field isolate, as well as it may be used for official routine drug monitoring programs (Fortes et al., 2013).

*Haemonchus contortus* is a hemophilic nematode which infects sheep and causes anemia and death to lambs. Benzimidazole drugs are used to remove these parasites, but the phenomenon of resistance has arisen worldwide. A sensitive test to detect resistance before treatment would be a useful tool to enable farmers to anticipate the efficiency of the drug before drenching the flock. In this study, we compared a test for benzimidazole resistance based on detection of genetic markers in *H. contortus* before treatment with the common method of fecal egg count reduction test (FECRT). We recruited 11 farms from different regions of Quebec for this study. Fecal samples from animals were collected per rectum before and after treatment in control and treated groups (10 animals per group). The 10 sheep were treated with fenbendazole at the recommended dose rate. Among the 11 farms participating in the study, we found *H. contortus* in 8 of them and it was the most predominant nematode species detected by egg count. Using the genetic test, we found benzimidazole resistance in each of these 8 farms. In 5 of these 8 farms there were sufficient sheep with an egg count for *H. contortus* above 150 eggs per gram to allow the FECRT test to be conducted. Benzimidazole resistance was observed in each of these 5 farms by the FECRT. When we compared the results from the genetic test for samples off pasture and from individual sheep, with the results from the FECRT, we concluded that the genetic test can be applied to samples collected off pasture to estimate benzimidazole resistance levels before treatment for *H. contortus* infections. (Barrere et al., 2013b)

Anthelmintic resistance has emerged as an important problem in animal industries. Understanding resistance mechanisms, especially against macrocyclic lactones (MLs), is the first step in developing better diagnostic tools. Effects of several MLs including ivermectins and milbemycins were tested using two well established *in vitro* assays: the larval development assay (LDA) and the larval migration inhibition assay (LMIA). These were performed on free-living stages of susceptible and ML-resistant isolates of three trichostrongyloid nematode species of sheep. In general, dose response curves shifted to the right in the resistant isolates. Data showed that resistance was present to ivermectin and its two components suggesting that both components contribute to action and resistance. There were no consistent patterns of potency and resistance of the tested substances for the different isolates in the LDA except that moxidectin (MOX) tended to have lower resistance ratios than ivermectin (IVM). MOX was the most potent inhibitor in the LMIA in susceptible *Haemonchus contortus* while being less potent in *Trichostrongylus colubriformis* and particularly in *Ostertagia circumcincta*. MOX showed high resistance ratios in the LMIA in all three species. Based on these results, resistance to MOX has unique characteristics and the LMIA may perform better in detecting resistance to MOX in these parasite species. (Demeler et al., 2013a)

The aim of this study was to evaluate the potential of the larval development test for the detection of ivermectin (IVM) resistance in *Haemonchus contortus* of sheep. Single infections with 5000 third-stage larvae of five resistant and two susceptible isolates of *H. contortus* were given to sheep. Fecal samples were collected four times during patency, and the micro-agar version of the larval development test (MALDT) was performed. Three macrocyclic lactone drugs (IVM, eprinomectin and IVM aglycone) were tested. The results of the tests are presented as LC50 and LC99 values. The MALDT was well able to distinguish between susceptible and resistant isolates. Resistance factors (RF) for the LC99 values were generally higher than those obtained by comparing LC50 values. The highly resistant isolates were
readily distinguishable from the susceptible isolates, particularly when using IVM aglycone and eprinomectin, with RFs above 20. (Dolinska et al., 2013)

**Mechanism of anthelmintic resistance**

In vertebrates, the function of P-glycoprotein (PGP) is to protect against toxic compounds through active efflux of the toxin from target tissues. In clinical oncology, the overexpression of PGP confers drug resistance. The function(s) of PGP in nematode physiology or in conferring drug resistance is less understood. Our results demonstrate that PGP s play a role in protecting *C. elegans* from IVM toxicity and inhibition of PGP enhances susceptibility to IVM. PGP may be a mechanism for multidrug resistance (MDR) in parasitic nematodes. (Ardelli and Prichard, 2013)

While the F200Y SNP in the beta-tubulin gene is most commonly associated with benzimidazole resistance in trichostrongyloid nematodes, other SNPs as well as drug efflux pathways have been implicated in the resistance. The relative contributions of all these mechanisms are not understood sufficiently to allow expected drug efficacy to be inferred from molecular data. As a component of developing better means to interpret molecular resistance tests, the present study utilised a drug resistant *Haemonchus contortus* isolate which possesses two of the principal benzimidazole resistance SNPs (E198A and F200Y) in order to assess the relative degree of resistance conferred by the two SNPs. We exposed larvae to a range of thiabendazole concentrations in *in vitro* development assays, and collected the surviving L3 larvae at each drug concentration to establish sub-populations showing increasing levels of resistance. We then sequenced the isotype 1 beta-tubulin gene in pooled larval samples, and measured allele frequencies at the two SNP positions. The frequency of the resistance allele at the 198 position increased as the thiabendazole concentration increased, while the frequency of the resistance allele at the 200 position decreased. Genotyping of individual larvae showed that the highest drug concentration was associated with the removal of all genotypes except for homozygous resistance at the 198 position alongside homozygous susceptible at the 200 position. This indicates that, at least for larval life stages, the E198A SNP is able to confer higher levels of resistance to benzimidazole drugs than the F200Y SNP, and that the homozygosity at 198 in the highly resistant individuals is mutually exclusive with heterozygosity or resistant homozygosity at the 200 position. This study illustrates the need to understand the relative contributions of different resistance mechanisms in order to maximise the degree to which molecular tests are able to inform on drug resistance phenotype. (Kotze et al., 2012)

The increased activity of drug-metabolizing enzymes can protect helminths against the toxic effect of anthelmintics. The aim of this study was to compare the metabolism of the anthelmintic drug albendazole (ABZ) and the activities of selected biotransformation and antioxidant enzymes in three different strains of *Haemonchus contortus*: the ISE strain (susceptible to common anthelmintics), the BR strain (resistant to benzimidazole anthelmintics) and the WR strain (multi-resistant). *H. contortus* adults were collected from the abomasum of experimentally infected lambs. *In vitro* (subcellular fractions of *H. contortus* homogenate) as well as *ex vivo* (living nematodes cultivated in flasks with medium) experiments were performed. HPLC with spectrofluorimetric and mass-spectrometric detection was used in the analysis of ABZ metabolites. The *in vitro* activities of oxidation/antioxidation and conjugation enzymes toward model substrates were also assayed. The *in vitro* data showed significant differences between the susceptible (ISE) and resistant (BR, WR) strains regarding the activities of peroxidases, catalase and UDP-glucosyltransferases. S-oxidation of ABZ was significantly lower in BR than in the ISE strain. *Ex vivo*, four ABZ metabolites were identified: ABZ sulphoxide and three ABZ glucosides. In the resistant strains BR and WR, the *ex vivo* formation of all ABZ glucosides was significantly higher than in the susceptible ISE strain. The altered activities of certain detoxifying enzymes might partly protect the parasites against the toxic effect of the drugs as well as contribute to drug-resistance in these parasites. (Vokral et al., 2013)

Benzimidazole-2-carbamate derivatives (BzCs) are the most commonly used antiparasitic drugs for the treatment of protozoan and helminthic infections. BzCs inhibit the microtubule polymerization mechanism through binding selectively to the beta-tubulin subunit in which
mutations have been identified that lead to drug resistance. Currently, the lack of crystallographic structures of beta-tubulin in parasites has limited the study of the binding site and the analysis of the resistance to BzCs. Recently, our research group has proposed a model to explain the interaction between the BzCs and a binding site in the beta-tubulin. Herein, we report the homology models of two susceptible (Haemonchus contortus and Giardia intestinalis) parasites and one unsusceptible (Entamoeba histolytica) generated using the structure of the mammal Ovis aries, considered as a low susceptible organism, as a template. Additionally, the mechanism by which the principal single point mutations Phe167Tyr, Glu198Ala and Phe200Tyr could lead to resistance to BzCs is analyzed. Molecular docking and molecular dynamics studies were carried out in order to evaluate the models and the ligand-protein complexes’ behaviors. This study represents a first attempt towards understanding, at the molecular level, the structural composition of beta-tubulin in all organisms, also suggesting possible resistance mechanisms. Furthermore, these results support the importance of benzimidazole derivative optimization in order to design more potent and selective (less toxic) molecules for the treatment of parasitic diseases. (Aguayo-Ortiz et al., 2013)

The completion of a number of nematode genomes has provided significant information on ABC systems in these organisms. Nematodes have more ABC systems genes and greater diversity than do mammalian species. Class 1 and class 2 ABC systems, more commonly known as ABC transporters, are present. As in other organisms, nematode ABC systems are characterized by a highly conserved ATP-binding domain (ABC_2) and a less conserved transmembrane domain (ABC_TM1/TM1F). Studies of drug resistance in nematodes have suggested that ABC transporters are part of the resistance mechanism. Evidence in support of this has been obtained from genetic studies where an association between anthelmintic selection and ABC transporters was shown by comparisons between unselected and drug selected, or resistant, populations of parasitic nematodes. In drug resistant populations, genetic polymorphism and diversity, genotype patterns, and linkage disequilibrium were disrupted. Multidrug resistance (MDR) reversing agents that inhibit ABC function improve efficacy in sensitive nematode populations and restore sensitivity in resistant populations. Similar to the situation in clinical oncology, overexpression of ABC systems occurs in drug resistant and sensitive populations following drug exposure, particularly those in the P-glycoprotein (PGP) subfamily. Deletion or disruption of ABC genes, particularly PGP and the multidrug resistance associated protein (MRP), increases sensitivity to some drugs, particularly ivermectin. These studies provide evidence that ABC transporters play a role in drug action and resistance in nematodes. (Ardelli, 2013)

A paper was published reviewing the genetic and genomic approaches to understanding drug resistance in parasites. (Devaney, 2013)

Anthelmintic resistance is a major problem for the control livestock parasites and a potential threat to the sustainability of community-wide treatment programmes being used to control human parasites in the developing world. Anthelmintic resistance is essentially a complex quantitative trait in which multiple mutations contribute to the resistance phenotype in an additive manner. Consequently, a combination of forward genetic and genomic approaches is needed to identify the causal mutations and quantify their contribution to the resistance phenotype. Therefore, there is a need to develop genetic and genomic approaches for key parasite species identified as relevant models. Haemonchus contortus, a gastro-intestinal parasite of sheep, has shown a remarkable propensity to develop resistance to all the drugs used in its control. Partly because of this, and partly because of its experimental amenability, research on this parasite has contributed more than any other to our understanding of anthelmintic resistance. H. contortus offers a variety of advantages as an experimental system including the ability to undertake genetic crosses, a prerequisite for genetic mapping. This review will discuss the current progress on developing H. contortus as a model system in which to study anthelmintic resistance. (Gilleard, 2013)

The rotation of different anthelmintic classes, on an approximately annual basis, has been widely promoted and adopted as a strategy to delay the development of anthelmintic resistance in nematode parasites. Part of the rationale for recommending this practice was the expectation that resistant genotype worms have a lower ecological fitness than
susceptible worms, at least in the early stages of selection, and so reversion towards susceptibility could be expected in those years when an alternative class of anthelmintic was used. The routine use of combination anthelmintics might be expected to negate this opportunity for reversion because multiple classes of anthelmintic would be used simultaneously. A simulation model was used to investigate whether the optimal strategy for use of multiple drug classes (i.e. an annual rotation of two classes of anthelmintic or continuous use of two classes in combination) changed with the size of the fitness cost associated with resistance. Model simulations were run in which the fitness cost associated with each resistance gene was varied from 0% to 15% and the rate at which resistance developed was compared for each of the drug-use strategies. Other factors evaluated were the initial frequency of the resistance genes and the proportion of the population not exposed to treatment (i.e. in refugia). Increasing the proportion of the population in refugia always slowed the development of resistance, as did using combinations in preference to an annual rotation. As the fitness cost associated with resistance increased, resistance developed more slowly and this was more pronounced when a combination was used compared to a rotation.

If the fitness cost was sufficiently high then resistance did not develop (i.e. the resistance gene frequency declined over time) and this occurred at lower fitness costs when a combination was used. The results, therefore, indicate that the optimal drug-use strategy to maximise the benefit of any fitness cost associated with resistance is the use of combinations of different anthelmintic classes. Manual calculations confirmed that, within the model, the only resistant genotypes capable of surviving treatment with a combination are those carrying multiple resistance genes. These individuals are less fit, resulting in the worm population surviving treatment having a lower overall ecological fitness. This is a previously unreported perspective on the use of combination anthelmintics and strengthens the argument that any new class of anthelmintic, for which resistance genes can be expected to be rare, should be brought to market in combination. (Leathwick, 2013)

A paper published in 2013 described F200Y polymorphism in the beta-tubulin gene in field isolates of *Haemonchus contortus* and risk factors of sheep flock management practices related to anthelmintic resistance (Meo Niciura et al., 2012)

A case study was published of multiple anthelmintic resistance of *Haemonchus contortus*, including a case of moxidectin resistance, in a Dutch sheep flock (Van den Brom et al., 2013)

**Anthelmintic resistance in alpacas**

Parasitic nematodes can cause substantial clinical and subclinical problems in alpacas and anthelmintics are regularly used to control parasitic nematodes in alpacas. Although anthelmintic resistance has been reported in ruminants worldwide, very little is known about anthelmintic resistance in alpacas. The present study was carried out to confirm a suspected case of anthelmintic resistance in *Haemonchus contortus* in alpacas in Australia. Post mortem examination of an alpaca was conducted to determine the cause of its death. To confirm a suspected case of macrocyclic lactone (ML) resistance in *H. contortus* in alpacas, a faecal egg count reduction test (FECRT) was performed using closantel (7.5 mg/kg) and ivermectin (0.2 mg/kg). Nematode species were identified by morphological and molecular methods. Post mortem examination of a 1-year-old female alpaca that had died following a brief period of lethargy, anorexia and recumbency revealed severe anaemia, hypoproteinaemia and gastric parasitism by adult *Haemonchus contortus*, despite recent abamectin (0.2 mg/kg) treatment. Based on these findings and the exclusive use of MLs in the herd over the preceding six years, ML resistance in parasitic nematodes of alpacas on this farm was suspected. FECRT revealed that the efficacy of closantel was 99% (95% CI 93-100), whereas that of ivermectin was 35% (95% CI 0-78), indicating that the treatment failure was likely due to the presence of ML-resistant nematodes. Larval culture of faecal samples collected following ivermectin treatment consisted of 99% *H. contortus* and 1% *Cooperia oncophora*, a result confirmed using a PCR assay. This study provides the first evidence of ML resistance in *H. contortus* in alpacas in Australia. Based on the extent of anthelmintic resistance in sheep gastrointestinal nematodes in Australia, veterinarians and alpaca owners should be encouraged to implement integrated parasite management strategies to improve nematode control in alpacas. (Jabbar et al., 2013)


Chemical control

Anthelmintic treatment of nematode infections remains the mainstay of worm control in farm and companion animals. However, control is threatened by the occurrence of drug resistant nematodes. In recent years, three new anthelmintics have been introduced to the market. Here, we describe the main features including mode of action, availability, spectrum, dose, tolerability, safety, and resistance of emodepside, monepantel, and derquantel (Epe and Kaminsky, 2013)

Monepantel (MOP) belongs to a new class of anthelmintic compounds, the amino-acetonitrile derivates, which have a different mode of action as the currently used anthelmintics. Many present studies confirmed the high efficacy of MOP against fourth larval and adult stages of Haemonchus contortus. The objective of this study was to determine in vitro efficacy of MOP against lower development stages (eggs, L-1-L-3 larvae) and to compare it between resistant and susceptible isolates of H. contortus. For this purpose, two in vitro tests - egg hatch test and micro-agar larval development test were used. Results were quantified as 50 % lethal concentration (LC50), 99 % lethal concentration (LC99) and resistance factor (RF). This study revealed the high efficacy against lower larval stages (L-1-L-3) of both resistant and susceptible strains of this parasite. Larval susceptibility was not dependent of the sensitivity status of the nematode isolate. On the other hand, ovicidal effect of MOP was very low.(Lecova et al., 2013)

As a result of the need to develop new active principles for the control of endoparasites in ruminants, the present in vivo study evaluated a formulation containing 24% Aurixazol (48mg/kg), a parasiticide molecule based on disophenolate of levamisole. Two experiments were conducted: one evaluating the anthelmintic efficacy of 24% Aurixazol (48mg/kg) against gastrointestinal nematodes in naturally infected sheep, compared to an association of ivermectin (0.2mg/kg)+albendazole (5.0mg/kg)+levamisole (7.5mg/kg) (IAL), and a second one which evaluated the persistent efficacy of the same formulation against immature stages (L4) and adults of Haemonchus contortus in experimentally infected animals. In experiment I, against H. contortus, the formulation of Aurixazol and the IAL association reached efficacies (arithmetic means) of 99.32% and 96.11%, respectively. For Trichostrongylus colubriformis, the efficacy values were 88.92% and 98.08% for Aurixazol and the IAL association, respectively. Both formulations were totally effective against Oesophagostomum columbianum (100%). The results of the statistical analysis demonstrated that the mean parasitic burden of treated animals was significantly different (P≤0.05) compared to the average number of helminths diagnosed in animals from the control group for H. contortus, T. colubriformis and O. columbianum. Comparing only the treated groups, it was possible to verify that the average number of H. contortus recovered from animals treated with Aurixazol was different (P≤0.05) when compared to the mean amount recovered from sheep treated with the IAL association. When evaluating the prevention of H. contortus infection in experiment II, Aurixazol did not present preventive efficacy. Up until 21 days after treatment the groups treated with Aurixazol contained less adults and L4 of H. contortus (P≤0.05) when compared to the non-medicated control group. However, future studies will be necessary to assess the effectiveness of Aurixazol against nematode strains resistant to levamisole and
disophenol, but the efficacy results described in this study allow to state that Aurixazol can, associated with other measures, become an important tool in the control of sheep nematodes. (Sakamoto et al., 2013)

Broad-spectrum antiparasitic macrocyclic lactones (MLs) are the principal nematicides used today, but drug resistance compromises the efficiency of ML-based therapy. Drug action in the parasite is essential for the effectiveness. Thus, to meet the needs of sustainable control of nematodes, the challenge is to maintain an effective drug concentration in the host tissues where parasites locate. This requires knowledge of the site of action of the drug, and processes that govern the pharmacokinetics of MLs in the host and in the parasites. These processes are primarily biotransformation, distribution, storage in fat, and elimination via ATP-binding cassette (ABC) transporters. This article describes how MLs are lipid-like compounds that differ in chemical substituents and in physicochemical properties. Furthermore, the degree of drug lipophilicity influences affinity for fat tissues and for transporters and possibly for target receptors. This opinion highlights how the structural particularities of widely used representative MLs impact on drug kinetics. Optimizing the efficacy of MLs relies on the choice of the drug on the degree of lipophilicity, taking into account the host species, the location of the parasites, and alterations in lipid status. Furthermore, lipid-based formulations can be used to improve intestinal drug absorption. Inhibiting ABC transporter offers an additional option for increasing intestinal M bioavailability. (Lespine, 2013)

The high level of resistance to the macrocyclic lactones has encouraged the search for strategies to optimize their potential as antiparasitic agents. There is a need for pharmacoperasitological studies addressing the kinetic-dynamic differences between various macrocyclic lactones under standardized in vivo conditions. The current work evaluated the relationship among systemic drug exposure, target tissue availabilities and the pattern of drug accumulation within resistant Haemonchus contortus for moxidectin, abamectin and ivermectin. Drug concentrations in plasma, target tissues and parasites were measured by high performance liquid chromatography. Additionally, the efficacy of the three molecules was evaluated in lambs infected with resistant nematodes by classical parasitological methods. Furthermore, the comparative determination of the level of expression of P-glycoprotein (P-gp2) in H. contortus recovered from lambs treated with each drug was performed by real time PCR. A longer persistence of moxidectin (P < 0.05) concentrations in plasma was observed. The concentrations of the three compounds in the mucosal tissue and digestive contents were significant higher than those measured in plasma. Drug concentrations were in a range between 452 ng/g (0.5 day post-treatment) and 32 ng/g (2 days post-treatment) in the gastrointestinal (GI) contents (abomasal and intestinal). Concentrations of the three compounds in H. contortus were in a similar range to those observed in the abomasal contents (positive correlation P = 0.0002). Lower moxidectin concentrations were recovered within adult H. contortus compared to abamectin and ivermectin at day 2 post-treatment. However, the efficacy against H. contortus was 20.1% (ivermectin), 39.7% (abamectin) and 89.6% (moxidectin). Only the ivermectin treatment induced an enhancement on the expression of P-gp2 in the recovered adult H. contortus, reaching higher values at 12 and 24 h post-administration compared to control (untreated) worms. This comparative pharmacological evaluation of three of the most used macrocyclic lactones compounds provides new insights into the action of these drugs. (Lloberas et al., 2013)

Lespine, A., 2013. Lipid-like properties and pharmacology of the anthelmintic macrocyclic lactones. Expert Opinion on Drug Metabolism & Toxicology 9, 1581-1595.
Alternative control

Papers continue to be published into the use of alternative forages to control gastro-intestinal parasites. Usually condensed tannins are thought to be the ingredient responsible for the effect on the parasites. Palatability and the practicalities of growing the forage are potential problems.

The high prevalence of anthelmintic resistance observed in recent years necessitates a reduced reliance on chemotherapy against endoparasites in small ruminants and increased reliance on other agents. Tannin-rich legume forages may assist with this need as they negatively impact endoparasites. The efficiency in the use of tannin-containing legumes in grazing systems may be improved if parasitized animals are allowed to self-select plants in pastures that offer multiple forages. The objectives of this study were to 1) evaluate the effects of the tanniferous legume Onobrychis viciifolia (sainfoin) on Haemonchus contortus infection in sheep, and 2) determine if parasitized sheep (group SAN) increased preferences for this legume after they experienced the antiparasitic effects of tannins. We compared their response with parasitized animals (group CIC) that grazed the non-tanniferous legume Astragalus cicer (cicer milkvetch). These results suggest exposure to sainfoin attenuated H. contortus parasitic burdens in sheep. The uniform use of sainfoin by parasitized lambs also suggests preferences for the legume would remain high even when grazed for several days and despite the availability of alternative legumes like cicer milkvetch. Exposure to sainfoin or cicer milkvetch by parasitized lambs affected subsequent preference for the two legumes in choice tests likely due to positive post digestive effects induced by tannins in nutrition and health.(Villalba et al., 2013)

This study was performed in Cashmere goats that were experimentally infected with Teladorsagia circumcincta to investigate the effects of heather consumption on the establishment of incoming infective larvae (experiment 1) and on an adult nematode population (experiment 2). The worm counts were similar in both groups, but the female length and fecundity were significantly (P < 0.001) lower in supplemented goats. These results show that heather consumption reduces the establishment of T. circumcincta larvae in goats and the development and fecundity of female adult parasites.(Moreno-Gonzalo et al., 2013c)

This study was carried out to evaluate the in vitro-effects of different heather species on Trichostrongylus colubriformis eggs, larvae and adult worms, and obtain scientific evidence to attribute these effects to the action of their phenolic compounds and/or tannins. Total phenolic extracts of three heather species (Calluna vulgaris, Erica cinerea, and Erica umbellata) and an equal mixture of these three extracts were tested in vitro in the three development stages of T. colubriformis using an egg hatching assay (EHA), larval exsheathment inhibition assay (LEIA), and adult motility inhibition assay (AMIA). All extracts significantly (P < 0.001) inhibited egg hatching and the effect was dose dependent. All extracts inhibited or delayed the exsheathment of T. colubriformis L3, and the effect was dose dependent for C. vulgaris. Incubation with heather extracts induced a reduction in adult worm motility compared to control, although significant (P < 0.05) differences were only found at the highest concentrations. Additional studies showed that purified tannins of the same heather species disturbed T. colubriformis larval exsheathment. All these results confirm the anthelmintic properties of heather against T. colubriformis, and suggest that not only tannins but also some other phenolic compounds might be involved.(Moreno-Gonzalo et al., 2013b)

The aim of the present study was to evaluate the in vitro effects of heather (Ericaceae) phenolic extracts on the abomasal nematodes Teladorsagia circumcincta and Haemonchus contortus. Extracts of three heather species (Calluna vulgaris, Erica cinerea, Erica umbellata and a balanced mixture of all three) were tested in vitro on different development stages of T.
circumcincta (eggs, infective larvae and adult worms) and H. contortus (eggs and infective larvae) using an egg hatching assay (EHA), a larval exsheathment inhibition assay (LEIA) and an adult motility inhibition assay (AMIA). Incubation with E. cinerea, E. umbellata and mixed heather extracts had a significant (P < 0.01) dose-dependent effect on T. circumcincta egg hatching. H. contortus egg hatching was significantly (P < 0.01) inhibited only by the E. cinerea extract. All extracts had a significant (P < 0.01) dose-dependent effect on the exsheathment of T. circumcincta and H. contortus infective larvae. The incubation with all heather extracts induced a reduction in adult T. circumcincta motility compared to the control, although significant (P < 0.05) differences were only found at the highest concentration (1200 μg/mL). These results show anthelmintic properties of heather phenolic extracts against T. circumcincta and H. contortus, thus confirming observations from previous in vivo studies (Moreno-Gonzalo et al., 2013a).

The study assessed the effect of dietary supplementation of leaf meal mixture (LMM) containing condensed tannins (CT) on feed intake, nutrient utilization and performance of sheep infected with Haemonchus contortus. Eighteen adult sheep of similar age and body weight (25.0±3.52) were included in this study and out of these, 12 sheep were infected with single dose of infective third stage larvae of H. contortus at 2,000 larvae per sheep. It may be concluded that dietary supplementation of CT through LMM significantly improved the N retention, and inhibited the different developmental stages of Haemonchus contortus in experimental sheep. (Pathak et al., 2013)


GASTRO-INTESTINAL PARASITES IN CATTLE

Epidemiology

Beef production, especially suckler beef production, has not received as much attention as dairy production with respect to nematode control. Spring-born suckler calves have several advantages compared to weaned dairy calves in that they receive a largely milk diet during their first few months at pasture. This confers a number of benefits, including a high plane of nutrition, a comparatively low herbage intake (and hence infective larvae intake) and an apparent resistance to the establishment of abomasal parasitic nematodes. Nevertheless, as the grazing season progresses, herbage intake increases and milk intake relatively declines and calves are exposed to the negative effects of parasitic gastroenteritis (PGE), albeit, rarely suffering from clinical disease. However, the notion that cows only play a positive role in the epidemiology of PGE in relation to young stock has been challenged by several studies. In summary, these authors conclude that each farm should be considered as an individual epidemiological unit, within which the resources on the farm and the abilities and goals of the
producer can be taken into consideration to devise appropriate parasite control approaches within the overarching herd-health action plan. (Forbes and Ellis, 2013)

The combined influence of (1) calving period (early or late) and (2) overwintering contamination by residual infective larvae (high or low) on subsequent exposure of suckler calves to gastrointestinal nematodes was investigated. We found that the effect of calving date was greater than the level of residual contamination. This was because the adult cows produced large quantities of manure containing small amounts of nematode eggs from turnout, which significantly contaminated the pasture, and thereby, reduced the effect of prior high-low contamination. Early born calves were found to be more heavily exposed to parasites, most likely due to ingesting more herbage than those born later. Late-born calves also had relatively high antibody levels at turnout, which first decreased and then increased again. We suggest that the high antibody levels at turnout reflect passive transfer of maternal antibodies through the milk. There was also a significant difference in animal performance, with the more heavily exposed early born calves having significantly lower daily weight gain than the late-born calves. However, this might not be entirely due to increased parasitism. (Hoglund et al, 2013c)

This study aimed to estimate the prevalence of gastrointestinal nematodes and the intensity of infection in grazing dairy cattle from small and medium-sized farms in southern Poland. The level of antibodies against Ostertagia ostertagi in the bulk tank milk (BTM) from the animals was also assessed. Rectal fecal samples collected from 361 cows on 20 farms were examined using Willis-Schlaaf flotation and the McMaster method. BTM samples were tested for the presence of O. ostertagi antibodies using ELISA. Multiplex PCR was used to identify the third-stage larvae (13) of gastrointestinal nematodes derived from the culture of pooled fecal samples from sampled farms. Gastrointestinal nematode eggs were found in the samples from 18 of the 20 herds with a prevalence range from 20.4 to 94.5%. The average number of eggs excreted in the feces of the herds was 200 eggs per gram (EPG). Antibodies to O. ostertagi were found in 20 of the examined herds (100%), of which 6 had optical density ratios (ODR) greater than 0.5. PCR results showed the presence of three nematode species: Ostertagia ostertagi, Cooperia oncophora and Oesophagostomum radiatum. (Piekarska et al., 2013)

Parasitism during development impairs normal growth and delays the onset of puberty through altered hormone profiles, including insulin-like growth factor one (IGF-1). As mammary gland development during prepuberty is strongly dependent on IGF-1, workers in the Americas determined if antiparasitic treatment during this stage of growth improved mammary gland development. One group of Holstein heifers was treated monthly, rotationally with antiparasitic drugs from birth to 70 weeks of age, a second group was untreated. Treated heifer calves had between 56% and 65% less EPG counts than untreated ones. Presence of Ostertagia, Cooperia, Haemonchus and Trichostrongylus was demonstrated. Treatment effectively advanced the onset of puberty and increased IGF-1 levels. At 20, 30, 40 and 70 weeks of age biopsies from the mammary gland were taken and histological sections were prepared and stained with hematoxylin-eosin. Pictures were analyzed to compare parenchyma area in relation to total mammary tissue between groups. Mammary samples from treated heifers had higher ratios of parenchyma/total area than untreated ones. As mammary development during prepuberty is crucial for mammary performance during lactation, these results add new evidence to the importance of gastrointestinal parasite control in heifers. (Perri et al., 2013)


Högland, J., Hessle, A., Dahlström 2013c. Calving season is a stronger determinant of worm burdens in pasture-based beef production than the level of residual larval contamination at turnout. Veterinary Record doi: 10.1136/vr.101077

Diagnostic tests

A quantitative real-time polymerase chain reaction (qPCR) based on hydrolysis (TaqMan (R)) probes is described for robust and sensitive detection of the infection levels with eggs and third stage larvae (L3) of Cooperia oncophora and Ostertagia ostertagi isolated from cattle faeces. The current microscopic method for identification of strongyle nematodes in cattle faeces is labour-intensive where reliable species determination also requires trained expertise, which is increasingly lacking. The goal of this study was to develop a sustainable non-labour intensive diagnostic qPCR assay to detect and determine the levels of infection with the two most common gastro-intestinal nematodes (GIN) in cattle faeces targeting the second internal transcribed spacer of nuclear ribosomal DNA (ITS2) region (rDNA). According to our results, this procedure allows to reliably detect the relative proportions of eggs and L3 for each of the two species. This assay produced consistent results when mixtures with known numbers of L3 of both species were tested, although it was also demonstrated that the calculated copy numbers of ITS2 between single L3 sometimes varied very much. In addition, a positive correlation ($r^2 = 0.23$) between the proportion of eggs and L3 in different paired samples collected in the field was observed for both species. Thus, for the first time a qPCR assay is reported, which can discriminate between the two most important cattle nematode parasites in temperate regions. This is of major importance to the livestock sector as it can be used with great precision to demonstrate strategic treatment efficacy that is important for the detection of anthelmintic resistance (AR). (Hoglund et al., 2013b)

In Western Europe, gastrointestinal nematodes are widespread in dairy cattle. This study was carried out to evaluate the relationship between optical density ratio (ODR) measured on bulk tank milk with an indirect Ostertagia ostertagi ELISA and reproduction/mortality parameters. Data were collected between 2008 and 2010 from monitoring carried out on 1643 dairy herds (Normandy, Western France). ODR values of 3 samples from each farm taken from November 2008 to 2010 were averaged and then transformed into a categorical variable. Reproductive and mortality data were obtained from 1444 herds using cow records from government databases. Statistical analysis was carried out using ordinary logistic regression (OLR). The outcome variables were the case-control status of a herd for reproductive factors, age at first calving and inter-calving intervals, and mortality ratios of various age classes. The effect of the categorical ODR variable was studied and several potential confounder herd factors were used to improve the model fit. A significant relationship was found between high Ostertagia ODR levels and a late age at the first calving (>34.5 months) (odds ratio (OR) = 1.94, $p < 0.001$). No significant relationship was observed with OLR for inter-calving intervals although bivariate analysis showed that herds with high ODR levels had longer inter-calving intervals than herds with low ODR level (first inter-calving interval in herds with low vs. high ODR levels = 412 days vs. 422 days, $p < 0.001$; other inter-calving intervals = 408 days vs. 413 days, $p < 0.01$). A high ODR level was also associated with high mortality of calves between 0 and 30 days of life (mortality ratio >6%) (OR = 1.43, $p < 0.05$) and between 91 and 365 days (ratio >3%) (OR= 1.72, $p < 0.01$). No significant relationship was observed with multivariate approach for mortalities in other classes by age, but bivariate analysis showed that herds with high ODR level had higher mortalities than herds with low ODR levels (mortality between 31 and 90 days in herds with low vs. high ODR levels = 1.89% vs. 2.91%, $p < 0.001$; mortality after 365 days = 1.67% vs. 2.93%, $p < 0.001$). In conclusion, our results confirm the usefulness of ELISA as an indicator for production losses in dairy herds. This inexpensive tool could be advantageous, used to aid farmers and veterinarians to carry out appropriate control measures (Delafosse, 2013)

We investigated the magnitude of temporal changes in activity, posture and feeding behaviour of cattle infected with Ostertagia ostertagi, and their reversal after treatment with an anthelmintic. Twenty-six, 3-month-old, Holstein-Friesian bulls were allocated to one of three treatment groups. Bulls in two of those (groups P and PA) received 100,000 larvae on three occasions (Days 0, 7 and 14) and the remaining animals served as controls (C). The PA
group also received an anthelmintic on Day 31. Parasite eggs appeared in the faeces of P and PA bulls from Day 17; from approximately the same time blood pepsinogen levels increased and body weight (BW) gain decreased (P < 0.001). The reduction in BW gain persisted until Day 45 for P animals only. There was a decrease in the number of steps taken for P and PA animals, as well as lying and standing episode frequency, by 41 and 44% respectively (P < 0.001) from Day 21 onwards. The average lying and standing episode duration increased by 52 and 55% respectively (P < 0.001) from the same time in P and PA compared to C bulls. In addition, meal frequency showed a tendency to decrease for P animals only (P=0.039) from Day 39, and this was the only aspect of feeding behaviour affected by parasitism. All behaviours, returned to control levels within a week of anthelmintic drenching of PA bulls, apart from the number of steps taken. Although BW gain and pepsinogen also started to recover after drenching, these had not returned to control levels by Day 45. The magnitude of the changes inactivity, and standing and lying episode frequency and duration suggest that these might have a diagnostic value, especially as all can now be monitored by automated means. However, these behaviours did not show the rapid changes we expected before parasitism manifested clinically and following recovery. (Szyszka et al., 2013)

Diagnosis of gastrointestinal nematodes relies predominantly on coproscopic methods such as flotation, Kato-Katz, McMaster or FLOTAC. Although FLOTAC allows accurate quantification, many nematode eggs can only be differentiated to genus or family level. Several molecular diagnostic tools discriminating closely related species suffer from high costs for DNA isolation from feces and limited sensitivity since most kits use only small amounts of feces (<1 g). A direct PCR from crude egg preparations was designed for full compatibility with FLOTAC to accurately quantify eggs per gram feces (epg) and determine species composition. Eggs were recovered from the flotation solution and concentrated by sieving. Lysis was achieved by repeated boiling and freezing cycles - only Trichuris eggs required additional mechanic disruption. Egg lysates were directly used as template for PCR with Phusion DNA polymerase which is particularly resistant to PCR inhibitors. Qualitative results were obtained with feces of goats, cattle, horses, swine, cats, dogs and mice. The finally established protocol was also compatible with quantitative real-time PCR in the presence of EvaGreen and no PCR inhibition was detectable when extracts were diluted at least fourfold. Sensitivity was comparable to DNA isolation protocols and spiked samples with five epg were reliably detected. For *Toxocara cati* a detection limit below one epg was demonstrated. It was possible to distinguish *T. cati* and *Toxocara canis* using high resolution melt (HRM) analysis, a rapid tool for species identification. In human samples, restriction fragment length polymorphism (RFLP) and HRM analysis were used to discriminate *Necator americanus* and *Ancylostoma duodenale*. The method is able to significantly improve molecular diagnosis of gastrointestinal nematodes by increasing speed and sensitivity while decreasing overall costs. For identification of species or resistance alleles, analysis of PCR products with many different post PCR methods can be used such as RFLP, reverse-line-blot, Sanger sequencing and HRM. (Demeler et al., 2013d)

The presence of gastrointestinal nematode eggs in faecal samples is diagnostic of infection by these parasites. However, this technique cannot be used to distinguish between species of importance. The faecal culture technique and subsequent microscopic analysis of developed larvae is currently used to determine which parasite species are present in the samples, but these techniques take a week to perform and have inherent limitations. To overcome these parasite detection and identification problems, we have developed a DNA extraction method for sheep faeces, and a quantitative multiplex PCR (qPCR) test which can both enumerate and identify Haemonchus, Trichostrongylus and Teladorsagia. We demonstrate that the technique is sensitive to 10 eggs per gram and that dilution of DNA to 0.1 fold can overcome PCR inhibition issues for samples obtained from the field, while maintaining assay sensitivity. Further development of these tests for commercial use is warranted, given their potential to provide consistently faster and more accurate diagnoses of these parasites using simple sample collection and laboratory methods which can be easily adapted for the detection of a variety of pathogens from the same faecal sample. (McNally et al., 2013)

This chapter provides an account of the significance of parasitic nematodes (order Strongylida), reviews conventional diagnostic techniques that are presently used routinely and describes advances in polymerase chain reaction (PCR)-based methods for the specific
The efficacy of moxidectin administered by different routes, against naturally acquired infections of gastrointestinal nematode parasites of cattle, was compared using faecal egg count reduction tests on 14 commercial farms throughout New Zealand. On each farm, groups of 15 calves were sampled for faecal nematode egg count and then treated with ivermectin administered orally, or with moxidectin administered either by the oral, subcutaneous injection or topical (pour-on) route. Samples were again collected 14 days after treatment and efficacy was calculated as the percentage reduction in group mean egg count between the pre- and post-treatment samples. In addition, efficacy was calculated for individual animals, in order to compare the variability of the different treatments. On four farms untreated control groups were run and five animals from each of the control and all of the moxidectin-treated groups were bled over time to estimate plasma-moxidectin concentrations. Averaged across all tests, the reduction in faecal egg count was significantly greater after treatment with moxidectin oral (91.1%) than following treatment with moxidectin injection (55.5%) or with moxidectin pour-on (51.3%). Low efficacies were invariably against Cooperia oncophora. The oral treatments were significantly less variable in efficacy than the injection and pour-on treatments. Moxidectin concentrations in plasma were highest following subcutaneous injection and lowest following pour-on administration. Plasma levels following oral administration were intermediate, being significantly lower than post-injection and significantly higher than post-pour-on. There was no evidence of transfer of moxidectin to
untreated animals through licking. Based on these results, along with those of other studies, it is proposed that oral administration of macrocyclic lactone anthelmintics results in higher concentrations of active reaching the target worms in the gastrointestinal tract than following either administration by injection or by pour-on. (Leathwick and Miller, 2013)

A three-year trial was performed in south-western Sweden to compare animal performance and levels of parasite control in three grazing groups, each with 18-24 first-season grazing (FSG) calves in similar set-stocked pasture enclosures. These groups were subjected to: (1) no parasite control (NT), (2) monthly repeated doramectin (Dectomax (R)) injections (SP), or (3) targeted selective weight gain-based anthelmintic treatments (TST) but only when individual calf performance was inferior to the average of the poorer 50% of those calves in group SP. In each year, weight and parasitological variables were measured at turn-out and then at predetermined intervals for 22-24 weeks during the grazing season. The dewormed calves in group SP had a higher average weight gain at housing (range 0.39-0.61 kg/day) than those in TST (0.36-0.50 kg/day), which in turn always exceeded the NT group (0.23-0.42 kg/day). This indicates that the parasite challenge in the NT group was sufficiently high to result in production loss. However, the average cumulative faecal egg counts (FEC) at housing in NT were in the range 1271-1953 eggs per gram faeces (epg) and in TST 1221-1968 epg. In contrast, parasite eggs were rarely recorded in group SP and then only during the first two years (on average 12 and 38 epg). There were also no significant differences in FEC or serum pepsinogen levels between FSG in groups NT and TST. The animals in SP received 7 doses of doramectin each year, whereas those in TST received an average of 0.5 doses. Thus, the TST approach represented a 92% reduction in anthelmintic use. The average weight gain in animals subjected to TST was always significantly lower than in animals dewormed regularly. In addition, there were no signs of short-term selection for anthelmintic resistance in the group SP animals, despite the fairly intensive use of injectable doramectin. (Hoglund et al., 2013a)

A study on the effect of topical macrocyclic lactones (ML) against gastrointestinal nematodes (GIN) in Swedish first season grazing cattle (FSG) was performed during the grazing seasons of 2009 and 2010. A questionnaire revealed that 64% of participating farmers dewormed their animals in previous years, and of these 76% used topical formulations with ML. Four to six weeks after turnout, 107 (2009) and 64 (2010) farmers sent in individual faecal samples from 6-10 FSG. Faecal egg counts (FEC) were determined by the FECPAK (R)-method in 2009 and the McMaster-method in 2010, when also larvae were cultured. Average FEC of >= 100 eggs per gram faeces (EPG) was seen in 39% of the herds in 2009 and 42% in 2010 and with arithmetic means of 258 +/- 110 and 252 +/- 350 EPG, respectively. Interestingly, FSG in dairy and beef herds had similar mean FEC. In herds with mean FEC of >= 100 EPG, farmers dewormed all FSG in the tested grazing group with ivermectin (IVM) or doramectin (DOR) pour-on. In 2009, 33 (31%), and in 2010, 26 (40%) of the herds were retested 7-16 days post treatment. Mean reduction was 89% and 88%, respectively, and in only 12 (36%) and 10 (38%) herds it was >= 95%. Beef herds had mean reductions similar to those of the dairy herds. No significant difference (P = 0.66) in reduction was seen between the groups treated with three different pour-on formulations, nor was there any correlation between the previous year's usage of anthelmintics and the efficacy. Larvae from post-treatment cultures analysed in 2010 with a species-specific ITS2 qPCR showed that Cooperia oncophora was the predominant species after deworming. Four (15%) groups also harboured surviving Ostertagia ostertagi post treatment. (Areskog et al., 2013)

Gastrointestinal nematodes, such as Ostertagia ostertagi and several species of Cooperia are ubiquitous in temperate climates and have been shown to have detrimental effects on production in adult dairy cattle. A published meta-analysis demonstrated that overall, producers lose approximately 0.35 kg of milk per parasitized cow per day. Enzyme-linked immunosorbent assays (ELISAs) have the ability to quantify nematode infections in cattle, and thus, could be used to estimate the amount of milk production loss due to differing levels of parasitism at the individual cow level. ELISA results from individual cow milk samples were used to predict milk production response following a randomized anthelmintic treatment in a large field trial in Canada. To increase statistical power, the data collected from this field trial was pooled with data from two other published field trials to form an individual patient data meta-analysis (IPDMA). The ability to predict the effect of anthelmintic treatment on milk
production depends on the level of parasitism quantified by an ELISA measuring milk antibodies against O. ostertagi, and reported as optical density ratios (ODRs). Therefore, the estimates from the interaction between ODR and treatment on milk production were used to determine how well the ODR predicted the response of the treatment. It was anticipated that the relationship between milk production and ODR was unlikely to be linear, so fractional polynomials were applied to the continuous ODR values. The interaction in the field trial showed a trend (p = 0.138) toward a beneficial treatment effect when the individual ODR values, measured in late lactation and using Svanovir (R), were greater than 0.12. When individual data from two other similar studies were included in an IPDMA, the interaction terms became statistically significant (p = 0.009) indicating that there is a beneficial treatment effect when ODR values are slightly elevated. A graph was used to demonstrate the treatment effect (the estimated difference of kg/cow/day of milk yield between the treated and placebo cows), with 95% confidence intervals, as the ODR values increase. It is important to note that the methods of quantifying the ODR values differed between the three studies in the IPDMA, therefore some caution should be used when using these final estimated values. However, the shape and magnitude of the treatment effects, as well as the other fixed model estimates, were very similar between the field trial and the IPDMA suggesting that any bias would likely be minimal. (Vanderstichel et al., 2013)


Extended Release Eprinomectin

A number of papers were published on this new formulation of eprinomectin, which is not yet on the market in the UK. It will not have a zero milk withdrawal.

This injection has been developed to provide up to 150 days control of parasites of cattle. The product can facilitate the achievement of two of the fundamental aims of parasite control. The first is protection of the host against the negative impact of susceptible parasites in order to ensure control of disease and to enhance performance. The second is to reduce parasite transmission and hence the challenge to animals when grazing. In addition, farmers and veterinarians can benefit from high levels of convenience and hence compliance from a single administration, which also limits handling stress in the cattle. This introductory paper provides some perspective on the practical applications for this extended-release product under various husbandry systems and in different classes of cattle and discusses its role in sustainable parasite control. (Forbes, 2013)

A series of 10 dose confirmation studies was conducted to evaluate the persistent activity of an extended-release injectable (ERI) formulation of eprinomectin against single point challenge infections of gastrointestinal and pulmonary nematodes of cattle. Treatments were administered once at a dose volume of 1 mL/50 kg bodyweight by subcutaneous injection in front of the shoulder. In each study, cattle were challenged with a combination of infective stages of gastrointestinal and/or pulmonary nematodes 100, 120 or 150 days after treatment and were processed for parasite recovery according to standard techniques 25-30 days after challenge. Based on parasite counts, Eprinomectin ERI (1 mg eprinomectin/kg bodyweight) provided >90% efficacy (p < 0.05) against challenge with Cooperia oncophora and Cooperia surnabada at 100 days after treatment; against challenge with Ostertagia ostertagi, Ostertagia lyrata, Ostertagia leptospicularis, Ostertagia circumcincta, Ostertagia trifurcata,
The therapeutic efficacy of eprinomectin in an extended-release injection (ERI) formulation was evaluated against induced infections of developing fourth-stage larval or adult gastrointestinal and pulmonary nematodes of cattle in a series of six studies under two identical protocols (three each for developing fourth-stage larvae or adults) conducted in the USA, Germany or the UK (two studies at each location, one per stage). Each study initially included 16 nematode-free cattle. The cattle were of various breeds or crosses, weighed 109-186.5 kg prior to treatment, and were approximately 4-7 months old. The animals were blocked based on pre-treatment bodyweight and then randomly allocated to treatment: eprinomectin ERI vehicle (control) at 1 mL/50 kg body weight or eprinomectin 5% ERI at 1 mL/50 kg bodyweight (1.0 mg eprinomectin/kg) for a total of eight and eight animals in each group. Treatments were administered once on Day 0 by subcutaneous injection in front of the shoulder. In each study, cattle were infected with a combination of infective third-stage larvae or eggs of gastrointestinal and pulmonary nematodes. Inoculation was scheduled so that the nematodes were expected to be fourth-stage larvae or adults at the time of treatment. For parasite recovery, all study animals were humanely euthanized and necropsied 14-15 (adult infections) or 21-22 days after treatment (developing fourth-stage larval infections). When compared with the vehicle-treated control counts, efficacy of eprinomectin ERI against developing fourth-stage larvae and adults was >= 98% (p <0.05) for the following nematodes: *Dictyocaulus viviparus, Bunostomum phlebotomum, Cooperia curvata, C oncophora, C surnabada, C. punctata, Haemonchus contortus, H. placei, Nematodirus helvetianus, Oesophagostomum radiatum, Oes. venutosum, Ostertagia leptospicularis, O. ostertagi, O. circumcincta, O. pinnata, O. trifurcata* (developing fourth-stage larval infections only), *Strongyloides papillosus, Trichostrongylus axei, T. colubriformis, and Trichuris ovis* (adult infections only). All animals accepted the treatment well. No adverse reaction to treatments was observed in any animal in any study (Rehbein et al., 2013b).

The efficacy of eprinomectin in an extended-release injection (ERI) formulation was evaluated against infections with third-stage larvae or eggs of gastrointestinal and pulmonary nematodes in cattle under 120-day natural challenge conditions in a series of five studies conducted in the USA (three studies) and in Europe (two studies). For each study, 30 nematode-free (four studies) or 30 cattle harboring naturally acquired nematode infections (one study) were included. The cattle were of various breeds or crosses, weighed 107.5-273 kg prior to treatment and aged approximately 4-11 months. For each study, animals were blocked based on pre-treatment bodyweight and then randomly allocated to treatment: ERI vehicle (control) at 1 mL/50 kg bodyweight or Eprinomectin 5% (w/v) ERI at 1 mL/50 kg bodyweight (1.0 mg eprinomectin/kg) for a total of 15 and 15 animals in each group. Treatments were administered once on Day 0 by subcutaneous injection in front of the shoulder. In each study, all animals grazed one naturally contaminated pasture for 120 days. At regular intervals during the studies, fecal samples from all cattle were examined for nematode egg and larval counts. In four studies pairs of tracer cattle were used to monitor pasture infectivity at 28-day intervals before and/or during the grazing period. All calves were weighed before turnout onto pasture and at regular intervals until housing on Day 120. For parasite recovery, all study animals were humanely euthanized 27-30 days after removal from pasture. Cattle treated with Eprinomectin ERI had significantly (p < 0.05) fewer strongylid eggs (<= 1 egg per gram; egg count reduction >= 94%) than the control cattle and zero lungworm larvae at each post-treatment time point. At euthanasia, cattle treated with Eprinomectin ERI had significantly (p < 0.05) fewer of the following nematodes than the ERI vehicle: treated (control) cattle with overall reduction of nematode counts by >92%: *Dictyocaulus viviparus* (adults and fourth-stage larvae (L4)), *Bunostomum phlebotomum, Cooperia curvata, Cooperia oncophora, Cooperia punctata, Cooperia surnabada, Cooperia spp. inhibited L4, Haemonchus contortus, Haemonchus placei, Haemonchus spp. inhibited L4, Nematodirus helvetianus, Nematodirus spp. inhibited L4, Oesophagostomum radiatum, Oesophagostomum spp. inhibited L4, Ostertagia leptospicularis, Ostertagia lyrata, Ostertagia ostertagi, Ostertagia spp. inhibited L4, Trichostrongylus axei, Trichostrongylus colubriformis, Trichostrongylus spp. inhibited L4, Trichuris discolor, and *Trichuris ovis*. Over the 120-day grazing period, Eprinomectin ERI-treated cattle gained between 4.8 kg and 31 kg more weight.
than the controls. This weight gain advantage was significant (p < 0.05) in three studies. (Rehbein et al., 2013a)

The efficacy of eprinomectin in an extended-release injection (ERI) formulation in the treatment of cattle harboring naturally acquired nematode populations (including inhibited nematodes) was evaluated. Five studies were conducted under a similar protocol in the USA, the UK, and in Germany. All study animals were infected by grazing naturally contaminated pastures. The adequacy of pasture infectivity was confirmed by examining tracer calves prior to allocation and treatment of the study animals. The cattle were of various breeds or crosses, weighing 79-491 kg, and aged approximately 6-15 months. In each study, 20 animals were infected by grazing, and then removed from pasture and housed in a manner to preclude further nematode infections for 8-16 days until treatment. Animals were blocked based on descending pre-treatment body weight and randomly allocated to one of two treatments: ERI vehicle (control) at 1 mL/50 kg body weight or eprinomectin 5% (w/v) ERI at 1 mL/50 kg body weight (1.0 mg eprinomectin/kg). Treatments were administered once on Day 0 by subcutaneous injection in front of the shoulder. For parasite recovery and count, all study animals were humanely euthanized 14/15 days after treatment. Cattle treated with eprinomectin ERI had significantly (p<0.05) fewer of the following nematodes than the controls with overall reduction of parasite counts of >= 94%: adult Dictyocaulus viviparus, Capillaria spp., Cooperia oncophora, Cooperia pectinata, Cooperia surinamabada, Haemonchus placei, Nematodirus helvetianus, Oesophagostomum radiatum, Ostertagia lyrata, Ostertagia ostertagi, Trichostrongyulus axei, Trichostrongyulus colubroformis, Trichuris discolor, Trichuris skrjabini, and Trichuris spp.; developing fourth-stage larvae of Ostertagia spp. and Trichostrongyulus spp.; and inhibited fourth-stage larvae of Cooperia spp., Haemonchus spp., Nematodirus spp., Oesophagostomum spp., Ostertagia spp., and Trichostrongyulus spp. The results of this series of controlled studies demonstrated high therapeutic efficacy and acceptability of eprinomectin ERI against pulmonary nematodes and a wide range of gastrointestinal parasitic infections, including inhibited gastrointestinal nematodes, in cattle. (Hunter et al., 2013)


**Anthelmintic resistance in cattle**

Ivermectin (IVM) resistance of Cooperia spp. in cattle has become an increasing and global problem. The early detection of anthelmintic resistance (AR) is important to propose strategies to slow down the development of resistance and requires sensitive, reliable, economic high-throughput and practical tests. The purpose of the present study was to apply a larval migration inhibition test (LMIT) for evaluating IVM and MOX efficacy against well-characterized field isolates of Cooperia spp. infecting cattle in Brazil. Eight isolates were used for IVM and seven for MOX. The following EC50 values of IVM were observed for the isolates: susceptible, 1.16 eta mol; Nova Alvorada do Sul I, 4.09 eta mol (RF = 3.52); Campo Grande BNA, 3.57 eta mol (RF = 3.07); Campo Grande TBR, 4.09 eta mol (RF = 3.52); Nova
Anthelmintic resistance of parasites in small ruminants, cattle and horses is increasing worldwide as a consequence of the over usage of the currently available products. In Belgium, Cooperia oncophora is the most common cattle nematode in which resistance, especially against macrocyclic lactones, occurs. Once resistance has been diagnosed, a change to another drug with a different mode of action is advised. However, effective anthelmintics will be hardly available in the near future. Therefore, it is important that farmers and veterinarians find a balance between achieving good parasite control and the sustainability of their control strategies. In this way, anthelmintic resistance may be delayed, and the effectiveness of anthelmintic drugs may be prolonged. This requires sensitive detection tools. With a sensitive detection technique, anthelmintic resistance can be diagnosed in a very early stage. Hence, the spread of resistance alleles in the parasite population may be prevented. In this review, different diagnostic assays for the detection of anthelmintic resistance are discussed, an overview is given of the current status of anthelmintic resistance in Belgian cattle, and measures are suggested to avoid or delay the development of anthelmintic resistance. (De Graef et al., 2013a)

Members of the ATP-binding cassette (ABC) transporter family (P-glycoproteins, Half-transporters and Multidrug Resistant Proteins) potentially play a role in the development of anthelmintic resistance. The aim of this study was to investigate the possible involvement of ABC transporters in anthelmintic resistance in the bovine parasite, Cooperia oncophora. Partial sequences of 15 members of the ABC transporter protein family were identified, by mining a transcriptome dataset combined with a degenerate PCR approach. Reverse transcriptase PCR showed that most of the ABC transporters identified were constitutively transcribed throughout the life cycle of C. oncophora. Constitutive differences in gene transcript levels between a susceptible and resistant isolate were only observed for Con-haf-9 and Con-mrp-1 in eggs of the resistant isolate, while no differences were observed in L3 or the adult life stage. Analysis of resistant adult worms, collected from calves 14 days after treatment with either ivermectin or moxidectin, showed a significant 3- to 5-fold increase in the transcript levels of Con-pgp-11 compared to non-exposed worms. Interestingly, a 4-fold transcriptional up-regulation of Con-pgp-11 was also observed in L3 of the resistant isolate, after in vitro exposure to different concentrations of ivermectin, whereas this effect was not observed in exposed L3 of the susceptible isolate. The results suggest that the worms of this particular resistant isolate have acquired the ability to up-regulate Con-pgp-11 upon exposure to macrocyclic lactones. Further work is needed to understand the genetic basis underpinning this process and the functional role of PGP-11. (De Graef et al., 2013b)

Resistance against macrocyclic lactones such as ivermectin is widespread among parasitic gastrointestinal nematodes of small ruminants and is rapidly increasing in cattle parasites. ABC transporters of the subfamily B, the so-called P-glycoproteins (Pgps) have been frequently implicated in ivermectin resistance and are a major cause of multi-drug resistance in protozoa and helminths. The Pgp inhibitor verapamil (VPL) dramatically enhanced susceptibility of the cattle parasitic nematode Cooperia oncophora to ivermectin in vitro as measured in a larval developmental assay and a larval migration inhibition assay using third stage larvae. Moreover, VPL completely restored susceptibility to ivermectin in a resistant isolate resulting in virtually identical dose-response curves of susceptible and resistant isolates in the presence of VPL. Further characterisation of the molecular mechanisms resulting in Pgp-mediated ivermectin resistance is still hampered by the lack of molecular and biochemical information for Pgps of parasitic nematodes. Using PCR with degenerate primers, fragments of four different C. oncophora Pgps could be amplified and the Conpgp-2,
previously implicated in macrocyclic lactone resistance in Haemonchus contortus, and Conpap-3 full-length cDNAs were obtained by RACE PCR. The pgp sequences presented here contribute important data required to systematically screen resistant C. oncophora isolates for up- or down-regulation of Pgps and for the detection of single nucleotide polymorphisms in Pgps to detect selection of specific Pgp alleles by anthelmintics as early as possible (Demeler et al., 2013b)

Control of helmint infections is a major task in livestock production to prevent health constraints and economic losses. However, resistance to established anthelmintic substances already impedes effective anthelmintic treatment in many regions worldwide. Thus, there is an obvious need for sensitive and reliable methods to assess the resistance status of at least the most important nematode populations. Several single nucleotide polymorphisms (SNPs) in the beta-tubulin isotype 1 gene of various nematodes correlate with resistance to benzimidazoles (BZ), a major anthelmintic class. Here we describe the full-length beta-tubulin isotype 1 and 2 and alpha-tubulin coding sequences of the cattle nematode Ostertagia ostertagi. Additionally, the Cooperia oncophora alpha-tubulin coding sequence was identified. Phylogenetic maximum-likelihood analysis revealed that both isotype 1 and 2 are orthologs to the Caenorhabditis elegans ben-1 gene which is also associated with BZ resistance upon mutation. In contrast, a Trichuris trichiura cDNA, postulated to be beta-tubulin isotype 1 involved in BZ resistance in this human parasite, turned out to be closely related to C. elegans beta-tubulins tbb-4 and mec-7 and would therefore represent the first non-ben-1-like beta-tubulin to be under selection through treatment with BZs. A pyrosequencing assay was established to detect BZ resistance associated SNPs in beta-tubulin isotype 1 codons 167, 198 and 200 of C. oncophora and O. ostertagi. PCR-fragments representing either of the two alleles were combined in defined ratios to evaluate the pyrosequencing assay. The correlation between the given and the measured allele frequencies of the respective SNPs was very high. Subsequently laboratory isolates and field populations with known resistance status were analyzed. With the exception of codon 167 in Cooperia, increases of resistance associated alleles were detected for all codons in at least one of the phenotypically resistant population. Pyrosequencing provides a fast, inexpensive and sensitive alternative to conventional resistance detection methods. (Demeler et al., 2013c)


POULTRY PARASITES

Faecal samples from 19 commercial, 65 week old free-range egg laying flocks were examined to assess the prevalence and number of parasitic nematode eggs. Data were collected to characterise the housing, husbandry, behaviour and welfare of the flocks to examine possible relationships with the egg counts. 2. Eggs of at least one genus of nematode were present in the faeces of all 19 flocks. Heterakis eggs were detected in 17 (89%) flocks, Ascaridia in 16
(84%), Trichostrongylus in 9 (47%), and Syngamus in 6 (32%). Faecal egg counts (FEC) were greatest for Ascaridia and Heterakis. 3. For each nematode genus, there was no significant difference in FEC between organic (N=9) and non-organic (N=10) flocks, or between static (N=8) and mobile (N=11) flocks. 4. FEC were correlated with a range of housing, husbandry and management practices which varied between the nematode genus and included depth of the litter, percentage of hens using the range, and number of dead hens. Statistical analysis indicated relationships with FEC that included light intensity above the feeder, indoor and outdoor stocking density, fearfulness in the shed and on the range, distance to the nearest shelter, and swollen toes. 5. None of the FEC for any of the genera was correlated with weekly egg production or cumulative mortality. 6. Although nematode FEC were highly prevalent among the flocks, the overall lack of relation to other welfare and production measures suggests that these infections were not severe. (Sherwin et al., 2013)

The population dynamics of Ascaridia galli was studied in 70 ISA Brown layer pullets, 42 of them were each experimentally infected with 500 embryonated A. galli eggs and 28 chickens were kept as uninfected controls. Six chickens from the infected group and 4 from the control group were necropsied at 3, 7, 10, 14, 21, 28 and 42 days post-infection (d.p.i.). The mean worm recovery varied from 11-20% of the infection dose with the highest recovery at 3 d.p.i. and the lowest at 21 and 42 d.p.i. (P<0.05). More larvae were recovered from the intestinal wall than from the content (P<0.0001) and intestinal content larvae were longer than those from the wall (mean length 1.6 and 1 mm, respectively, P<0.0001). Although larvae were growing over time, a population of small-sized larvae (length <1 mm) was recovered at all d.p.i. During the first week of infection most of the larvae were located in the anterior half of the jejunileum but they moved posteriorly with the age of infection. Thus, a subpopulation of larvae mainly in the lumen grew with time while another subpopulation remained small and associated with the mucosa. During the infection both subpopulations moved to a more posterior localization in the gastrointestinal (GI) tract. (Ferdushy et al., 2013)


PIG PARASITE CONTROL

The goal of the current experiment was to assess the clinical efficacy of oxfendazole (OFZ) administered as a single oral dose (30 mg/kg) to pigs naturally parasitized with Ascaris suum, Oesophagostomum spp., Metastrongylus spp. and Trichuris suis. Thirty-six local ecotype piglets were divided into three independent experiments, named I, II and III (n =12 each), respectively. Each experiment involved two different groups (n=6): Untreated Control and OFZ treated. Animals were naturally parasitized with A. suum (Experiments I, II and III), Oesophagostomum spp. (Experiments I and II), T. suis (Experiments II and III) and Metastrongylus spp. (Experiment I). Pigs in the treated group received OFZ (Synanthice, Merial Ltd., 9.06% suspension) orally at 30 mg/kg dose. At five (5) days post-treatment, animals were sacrificed and the clinical efficacy of the OFZ treatment was established following the currently available WAAVP guidelines for a controlled efficacy test. None of the animals involved in this experiment showed any adverse events during the study. OFZ treatment given as a single 30 mg/kg oral dose showed a 100% efficacy against all the nematode parasites present in the three experiments. In conclusion, under the current experimental conditions, OFZ orally administered to naturally parasitized piglets at a single dose of 30 mg/kg was safe and highly efficacious (100%) against adult stages of A. suum, Oesophagostomum spp., T. suis and Metastrongylus spp. (Alvarez et al., 2013)

gastrointestinal nematodes in naturally infected pigs. Veterinary Parasitology 194, 70-74.

PARASITES OF WILDLIFE

The aim of the present study was to determine and compare prevalence and intensity of parasitic infections in European brown hares from seven hunting grounds of the Czech Republic. A total of 296 European brown hares (Lepus europaeus) killed during the autumn season of 2007, 2008 and 2009 were examined using necropsy and coprology samples of the entire gastrointestinal tract, lungs and liver. The prevalence of findings was very high and uniform in these years and amounted to 89.1%, 86.9% and 90%, respectively. A significant difference, however, was found in the prevalence and intensity of infection in juvenile and adult hares, especially coccidia (95.1% and 69.2%, respectively) and nematodes of the gastrointestinal tract, i.e. *Trichostrongylus retortaeformis* (91.5% and 73.6%, respectively) and *Graphidium strigosum* (21.9% and 2.3%, respectively). In contrast, there was a much higher prevalence of lungworms *Protostrongylus pulmonalis* (33% and 14.1%, respectively) and *Trichuris leporis* (37.6% and 13.2%, respectively) in adult hares. Tapeworms were found rarely and only in juvenile hares. Differences in the infection prevalence and intensity in all diagnosed parasitoses, which were compared with three body mass groups of adult hares, were highly demonstrative. In the group of the lowest body mass (<3.5 kg live weight) 90-100% prevalence and high intensity of findings in both lungs and the gastrointestinal roundworms and coccidian was demonstrated. In contrast, the lowest prevalence (20-46.7%) was found in the highest body mass group (>4.5 kg live weight). Comparison of the relation between the intensity of infection and body mass of adult hares revealed negative correlations in *Protostrongylus pulmonalis* (r=-0.67), *Eimeria* spp. coccidia (r=-0.64) and *Trichostrongylus retortaeformis* (r=-0.78). Results of the study document significant negative impacts of parasitic infections on health, physical condition and body mass of hares. (Chroust et al., 2013)


**DICTYOCAULUS VIVIPARUS**

Lungworm antibody ELISAs developed in Germany (DE) and The Netherlands (NL) were compared using four sets of serum (S) and bulk-tank milk (BTM) samples from adult dairy cows. The samples originated from 37 farms with or without a suspected clinical lungworm infection during August-October 2010 (Dataset 1), from cows excreting lungworm larvae or not during August-October 2010 (n=59) or May-June 2011 (n=164) (Dataset 2), from 305 farms in a national survey during October 2010 (Dataset 3), and 14 zero-grazing farms during February-April 2011 (Dataset 4). During August-October 2010, covering the second half of the grazing season, the NL-S and NL-BTM ELISA outperformed the DE-S and DE-BTM ELISAs in terms of sensitivity. For at least the NL-S and DE-S ELISA the opposite was found during May-June 2011, covering the end of the winter housing period and the early grazing season. Of the 305 farms in the survey 62.6% were found positive with the NL-BTM ELISA, whereas 2.6% was found positive with the DE-BTM ELISA. ODR values for the zero-grazing farms indicated that a cut-off value of 30% for the DE-BTM ELISA might be more appropriate than the currently used 41%. Results suggest that the NL ELISAs also respond to lungworm antigens other than Major Sperm Protein as used in the DE ELISAs. It is concluded that the generally higher sensitivity of the NL-BTM ELISA makes it better suited for large-scale prevalence studies and herd health monitoring programmes than the DE-BTM ELISA, positivity of which is more associated with the presence of clinical lungworm infection. Reducing the cut-off value of the DE-BTM ELISA from the original 49.3% to the current 41% or the possibly more appropriate 30% increased its sensitivity for detecting lungworm infections, but did not lead to similar sensitivity estimates as found for the NL-BTM ELISA. (Ploeger et al., 2014)

In November 2008, a total of 19,910 bulk tank milk (BTM) samples were obtained from dairy farms from all over Germany, corresponding to about 20% of all German dairy herds, and
analysed for antibodies against the bovine lungworm *Dictyocaulus viviparus* by use of the recombinant MSP-ELISA. A total number of 3,397 (17.1%; n = 19,910) BTM samples tested seropositive. The prevalences in individual German federal states varied between 0.0% and 31.2% positive herds. A geospatial map was drawn to show the distribution of seropositive and seronegative herds per postal code area. ELISA results were further analysed for associations with land-use and climate data. Bivariate statistical analysis was used to identify potential spatial risk factors for dictyocaulosis. Statistically significant positive associations were found between lungworm seropositive herds and the proportion of water bodies and grassed area per postal code area. Variables that showed a statistically significant association with a positive BTM test were included in a logistic regression model, which was further refined by controlled stepwise selection of variables. The low Pseudo R-2 values (0.08 for the full model and 0.06 for the final model) and further evaluation of the model by ROC analysis indicate that additional, unrecorded factors (e.g. management factors) or random effects may substantially contribute to lungworm infections in dairy cows. Monitoring of herds through BTM screening for antibodies can help farmers and veterinarians plan and implement appropriate control measures.(Schunn et al., 2013)

The risk factors, disease prevalence, economic costs, limited antigenic stimulus leading to deficient immune response; epidemiological-based control strategy; and vaccination strategies in preventing lungworms (*Dictyocaulus viviparus*) infection in adult cattle are discussed.(Murphy, 2013)

Murphy, T.M., 2013. Preventing lungworm infection in adult cattle. Veterinary Ireland Journal 3, 196-200.

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**FASCIOLA HEPATICA**

This review is divided into different topics covering diagnostics, therapeutics, molecular research, epidemiology, human fasciolosis and a section referred to as worldwide disease because of the increase in studies of the impact of fasciolosis in other countries.

**DIAGNOSTICS**

The diagnostic tests for *F. hepatica* are well established but there is increasing research into PCR techniques which should prove more sensitive in detecting infection at an earlier stage, and before other diagnostic tests begin to work.

Afshan and others looked at the use of serological and coprological tests to diagnose *Fasciola hepatica* infection in small ruminants in Pakistan. The results showed that there is a significance difference in prevalence between breeds of sheep and goats. In goats there was no significant difference between age and sex in groups examined. They concluded that the indirect serum ELISA was a more efficient technique for early diagnosis compared to coprological tests and that the serum ELISA test should be used for epidemiological surveys and to monitor anthelmintic treatment.

A Brazilian paper by Bernardo et al compared commercial ELISA kits for antibodies in serum and milk with faecal tests in naturally infected cattle. There unusual conclusion was that, despite the increased sensitivity of ELISA kits, when treatment was taken into consideration the faecal egg sedimentation test was the more efficient and easy to perform. Brockwell and others looked at the kinetics of serological copper antigen ELISA tests for *F. hepatica*
infection in animals treated with triclabendazole. The coproantigen ELISA test was more sensitive than the faecal egg count where they had concern about false negatives and infection was also detected earlier. There was good correlation between the ELISA and fluke burdens in cattle and that this was a very efficient test for detecting low fluke burdens.

Charlier monitored the level of gastro-intestinal nematode and fluke infection in Belgium. The conclusion was that there were higher levels of exposure in Belgium compared to surrounding countries, particularly for Nematodes. They concluded that there was a need to decrease levels of exposure and alter timings of treatment and changes to pasture management to reduce the levels of these infections.

Robles-Perez looked at the diagnosis of ovine fasciolosis by PCR test and also to detect anthelmintic resistance in naturally infected flocks. They developed a PCR for a ribosomal internal transcribed spacer for the diagnosis of *F. hepatica* in sheep faeces. The percentage positive sheep was always higher by PCR than by the sedimentation fluke egg detection and that the PCR was more sensitive in detecting resistance to triclabendazole treatment compared to egg detection.

References


EPIDEMIOLOGY

In this section there are papers on the prevalence of fasciolosis in different countries, the prevalence has been estimated by different diagnostic techniques. Also an interesting paper on infecting French intermediate host snails with Argentinian miracidia was published.

Kuerpick measured the sero-prevalence compared to risk factors in dairy herds in Germany. Bulk tank ELISA results from a total of 20,749 bulk tank samples were recorded in 2008. The sero-prevalence was 23.6%. The sero-prevalence did however vary between different federal states and the highest was 38.4%. The conclusion was that in areas of very high sero-prevalence the economic impact of fasciolosis should be publicised and farmers and veterinarians strongly advised to implement effective liver fluke control programmes.

Martinez Valladares looked at the sero-prevalence of *F. hepatica* in sheep in North West Spain. Faecal samples from 110 flocks located in four different provinces were examined. The prevalence for Fasciolosis was 59.3% with a mean egg per gram test of 17.5 per flock. He compared prevalence to weather conditions including temperature and humidity. There was a close correlation between humidity and rainfall and prevalence of *F. hepatica*. There was an increase in prevalence since a similar survey in the early 1990's and the higher
prevalence now could be related to change in climatic conditions. There was also a higher prevalence in areas where there was irrigation of the land.

Mezo looked at the possibility of wild boar as a wildlife reservoir of F. hepatica infection. Using the coproantigen ELISA he noted a very high prevalence in wild boar in the areas of Spain sampled. 62.9% of wild boar sampled were positive for F. hepatica infection by the coproantigen ELISA. Because there was a loss of wild habitat there was encroachment of wild boar on agricultural land and this increased the risk of infection to domesticated animals.

Sanabria undertook a comparative study of the success of infection of French Galba truncatula (intermediate snail host) by French and Argentinian miracidia of F. hepatica. The Argentinian miracidia of F. hepatica were less pathogenic than the French ones and produced a lower prevalence of infection in these snails. The longer patent period and high numbers of metacercariae noted might be the consequence of adaptive mechanism used by the parasite introduced to Argentina where they would have to infect new intermediate snail hosts.

References


WORLDWIDE DISEASE

There were a number of papers from Brazil and other South American countries. Large areas of S America are suitable for the life cycle of F. hepatica. Adrien and others described acute fasciolosis in cattle in Southern Brazil. Fifteen out of 70, three year old pregnant cows lost weight over a 30-40 day period prior to calving. Clinical signs included diarrhoea, weakness, mild anaemia and jaundice. The pathology is described and the outbreak occurred in an area of endemic fasciolosis, although the acute disease was said to be uncommon in this region. They described treatment measures to control further outbreaks.

Dias described the use of Pochonia chlamydosporia as a biological control for F. hepatica in cattle in South Eastern Brazil. This is a fungus which can infect F. hepatica eggs in faeces, excreted by the primary host. His results demonstrated that the fungal infection can be effective in reducing the availability of eggs in the environment for re-infection of calves in natural conditions.

Fiss and others described sub-acute and acute fasciolosis in sheep in Southern Brazil. Eight outbreaks were reported in areas that were irrigated for rice production. These areas were frequently flooded and therefore provided an ideal environment for the parasite. Cases of acute and sub-acute fasciolosis were described in sheep that grazed the rice stubble following harvesting.
**Fruet** described economic losses due to condemnation of cattle viscera in slaughter houses in a region of Brazil. Losses amounted to 58,000 Brazilian dollars during a 12 month period because of liver condemnation due to *F. hepatica*.

**Carnevale** calculated the prevalence of fasciolosis in humans, animals and snails in a region of Argentina. Immunological and molecular techniques were described and the prevalence was found to be 11.9% in humans, 5.26% in cattle by coprological analysis and 61.76% in snails by PCR.

One paper from Egypt (Khalifa) was of note describing the hybrid or intermediate form of the parasite between *F. hepatica* and *F. gigantica*. Characterisation of DNA sequences confirmed that the three species existed in the region of Egypt tested. The intermediate form which they named *Fasciola hepatogigantica* had characters of *F. hepatica* in terms of length and pattern of uterine coils but was genetically more related to *F. gigantica*.

**References**


**MOLECULAR TECHNOLOGY**

Because of the importance of *F. hepatica* as a parasite that causes production losses in sheep and cattle, and is a zoonosis, there was a large amount of research focused on molecular techniques to investigate the parasite. A selection of these papers is summarised below, others are listed, because of the specialist nature of these papers, and the specialist knowledge required to describe them.

**Banford** and others looked at the DNA sequence of a protein that bound calcium within the parasite. He concluded that there were different biochemical properties to this protein compared to other similar *F hepatica* proteins, and it therefore had a discreet functional role.

**Corvo** looked at the protein sequences of the L3 protease enzyme of the invasive stage of *F. hepatica*. Because they now understood the nature of this protein enzyme it was possible to design specific inhibitors to interfere with the action of this enzyme, and therefore the infective stage of the parasite.
Dalton produced a very good review paper of possible molecular candidates for vaccine and immuno-therapeutic developments. Dalton has worked for many years on identifying candidates for vaccines and this paper should be read in its entirety.

Farahnak looked at the superoxide dismutase enzyme in excretory secretory products of F. hepatica and F. gigantica. He concluded that both species had comparable biochemical defence enzymes that explained their survival in the host tissue.

Fuchs looked at the differential expression of F. hepatica beta tubulin iso-types at selected lifecycle stages. The conclusion was that there was tissue specific expression of tubulin isotypes in the liver fluke but the development of resistance to TCBZ is not associated with changes in its presumed target molecule.

Hacariz posed the question where was the 28S ribosomal RNA in F. hepatica as denaturing gel electrophoresis studies should demonstrate the presence of this important molecule. He found that the intensity of the 28S R RNA band is reduced by the effect of this technique.

Hannah continued his research using histology to look at the reproductive organs of TCBZ sensitive and TCBZ resistant isolates of F. hepatica. Use of histology and in-situ hybridisation to visualise endonuclease generated DNA strand breaks could be used to diagnose TCBZ resistance.

Hodgkinson identified markers of TCBZ resistant by geno-wide analysis of genetically recombinant F. hepatica. He described an approach to mapping the major genetic loci involved in conferring TCBZ resistance in F. hepatica.

Kuerpick looked at different ELISA tests for the detection of antibodies to F. hepatica. The Pourquier was superior to another ELISA kit referred to as the ES ELISA.

References


THERAPEUTICS

Cawderey described the action of the flukicide Rafoxanide, its mode of action, pharmacokinetics, dosage, excretion rate, toxicity and tolerance. No conclusions were provided in the abstract.

Keser used iso-thermal microcalorimetry as an analytical tool to study the activity of triclabendazole on juvenile F. hepatica. They concluded that this method was a useful tool for documenting drug effects on juvenile F. hepatica, and probably in the future for adult F. hepatica.

Martinez-Perez studied the effect of dietary supplements such as flax oil and alpha tocopherol on ovine experimental fasciolosis. There was a significant difference in body weight and biochemical values in sheep with these supplements added to the diet compared to a control group at 4 weeks post infection.

The same author also looked at the effect of lipopolysaccharides on sheep experimentally infected with F. hepatica. Again he found that the administration of these compounds increased the non-specific resistance to F. hepatica in experimentally infected sheep. A similar set of experiments was conducted by Meister where he used intramusculature injections of synthetic peroxides in sheep treated with triclabendazole to study the outcomes. Treatment with one of these peroxides had no effect on egg burden and adult worm counts, but another one referred to as MT04 displayed a significant egg count reduction of 98% and worm burden of 92%. He concluded that the peroxide MT04 had excellent activity against F. hepatica in naturally infected sheep.
Ortiz described resistance to *F. hepatica* against triclabendazole in cattle in Cajamarca in Peru in a clinical trial with naturally infected sheep and cattle. The efficacy following treatment and slaughter was only 25%.

Somewhat surprisingly, Sanabria described a strain of *F. hepatica* that was resistant to albendazole but not triclabendazole.

There were a series of papers from Ian Fairweather’s group at St. Mary’s University, Belfast – two papers by Savage et al. Many of these look at the effect of glycoprotein inhibitors, such as verapamil, on the outcome of treatment with triclabendazole in *F. hepatica* strains. The results indicated that verapamil had an effect on TCBZ efflux from the parasite. They concluded that the transport mechanism may have a part to play in drug resistance.

There was concern during 2013 about the imposition of maximum residue limits for a number of flukicides, including TBZ. New techniques such as high pressure liquid chromatography and mass spectrometry can be used to detect these flukicides in milk - Whelan et al. These lead to the withdrawal of a number of flukicide products during 2013 in order that maximum residue limits (MRLs) could be established for these drugs before they were re-introduced. This had an important knock on effect on the choice of flukicide treatment on farms during 2013.

References


HUMAN INFECTION

Most human infection occurs as a result of eating aquatic plants that have on them encysted *F. hepatica* metacercariae. Populations at risk are therefore more likely to be indigenous to countries with suitable fluke habitats.

Infection has also occurred by eating of the plant khat which has hallucinogenic properties. The plant is eaten in the horn of Africa and cases have occurred in the UK in immigrants from this area. One case was described by De Bree in the Netherlands. This was in a Somalian man admitted with jaundice and abdominal discomfort.

The cytotoxic effect of *F. hepatica* was investigated by Baska who looked at the effect on human liver cells in tissue culture of the excretory/secretory protein and a recombinant phosphoglycerate kinase. There was a dose dependence cytotoxicity with the secretory/excretory protein but not with the phosphoglycerate enzyme. He concluded that the excretory/secretory protein had a role in destroying human liver cells.

Cases of fasciolosis in humans as a result of eating water cress do still occur; one was described in California by Weisenberg in a patient with a febrile illness for two months. Diagnosis was made by serology and treatment with triclabendazole was successful.

A case of fasciolosis was described in Iran which was unusual in that the subject lived in a dry part of the country with no endemic sheep population to be a reservoir of infection. Alavi-Naini described this case where humans usually become infected by ingesting contaminated drinking water or plants in endemic areas, mainly Northern Iran. There was no history of travel in this case. Another paper described a low incidence of fasciolosis in animals the South of Iran which was very dry with very few snails to propagate infection – Abdi et al.

Fasciolosis in humans is becoming more recognised and diagnostic tests are improving. Two diagnostics tests were described in papers that evaluated ELISA kits designed to detect Fasciola antibodies in serum usually to detect antibodies to the cathepsin protease enzymes (Gonzales).

Mas-Coma described unusual manifestations of human fasciolosis where there were neurological signs. There were two possible causes of this – aberrant migration of larvae through the brain, or indirect immuno-allergic and toxic effects at distance from flukes in the liver.

References

Abdi, J., et al. (2013). "New features of fascioliasis in human and animal infections in Ilam province, Western Iran." Gastroenterology and Hepatology - From Bed to Bench 6(3): 152-155


**PARAMPHISTOMES**

*Calicophoron daubneyi* infection is becoming more recognised both in Europe and in the UK. There were a number of papers referring to this parasite in 2013. Treatment is an issue as many of the flukicides active against *F. hepatica* are not as effective against *C. daubenyi*. One paper by Arias looked at the efficacy of four anthelmintics against *C. daubneyi* in naturally infected cattle. Seventy Friesian cows were treated at drying off with albendazole, netobimin, closantel, and oxfenclazole. Untreated controls were included in the trial. The egg output of *C. daubneyi* was not fully suppressed following administration of any of these flukicides. The faecal egg count reduction rate ranged from 0-26% in cows receiving albendazole or netobimin with 11-39% of these cattle becoming negative after therapy. Better results were received with closantel and oxfenclazole with a faecal egg count reduction of 97-99%. He recommended that closantel could be used as a treatment for this parasite particularly in countries where oxfenclazole was not available.

Ausruf looked at the comparative efficacy of Ivermectin plus Clorsulon and Nitroxinyl in cattle naturally infected with strongyles and paramphistomum species. Where treatment against paramphistomes was successful, there were no eggs in faeces by 60 days post treatment. The efficacy of combined drug treatment was significantly higher than Ivermectin plus clorsulon treatment against paramphistome sp. A combination of ivermectin, closantel, and nitroxinyl was recommended for treatment of this parasite.

A paper by Shaheen described paramphistome infections in buffaloes in the tropics as causing enteritis and anaemia, and resulted in substantial production and economic loss. He looked at the efficacy of oxfenclazole and nicosamide as combination drugs for the treatment of Paramphistome infection. The efficacy of the treatment was estimated by reduction of body weight, egg count and haematological and biochemical parameters. Use of the drug combinations did reduce the effect of infection as evidenced by haematology and decreased egg production.

Vago and others looked at the parasite *Fascioloides magna* infection in deer in Slovakia. This parasite is endemic for the species of deer. In faeces sample *F. magna* was found in 67.9% of samples and as an incidental finding, paramphistomes were found in 64.3% of samples. In a 1 gram sample of faeces there were usually 68 eggs of *F. magna* and 46 of paramphistome sp. It remains to be seen whether the Paramphistomes were in fact *C. daubnyi*. Pathology of the red deer that were slaughtered is described in this paper.

A paper by Yan and others described the mitochondrial DNA genome of *P. cervi*. They concluded that the complete mitochondrial DNA sequence of *P. cervi* provided important genetic markers for diagnostics, ecological and evolutionary study of this parasite.

Young and others described parasite infections of large ruminants in Cambodia. Large numbers of parasites were found including *Fasciola hepatica* and *F. gigantica* and *Paramphistomum sp* were found in nearly all cattle sampled according to their data.

**References**

DICROCOELIUM SPECIES

There were a few papers of note describing *Dicrocoelium dendriticum* infection in 2013. The first was by Gordon who looked at the prevalence of fluke in ruminants in the west of Ireland. He looked at ten different farms and sampled faeces over several weeks during August 2012. Large numbers of paramphistome eggs were detected in all but two farms with a concurrent low level of detection of mature *F. hepatica* on four farms while *D. dendriticum* was an incidental finding on two farms. They concluded that environmental factors such as flooding, type of pasture, particularly wet and low lying land, the out-wintering of animals were risk factors on farms with a significant burden of paramphistome and mature *F. hepatica*.

Eichenberger looked at estimation of prevalence of tape worms in naturally infected dairy cows in Switzerland. This was an abattoir survey of a total of 793 slaughtered dairy cows and an incidental finding was a high prevalence of *D.dendriticum* infection (60.8%) and *Fasciola hepatica* (30.5%).

Martinez-Ibeas looked at proteomic analysis of the tegument and excretory secretory products of *D.dendriticum*. Few studies of the antigens of this trematode have been undertaken. This was a useful paper, 29 proteins in the Excretory/Secretory group, and 43 tegument proteins were identified, of which 23 were strongly antigenic. Their aim was to understand the complex parasite host relationship and improve the diagnosis of Dicroceliosis.

Sandoval expanded on this work to identify a PCR test for the diagnosis of *D. dendriticum* infection. DNA primers were designed from an eighteen sRNA sequence from this parasite. Their PCR test was able to identify a minimum of 40 D. dendriticum eggs in host faeces samples. This is therefore a useful test for the diagnosis of this parasite.

References


**Coccidia in Birds**

**Control**

Lasalocid – following the finding of Lasalocid above maximum residual limit in five samples of eggs in 2013, the British Egg Industry Council has taken the decision to withdraw the degradation on the use of Lasalocid in pullet rearing diets with immediate effect.

Emilio del Cacho from Spain published a paper on dendritic cells expressing tspan-3 protein regulating protective immunity. They are looking into the possibility of using these cells as a possible vaccine.


Chenzhong Fei has written about the evaluation of a novel anticoccidial nitromezuril. The results demonstrate an equal effect to that of diclazuril, but the mechanism of action remains unknown. The immersgence of new anticoccidial drugs is always welcome to help with resistant strains.


**Technical**

A paper written by Kyung-Woo Lee compared the growth rates of broilers fed on an antibiotic/ ionophore - anticoccidial diet and a live attenuated vaccine. The findings demonstrate that the vaccines give a more stable and in most cases better growth rate, but the ionophores have the added ability to keep separate infections such clostridium at bay.


An interesting article by Raymond H Fetterer on the use of dual-energy x-rays to determine the effect of an *Eimeria* infection on broiler body composition. This was a relatively small trial, but could be a useful tool in future vaccine/infection evaluation.


**Wildlife**

The Wetlands and Wildlife Trust continues to battle with coccidiosis in its wild and captive cranes population.

**Captive**

Work has started to fully investigate the *Eimeria* species found in red-legged partridges. The two main species are *E. legionensis* and *E. kofoidi*. More species have been seen, but not fully investigated.

Andrew Morris, AHVLA Weybridge

**Ectoparasites**

Dairy cattle are becoming increasingly complicated to treat in the USA due to the great limitation of approved drugs. Additionally, most drugs require withdrawal times for milk that
are not viable for treating entire dairy herds. The objective of this field trial was to determine the efficacy of eprinomectin, for eradication of naturally occurring chorioptic mange on a commercial dairy farm. All animals present on the farm were treated on the same day and, later, new animals introduced to the premises were treated on arrival. All cows were retreated at dry-off. Lesion scoring was performed five times over a period of 12 months. A reduction in the proportion of cows with lesions was apparent 3 months after treatment and, although the proportion stayed low, it increased again at 12 months post-treatment. Logistic regression to evaluate factors associated with the presence of mange lesions showed that older cows, late lactation, and recent treatment, were associated with presence of lesions. It also showed that multiple treatments (whole-herd treatment and at dry-off) helped to reduce the presence of lesions. No increase in milk production could be measured, but animal wellbeing improved. The results of this study show that chorioptic mange can be controlled in entire herds, although multiple treatments will be required to potentially eradicate the parasite. The value of the study is that it shows that mange can be controlled in dairy cattle with approved drugs, eliminating the need to use non-approved agents. (Villarroel and Halliburton, 2013)

A paper was also published showing the efficacy of eprinomectin long acting injection against *Sarcotes scabiei* in cattle (Vissera et al, 2013)


A chapter covering the taxonomy, morphology, life cycle, behaviour, biology, medical and veterinary importance, prevention and control of chewing lice (*Amblycera* and *Ischnocera*). Focus is given on their role as parasites of birds (*Culcotogaster heterographa*, *Goniocotes gallinae*, *Goniodes dissimilis*, *Goniodes gigas* and *Lipeurus caponis*) and mammals (*Felicola subrostratus* in cats, *Trichodectes canis* in dogs and wild canids, *Bovicola ovis* in sheep and *B. bovis* in cattle) and as intermediate host of *Acanthocheilonema reconditum* and *Dipylidium caninum* in dogs. (Russell et al., 2013)


Sian Mitchell, AHVLA Carmarthen Investigation Centre

**VECTOR BORNE DISEASE**


Several studies provide evidence of a link between vector-borne disease outbreaks and El Niño driven climate anomalies. Less investigated are the effects of the North Atlantic Oscillation (NAO). Here, we test its impact on outbreak occurrences of 13 infectious diseases over Europe during the last fifty years, controlling for potential bias due to increased surveillance and detection. NAO variation statistically influenced the outbreak occurrence of eleven of the infectious diseases. Seven diseases were associated with winter NAO positive phases in northern Europe, and therefore with above-average temperatures and precipitation. Two diseases were associated with the summer or spring NAO negative phases in northern Europe, and therefore with below-average temperatures and precipitation. Two diseases were associated with summer positive or negative NAO phases in southern Mediterranean countries. These findings suggest that there is potential for developing early warning systems, based on climatic variation information, for improved outbreak control and management.
BESNOITIOSIS IN CATTLE


- Besnoitia besnoiti infection in water buffalo (Egypt)- water buffalo are a potential source/carrier of the disease.

The present study evaluated the presence of specific antibodies against Besnoitia besnoiti in cattle and water buffalo (Bubalus bubalis) in Egypt. The highest infection rate of B. besnoiti was significantly higher in cattle (17.13 %) than in water buffaloes (9.02 %). Positive cases were observed in all age categories. While the highest infection rate (17.13 %) was recorded in the age group 5-10 years followed by the age group 1-5 years (15.38 %), and only one positive case (1.58 %) was recorded in the age group less than 1 year. The highest infection rate of B. besnoiti infection was recorded in the female animals (14.95 %) followed by the male animals (8.33). This is the first report on the detection of B. besnoiti in cattle and water buffaloes in Egypt.


- B. besnoiti screening of cattle in Switzerland was carried out to investigate whether B. besnoiti could be introduced and remain unnoticed and become established in Switzerland. A total of 767 animals (650 cattle imported from France and 117 cattle that had contact with B. besnoiti positive cattle on Swiss farms) were tested. Eight were confirmed seropositive by western blot, seven were further confirmed using PCR and histopathology. The risk of establishment of the disease with imported subclinically infected cattle is suggested from these results.

Bovine besnoitiosis is an economically important disease of cattle, caused by Besnoitia besnoiti (Protozoa, Apicomplexa). A considerable spreading of this parasitic infection has been observed in Europe in the last ten years, mainly related to animal trade. In order to investigate the possibility of B. besnoiti being unnoticed introduced and getting established in Switzerland through the import of breeding cattle from France, a total of 767 animals (650 cattle imported from France and 117 cattle that had contact with B. besnoiti positive cattle in Swiss farms) were screened for antibodies against B. besnoiti by both a commercial ELISA and by the indirect fluorescent antibody test (IFAT). A total of 101 (13.17%) samples showed a positive reaction in ELISA (cut-off: percent of positivity [PP] ≥ 15) and 16 (2.09%) samples had IFAT titers ≥ 1:100. Eight of those samples reacted positive in Western blot (WB), corresponding to five imported Limousin cattle (two cows and one bull from France and two cows from Germany) and to three cattle born in Switzerland (one Limousin heifer born from one of the positive German cows, and two adult Braunvieh cows, that had been in contact with one of the French cows at a Swiss farm). Seven of those animals were subclinically infected and one animal showed only very mild signs. They were subsequently slaughtered, and the serological diagnosis could be confirmed by real-time PCR and/or histopathology in seven animals. The most frequent parasite localizations were the tendons and surrounding connective tissue of the distal limbs and the skin of the head region. Furthermore, B. besnoiti could be successfully isolated in vitro from one French, one German and one Swiss cattle (isolates Bb-IPZ-1-CH, Bb-IPZ-2-CH and Bb-IPZ-3-CH). In the current situation in Switzerland, prophylactic and control measures should include a serological examination of cattle to be imported from endemic areas and the culling of all confirmed positive animals from the herd. The evidence of B. besnoiti infection in both imported and locally born cattle shows
that the conditions for the establishment and dissemination of this parasite in Switzerland seem to be adequate.


- A study in Spain was carried out to identify the presence of specific antibodies against Besnoitia spp. in wild ruminants, to help provide more information on possible sylvatic and domestic lifecycles for the parasite. Specific antibodies were found in red deer and roe deer.

Besnoitia besnoiti has been reported to affect cattle, wildebeest, kudu and impala, and B. tarandi other wild ruminants (caribou, reindeer, mule deer and musk ox), causing similar characteristic clinical signs and lesions. However, both Besnoitia species have been reported in different geographical areas and the link between the sylvatic and domestic life cycles of Besnoita spp. in wild ruminants and cattle remains unknown. The aim of this study was to evaluate the presence of specific antibodies against Besnoitia spp. in wild ruminants in Spain. A wide panel of sera from red deer (Cervus elaphus) (n=734), roe deer (Capreolus capreolus) (n=124), chamois (Rupicapra pyrenaica) (n=170) and mouflon (Ovis musimon) (n=20) collected from different locations of Spain was analyzed. Beef cattle were present in all sampled areas and, interestingly, bovine besnoitiosis has been widely reported in some of them (e.g., Pyrenees and Central Spain). Sera samples were first examined with an Enzyme-Linked Immunosorbent Assay (ELISA). For red deer and roe deer, the ELISA was standardized with positive and negative control sera from several Cervidae species (100% Se and 98% Sp). Chamois and mouflon sera samples were tested with a previously reported ELISA validated for bovine sera (97% Se and 95% Sp) using protein G as a conjugate. Positive results by ELISA were confirmed a posteriori with a tachyzoite-based Western blot. Sixty-one sera samples from red deer and 17 sera samples from roe-deer were seropositive or doubtful by ELISA. All samples from mouflon were seronegative and 15 sera samples from chamois were considered doubtful. B. besnoiti exposure was only confirmed clearly by Western blot in one red deer and one roe deer from the Spanish Pyrenees where the disease is traditionally endemic. This is the first serological report of Besnoitia spp. infection carried out in European wild ruminants and the results show that specific antibodies are present at least in red deer and roe-deer. Thus, wild ruminants from endemic regions of bovine besnoitiosis should be further studied because they may be putative reservoirs of the parasite.


- The aim of the study was to assess the intra-organ parasite distribution, the parasite load and the parasite-associated lesions in seropositive but subclinically infected animals. The tissue cysts were located primarily in the upper respiratory tract, i.e., in the rhinarium and larynx/pharynx (four cows), followed by the distal genital tract (vulva/vagina) and the skin of the neck (three and two cows, respectively, out of the four cows with cysts in the respiratory tract).

Bovine besnoitiosis caused by Besnoitia besnoiti is a chronic and debilitating disease. The most characteristic clinical signs of chronic besnoitiosis are visible tissue cysts in the scleral conjunctiva and the vagina, thickened skin and a generally poor body condition. However, many seropositive animals remain subclinically infected, and the role that these animals may play in spreading the disease is not known. The aim of the present study was to assess the intra-organ parasite distribution, the parasite load and the parasite-associated lesions in
seropositive but subclinically infected animals. These animals were seropositive at the time of several consecutive samplings, had visible tissue cysts in the past and, at time of slaughter, had detectable specific anti-Besnoitia spp. antibody levels, but they did not show evident clinical signs at culling. Thus, histopathological, immunohistochemical and molecular analyses of several samples from the respiratory tract, reproductive tract, other internal organs and skin from six cows were performed. The tissue cysts were located primarily in the upper respiratory tract, i.e., in the rhinarium and larynx/pharynx (four cows), followed by the distal genital tract (vulva/vagina) and the skin of the neck (three and two cows, respectively, out of the four cows with cysts in the respiratory tract). We were unable to detect any parasites in the two remaining cows. Cysts were associated with a significant non-purulent inflammatory infiltrate consisting predominantly of T lymphocytes and activated monocytes/macrophages in two cows. The parasite burden, estimated by quantitative real-time PCR, was very low. It is noteworthy that the only animal that showed a recent increase in the antibody titre had the highest parasite burden and the most conspicuous inflammatory reaction against the cysts. In conclusion, although these cows no longer displayed any visible signs of besnoitiosis, they remained infected. Therefore, cows without visible signs of disease may still be able to transmit the parasite.


- The South African paper describes B.besnoiti tissue cyst development in a bull. The bull died of nephrotic syndrome; anasarca in acute besnoitiosis due to protein-losing glomerulopathy has not previously been reported in cattle.

Besnoitia besnoiti is an apicomplexan that causes serious economic loss in cattle in many countries and the disease is now spreading in Europe. At least 2 phases of bovine besnoitiosis are recognized clinically. An acute febrile phase characterized by anasarca and necrosis of skin is associated with multiplication of tachyzoites in vascular endothelium; this phase is short-lived and rarely diagnosed. Chronic besnoitiosis characterized by dermal lesions is associated with the presence of macroscopic tissue cysts and is easily diagnosed. Here we report the development of early B. besnoiti tissue cysts in a naturally infected Hugenoot bull from South Africa. Tissue cysts were 10-70 μm in diameter, contained 1-12 bradyzoites, and were most numerous in the dermis, testicles, and pampiniform venous plexus. Amylopectin granules in bradyzoites stained red with periodic acid Schiff (PAS) reaction. Bradyzoites varied in size and in the intensity of PAS reaction (some were PAS-negative, some were plump, and others were slender. With immunohistochemical staining with Besnoitia oryctofelisi and bradyzoite-specific antibodies (BAG-1 made against Toxoplasma gondii bradyzoites), the staining was confined to parasites, and all intracyclic organisms were BAG-1 positive. With Gomori's silver stain only bradyzoites were stained very faintly whereas the rest of the tissue cyst was unstained. Ultrastructurally many tissue cysts contained dead bradyzoites and some appeared empty. Unlike bradyzoites from mature cysts, bradyzoites in the present case contained few or no micronemes. These findings are of diagnostic significance. Ultrastructurally host cyst cells had features of myofibroblasts, and immunohistochemistry using antibodies against MAC387, lysozyme, vimentin, Von Willebrand factor, and smooth muscle actin confirmed this. Polymerase chain reaction on DNA extracted from lymph node of the bull confirmed B. besnoiti diagnosis. Associated clinical findings, lesions, and histopathology are briefly presented. The bull died of nephrotic syndrome; anasarca in acute besnoitiosis due to protein-losing glomerulopathy is a finding not previously reported in cattle.

• **Serological survey for B. besnoiti in Italy.** The farm prevalence was 83.0% (73/88), and the individual animal prevalence was 44.1% (233/528).

A cross-sectional serological survey was conducted to evaluate the prevalence of besnoitiosis in cattle farms located in a region of southern Italy. A geographical information system (GIS) was used in order to uniformly sample the bovine farms (n=88) throughout the entire region. Blood samples were collected from 528 autochthonous cattle and sera were tested for antibodies to Besnoitia besnoiti using an enzyme-linked immunosorbent assay test. The farm prevalence was 83.0% (73/88), and the individual animal prevalence was 44.1% (233/528). The availability of geo-referenced point or areal data on bovine besnoitiosis and the construction of prevalence maps by GIS are suggested for dissemination of information to veterinarians on this emerging infection in cattle.

Alvarez-Garcia G, Frey CF, Mora LM, Schares G. A century of bovine besnoitiosis: an unknown disease re-emerging in Europe. *Trends Parasitol.* 2013 Aug;29(8):407-15. Bovine besnoitiosis, which is caused by the cyst-forming apicomplexan parasite Besnoitia besnoiti, is a chronic and debilitating vector-borne disease characterized by both cutaneous and systemic manifestations. In Europe, this parasitic disease appeared in a few restricted areas in France and Portugal since the first recorded cases in the beginning of the 20th century. However, at present, the disease is considered to be re-emerging by the European Food Safety Authority due to an increased number of cases and the geographic expansion of besnoitiosis into cattle herds in several European countries. In this review, we will provide an update of the epidemiology and impact of B. besnoiti infection. Strategies to control this parasitic disease will also be discussed.

![World-wide distribution of Besnoitia spp. infections in ungulates.](image)

In areas where the disease is emerging the incidence of clinical cases is approximately 15–40% per year versus 1–10% per year in areas with endemic bovine besnoitiosis.

Once bovines are infected by B. besnoiti, the incubation period may last from 2 weeks up to 2 months. The acute anasarca period may last from 3 up to 10 days.

Disease has been endemic in France, Spain and Portugal for more than a century. In Italy, a few positive animals were discovered among imported cattle from France, but it was 2009 when the disease was noted to be endemic in the Northern Apennine Mountains due to autochthonous outbreaks of the disease. In Germany, an outbreak occurred in a beef cattle herd in Bavaria that was most likely caused by importation of Charolais or Limousin cattle from France. In a beef cattle herd in Switzerland, four imported cows from France and Germany were seropositive for besnoitiosis, one of them displaying the typical clinical signs

Suggested reasons for spread to new countries: animal trade; beef cattle sharing pastures during the summer during peak vector activity time (from different farms) and natural mating; frequent trade of Limousin and Charolais bulls mainly from France to neighboring countries; climatic change might positively influence arthropod populations, which may transmit the parasite.

It has been experimentally demonstrated that cyst stages (bradyzoites) inoculated into nostrils are infectious for cattle, indicating that the cyst stage of B. besnoiti is able to cross mucous membranes.

Chronically infected animals may recover, at least partially, and may become resistant to re-infections, but are thought to remain infected for the rest of their lives. Recently, this finding was corroborated in cows that no longer displayed visible signs of besnoitiosis but presented remaining tissue cysts mainly located in the upper respiratory tract.

How to control bovine besnoitiosis? First, avoiding the entrance of the infection into a herd should be a priority so the best approach for maintaining a herd free of the disease is to rigorously test all new animals prior to entry. Culling/isolating infected animals.

In valuable animals, the confirmation of an ELISA positive result with an a posteriori western blot is recommended. Serological tests have two main limitations - there have been specificity problems and the time that passes between infection and the appearance of antibodies is an issue.

A multicenter ring trial confirmed the lack of sensitivity of serology in serum samples from acutely infected animals. Some infected animals produce very low antibody levels that are below the detection limits of the tests used.

Both real-time and conventional PCR methods have been developed and have superior sensitivity compared with serology in acutely infected cattle. Valuable animals or animals that might be in the acute phase of infection should be tested by serology followed by PCR performed on blood (acute phase suspected), or on vaginal or skin biopsies (chronic phase).


A preliminary trial showed that stable flies could transmit tachyzoites from bovine artificially parasite-enriched blood to B. besnoiti-free blood. Parasite DNA was detected at both 1 and 24 hour intervals in blood recipient, mouthparts, and gut contents of stable flies.

Cattle besnoitiosis due to the cyst-forming coccidian parasite Besnoitia besnoiti has recently been reported in expansion in Europe since the end of the twentieth century. The B. besnoiti life cycle and many epidemiological traits are still poorly known. Hematophagous flies, including the worldwide-distributed Stomoxys calcitrans, could be mechanical vectors in the contamination of mouthparts after the puncture of cutaneous cysts or ingestion of infected
blood. In this study, a protocol is presented to assess more deeply the role of S. calcitrans, reared in laboratory conditions, in parasite transmission. A preliminary trial showed that stable flies could transmit tachyzoites from bovine artificially parasite-enriched blood to B. besnoiti-free blood using glass feeders. Evidence of transmission was provided by the detection of parasite DNA with Ct values ranging between 32 and 37 in the blood recipient. In a second time, a B. besnoiti-infected heifer harboring many cysts in its dermis was used as a donor of B. besnoiti. An interruption of the blood meal taken by 300 stable flies from this heifer was performed. Immediately after the blood meal was interrupted, they were transferred to a glass feeder containing B. besnoiti-free blood from a non-infected heifer. Quantitative PCR and modified direct fluorescence antibody test (dFAT) were used to detect B. besnoiti DNA and entire parasites, respectively, in the blood recipient, the mouthparts, and the gut contents of S. calcitrans at two time intervals: 1 and 24 h after the interrupted blood meal. Parasite DNA was detected at both time intervals (1 and 24 h) in all samples (blood recipient, mouthparts, and gut contents of stable flies) while entire parasites by dFAT were only found in the abdominal compartment 1 h after the interrupted blood meal. Then, S. calcitrans were able to carry B. besnoiti from chronically infected cattle to an artificial recipient in the conditions of the protocol.


- The aim of this study was to compare the serological tests available in Europe in a multi-centred study. PrioCHECK Besnoitia Ab V2.0 showed 100% Se and 98.8% Sp, whereas ID Screen Besnoitia indirect IDVET showed 97.2% Se and 100% Sp. The in-house ELISA and INGEZIM BES 12.BES.K1 INGENASA showed 97.3% and 97.2% Se; and 94.6% and 93.0% Sp, respectively. IFAT FLI-Wusterhausen performed better than IFAT SALUVET-Madrid, with 100% Se and 95.4% Sp. All ELISAs performed very well and could be used in epidemiological studies; however, Western blot tests performed better and could be employed as a posteriori tests for control purposes in the case of uncertain results from valuable samples.

Bovine besnoitiosis is considered an emerging chronic and debilitating disease in Europe. Many infections remain subclinical, and the only sign of disease is the presence of parasitic cysts in the sclera and conjunctiva. Serological tests are useful for detecting asymptomatic cattle/sub-clinical infections for control purposes, as there are no effective drugs or vaccines. For this purpose, diagnostic tools need to be further standardized. Thus, the aim of this study was to compare the serological tests available in Europe in a multi-centred study. A coded panel of 241 well-characterized sera from infected and non-infected bovines was provided by all participants (SALUVET-Madrid, FLI-Wusterhausen, ENV-Toulouse, IPB-Berne). The tests evaluated were as follows: an in-house ELISA, three commercial ELISAs (INGEZIM BES 12.BES.K1 INGENASA, PrioCHECK Besnoitia Ab V2.0, ID Screen Besnoitia indirect IDVET), two IFATs and seven Western blot tests (tachyzoite and bradyzoite extracts under reducing and non-reducing conditions). Two different definitions of a gold standard were used: (i) the result of the majority of tests ('Majority of tests') and (ii) the majority of test results plus pre-test information based on clinical signs ('Majority of tests plus pre-test info'). Relative to the gold standard 'Majority of tests', almost 100% sensitivity (Se) and specificity (Sp) were obtained with SALUVET-Madrid and FLI-Wusterhausen tachyzoite- and bradyzoite-based Western blot tests under non-reducing conditions. On the ELISAs, PrioCHECK Besnoitia Ab V2.0 showed 100% Se and 98.8% Sp, whereas ID Screen Besnoitia indirect IDVET showed 97.2% Se and 100% Sp. The in-house ELISA and INGEZIM BES 12.BES.K1 INGENASA showed 97.3% and 97.2% Se; and 94.6% and 93.0% Sp, respectively. IFAT FLI-Wusterhausen performed better than IFAT SALUVET-Madrid, with 100% Se and 95.4% Sp. Relative to the gold standard 'Majority of test plus pre-test info', Sp significantly decreased; this result was expected because of the existence of seronegative animals with clinical signs. All ELISAs performed very well and could be used in epidemiological studies; however, Western blot tests performed better and could be employed as a posteriori tests for control purposes in the case of uncertain results from valuable samples.
VECTOR-BORNE VIRUSES


- Alternative mechanisms for the introduction of bluetongue other than the importation of infected hosts and the arrival of windborne infected Culicoides were assessed. Air, sea and land transport networks continue to expand increasing the risk of vector-borne pathogen importation. A risk assessment model was constructed to assess the probability of a BTV outbreak following the introduction of Culicoides to Spain from northern European countries via these networks. For this mechanism to pose a significant risk to BTV-free countries, a large number of vectors would have to be transported.

The importation of infected hosts and the arrival of windborne infected Culicoides (Diptera: Ceratopogonidae) were considered unlikely mechanisms for bluetongue virus (BTV) incursion into a BTV-free area during the recent BTV serotype 8 (BTV-8) epidemic in northern Europe. Therefore, alternative mechanisms need to be considered. Air, sea and land transport networks continue to expand, and an important consequence of this is vector-borne pathogen importation. One important aspect of bluetongue (BT) epidemiology not yet addressed is the potential movement of infected Culicoides via transport and trade networks. Therefore, a risk assessment model was constructed to assess the probability of a BTV outbreak as a consequence of the introduction of Culicoides via these networks. The model was applied to calculate the risk for a BTV-8 epidemic in Spain in 2007 caused by the introduction of Culicoides from affected northern European countries. The mean weighted annual risk for an outbreak caused by transportation of a single vector from an affected northern European country varied from $1.8 \times 10^{-7}$ to $3.0 \times 10^{-13}$, with the highest risks associated with Culicoides imported from Belgium, the Netherlands, Germany and France. For this mechanism to pose a significant risk to BTV-free countries, a large number of vectors would have to be transported.


- A Bluetongue risk assessment based on the basic reproduction number was developed in Austria. The basic reproduction number is defined as the number of secondary cases caused by one primary case in a fully susceptible host population, in which values greater than one indicated the possibility, i.e., the risk, for a major disease outbreak. Basic reproduction numbers above one were generally found between June and August except in the mountainous regions of the Alps.

Bluetongue is an arboviral disease of ruminants causing significant economic losses. Our risk assessment is based on the epidemiological key parameter, the basic reproduction number. It is defined as the number of secondary cases caused by one primary case in a fully susceptible host population, in which values greater than one indicate the possibility, i.e., the risk, for a major disease outbreak. In the course of the Bluetongue virus serotype 8 (BTV-8) outbreak in Europe in 2006 we developed such a risk assessment for the University of Veterinary Medicine Vienna, Austria. Basic reproduction numbers were calculated using a well-known formula for vector-borne diseases considering the population densities of hosts (cattle and small ruminants) and vectors (biting midges of the Culicoides obsoletus spp.) as well as temperature dependent rates. The latter comprise the biting and mortality rate of
midges as well as the reciprocal of the extrinsic incubation period. Most important, but generally unknown, is the spatio-temporal distribution of the vector density. Therefore, we established a continuously operating daily monitoring to quantify the seasonal cycle of the vector population by a statistical model. We used cross-correlation maps and Poisson regression to describe vector densities by environmental temperature and precipitation. Our results comprise time series of observed and simulated Culicoides obsoletus spp. counts as well as basic reproduction numbers for the period 2009-2011. For a spatio-temporal risk assessment we projected our results from the location of Vienna to the entire region of Austria. We compiled both daily maps of vector densities and the basic reproduction numbers, respectively. Basic reproduction numbers above one were generally found between June and August except in the mountainous regions of the Alps. The highest values coincide with the locations of confirmed BTV cases.


- Rift Valley fever (RVF) is an important vector-borne zoonotic viral disease. Transmitted by mosquitoes or by direct contact with viraemic products. RVF affects both livestock and humans. It causes abortion storms in pregnant ruminants; high mortality in newborn livestock and deaths in older animals. The disease can cause flu-like signs in humans, and in some severe encephalitic or haemorrhagic forms and death. The disease recently spread to the Arabian Peninsula and Indian Ocean from the African continent. Animal movements, legal or illegal, contribute to viral spread, threatening the Mediterranean basin and Europe where there are competent vectors. Further research, surveillance and predictive models are needed to provide early warning methods for this virus.

Rift Valley fever (RVF), a vector-borne zoonotic disease caused by a phlebovirus (family Bunyaviridae), is considered to be one of the most important viral zoonoses in Africa. It is also a potential bioterrorism agent. Transmitted by mosquitoes or by direct contact with viraemic products, RVF affects both livestock and humans, causing abortion storms in pregnant ruminants and sudden death in newborns. The disease provokes flu syndrome in most human cases, but also severe encephalitic or haemorrhagic forms and death. There is neither a treatment nor a vaccine for humans. The disease, historically confined to the African continent, recently spread to the Arabian Peninsula and Indian Ocean. Animal movements, legal or illegal, strongly contribute to viral spread, threatening the Mediterranean basin and Europe, where competent vectors are present. Given the unpredictability of virus introduction and uncertainties about RVF epidemiology, there is an urgent need to fill the scientific gaps by developing large regional research programmes, to build predictive models, and to implement early warning systems and surveillance designs adapted to northern African and European countries.


- Recently the distribution of Rift Valley Fever widened, threatening Europe. The probability of the introduction and a large-scale spread of Rift Valley fever virus (RVFV) in Europe is thought to be low, but localized RVF outbreaks may occur in areas where populations of ruminants and potential vectors are present. A system of modeling is described using vector distribution maps and transmission risk area maps for sensitive hosts. This could allow for areas to be identified for targeted surveillance.
Rift Valley fever (RVF) is a severe mosquito-borne disease that is caused by a Phlebovirus (Bunyaviridae) and affects domestic ruminants and humans. Recently, its distribution widened, threatening Europe. The probability of the introduction and large-scale spread of Rift Valley fever virus (RVFV) in Europe is low, but localized RVF outbreaks may occur in areas where populations of ruminants and potential vectors are present. In this study, we assumed the introduction of the virus into Italy and focused on the risk of vector-borne transmission of RVFV to three main European potential hosts (cattle, sheep and goats). Five main potential mosquito vectors belonging to the Culex and Aedes genera that are present in Italy were identified in a literature review. We first modelled the geographical distribution of these five species based on expert knowledge and using land cover as a proxy of mosquito presence. The mosquito distribution maps were compared with field mosquito collections from Italy to validate the model. Next, the risk of RVFV transmission was modelled using a multicriteria evaluation (MCE) approach, integrating expert knowledge and the results of a literature review on host sensitivity and vector competence, feeding behaviour and abundance. A sensitivity analysis was performed to assess the robustness of the results with respect to expert choices. The resulting maps include (i) five maps of the vector distribution, (ii) a map of suitable areas for vector-borne transmission of RVFV and (iii) a map of the risk of RVFV vector-borne transmission to sensitive hosts given a viral introduction. Good agreement was found between the modelled presence probability and the observed presence or absence of each vector species. The resulting RVF risk map highlighted strong spatial heterogeneity and could be used to target surveillance. In conclusion, the geographical information system (GIS)-based MCE served as a valuable framework and a flexible tool for mapping the areas at risk of a pathogen that is currently absent from a region.


- Asian bush mosquito Aedes japonicus japonicus spread within Germany. The Asian bush mosquito is well adapted to moderate climates and appears to be further expanding its distribution area in Central Europe.

After its first detection in 2008 in the south German federal state of Baden-Wuerttemberg, another distinct population of the invasive Asian bush mosquito Aedes japonicus japonicus was unexpectedly found in western Germany in 2012. Range expansion had already been observed for the southern German population and was anticipated for the western German one. Here, we report on a third, apparently independent and even more northerly German colonization area of Aedes j. japonicus in southern Lower Saxony and northeastern North Rhine-Westphalia, which was discovered in spring 2013. In a snapshot study, intended to determine the presence or absence of Aedes j. japonicus in an area close to Hanover, the capital of the northern German federal state of Lower Saxony, where a specimen had been collected in late 2012, central water basins of cemeteries were checked for pre-imaginal mosquito stages at the beginning of the mosquito season 2013. Almost 20% of the inspected cemeteries were found positive (25 out of 129), with many of them being located in towns and villages close to the motorways A2 and A7. Being of Far Eastern origin, the Asian bush mosquito is well adapted to moderate climates and appears to be further expanding its distribution area in Central Europe. As it is a proven laboratory vector of several mosquito-borne disease agents, its present and future distribution areas should be carefully monitored.


- In order to support and harmonize surveillance activities in Europe, the European Centre for Disease Prevention and Control (ECDC) launched the production of ‘Guidelines for the surveillance of invasive mosquitoes in Europe’. This article describes these guidelines in the context of the key issues surrounding invasive mosquitoes surveillance in Europe.
The recent notifications of autochthonous cases of dengue and chikungunya in Europe prove that the region is vulnerable to these diseases in areas where known mosquito vectors (Aedes albopictus and Aedes aegypti) are present. Strengthening surveillance of these species as well as other invasive container-breeding aedine mosquito species such as Aedes atropalpus, Aedes japonicus, Aedes koreicus and Aedes triseriatus is therefore required. In order to support and harmonize surveillance activities in Europe, the European Centre for Disease Prevention and Control (ECDC) launched the production of 'Guidelines for the surveillance of invasive mosquitoes in Europe'. This article describes these guidelines in the context of the key issues surrounding invasive mosquitoes surveillance in Europe. Based on an open call for tender, ECDC granted a pan-European expert team to write the guidelines draft. It content is founded on published and grey literature, contractor’s expert knowledge, as well as appropriate field missions. Entomologists, public health experts and end users from 17 EU/EEA and neighbouring countries contributed to a reviewing and validation process. The final version of the guidelines was edited by ECDC (Additional file 1). The guidelines describe all procedures to be applied for the surveillance of invasive mosquito species. The first part addresses strategic issues and options to be taken by the stakeholders for the decision-making process, according to the aim and scope of surveillance, its organisation and management. As the strategy to be developed needs to be adapted to the local situation, three likely scenarios are proposed. The second part addresses all operational issues and suggests options for the activities to be implemented, i.e. key procedures for field surveillance of invasive mosquito species, methods of identification of these mosquitoes, key and optional procedures for field collection of population parameters, pathogen screening, and environmental parameters. In addition, methods for data management and analysis are recommended, as well as strategies for data dissemination and mapping. Finally, the third part provides information and support for cost estimates of the planned programmes and for the evaluation of the applied surveillance process. The 'Guidelines for the surveillance of invasive mosquitoes in Europe' aim at supporting the implementation of tailored surveillance of invasive mosquito species of public health importance. They are intended to provide support to professionals involved in mosquito surveillance or control, decision/policy makers, stakeholders in public health and non-experts in mosquito surveillance. Surveillance also aims to support control of mosquito-borne diseases, including integrated vector control, and the guidelines are therefore part of a tool set for managing mosquito-borne disease risk in Europe.


- The recent emergence of bluetongue virus in northern Europe, large scale outbreaks of West Nile fever in North America, and an outbreak of chikungunya fever in Italy, there is a very real risk of exotic pathogens being transported to Europe throughout the rapidly-changing, interconnected world. Such connectivity also increases the possibilities for movement of disease vectors, particularly mosquitoes and ticks, facilitating the transmission and spread of previously tropical and subtropical pathogens in Europe.

Continued vector importation events, in combination with climatic and environmental changes, increase the likelihood of the establishment and adaptation of vectors to new environments. In the case of exotic invasive mosquitoes, new disease vector species are frequently being recorded in Europe, and the spread of these species within Europe is evidenced by the growing list of countries known to harbor certain species.

Current invasive species of mosquito:

- **Aedes albopictus**: Of the invasive mosquito species discovered in Europe, the Asian Tiger mosquito, *Aedes (Stegomyia) albopictus*, probably presents the major threat to public health in Europe. *Aedes albopictus* originated in Southeast Asia to North, Central and South America, parts of Africa, northern Australia, and numerous countries in Europe. *A. albopictus* has been reported in 20 European countries including Albania, Belgium, Bosnia and
Herzegovina, Croatia, France (including Corsica), Germany, Greece, Italy (including Sardinia and Sicily), Malta, Monaco, Montenegro, the Netherlands, San Marino, Serbia, Slovenia, Spain, Switzerland, the Vatican City, Bulgaria and Turkey. It is an important/suspected vector of chikungunya virus (CHIKV), dengue virus, Dirofilaria (the filarial nematodes D. immitis and D. repens), Eastern equine encephalitis virus, La Crosse virus, Venezuelan equine encephalitis virus, West Nile virus, and Japanese encephalitis virus.

- **Aedes aegypti:** It is found throughout tropical and subtropical regions of the Americas, Africa, and Asia, as well as the southeastern U.S., the Indian Ocean islands, and northern Australia. This species was previously established in eastern Europe. It was reported for the first time in the Netherlands in 2010, associated with imported used tyres, but this species is unlikely to become established there. It is an important/suspected vector of chikungunya virus (CHIKV), dengue virus, Yellow fever virus and Zika virus.

- **Aedes japonicus:** It originated in eastern Asia and the Far East but has become widely established in North America and central Europe. Populations are now widely established in northern Switzerland and southern Germany and Belgium. Recently it has been found established in a wide area of southeastern Austria and neighboring Slovenia. It is thought to transmit West Nile Virus; Japanese encephalitis virus; La Crosse virus; Eastern equine encephalitis virus and St. Louis encephalitis virus.

- **Aedes (Ochlerotatus) atropalpus:** is a North American mosquito that has recently been reported in Europe. It is thought to transmit La Crosse virus, West Nile Virus and other arboviral encephalitides, but its importance as a vector of infectious diseases is still not clear. The tendency of this species to feed on a range of hosts (including humans), and studies demonstrating its competence as a vector of a number of arboviruses suggests that this species has the potential to become involved in disease transmission.

- **Aedes (Finlaya) koreicus:** originating from Korea, but has been found in Belgium and Italy. It transmits Japanese encephalitis virus.

- **Culex (Culex) vishnui:** Originating from Asia, this has been reported in Albania. It is a major vector of Japanese encephalitis virus and has been found carrying West Nile Virus.


- Lineage-2 West Nile Virus has now been found in Hungary, Austria, the Balkan states and Greece. Goshawk, birds of prey, other wild birds, horses and humans have been infected during outbreaks of WNV in these countries. The first occurrence of this strain outside of sub-Saharan Africa was in Hungary in 2004. The dramatic dispersal of the virus and outbreaks are described in this paper.

For the first time outside sub-Saharan Africa, a lineage 2 West Nile virus (WNV) emerged in Hungary in 2004. It caused sporadic cases of encephalitis in goshawks (Accipiter gentilis), other predatory birds, and in mammals. As a consequence, a surveillance program was initiated in Hungary and in Austria, which included virological, molecular, serological and epidemiological investigations in human beings, birds, horses, and mosquitoes. The virus strain became endemic to Hungary, however only sporadic cases of infections were observed between 2004 and 2007. Unexpectedly, explosive spread of the virus was noted in 2008, when neuroinvasive West Nile disease (WND) was diagnosed all over Hungary in dead goshawks and other birds of prey (n=25), in horses (n=12), and humans (n=22). At the same time this virus also spread to the eastern part of Austria, where it was detected in dead wild birds (n=8). In 2009, recurrent WND outbreaks were observed in Hungary and Austria, in wild birds, horses, and humans in the same areas. Virus isolates of both years exhibited closest genetic relationship to the lineage 2 WNV strain which emerged in 2004. As we know today, the explosive spread of the lineage 2 WNV in 2008 described here remained not restricted to
Hungary and Austria, but this virus dispersed further to the south to various Balkan states and reached northern Greece, where it caused the devastating neuroinvasive WND outbreak in humans in 2010.


- Two strains of West Nile Virus have been identified in Hungary. Lethal encephalitis due to WNV lineage 1 in geese, and WNV lineage 2 was identified in a goshawk. Overwintering of West Nile virus in Europe was suspected.

Two different West Nile virus (WNV) strains caused lethal encephalitis in a flock of geese and a goshawk in southeastern Hungary in 2003 and 2004, respectively. During the outbreak in geese, 14 confirmed human cases of WNV encephalitis and meningitis were reported in the same area. Sequencing of complete genomes of both WNV strains and phylogenetic analyses showed that the goose-derived strain exhibits closest genetic relationship to strains isolated in 1998 in Israel and to the strain that emerged in 1999 in the United States. WNV derived from the goshawk showed the highest identity to WNV strains of lineage 2 isolated in central Africa. The same strain reemerged in 2005 in the same location, which suggests that the virus may have overwintered in Europe. The emergence of an exotic WNV strain in Hungary emphasizes the role of migrating birds in introducing new viruses to Europe.

TICK-BORNE DISEASES


- Several tick-borne pathogens have been reported in new geographical regions while new species, strains or genetic variants of tick-borne microorganisms are continually being detected. Tick-borne pathogens are still poorly understood, and it is estimated that half of all human tick-borne disease has an unknown origin. Next Generation Sequencing (NGS) was used to extend the inventory of pathogenic bacteria carried by Ixodes ricinus in France. The data obtained from this study has proven that NGS has an enormous potential to detect the unexpected and provides a means of monitoring pathogen occurrence.

Ticks are highly susceptible to global environmental and socio-economical changes. Several tick-borne pathogens have been reported in new geographical regions while new species, strains or genetic variants of tick-borne microorganisms are continually being detected. However, tick-borne pathogens are still poorly understood, and it is estimated that half of all human tick-borne disease has an unknown origin. Therefore in order to prevent these diseases, more effort is required to identify unknown or unexpected tick-borne pathogens. Ixodes ricinus is the vector for a broad range of bacterial pathogens and the most prevalent tick in Europe. The aim of the present study was to evaluate the capability of Next Generation Sequencing (NGS) to extend the inventory of pathogenic bacteria carried by this species of tick in France. RNA and DNA were extracted from 1450 I. ricinus questing nymphs collected by flagging in Alsace, France. RNA was pooled and used for NGS. Following de novo assembly, bacterial contigs were assigned to the closest known taxonomy. DNA was used for real time PCR to confirm taxonomic species assignment of NGS-derived contigs for the doubtful cases, and for determination of prevalence. We have generated a global in-depth picture of tick-borne bacteria. We identified RNA from the main pathogenic bacterial species known to be transmitted by I. ricinus. In addition we also identified unanticipated bacterial species for which we have estimated the prevalence within those ticks inhabiting the studied
areas. The data obtained from this study has proven that NGS has an enormous potential to detect the unexpected and provides the means to monitor pathogen occurrence.


- Transplacental transmission of Anaplasma phagocytophilum was demonstrated in an experimentally infected sheep. Transplacental transmission should be considered as a means of A. phagocytophilum transmission and may contribute to the epidemiology of tick-borne fever in sheep and other mammals, including humans.

Anaplasma phagocytophilum, first identified as a pathogen of sheep in Europe, more recently has been recognized as an emerging tick-borne pathogen of humans in the U.S. and Europe. Transmission of A. phagocytophilum is reported to be by ticks, primarily of the genus Ixodes. While mechanical and transplacental transmission of the type genus organism, A. marginale, occur in addition to tick transmission, these modes of transmission have not been considered for A. phagocytophilum. Recently, we developed a sheep model for studying host-tick-pathogen interactions of the human NY-18 A. phagocytophilum isolate. Sheep were susceptible to infection with this human isolate and served as a source of infection for I. scapularis ticks, but they did not display clinical signs of disease, and the pathogen was not apparent in stained blood smears. In the course of these experiments, one sheep unexpectedly gave birth to a lamb 5 weeks after being experimentally infected by inoculation with the pathogen propagated in HL-60 cells. The lamb was depressed and not feeding and was subsequently euthanized 18 h after birth. Tissues were collected at necropsy for microscopic examination and PCR to confirm A. phagocytophilum infection. At necropsy, the stomach contained colostrum, the spleen was moderately enlarged and thickened with conspicuous lymphoid follicles, and mesenteric lymph nodes were mildly enlarged and contained moderate infiltrates of eosinophils and neutrophils. Blood, spleen, heart, skin and cervical and mesenteric lymph nodes tested positive for A. phagocytophilum by PCR, and sequence analysis confirmed that the lamb was infected with the NY-18 isolate. Transplacental transmission should therefore be considered as a means of A. phagocytophilum transmission and may likely contribute to the epidemiology of tick-borne fever in sheep and other mammals, including humans.


- Natural intra-uterine (congenital) infection of a calf with Anaplasma phagocytophilum.

Anaplasma phagocytophilum is a Gram-negative, obligate intracellular tick-transmitted bacterium that replicates in neutrophils. It causes tick-borne fever (TBF) in sheep and cattle, but also elicits febrile disease in humans as well as in other domestic animals such as dogs, horses, and cats. Although increasingly recognized in Europe, the first laboratory-confirmed case of TBF in cattle from Germany has been published only recently. We here present the unusual case of an intrauterine transmission of A. phagocytophilum in a calf from northern Germany. To our knowledge, this is the first report of such an event occurring under field conditions in cattle.
'Candidatus Neoehrlichia mikurensis' was confirmed for the first time in Spain. 'Candidatus Neoehrlichia mikurensis' is a relatively newly identified tick borne bacterium that has been associated with serious infections in immunocompromised humans. It has been identified as one of the most prevalent pathogenic agents in I. ricinus ticks throughout Europe. Little is known about the range of reservoir hosts; however studies have so far identified a range of potential rodent hosts.

'Candidatus Neoehrlichia mikurensis' is a tick-borne bacteria implicated in human health. To date, 'Ca. Neoehrlichia mikurensis' has been described in different countries from Africa, Asia and Europe, but never in Spain. However, according to the epidemiological features of the main vector in Europe, Ixodes ricinus, its circulation in our country was suspected. A total of 200 I. ricinus ticks collected in the North of Spain were analyzed. DNAs were extracted and used as templates for PCRs targeting fragment genes for Anaplasma/Ehrlichia detection. The amplified products were sequenced and analyzed. 'Ca. Neoehrlichia mikurensis' was amplified in two specimens. Furthermore, Anaplasma phagocytophilum was detected in 61 samples analyzed. The detection of 'Ca. Neoehrlichia mikurensis' in I. ricinus ticks from Spain indicates its circulation and the potential risk of contracting a human infection in this country.


Neoehrlichiosis caused by Candidatus Neoehrlichia mikurensis is an emerging zoonotic disease. In total, six patients have been described in Europe, with the first case detected in 2007. In addition, seven patients from China were described in a report published in October 2012. In 2009, the first human case of Ca. Neoehrlichia mikurensis infection was diagnosed in the Zurich area of Switzerland. This paper reports two additional human cases from the same region, which were identified by broad-range 16S rRNA gene PCR. Both patients were immunocompromised and presented with similar clinical syndromes, including fever, malaise, and weight loss. A diagnostic multiplex real-time PCR was developed for specific detection of Ca. Neoehrlichia mikurensis infections. The assay is based on the signature sequence of a 280-bp fragment of the Ca. Neoehrlichia mikurensis 16S rRNA gene and incorporates a Ca. Neoehrlichia mikurensis species, a Ca. Neoehrlichia genus, and an Anaplasmataceae family probe for simultaneous screening. The analytical sensitivity was determined to be below five copies of the Ca. Neoehrlichia mikurensis 16S rRNA gene. The assay is suitable for the direct detection of Ca. Neoehrlichia mikurensis DNA in clinical samples from tissue such as blood and bone marrow. In addition, it can be used to monitor treatment response during antibiotic therapy. Using the same assay, DNA extracts from 1,916 ticks collected in four forests in close proximity to the patients' residences (<3 km) were screened. At all sampling sites, the minimal prevalence of Ca. Neoehrlichia mikurensis was between 3.5 to 8% in pools of nymphs, males, or females, showing a strong geographic association between the three patients and the assumed vector.


- The risks of new tick species and tick borne diseases being introduced by wild bird migration is discussed. Turdus sp. birds, particularly blackbirds, appear to be important for harboring ticks. Birds can potentially transport ticks long distances, including ticks infected with tick-borne pathogens, or could act as hosts allowing transfer of pathogens between ticks. Knowledge of the bird migration routes and of the spatial distribution of tick species and tick-borne pathogens is crucial for understanding the possible impact of birds as spreaders of ticks and tick-borne pathogens.
Birds, particularly passerines, can be parasitized by Ixodid ticks, which may be infected with tick-borne pathogens, like Borrelia spp., Babesia spp., Anaplasma, Rickettsia/Coxiella, and tick-borne encephalitis virus. The prevalence of ticks on birds varies over years, season, locality and different bird species. The prevalence of ticks on different species depends mainly on the degree of feeding on the ground. In Europe, the Turdus spp., especially the blackbird, Turdus merula, appears to be most important for harboring ticks. Birds can easily cross barriers, like fences, mountains, glaciers, desserts and oceans, which would stop mammals, and they can move much faster than the wingless hosts. Birds can potentially transport tick-borne pathogens by transporting infected ticks, by being infected with tick-borne pathogens and transmit the pathogens to the ticks, and possibly act as hosts for transfer of pathogens between ticks through co-feeding. Knowledge of the bird migration routes and of the spatial distribution of tick species and tick-borne pathogens is crucial for understanding the possible impact of birds as spreaders of ticks and tick-borne pathogens. Successful colonization of new tick species or introduction of new tick-borne pathogens will depend on suitable climate, vegetation and hosts. Although it has never been demonstrated that a new tick species, or a new tick pathogen, actually has been established in a new locality after being seeded there by birds, evidence strongly suggests that this could occur.


- The prevalence of Anaplasma spp. and Babesia spp. pathogens in Ixodes ticks, collected from dogs in the UK in 2009, was evaluated using PCR. 0.74% contained rDNA consistent with Anaplasma phagocytophilum (from Ixodes ricinus and Ixodes hexagonus). 2.4% were positive for Babesia, and of these 11 were consistent with B. gibsoni, all from I. ricinus. One sample from I. ricinus, showed 99% homology for B. divergens. Four of the Babesia spp sequences were of the "venatorum" or EU1 type, three from I. ricinus and one from an Ixodes canisuga. Babesia venatorum (EU1) is related to but has distinct differences from B. divergens, and is found naturally in deer. It has been associated with cases of human babesiosis in Austria, Italy and Germany. A further 246 positive results were shown by sequence analysis to be derived from the bacterium Candidatus "Midichloria mitochondrii", which to date has been assumed to be non-pathogenic.

Ticks are important vectors of disease in companion animals and transmit an extensive range of viral, bacterial and protozoan pathogens to dogs and cats. They may also be vectors of zoonotic pathogens which affect the health of in-contact owners. In recent years, babesiosis, and anaplasmosis have all shown signs of increased prevalence and distribution in various parts of Europe. Here, the prevalence of Anaplasma spp. and Babesia spp. pathogens in Ixodes ticks, collected from dogs in the UK in 2009, were evaluated using PCR and sequence analysis of the 16S rDNA or 18S rDNA regions respectively. Species identification was performed by alignment with existing sequences in GenBank. After sequencing, 5 out of 677 tick samples (0.74%) contained rDNA which shared 97-100% sequence homology with Anaplasma phagocytophilum. Of these, three samples came from Ixodes ricinus and two from Ixodes hexagonus. Sixteen out of 742 ticks (2.4%) were positive for Babesia and of these 11 showed 97-100% homology with B. gibsoni. All of these 11 samples were derived from I. ricinus. One sample, again from I. ricinus, showed 99% homology for B. divergens. Four of the Babesia spp sequences were of the "venatorum" or EU1 type, three of which came from I. ricinus and one from an Ixodes canisuga. This strain has been associated with severe human cases of babesiosis. A further 246 positive results, which appeared to show the presence of Anaplasma following PCR, were shown by sequence analysis to be derived from the bacterium Candidatus "Midichloria mitochondrii", which to date has been assumed to be non-pathogenic. The results are of interest because the presence of B. gibsoni in the UK further confirms the worldwide distribution of this piroplasm and supports the inference that I. ricinus may act as a vector for Babesia of the gibsoni-complex.
Tick borne diseases in The Netherlands. Collated data of this and previous studies were used to generate, for each pathogen, a presence/absence map and to analyse their spatiotemporal variation. R. helvetica (31.1%) and B. burgdorferi sensu lato (11.8%) had the highest overall prevalence and were detected in all areas. N. mikurensis (5.6%), A. phagocytophilum (0.8%), and Babesia spp. (1.7%) were detected in most, but not all areas.

Ixodes ricinus transmits Borrelia burgdorferi sensu lato, the etiological agent of Lyme disease. Previous studies have also detected Rickettsia helvetica, Anaplasma phagocytophilum, Neoehrlichia mikurensis, and several Babesia species in questing ticks in The Netherlands. In this study, we assessed the acarological risk of exposure to several tick-borne pathogens (TBPs), in The Netherlands. Questing ticks were collected monthly between 2006 and 2010 at 21 sites and between 2000 and 2009 at one other site. Nymphs and adults were analysed individually for the presence of TBPs using an array-approach. Collated data of this and previous studies were used to generate, for each pathogen, a presence/absence map and to further analyse their spatiotemporal variation. R. helvetica (31.1%) and B. burgdorferi sensu lato (11.8%) had the highest overall prevalence and were detected in all areas. N. mikurensis (5.6%), A. phagocytophilum (0.8%), and Babesia spp. (1.7%) were detected in most, but not all areas. The prevalences of pathogens varied among the study areas from 0 to 64%, while the density of questing ticks varied from 1 to 179/100 m². Overall, 37% of the ticks were infected with at least one pathogen and 6.3% with more than one pathogen. One-third of the Borrelia-positive ticks were infected with at least one other pathogen. Coinfection of B. afzelii with N. mikurensis and with Babesia spp. occurred significantly more often than single infections, indicating the existence of mutual reservoir hosts. Alternatively, coinfection of R. helvetica with either B. afzelii or N. mikurensis occurred significantly less frequent. The diversity of TBPs detected in I. ricinus in this study and the frequency of their coinfections with B. burgdorferi s.l., underline the need to consider them when evaluating the risks of infection and subsequently the risk of disease following a tick bite.

The spotted fever rickettsias (Rickettsia helvetica and Rickettsia raoultii amongst others) can result in disease in humans. Clinical signs include fever, myalgia, lymphadenopathy and skin rashes. It is currently unknown if clinical signs may occur in animals. Rickettsia helvetica DNA has been identified in another study in blood from mice, deer and wild boar.
The occurrence of SFG rickettsiae in *D. reticulatus* in the UK appears to be confined only to Welsh and Essex populations, with no evidence so far from Devon. Similarly, the occurrence of SFG rickettsiae in *H. punctata* appears confined to one of two farms known to be infested with this tick in North Kent, with no evidence so far from the Sussex populations. *A. phagocytophilum*, *N. mikurensis*, *C. burnetii* and *B. burgdorferi sensu latu* DNA was not detected in any of the ticks. These two tick species are highly restricted in their distribution in England and Wales, but where they do occur they can be abundant. Following detection of these SFG rickettsiae in additional UK tick species, as well as *I. ricinus*, research should now be directed towards clarifying firstly the geographic distribution of SFG rickettsiae in UK ticks, and secondly to assess the prevalence rates in ticks, wild and domesticated animals and humans to identify the drivers for disease transmission and their public health significance.


- *Ixodes ricinus* (76.4%) and *I. hexagonus* (22.6%) were the predominant species. *Rhipicephalus sanguineus* (0.3%) and *D. reticulatus* (0.8%) were found in low numbers only. All dogs infested with *R. sanguineus* had a recent travel history, but *D. reticulatus* were collected from a dog without a history of travelling abroad. Of the collected *Ixodes* ticks, 19.5% were positive for *A. phagocytophilum* and 10.1% for *Borrelia* spp. (*B. afzelii*, *B. garinii*, *B. burgdorferi s.s.*, *B. lusitaniae*, *B. valaisiana* and *B. spielmani*). *Rickettsia helvetica* was found in 14.1% of *Ixodes* ticks. One *R. sanguineus* tick was positive for *Rickettsia massiliae* (also a member of the spotted fever group). *D. reticulatus* was confirmed to be present as an indigenous parasite in Belgium. *B. lusitaniae* and *R. helvetica* were detected in ticks in Belgium for the first time.

Although *Ixodes* spp. are the most common ticks in North-Western Europe, recent reports indicated an expanding geographical distribution of Dermacentor reticulatus in Western Europe. Recently, the establishment of a *D. reticulatus* population in Belgium was described. *D. reticulatus* is an important vector of canine and equine babesiosis and can transmit several *Rickettsia* species. *Coxiella burnetii* and tick-borne encephalitis virus (TBEV), whilst *Ixodes* spp. are vectors of pathogens causing babesiosis, borreliosis, anaplasmosis, rickettsiosis and TBEV.

A survey was conducted in 2008-2009 to investigate the presence of different tick species and associated pathogens on dogs and cats in Belgium. Ticks were collected from dogs and cats in 75 veterinary practices, selected by stratified randomization. All collected ticks were morphologically determined and analysed for the presence of *Babesia* spp., *Borrelia* spp., *Anaplasma phagocytophilum* and *Rickettsia* DNA.

In total 2373 ticks were collected from 647 dogs and 506 cats. *Ixodes ricinus* (76.4%) and *I. hexagonus* (22.6%) were the predominant species. *Rhipicephalus sanguineus* (0.3%) and *D. reticulatus* (0.8%) were found in low numbers on dogs only. All dogs infested with *R. sanguineus* had a recent travel history, but *D. reticulatus* were collected from a dog without a history of travelling abroad. Of the collected *Ixodes* ticks, 19.5% were positive for *A. phagocytophilum* and 10.1% for *Borrelia* spp. (*B. afzelii*, *B. garinii*, *B. burgdorferi s.s.*, *B. lusitaniae*, *B. valaisiana* and *B. spielmani*). *Rickettsia helvetica* was found in 14.1% of *Ixodes* ticks. *Dermacentor* ticks were negative for all the investigated pathogens, but one *R. sanguineus* tick was positive for *Rickettsia massiliae*. *D. reticulatus* was confirmed to be present as an indigenous parasite in Belgium. *B. lusitaniae* and *R. helvetica* were detected in ticks in Belgium for the first time.

**Overzier E, Pfister K, Herb I, Mahling M, Böck G Jr, Silaghi C**. Detection of tick-borne pathogens in roe deer (*Capreolus capreolus*), in questing ticks (*Ixodes ricinus*), and in

- Babesia spp.: Sequencing revealed *B. venatorum*, *B. capreoli*, and *B. microti*.;
- *A. phagocytophilum*; *Rickettsia* spp.: Sequencing revealed *R. helvetica*. The high prevalence rates of *B. capreoli* and *A. phagocytophilum* in roe deer support their role as reservoir hosts for these pathogens, but no evidence for a role of roe deer in the life cycle of *R. helvetica* could be provided. One-third of the *Borrelia*-positive ticks were infected with at least one other pathogen. Coinfection of *B. afzelii* with *N. mikurensis* and with Babesia spp. occurred significantly more often than single infections, indicating the existence of mutual reservoir hosts. Alternatively, coinfection of *R. helvetica* with either *B. afzelii* or *N. mikurensis* occurred significantly less frequent. The diversity of TBPs detected in *I. ricinus* in this study and the frequency of their coinfections with *B. burgdorferi* s.l., underline the need to consider them when evaluating the risks of infection and subsequently the risk of disease following a tick bite.

The hard tick *Ixodes ricinus* is the most common tick in Central Europe and plays an important role as a vector of several pathogens. In the complex life cycles of these pathogens, the role of wild animals as natural reservoirs has been discussed. The aims of this study were to investigate the role of roe deer (*Capreolus capreolus*) as a potential reservoir host for Babesia spp., *Anaplasma phagocytophilum*, and *Rickettsia* spp. Therefore, we explored the differences in the infection rates of roe deer and engorged and questing ticks with these pathogens from a single forest site with special attention to coinfection. Blood, spleen, and skin samples of a total of 95 roe deer individuals were screened by molecular methods for these pathogens from September 2010 to January 2012 in the 'Angelberger Forst' (Bavaria, Germany). Moreover, 331 engorged ticks from 44 roe deer individuals and 199 host-seeking ticks from the same area were screened. Altogether, the following prevalence rates and a high diversity of species were detected for the respective pathogens in individual animals and ticks: (i) Babesia spp.: roe deer, 89.5%; engorged ticks, 7.3%; questing ticks: adults, 2.5%, nymphs, 3.3%. Sequencing revealed *B. venatorum*, *B. capreoli*, and *B. microti*. (ii) *A. phagocytophilum*: roe deer 98.9%; engorged ticks, 86.1%; questing ticks: adults, 8.9%, nymphs, 0.8%. (iii) *Rickettsia* spp.: roe deer, 0%; engorged ticks, 16.6%; questing ticks: adults, 13.9%, nymphs, 17.5%. Sequencing revealed *R. helvetica*. Furthermore, several coinfections were detected in both roe deer and ticks. The high prevalence rates of *B. capreoli* and *A. phagocytophilum* in roe deer support their role as reservoir hosts for these pathogens, but no evidence for a role of roe deer in the life cycle of *R. helvetica* could be provided.

Ticks varied from 1 to 179/100 m². Overall, 37% of the ticks were infected with at least one pathogen and 6.3% with more than one pathogen. One-third of the *Borrelia*-positive ticks were infected with at least one other pathogen. Coinfection of *B. afzelii* with *N. mikurensis* and with Babesia spp. occurred significantly more often than single infections, indicating the existence of mutual reservoir hosts. Alternatively, coinfection of *R. helvetica* with either *B. afzelii* or *N. mikurensis* occurred significantly less frequent. The diversity of TBPs detected in *I. ricinus* in this study and the frequency of their coinfections with *B. burgdorferi* s.l., underline the need to consider them when evaluating the risks of infection and subsequently the risk of disease following a tick bite.

**Ionita M, Mitrea IL, Pfister K, Hamei D, Silaghi C. Molecular evidence for bacterial and protozoan pathogens in hard ticks from Romania. *Vet Parasitol.* 2013 Sep 1;196(1-2):71-6.**

- Tick species analyzed included *Ixodes ricinus*, *Dermacentor marginatus*, *Hyalomma marginatum*, *Rhipicephalus bursa*, and *Rhipicephalus sanguineus*. Four rickettsiae of the spotted fever group of zoonotic concern were identified for the first time in Romania: *Rickettsia monacensis* and *Rickettsia helvetica* in *I. ricinus*, and *Rickettsia slovaca* and *Rickettsia raoultii* in *D. marginatus*. Other zoonotic pathogens such as *A. phagocytophilum*, *Borrelia afzelii*, and Babesia microti were found in *I. ricinus*. Pathogens of veterinary importance were also identified, including *Theileria equi* in *H. marginatum*, Babesia occultans in *D. marginatus*.**
marginatus and H. marginatum, Theileria orientalis/sergenti/buffeli-group in I. ricinus and in H. marginatum and E. canis in R. sanguineus. These findings show a wide distribution of very diverse bacterial and protozoan pathogens at the domestic host-tick interface in Romania, with the potential of causing both animal and human diseases.

- Babesia occultans was previously considered as a low pathogenicity, but now has been reported in cases of bovine babesiosis. Theileria orientalis/sergenti/buffeli-group is an important cause of anaemia in cattle and can produce latent infection in sheep.

The aim of the present study was to provide a preliminary insight into the diversity of tick-borne pathogens circulating at the domestic host-tick interface in Romania. For this, feeding and questing ticks were analyzed by real-time polymerase chain reaction (PCR) for the presence of Anaplasma phagocytophilum, Anaplasma platys, Ehrlichia canis, Borrelia burgdorferi sensu latu, and by PCR and subsequent sequencing for Rickettsia spp., Babesia spp. and Theileria spp. A total of 382 ticks, encompassing 5 species from 4 genera, were collected in April-July 2010 from different areas of Romania; of them, 40 were questing ticks and the remainder was collected from naturally infested cattle, sheep, goats, horses or dogs. Tick species analyzed included Ixodes ricinus, Dermacentor marginatus, Hyalomma marginatum, Rhipicephalus bursa, and Rhipicephalus sanguineus. Four rickettsiae of the spotted fever group of zoonotic concern were identified for the first time in Romania: Rickettsia monacensis and Rickettsia helvetica in I. ricinus, and Rickettsia slovaca and Rickettsia raoultii in D. marginatus. Other zoonotic pathogens such as A. phagocytophilum, Borrelia afzelii, and Babesia microti were found in I. ricinus. Pathogens of veterinary importance were also identified, including Theileria equi in H. marginatum, Babesia occultans in D. marginatus and H. marginatum, Theileria orientalis/sergenti/buffeli-group in I. ricinus and in H. marginatum and E. canis in R. sanguineus. These findings show a wide distribution of very diverse bacterial and protozoan pathogens at the domestic host-tick interface in Romania, with the potential of causing both animal and human diseases.


- The prevalence for A. phagocytophilum (3%) was similar to that found in I. ricinus in Europe. Other pathogens present in D. marginatus included A. marginale (0.5%), Bartonella spp. (9%), C. burnetii (12%), F. philomiragia (1.3%), and Theileria annulata/Babesia bovis (0.3%), which were detected for the first time in France.

- T. annulata is highly pathogenic to cattle and can cause significant mortality among susceptible animals. Anaplasma marginale can cause clinical anaplasmosis in cattle, and Babesia bovis is considered a tropical Babesia which can cause significant clinical disease.

The importance of Dermacentor spp. in the transmission of tick-borne pathogens is not well recognized in Europe. To investigate the role of Dermacentor spp. in the transmission of tick-borne pathogens, questing ticks were collected in 9 sites from southern to northwestern France (Camargue Delta to Eastern Brittany) where Dermacentor spp. exist and tick-borne diseases had occurred previously. Three tick species were collected during the spring and autumn of 2009. Collected ticks (both males and females) included D. marginatus (n=377), D. reticulatus (n=74), and I. ricinus (n=45). All ticks were analyzed by PCR or reverse line blot for the presence of pathogens’ DNA. Pathogens analyzed were based on veterinarian reports and included Anaplasma phagocytophilum, Coxiella burnetii, Anaplasma marginale, Borrelia burgdorferi, Bartonella spp., Babesia spp., Theileria spp., and Francisella sp. Francisella tularensis was not detected in any of the analyzed ticks. In D. marginatus, infection
prevalence for A. phagocytophilum (3%) was similar to that found in I. ricinus in Europe. Other pathogens present in D. marginatus included A. marginale (0.5%), Bartonella spp. (9%), C. burnetii (12%), F. philomiragia (1.3%), and Theileria annulata/Babesia bovis (0.3%), which were detected for the first time in France. Pathogens detected in D. reticulatus included A. marginale (1%), Bartonella spp. (12%), C. burnetii (16%), Borrelia spp. (1.5%), and F. philomiragia (19%). Pathogens detected in I. ricinus included A. phagocytophilum (41%), Bartonella spp. (9%), C. burnetii (18%), A. marginale (1%), Borrelia spp. (4.5%), and Babesia sp. (7%). This study represents the first epidemiological approach to characterize tick-borne pathogens infecting Dermacentor spp. in France and that may be transmitted by ticks from this genus. Further experiments using experimental infections and transmission may be now conducted to analyze vector competency of Dermacentor spp. for these pathogens and to validate such hypothesis.


- Theileria sp. ZS TO4 in red deer

Theileria spp. are intracellular protozoa transmitted by ixodid ticks. T. parva and T. annulata are highly pathogenic and responsible for serious disease in domestic ruminants in tropical and subtropical countries. However, asymptomatic findings of Theileria sp. in wild ungulates lead to the suggestion that wild ruminants play a role as reservoirs for these piroplasms. In a game enclosure in Eastern Austria (Federal county of Burgenland), piroplasms were detected with molecular analysis in blood samples of all 80 examined asymptomatic red deer (Cervus elaphus). Furthermore, piroplasms were detected in four out of 12 questing nymphs of Haemaphysalis concinna. In 32 Ixodes ticks sampled on-site, no Theileria DNA was detected. Sequence analysis identified these samples from both red deer and ticks as Theileria sp. ZS TO4. Our findings indicate that farmed red deer serve as asymptomatic carriers and adapted intermediate hosts of Theileria sp. in Central Europe and H. concinna was identified as a possible vector species of Theileria sp. ZS TO4.


- Theileria annulata was the most frequently found species with a prevalence of 21.3%. The second most prevalent species was T. buffeli that infected 10.1% of the bovines. A low prevalence was found for Babesia infections (7.9%) with B. bigemina as the most frequent species. Some animals were infected with T. ovis and B. occultans. Veterinary practitioners and stakeholders should be made aware of the existence of a relatively high prevalence of carrier animals infected with T. annulata in Portugal, given the potential threat this pathogenic parasite represents to the cattle industry.

Piroplasmosis caused by tick-borne hemoproteozoa of several Theileria and Babesia species has a major impact on livestock production worldwide. A reverse line blotting assay that includes genus- and species-specific probes for Theileria and Babesia species was used to assess the occurrence of these parasites in blood samples collected from 1407 healthy bovines throughout mainland Portugal. The global prevalence of piroplasm-infected animals was 36.8%, although significant differences were found between various regions. Higher
prevalence was found in the southern regions (42.4% in Lisbon and Tagus Valley, 51.6% in Alentejo, and 40.0% in Algarve) compared to central (23.1%) and northern (12.8%) Portugal. Theileria annulata was the most frequently found species with a prevalence of 21.3%. The prevalence values of this pathogenic species were higher in the southern regions. The second most prevalent species was T. buffeli that infected 10.1% of the bovines. A low prevalence was found for Babesia infections (7.9%) with B. bigemina as the most frequent species. Some animals were infected with T. ovis and B. occultans. Veterinary practitioners and stakeholders should be made aware of the existence of a relatively high prevalence of carrier animals infected with T. annulata in Portugal, given the potential threat this pathogenic parasite represents to the cattle industry.


- A clinical outbreak of bovine piroplasmosis due to Babesia occultans, a parasite previously regarded as apathogenic and never detected before in continental Europe.

A clinical outbreak of bovine piroplasmosis was reported in Italy. The etiological agent was characterized as Babesia occultans, a parasite regarded as apathogenic and never detected before in continental Europe. This report paves the way for further studies to assess the occurrence of this tick-transmitted protozoan in other European regions.


- Lyme borreliosis is rapidly emerging in the United Kingdom, with over 1000 cases per annum now reported. Lyme borreliosis is caused by the Borrelia burgdorferi sensu lato (s.l.) group of spirochetes, which are transmitted by ixodid ticks. At 8 sites, large numbers (>160) ticks were collected, and at 3 of these sites B. burgdorferi infection prevalence was significantly higher (>7.5%) than the other 5 (<1.0%). Identification of infecting Borrelia species indicated that Borrelia valaisiana was the most common, B. garinii and B. afzelii were also found.

Lyme borreliosis is rapidly emerging in the United Kingdom, with over 1000 cases per annum now reported. Lyme borreliosis is caused by the Borrelia burgdorferi sensu lato (s.l.) group of spirochetes, which are transmitted by ixodid ticks. In the United Kingdom, Ixodes ricinus is recognized as the principal vector of the spirochetes, and this tick species is widely distributed across the country. However, as yet, it is unclear whether the distribution of B. burgdorferi essentially mirrors that of its vector, or if there are marked differences between the 2. The aim of this survey was to investigate the prevalence of B. burgdorferi in I. ricinus populations across northern England, north Wales, and the Scottish Border region. We surveyed for questing I. ricinus nymphs and adults at 17 sites, encountering ticks at 12. At 8 sites, large numbers (>160) ticks were collected, and at 3 of these sites B. burgdorferi infection prevalence was significantly higher (>7.5%) than the other 5 (<1.0%). Habitat type was associated with B. burgdorferi prevalence, with ticks from deciduous and mixed woodland being significantly more likely to be infected than those from other habitat types. Identification of infecting Borrelia species indicated that Borrelia valaisiana was the most common and widespread species encountered. B. garinii was common at sites where infection prevalence was high, and B. afzelii was also occasionally encountered at these sites. The survey revealed a surprisingly uneven distribution of B. burgdorferi s.l. across the region, thereby indicating that the presence of ticks does not necessarily mean the presence of the pathogen. Indeed, the spirochete appears to be absent or very rare at some sites where ticks are abundant.
The zoonotic disease tularemia is caused by the bacterial pathogen *Francisella tularensis*. Although the causative agent is known for 100 years, knowledge of its enzootic cycles is still rudimentary. Apart from tabanids and mosquitoes, hard ticks have been described as important vectors and potential reservoirs for *F. tularensis*. Available data on the incidence of human tularemia indicate an increase in cases in the federal state of Baden-Wuerttemberg in Germany.

HUMAN DISEASE

Transfusion-transmitted anaplasmosis from leukoreduced red blood cells


Human granulocytic anaplasmosis (HGA) caused by *Anaplasma phagocytophilum* is a tick-borne rickettsial infectious disease. To date four cases of transfusion-transmitted anaplasmosis (TTA) have been described in the literature, and only one from leukoreduced red blood cells (RBCs). A 64-year-old patient with acute gastrointestinal blood loss was admitted to hospital in USA and received 5 units of prestorage leukoreduced RBCs. He was stabilized and discharged. He developed headache, fever, and chills 2 days after discharge and was readmitted. On Day 5 of his second admission polymorphonuclear leukocytes containing morulae consistent with HGA were reported in the peripheral blood smear. Samples from the recipient tested positive by PCR for *A. phagocytophilum* and a sample from one of the five donors tested positive by both serology and PCR for *A. phagocytophilum*. The authors conclude that leukoreduction reduces the risk of TTA but does not interdict all infections and that TTA requires consideration in recipients of RBC transfusion with unexplained fever.

Humans parasitized by the hard tick *Ixodes ricinus* are seropositive to *Midichloria mitochondrii*: is *Midichloria* a novel pathogen, or just a marker of tick bite?


*Midichloria mitochondrii* is an intracellular bacterium found in the hard tick *Ixodes ricinus*. In this arthropod, *M. mitochondrii* is observed in the oocytes and in other cells of the ovary, where this symbiont is present in the cell cytoplasm and inside the mitochondria. No studies have yet investigated whether *M. mitochondrii* is present in the salivary glands of the tick and is observed in the oocytes and in other cells of the ovary, where this symbiont is present in the cell cytoplasm and inside the mitochondria. No studies have yet investigated whether *M. mitochondrii* is present in the salivary glands of the tick and
whether it is transmitted to vertebrates during the tick blood meal. To address the above issues, the authors developed a recombinant antigen of *M. mitochondrii* (to screen human sera) and antibodies against this antigen (for immunostaining the symbiont). The authors used these reagents to demonstrate the presence of *M. mitochondrii* in the salivary glands of *I. ricinus* and determined that 58% of humans reporting *I. ricinus* tick bite were seropositive for *M. mitochondrii* while only 1.2% of healthy individuals who had not reported tick bite were seropositive for *M. mitochondrii*. These results provide evidence that *M. mitochondrii* is released with the tick saliva and raises the possibility that *M. mitochondrii* is infectious to vertebrates. This study also indicates that *M. mitochondrii* should be regarded as a package of antigens inoculated into the human host during tick bite and implies that the immunological response toward saliva of *I. ricinus* should be reconsidered on the basis of potential effects of *M. mitochondrii*.

Oteo, JA; Portillo, A. (2012) TICKS AND TICK-BORNE DISEASES, 3 (5-6):270-277
Rickettsioses are caused by obligate intracellular bacteria within the genus *Rickettsia*, mainly transmitted by arthropods. Until recently, Mediterranean spotted fever (MSF) caused by *Rickettsia conorii* was considered the only tick-borne rickettsiosis in Europe. However, 'new' TBR have been reported to cause MSF like symptoms in Europe including the sub-species *R. conorii* caspia and *R. conorii* israelensis. Dermacentor-borne necrosis erythema and lymphadenopathy/tick-borne lymphadenopathy (DEBONEL/TIBOLA) cases caused by *R. slovaca*, *R. raoultii*, and *R. riou* been described in several countries where Dermacentor marginatus ticks are present. *Rickettsia helvetica* has also been described as a human pathogen in cases of fever with and without rash and in patients with meningitis and carditis. Other TBR such as lymphangitis-associated rickettsioses (LAR), caused by *R. sibirica mongolitimonae*, have been diagnosed in France, Spain, Portugal and Greece. *Rickettsia massiliae* is also considered an etiological agent of MSF-like illness in the Mediterranean basin and *R. monacensis* has been isolated from patients with MSF-like illness in Spain. Although *R. aeschlimannii* has been associated with MSF-like disease in Africa and is distributed in the Mediterranean area, no autochthonous human cases have been reported in Europe. Climate change, among other factors, may contribute to the emergence of other rickettsioses or changes in their distribution.

Seroreactivity for spotted fever rickettsiae and co-infections with other tick-borne agents among habitants in central and southern Sweden
Patients seeking medical care with erythema migrans or flu-like symptoms after suspected or observed tick bite in the southeast of Sweden and previously investigated for *Borrelia spp.* and/or *Anaplasma sp.* were retrospectively examined for serological evidence of rickettsial infection. Twenty of 206 patients had IgG and/or IgM antibodies to *Rickettsia spp.* equal to or higher than the cut-off titre of 1:64. Seven of these 20 patients showed seroconversion indicative of recent or current infection and 13 patients had titres compatible with past infection, of which five patients were judged as probable infection. Of 19 patients with medical records, 11 were positive for *Borrelia spp.* as well, and for *Anaplasma sp.*, one was judged as positive. Five of the 19 patients had antibodies against all three pathogens. Erythema migrans or rash was observed at all combinations of seroreactivity, with symptoms including fever, muscle pain, headache and respiratory problems. Of a further 159 patients primarily sampled for the analysis of *Borrelia spp.* or *Mycoplasma pneumoniae*, sixteen were seroreactive for *Rickettsia spp.*, of which five were judged as recent or current infection. Symptoms of arthritis, fever, cough and rash were predominant. In 80 blood donors without clinical symptoms, approximately 1% were seroreactive for *Rickettsia spp.*, interpreted as past infection. The study shows that both single and co-infections occur illustrating the complexity in the clinical picture and a need for further studies to fully understand how these patients should best be treated.

Lyme disease is transmitted by the bite of certain Ixodes sp ticks, which can also transmit *Anaplasma phagocytophilum*, the cause of human granulocytic anaplasmosis (HGA).
Although culture can be used to identify patients infected with \textit{A. phagocytophilum} and is the microbiologic gold standard, few studies have evaluated culture-confirmed patients with HGA. We conducted a prospective study in which blood culture was used to detect HGA infection in patients with a compatible clinical illness. Early Lyme disease was defined by the presence of erythema migrans. The epidemiologic, clinical, and laboratory features of 44 patients with culture-confirmed HGA were compared with those of a convenience sample of 62 patients with early Lyme disease. Co-infected patients were excluded. Patients with HGA had more symptoms ($P = 0.003$) and had a higher body temperature on presentation ($P < 0.001$) than patients with early Lyme disease. HGA patients were also more likely to have a headache, dizziness, myalgias, abdominal pain, anorexia, leukopenia, lymphopenia, thrombocytopenia, or elevated liver enzymes. A direct correlation between the number of symptoms and the duration of illness at time of presentation ($\rho = 0.389$, $P = 0.009$) was observed for HGA patients but not for patients with Lyme disease. In conclusion, although there are overlapping features, culture-confirmed HGA is a more severe illness than early Lyme disease.

**Human Babesiosis in Europe: what clinicians need to know.**

Hildebrandt, A; Gray, JS; Hunfeld, KP (2013) \textit{INFECTION, 41} (6):1057-1072

Although best known as an animal disease, human babesiosis is attracting increasing attention as a worldwide emerging zoonosis. Humans are commonly infected by the bite of ixodid ticks but transmission may occur via transplacental, perinatal and blood transfusion routes. Infection of the human host can cause a very severe host-mediated pathology including fever, and haemolysis leading to anaemia, hyperbilirubinuria, haemoglobinuria and possible organ failure. In recent years, apparently owing to increased medical awareness and better diagnostic methods, the number of reported cases in humans is rising steadily worldwide. Hitherto unknown zoonotic \textit{Babesia spp.} are now being reported from geographic areas where babesiosis was not previously known to occur. The increase in global travel and numbers of immunocompromised individuals suggest that the frequency of cases in Europe will also continue to rise. This review is intended to provide clinicians with practical information on the clinical management of this rare, but potentially life-threatening zoonotic disease. It covers epidemiology, phylogeny, diagnostics and treatment of human babesiosis and the potential risk of transfusion-transmitted disease with a special focus on the Europeansituation.

**Effects of tick saliva on the migratory and invasive activity of Saos-2 osteosarcoma and MDA-MB-231 breast cancer cells**

N.M.Poole et al (2013) \textit{TICKS AND TICK-BORNE DISEASES, 4} (1-2):120-127

In previous studies these authors showed that tick saliva modulates the migratory activity of cells involved in the wound healing response. Since cell migration is a prerequisite for tumor invasion and metastasis, they examined the effects of tick saliva on the migratory and invasive activity of Saos-2 osteosarcoma and MDA-MB-231 (MB-231) breast cancer cells and the potential signalling pathways that may be affected. Saliva inhibited basal and agonist-induced Saos-2 and MB-231 migration and invasion through a matrigel-coated filter. In the Saos-2 cells, saliva suppressed epidermal growth factor (EGF)-activation of Akt/Protein Kinase B, however, only basal extracellular signal-regulated kinase (ERK) activity was affected in MB-231 cells. EGF receptor (EGFR) over expression masked the effect of saliva on MB-231 cells, but its ability to inhibit MB-231 migration was enhanced by the EGFR inhibitor PD 168393 and MEK inhibitor U0126. These data indicate that the mechanisms ticks have evolved to regulate the wound healing response have generalized effects on the migratory and invasive activities of metastatic cancer cells.

**DISEASE IN DOGS, CATS AND HORSES**

**Isolation of canine \textit{Anaplasma phagocytophilum} strains from clinical blood samples using the \textit{Ixodes ricinus} cell line IRE/CTVM20**

V. Dyachenko et al (2013) \textit{VETERINARY MICROBIOLOGY, 162} (2-4):980-986

\textit{Anaplasma phagocytophilum} has been isolated and propagated in cell lines derived from the tick \textit{Ixodes scapularis} and in the human promyelocytic cell line HL60. In this study the authors used the \textit{I. ricinus}-derived cell line IRE/CTVM20 to isolate and propagate two new canine strains of \textit{A. phagocytophilum}. Blood samples were collected by veterinarians from two dogs with clinical canine anaplasmosis confirmed by PCR, one from Germany and the other from
Austria. Buffy coat cells from both dogs were isolated and co-cultivated with IRE/CTVM20 cells maintained at 28 degrees C in L15/L15B medium. In the tick cells, rickettsial inclusions were first recognised after 86 days of incubation. Electron microscopic examination of tick cells infected with one of the isolates revealed cytoplasmic vacuoles containing pleomorphic organisms with individual bacteria enveloped by a bilayer membrane. Sequencing of 16S rRNA genes confirmed the isolation of *A. phagocytophilum* and showed the highest identity to the *A. phagocytophilum* human H2 strain. The two *A. phagocytophilum* isolates were passaged several times in IRE/CTVM20 cells and transferred to the *I. scapularis* cell line ISE6. This represents the first successful establishment and continuous cultivation of this pathogen in *I. ricinus* cells as well as infectivity of these canine strains for *I. scapularis* cells.


This study describe *Anaplasma phagocytophilum* infection of three cats in Poland showing signs of fever, swollen and painful joints, pale mucous membranes and epistaxis. Morulae consistent with *A. phagocytophilum* were present within the neutrophils of two of the cats. A PCR targeting the 16S rRNA gene amplified DNA consistent with *A. phagocytophilum* in the blood of all three cats. The sequence of the PCR product obtained showed 99.6-100% homology with the sequence of *A. phagocytophilum*, gene number EU 090186 from Genbank. Applied therapy including administration of tetracyclines for 3 weeks resulted in a gradual clinical recovery.


Equine granulocytic anaplasmosis (EGA) is a seasonal rickettsial disease of horses transmitted by *Ixodes spp.* ticks. The aetiological agent is *Anaplasma phagocytophilum*, a coccobacillary gram-negative organism with a tropism for granulocytes. Clinical manifestations include fever, partial anorexia, depression, distal limb oedema, petechiation, icterus, ataxia, and reluctance to move. Haematologic changes observed are thrombocytopenia, decreased packed-cell volume and marked leukopenia involving first lymphocytes and then granulocytes. Diagnosis is based on clinical signs, serology, specific PCR and demonstration of characteristic morulae in the cytoplasm of neutrophils and eosinophils in a peripheral blood smear. Treatment consists of the administration of tetracycline. The disease is being diagnosed with increasing frequency in the United States, Canada, Brazil and northern Europe.


A two-year-old female neutered Tibetan terrier was referred following a one-month history of lethargy, inappetence and pancytopenia, which had been poorly responsive to immunosuppressive and fluoroquinolone treatment. The dog was diagnosed with pure red cell aplasia and was found to be positive for *Ehrlichia canis* by both antibody titre measurement and polymerase chain reaction. The dog lived in London and had not travelled outside the UK. The dog was treated with doxycycline, prednisolone and ciclosporin, but died as a result of gastrointestinal tract haemorrhage. To the authors' knowledge, this represents the first reported case of *E. canis* in a dog in the UK with no previous travel history.

*Babesia vogeli* in a quarantined dog


As part of a validation of an established PCR for the detection of canine babesiosis in blood samples, the AHVLA has tested a random panel of blood samples originally submitted for testing for a range of canine diseases unrelated to babesiosis. Of 50 samples tested, 49 were negative for *Babesia* species, but one sample produced a faint band of approximately 400 base pairs (bps), equivalent in size to that of a *Babesia gibsoni* control sample. Subsequent DNA sequencing generated 387 bps of readable sequence. This sequence showed 100 per cent identity with existing NCBI GenBank *Babesia vogeli* sequences AY072925 (France), DQ439545 (Spain) and AY371198 (USA). This evidence suggests that the dog was cryptically infected with *B. vogeli* of unknown origin. Subsequent investigation revealed that the dog was in UK quarantine, having entered the country from North Africa. Due to it having a poor skin condition, a serum sample had been submitted for *Leishmania* testing. This investigation
confirms the suspicion that the UK is under constant risk of introduction of exotic tick borne diseases. There have been a number of reports of babesiosis in dogs in the UK, including one with no travel history outside of the UK. B. vogeli is endemic in countries across Europe and is intimately associated with its vector, the brown dog tick (Rhipicephalus sanguineus). With harmonisation of companion animal movements within the EU, tick treatment is now recommended but not compulsory. This has led to concern that exotic pathogens and their vectors could be introduced into the UK with increasing frequency. Continued surveillance for both is needed to ensure that the UK can respond adequately to changes in vector ecology and the disease profile of companion animals.


(Although the piroplasm in this case is described as Babesia equi this parasite is now more commonly known as Theileria equi based on the formation of schizonts in equine monocytes and subsequent release of daughter merozoites to become intraerythrocytic forms)

A 7-year-old Quarter Horse gelding used for unsanctioned racing was examined because of fever and anorexia. Physical examination revealed fever, tachycardia, and tachypnea. Results of a blood cell count indicated anaemia and mild thrombocytopenia. Results of microscopic examination of a blood smear indicated piroplasms in erythrocytes, consistent with Babesia spp. Regulatory authorities were contacted, and results of serology at the National Veterinary Services Laboratories, Ames, Iowa confirmed acute Babesia equi infection. Equids on the home premises of the index horse were placed under quarantine. Those equids were tested for piroplasmosis and 6 of 63 horses had positive results for B equi. Another horse that had previously been housed on the index premises also had positive results for B equi.

Competent tick vectors were not identified. All 8 horses with piroplasmosis were Quarter Horses that participated in unsanctioned racing and were trained by the same person. Two of the horses were illegally removed from the index premises; these 2 horses and the other horse with piroplasmosis that was previously housed on the index premises could not be found. The other 5 horses with piroplasmosis were euthanased. Investigators concluded that transmission of B equi among horses was most likely iatrogenic. Veterinary surgeons should consider piroplasmosis in horses with clinical signs similar to those of the index horse of this report.

**Serum Antibodies from a Subset of Horses Positive for Babesia caballi by Competitive Enzyme-Linked Immunosorbent Assay Demonstrate a Protein Recognition Pattern That Is Not Consistent with Infection**


Tick-borne pathogens that cause persistent infection are of major concern to the livestock industry because of transmission risk from persistently infected animals and the potential economic losses they pose. The recent re-emergence of Theileria equi in the United States prompted a widespread national survey resulting in identification of limited distribution of equine piroplasmosis (EP) in the U. S. horse population. This program identified Babesia caballi seropositive horses using rhoptry-associated protein 1 (RAP-1) competitive enzyme-linked immunosorbent assay (cELISA), despite B. caballi being considered non-endemic on the US mainland. The purpose of the present study was to evaluate the suitability of RAP-1-cELISA as a single serological test to determine the infection status of B. caballi in US horses. Immunoblotting indicated that sera from US horses reacted with B. caballi lysate and purified B. caballi RAP-1 protein. Antibody reactivity to B. caballi lysate was exclusively directed against a single protein similar to 50-kDa band corresponding to a native B. caballi RAP-1 protein. In contrast, sera from experimentally and naturally infected horses from regions where B. caballi is endemic bound multiple proteins ranging from 30 to 50 kDa. Dilutions of sera from US horses, positive by cELISA, revealed low levels of antibodies while sera from horses experimentally infected with B. caballi and from areas where B. caballi is endemic had comparatively high antibody levels. Finally, blood transfusion from seropositive US horses into naive horses demonstrated no evidence of B. caballi transmission, confirming that antibody reactivity in cELISA-positive US horses was not consistent with infection. Therefore, the authors conclude that a combination of cELISA and immunoblotting is required for the accurate serodiagnosis of B. caballi infection.
*Theileria annae* in a young Swedish dog.


A severe regenerative anemia was detected in a 12-week-old mixed breed puppy in Sweden. A small protozoan parasite was observed in erythrocytes on a blood smear. It was initially suspected to be *Babesia gibsoni* based on its size and because *B. gibsoni* was previously recorded in Sweden. Surprisingly, specific polymerase chain reaction analysis identified the protozoan as *Theileria annae*. *T. annae* is endemic in Northwest Spain, is very uncommonly reported elsewhere and has never been recorded in Scandinavia. *T. annae* has been identified in dogs used for dog fighting, and it is thought to be transmitted by dog bites. This puppy was a mixed pit bull terrier. Pit bull terriers are sometimes used for dog fighting. *T. annae* has been reported to be transmitted vertically, and in light of the puppy’s age, this mode of transmission was suspected in the present case.

*Hepatozoon canis* infection in three dogs imported from Spain

Criel, D; Vandenberghe, A. (2013) VLAAMS DIERGENEESKUNDIG TIJDSSCHRIFT, 82 (2):69-74

This case report describes three dogs imported into Belgium from Malaga, Spain and subsequently determined to be infected with *Hepatozoon canis*. The dogs were presented to a veterinarian with a fever of unknown origin. Numerous *H. canis* gamonts were seen inside neutrophilic granulocytes on blood smears of the three dogs. The animal with the most serious clinical signs was also infected with *Ehrlichia canis*. The life cycle, clinical findings, diagnostic techniques, treatment options and epidemiology of *H. canis* are reviewed in this article. With the increasing risk of importation of canine exotic diseases, *H. canis* infection should be considered in a differential diagnosis in animals that originate from, or have travelled to endemic areas.

WILDLIFE

Detection of *Rickettsia helvetica* in ticks collected from European hedgehogs *(Erinaceus europaeus*, Linnaeus, 1758)


The role of wild mammals in the dissemination and maintenance of *Rickettsia* in nature is still under investigation. European hedgehogs (*Erinaceus europaeus*) are often heavily infested by tick and flea species that are known to harbour and transmit different *Rickettsia* spp. We investigated ixodid ticks sampled from European hedgehogs for the presence of *Rickettsia*. A total of 471 *Ixodes ricinus* and 755 *I. hexagonus* were collected from 26 German and 7 British hedgehogs. These were tested by a genus-specific real-time PCR assay targeting the citrate synthase gene (gltA). The *Rickettsia sp* minimum infection rate was 11.7% with an increase detected with each parasitic tick stage. No significant difference in *Rickettsia* prevalence was detected in either *Ixodes* species. Using sequencing of partial ompB, *R. helvetica* was the only species identified. More than half of the hedgehogs carried *Rickettsia*-positive ticks. In addition, tissue samples from 2/5 hedgehogs (where tissue DNA was available) were PCR-positive. These results show that European hedgehogs are exposed to *R. helvetica* via infected ticks and might be involved in the natural transmission cycle of this zoonotic *Rickettsia* species.

Transmission dynamics of *Borrelia burgdorferi* s.l. in a bird tick community


This work describes the circulation of *Borrelia burgdorferi* sensu lato in a tick community consisting of three species (*Ixodes ricinus, I. frontalis* and *I. arboricola*) with contrasting ecologies, but sharing two European songbird hosts (*Parus major* and *Cyanistes caeruleus*). *Parus major* had the highest infestation rates, primarily due to larger numbers of *I. ricinus*, and probably because of their greater low-level foraging. The prevalence of *Borrelia* in feeding ticks did not significantly differ between the two bird species; however, *P. major* in particular hosted large numbers of *Borrelia*-infected *I. frontalis* and *I. ricinus* larvae, suggesting that the species facilitates *Borrelia* transmission. The low but significant numbers of *Borrelia* in questing *I. arboricola* ticks also provides the first field data to suggest that it is competent in maintaining *Borrelia*. Aside from *B. garinii*, a high number of less dominant genospecies was observed, including several mammalian genospecies and the first record of *B. turdi* for North-
Western Europe. *Borrelia burgdorferi* sensu lato IGS genotypes were shared between *I. arboricola* and *I. ricinus* and between *I. frontalis* and *I. ricinus*, but not between *I. arboricola* and *I. frontalis*. This suggests that the *Borrelia* spp. transmission cycles can be maintained by bird-specific ticks, and bridged by *I. ricinus* to other hosts outside bird-tick cycles. *Ixodes ricinus*, *I. frontalis* and *I. arboricola* infest passerine species as well as raptors in UK, however very little information is available on the co-feeding of these three tick vectors.

A golden jackal (*Canis aureus*) from Austria bearing *Hepatozoon canis*- import due to immigration into a non-endemic area?  
The protozoan *Hepatozoon canis*, which is transmitted via ingestion of infected ticks by canine hosts, is not endemic to mid-latitude regions in Europe. Its distribution is supposed to be linked to the occurrence of its primary tick vector *Rhipicephalus sanguineus*. A young male golden jackal (*Canis aureus*) found as road kill close to Vienna, Austria, was infected by this pathogen. Cloning and sequencing of the PCR product revealed 6 different haplotypes of *H. canis*. Based on the sequences, no clear relationship to the origin of infection could be traced. This is the first report of *H. canis* in Austria, and wild canines such as the jackal in this case may provide a source of natural spread of this parasite into non-endemic areas. This natural immigration of wild animals represents a way of pathogen introduction, which has to be considered in disease prevention in addition to human-made introduction due to animal import and export.

DETECTION, CULTURE AND VACCINE STUDIES

**Effect of Sex Steroids on Babesia microti Infection in Mice**  
Sex-based-differences are known to affect susceptibility to protozoan infections but their effects on *Babesia* sp parasitaemia and clinical signs of infection remain unclear. We examined the sex-based susceptibility of various mouse strains to *Babesia microti* Munich strain infection. In all mouse strains, males exhibited significantly higher parasitaemia and more severe anaemia than female mice. Testosterone and estradiol-17 beta treatment caused an increase in parasitaemia and aggravation of anaemia. Orchidectomized male mice receiving testosterone exhibited smaller splenic macrophage populations three days after infection, smaller B cell populations 10 days after infection, and reduced splenic tumor necrosis factor-alpha and interferon-gamma mRNA expression than mice that did not receive testosterone. Mice receiving estradiol-17 beta did not exhibit immunosuppressive effects. Thus, a weakened and delayed innate immunity response may lead to acquired immunity failure. The results suggested that testosterone directly affects T or B cells, leading to delayed acquired immunity, dramatically increased parasitaemia, and severe anaemia.

**Validation of BdCCp2 as a marker for Babesia divergens sexual stages in ticks.**  
Transmission of *Babesia* parasites from the vertebrate host to the tick is mediated by sexual stages, the gametocytes which are the only intraerythrocytic stages that survive and develop inside the vector. Very little data is available concerning these parasite stages and some markers are needed in order to refine our knowledge of *Babesia* life cycle inside the tick and to permit the monitoring of parasite transmission from vertebrate to vector. Potential protein markers of *B. divergens* gametocytes were identified using an in-silico post-genomic approach based on sequence identity between the available genomes of *Plasmodium* and *Babesia* spp. One of the proteins, BdCCp2, was validated as a marker of sexual stages of infected *I. ricinus* ticks challenged with antiserum directed against recombinant BdCCp2 protein. The BdCCp2 protein was detected by Western blot in infected ticks as a discrete band of approximately 171 kDa, whilst no signal was detected in laboratory-reared non-infected ticks. BdCCp2 was also detected by immunohistochemistry in piriform or ovoid *Babesia* forms measuring 2.5-4.5 µm in diameter in the gut of partially engorged, experimentally infected ticks. This molecular marker will be used in the future to characterize the biology of *B. divergens* gametocytes in both host blood and in the tick vector.
In vitro culture systems for the study of apicomplexan parasites in farm animals. Muller, J; Hemphill, A. (2013) INTERNATIONAL JOURNAL FOR PARASITOLOGY, 43 (2):115-124

In vitro culture systems represent powerful tools for the study of apicomplexan parasites such as Cryptosporidium, Eimeria, Sarcocystis, Neospora, Toxoplasma, Besnoitia, Babesia and Theileria sp which may infect farm animals. Proliferative stages of these parasites have been cultured in vitro using a large variety of cell culture and explant approaches. For some, such as Cryptosporidium and Eimeria sp, sexual development has been reproduced in cell cultures, while for others, animal experimentation is required to complete the life cycle. In-vitro cultures have paved the way to examine the basic biology of these organisms, and are significant in the development of tools for diagnostic purposes. With the aid of in-vitro cultivation, studies on host-parasite interactions, on factors involved in innate resistance, stage conversion and differentiation, genetics and transfection technology, vaccine candidates and drug trials may be carried out. The use of transgenic parasites has facilitated high-throughput screening of anti-microbial compounds that are active against the proliferative stages. This work reviews cell culture-based in-vitro systems for farm animal apicomplexan parasites and their applications with a focus on drug identification and studies of stage differentiation is discussed.


Diseases transmitted by arthropod vectors such as mosquitoes, ticks and sandflies greatly impact human and animal health, and therefore, their control is important for the eradication of vector borne diseases (VBD). Vaccination is an environmentally friendly alternative for vector control that allows control of several VBD by targeting their common vector. Recent results have suggested that subolesin (SUB) and its orthologue in insects, akirin (AKR) are good candidate antigens for the control of arthropod vector infestations and pathogen infection. SUB was discovered as a tick-protective antigen in Ixodes scapularis. Vaccination trials with recombinant SUB/AKR demonstrated effective control of arthropod vector infestations in various hard and soft tick species, mosquitoes, sand flies, poultry red mites and sea lice by reducing their numbers, weight, oviposition, fertility and/or moulting. SUB/AKR vaccination also reduced tick infection with tick borne pathogens, Anaplasma phagocytophilum, A.marginale, Babesia bigemina and Borrelia burgdorferi. The effect of vaccination on different hosts, vector species, developmental stages and vector borne pathogen infections demonstrate the feasibility of SUB/AKR universal vaccines for the control of multiple vector infestations and for reduction of infection by VBD.

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CESTODES

A textbook that reviews the zoonotic infections humans share with dogs was published with updated reviews on all the major viral, bacterial, protozoan, and helminth parasitic zoonoses shared by humans and dogs. Dogs and protozoan zoonoses; dogs and trematode zoonoses; dogs and cestode zoonoses; dogs and nematode zoonoses; dogs and ectoparasitic zoonoses, zoonoses prevention, control, and elimination in dogs are included. (Macpherson et al., 2013). A chapter in this book reviews the complex role dogs play in the cestode zoonoses, with a focus on the most important from a public health point of view, including Echinococcus granulosus and E. multilocularis, to the less common Echinococcus, Diphyllobothrium, Dipylidium, Taenia, Spirometra spp. and Mesocestoides. The transmission, diagnosis, treatment, epidemiology and public health aspects, and the control and surveillance of these diseases are highlighted.(Macpherson and Torgerson, 2013)

The status and emerging issues in the use of praziquantel for treatment of human trematode and cestode infections were briefly reviewed in 2013. Since praziquantel was first introduced as a broad spectrum anthelmintic in 1975, innumerable articles describing its successful use in the treatment of the majority of human-infecting trematodes and cestodes have been
published. The target trematode and cestode diseases include schistosomiasis, clonorchiasis and opisthorchiasis, paragonimiasis, heterophyidiasis, echinostomiasis, fasciolopsiasis, neodiplostomiasis, gymnothiridiasis, taeniasis, diphyllobothriasis, hynemolepiasis, and cysticercosis. However, *Fasciola hepatica* and *Fasciola gigantica* infections are refractory to praziquantel, for which triclabendazole, an alternative drug, is necessary. In addition, larval cestode infections, particularly hydatid disease and sparganosis, are not successfully treated by praziquantel. The precise mechanism of action of praziquantel is still poorly understood.

There are also emerging problems with praziquantel treatment, which include the appearance of drug resistance in the treatment of *Schistosoma mansoni* and possibly *Schistosoma japonicum*, along with allergic or hypersensitivity reactions against praziquantel treatment. To cope with and overcome these problems, combined use of drugs, i.e., praziquantel and other newly introduced compounds such as triclabendazole, artemisinins, and tribendimidine, is being tried (Chai, 2013).


**TAENIA SPP**

Reviews of the life cycle of *Taenia solium*, *T. saginata* and *T. asiatica*, were published in this book chapter and the pathogenesis, clinical features and epidemiology of diseases caused by these parasites discussed. It also described available diagnostic methods, as well as measures to prevent and control foodborne *Taenia* infections. (Steinbruch, 2013)


*Taenia saginata*

Bovine cysticercosis (BC) is a zoonotic, parasitic infection in cattle. Under the current EU meat inspection regulation, every single carcass from all bovines above 6 weeks of age is examined for BC. This method is costly and makes more sense in countries with higher number of BC-infected animals than in countries with few lightly infected cases per year. The aim of the present case control study was to quantify associations between potential herd-level risk factors and BC in Danish cattle herds. Risk factors can be used in the design of a risk-based meat inspection system targeted towards the animals with the highest risk of BC. Cases (n=77) included herds that hosted at least one animal diagnosed with BC at meat inspection, from 2006 to 2010. Control herds (n=231) consisted of randomly selected herds that had not hosted any animals diagnosed with BC between 2004 and 2010. The answers from a questionnaire and register data from the Danish Cattle Database were grouped into meaningful variables and used to investigate the risk factors for BC using a multivariable logistic regression model. Case herds were almost three times more likely than control herds to let all or most animals out grazing. Case herds were more than five times more likely than control herds to allow their animals’ access to risky water sources with sewage treatment plant effluent in proximity. Case herds were also more likely to share machinery or hire contractors than control herds. The risk decreased with increasing herd size probably because the larger herds generally tend to keep cattle indoors in Denmark. The results are useful to guide future data recording that can be supplied by the farmer as food chain information and then be used for differentiated meat inspection in low- and high-risk groups, enabling development of risk-based meat inspection systems. (Calvo-Artavia et al., 2013)

The beef tapeworm (*Taenia saginata*) reaches a length of 10 m and a width up to 7 mm. With the obligatory and only definitive host of humans, the beef tapeworm stage comprises a stage in the intermediate host. After recording of worm eggs from contaminated food or water with
faeces, the worm develops in the muscles of cattle. Humans are infected by eating meat that was insufficiently frozen or heated. Recent studies found no regional differences in disease incidence. Only a very heavy infestation of cattle, the so-called generalized cysticercosis, can lead to loss of performance. According to the Ordinance of the FDEA on hygiene, all cattle must be inspected for cysticercosis during slaughter. If at a prescribed muscle cut a cyst is discovered, the regulations provide a defreezing of the whole carcass. With a massive infestation, the entire carcass is declared unfit for human consumption and disposed of as an animal byproduct. 30-50% of infected animals for slaughter are diagnosed at routine meat inspection, thereby incurring great losses for the farmers. The following risk factors have rarely been observed: in recreational areas, as well as larger events on the farm, is the risk that cattle are contaminated with tapeworm eggs, especially when forage areas are adjacent to parking, railway lines or recreational areas such as sports field or beach. Internal risk factors are the presence of many people on average over the operation (e.g. on the occasion of festivals, sightseeing, sleeping on straw). If there is no sewer connection of toilets in the house, but also a separate apartment, a house or of public toilets it must be expected that tapeworm segments and eggs come with the manure to forage crops. In addition, the use of foreign manure can pose a risk. For prevention of cysticercosis, the following preventive measures are presented. Risky pasture and hay forage and hay stored less than ten weeks (tapeworm eggs are sensitive to drought). For areas such as bathrooms or beach picnic areas, apply for the installation of a toilet in the community. For major events, set up mobile toilets and do not allow wastewater in the pasture. New employees (including trainees) must be tested for tapeworm infestation. An information leaflet on tapeworm cysts in cattle which is available and includes an analysis of affected farms.(Strabel, 2013)

Laboratory confirmation methods are important in bovine cysticercosis diagnosis as other pathologies can result in morphologically similar lesions resulting in false identifications. The development of a probe-based real-time PCR assay to identify *Taenia saginata* in suspect cysts encountered at meat inspection was described and compared with the traditional method of identification, histology, as well as a published nested PCR. The assay simultaneously detects *T. saginata* DNA and a bovine internal control using the cytochrome c oxidase subunit 1 gene of each species and shows specificity against parasites causing lesions morphologically similar to those of *T. saginata*. A loss in PCR sensitivity was observed with increasing cyst degeneration as seen in other molecular methods. In comparison to histology, the assay offered greater sensitivity and accuracy with 10/19 (53%) *T. saginata* positives detected by real-time PCR and none by histology. When the results were compared with the reference PCR, the assay was less sensitive but offered advantages of faster turnaround times and reduced contamination risk. Estimates of the assay’s repeatability and
reproducibility showed the assay is highly reliable with reliability coefficients greater than 0.94. (Cuttell et al., 2013)

The diagnostic values of seven serological tests (ELISAs) and of the obligatory European Union-approved routine visual meat inspection for the detection of *Taenia saginata* cysticercosis were investigated. A total of 793 slaughtered dairy cows were selected in three European Union approved abattoirs in Switzerland, an endemic area (apparent prevalence by enhanced meat inspection up to 4.5%) with typically low parasite burdens. ELISAs based on a somatic larval antigen, isoelectric focused somatic larval antigen, larval excretory/secretory antigens, peptide HP6-2, peptide Ts45S-10, pooled peptide solution and a monoclonal antibody antigen capture assay were initially screened. As there is no perfect diagnostic ‘gold standard’ reference test, the obligatory meat inspection and four selected serological tests were further analysed using Bayesian inference to estimate the "true" prevalence and the diagnostic test sensitivities and specificities. The ELISA for specific antibody detection based on excretory/secretory antigens showed highest sensitivity and specificity with 81.6% (95% credible interval: 70-92) and 96.3% (95% credible interval: 94-99), respectively. The Bayesian model estimated the specificity of the ELISA, based on the synthetic peptide Ts45S-10 as 55.2% (95% credible interval: 46-65) and sensitivity as 84.7% (95% credible interval: 82-88). The sensitivity of the ELISA based on mAbs, detecting circulating antigen, was 14.3% (95% credible interval: 9-23) with a specificity of 93.7% (95% credible interval: 92-96). The diagnostic sensitivity of the obligatory standard European Union meat inspection procedure for the detection of *T. saginata* cysticercus infection at the abattoir was estimated to be 15.6% (95% credible interval: 10-23). Based on these data, the modelled prevalence of cysticercosis in dairy cows presented at abattoirs in Switzerland was estimated to be 16.5% (95% credible interval: 13-21). These cattle also had a high prevalence of infection with *Dicrocoelium dendriticum* (60.8%) and *Fasciola hepatica* (13.5%) (Eichenberger et al., 2013)

A study to describe the prevalence of *C bovis* cases within Northern Ireland, to analyse the differences in the recorded number of cases of *C bovis* across Northern Ireland over the past decade, and to ascertain whether or not there is any difference in the number of cases of *C bovis* detected in abattoirs with different techniques for muscle examination was published in a short communication. Between 2001 and 2011, there were 536 cases giving an overall prevalence of 0.011 per cent (95% CI 0.011 per cent to 0.012 per cent). Since 2002, the prevalence has remained very low. No link to examination practices could be made (McBrien and Courcier, 2013).


*Taenia solium*

Cysticercosis is caused by the invasion of human or pig tissues by the metacestode larval stage of *Taenia solium*. In Europe, the disease was endemic in the past but the autochthonous natural life cycle of the parasite is currently completed very rarely. Recently, imported cases have increased in parallel to the increased number of migrations and
international travels. The lack of specific surveillance systems for cysticercosis leads to underestimation of the epidemiological and clinical impacts. A review of literature on human cysticercosis and *T. solium* taeniasis in Europe published between 1990-2011 was conducted. Out of 846 cysticercosis cases described in the literature, 522 cases were autochthonous and 324 cases were imported. The majority (70.1%) of the autochthonous cases were diagnosed in Portugal from 1983 and 1994. Imported cases of which 242 (74.7%) diagnosed in migrants and 57 (17.6%) in European travellers, showed an increasing trend. Most of imported cases were acquired in Latin America (69.8% of migrants and 44.0% of travellers). The majority of imported cases were diagnosed in Spain (47.5%), France (16.7%) and Italy (8.3%). One third of neurosurgical procedures were performed because the suspected diagnosis was cerebral neoplasm. Sixty eight autochthonous and 5 imported *T. solium* taeniasis cases were reported. Cysticercosis remains a challenge for European care providers, since they are often poorly aware of this infection and have little familiarity in managing this disease. Cysticercosis should be included among mandatory reportable diseases, in order to improve the accuracy of epidemiological information. European health care providers might benefit from a transfer of knowledge from colleagues working in endemic areas and the development of shared diagnostic and therapeutic processes would have impact on the quality of the European health systems. (Zammarchi et al., 2013)

Neurocysticercosis continues to be a major health burden on humans living in many regions of the world, despite the availability of highly effective taeniacides and identification of the cause, *Taenia solium*, as being potentially eradicable. Several *T. solium* control trials have been undertaken, generally achieving limited success and none that has been fully documented has achieved what was demonstrated to be a sustainable level of disease control. Pigs act as intermediate hosts for *T. solium* and two new control tools have become available for application in pigs - single-dose oxfendazole treatment of porcine cysticercosis and the TSOL18 vaccine. Three potential intervention scenarios for pigs are compared for control of cysticercosis, using either oxfendazole or vaccination. A control scenario involving vaccination plus oxfendazole treatment delivered at 4 monthly intervals was predicted to achieve the best outcome, with no pigs slaughtered at 12 months of age having viable *T. solium* cysticerci. Now that new control tools are available, there are opportunities to concentrate research attention on evaluation of novel control scenarios leading to the implementation of effective and sustainable control programmes and a reduction in the global burden of neurocysticercosis. (Lightowlers, 2013)

Porcine cysticercosis, an infection caused by *Taenia solium* metacestodes, is continuously being reported in low-income countries of Latin America, Asia, and sub-Saharan Africa. The disease was declared eradicable by the International Task Force for Diseases Eradication (ITFDE) in 1993, and it is listed among the 17 WHO Neglected Tropical Diseases and Neglected Zoonoses that are potentially eradicable. In view of that, WHO has proposed a step-wise approach to its elimination, including chemotherapy of infected pigs. Different drugs have been tested on porcine cysticercosis with varying efficacies. These include flubendazole, fenbendazole, albendazole, albendazole sulphoxide, oxfendazole, praziquantel, and nitazoxanide. This review summarises available information on the efficacies and adverse effects shown by these drugs in pigs. Oxfendazole has shown to be effective for the control of porcine cysticercosis; however, it needs to be integrated with other control approaches. There is a need for standardised guidelines for evaluating the efficacy of anthelmintics against porcine cysticercosis, and more efficacy studies are needed since the conclusions so far are based on a limited number of studies using few infected pigs. (Mkupasi et al., 2013)

Human cysticercosis, caused by accidental ingestion of eggs of *Taenia solium*, is one of the most pathogenic helminthiases and is listed among the 17 WHO Neglected Tropical Diseases. Controlling the life-cycle of *T. solium* between humans and pigs is essential for eradication of cysticercosis. One difficulty for the accurate detection and identification of *T. solium* species is the possible co-existence of two other human *Taenia* tapeworms (*T. saginata* and *T. asiatica*, which do not cause cysticercosis in humans). Several key issues for taeniasis/cysticercosis (T/C) evidence-based epidemiology and control are reviewed: (1) advances in immunological and molecular tools for screening of human and animals hosts and identification of *Taenia* species, with a focus on real-time detection of taeniasis carriers.
and infected animals in field community screenings, and (2) spatial ecological approaches that have been used to detect geospatial patterns of case distributions and to monitor pig activity and behaviour. Most recent eco-epidemiological studies undertaken in Sichuan province, China, are introduced and reviewed. (Raoul et al., 2013)


**Echinococcus spp**

Echinococcosis, resulting from infection with tapeworms *Echinococcus granulosus* and *E. multilocularis*, has a global distribution with 23 similar to million people affected and 200,000 new cases diagnosed annually. Costs of treatment for humans and economic losses to the livestock industry have been estimated to exceed $2 similar to billion. These figures are likely to be an underestimation given the challenges with its early detection and the lack of mandatory official reporting policies in most countries. Despite this global burden, echinococcosis remains a neglected zoonosis. The importance of environmental factors in influencing the transmission intensity and distribution of *Echinococcus* spp. is increasingly being recognized. With the advent of climate change and the influence of global population expansion, food insecurity and land-use changes, questions about the potential impact of changing temperature, rainfall patterns, increasing urbanization, deforestation, grassland degradation and overgrazing on zoonotic disease transmission are being raised. This study is the first to comprehensively review how climate change and anthropogenic environmental factors contribute to the transmission of echinococcosis mediated by changes in animal population dynamics, spatial overlap of competent hosts and the creation of improved conditions for egg survival. We advocate rigorous scientific research to establish the causal link between specific environmental variables and echinococcosis in humans and the incorporation of environmental, animal and human data collection within a sentinel site surveillance network that will complement satellite remote-sensing information. Identifying the environmental determinants of transmission risk to humans will be vital for the design of more accurate predictive models to guide cost-effective pre-emptive public health action against echinococcosis. (Atkinson et al., 2013)

Canine echinococcosis is a potential zoonotic infection caused by the adult form of several cestode species belonging to the genus Echinococcus, of which *E. granulosus sensu lato* and *E. multilocularis* are the most epidemiologically relevant. Dogs infected with *E. granulosus* and *E. multilocularis* are widely regarded as the main source of infection for human cystic and alveolar echinococcosis, diseases that cause substantial morbidity and socio-economic burden in several regions of the world. This summarizes here current knowledge on the global epidemiology, geographical distribution and molecular diversity of *Echinococcus* spp. infection in dogs. The implications of the increasing urbanization of wildlife species such as foxes, coyotes, and dingoes in the establishment of urban cycles of *Echinococcus* spp., or the rising concerns regarding the role of unsupervised translocation of infected dogs in spreading the infection to Echinococcus-free areas are all discussed. The involvement of wildlife species as natural reservoirs of disease to domestic animals and humans and the epidemiological
significance of the sympatric occurrence of different *Echinococcus* species in the same geographical region are also debated. (Carmena and Cardona, 2013)

Currently, dogs infected with *Echinococcus* spp. are the main contagium of hydatid disease. Therefore, undertaking dynamic monitoring and reasonable deworming of the dogs infected with *Echinococcus* spp. are some of the key measures for preventing and eliminating hydatid disease in epidemic areas. In recent years, scientists intend to establish a fast coproantigen detection system for diagnosis of dogs infected with *Echinococcus* spp. by using sandwich-ELISA method. This article reviews the most recent advances in the establishment of specific coproantigen detection techniques for dogs infected with *Echinococcus* spp. (Zhang and Mamuti, 2013)

*E. multilocularis*

*Echinococcus multilocularis* is a tapeworm of canids and is ranked among the world's most lethal zoonoses. Red foxes (*Vulpes vulpes*) provide a natural reservoir of infection but the urbanisation of the red fox and it's increase in numbers across Europe have brought *E. multilocularis* into increased contact with domestic dogs that may also harbor infection. The UK remains free of *E. multilocularis* but the lack of adverse clinical signs and difficulty of diagnosing the parasite in canids without post-mortem means that introduction of the parasite from pets travelling abroad is a constant threat. The Pet Travel Scheme (PETS) includes a compulsory requirement for praziquantel treatment for dogs 1-5 days before entry into the UK to prevent *E. multilocularis* being introduced. This article discusses the importance of maintaining this requirement as well as reviewing the life cycle, epidemiology, diagnostic tests and treatment of the parasite. (Wright, 2013)

In Europe, most cities are currently colonized by red foxes (*Vulpes vulpes*), which are considered to be the main definitive host of the zoonotic cestode *Echinococcus multilocularis*. The risk of transmission to humans is of particular concern where high fox populations overlap with high human populations. The distribution of baits containing praziquantel has successfully reduced the infection pressure in rural areas and in small plots within large cities. The purpose of this study was to assess its efficiency in two medium size cities (less than 100,000 inhabitants) in areas of high human alveolar echinococcosis incidence. From August 2006 to March 2009, 14 baiting campaigns of praziquantel treatment were run in Annemasse and Pontarlier (Eastern France), each of which encompassed 33 km(2), with a density of 40 baits/km(2). The bait consumption appeared to be lower in strictly urban context compared to suburban areas (78.9% vs. 93.4%) and lower in Annemasse than in Pontarlier (82.2% vs. 89.5%). During our study, the prevalence of *E. multilocularis*, as assessed by EM-ELISA on fox faeces collected in the field in Annemasse, was lower within the treated area than in the rural control area. A “before/during” treatment comparison revealed a significant decrease of spring prevalence from 13.3% to 2.2%. No significant change in prevalence was detected in Pontarlier (stable prevalence: 9.1%) where the contamination of the treated area followed the temporal trend observed in the control area. There, a greater resilience of the parasite's life cycle, probably due to a strong pressure of recontamination from outside the treated area, may have counteracted the prophylaxis treatment. These contrasted outcomes suggest that the frequency of fox anthelmintic treatment should be adapted to the local situation. (Comte et al., 2013)

*Echinococcus multilocularis*, the zoonotic agent of human alveolar echinococcosis, has considerably extended its range and became more prevalent in many parts of the endemic areas. Accordingly, there is an increasing demand for measures to prevent human infections. Rising public awareness of this zoonosis and individual protective actions should be part of every prevention program. Considering the high reproduction of *E. multilocularis* in domestic dogs which live in close contact to humans, a monthly deworming scheme for domestic dogs with access to rodents is likely to be of high importance. This holds true if only low prevalences in domestic dogs are recorded, as high densities of these pets can easily outweigh low infections rates. Thus, in central Europe their estimated contribution to environmental contamination with *E. multilocularis* eggs ranges between 4% and 19%. The estimated contribution of domestic cats is insignificant (<0.3%) due to low parasite reproduction in this species. Control of the parasite by reducing its main wildlife hosts (foxes,
vole species) is barely achievable on a larger scale and is generally not well accepted due to ecological considerations and animal welfare concerns. In general, the frequency of the parasite sharply decreases when anthelmintic baits are regularly distributed to foxes. However, eradication of the parasite is unlikely and long-term baiting campaigns are actually the most effective tool to significantly lower the infection pressure with parasite eggs. According to ecological considerations and animal welfare concerns. In general, the frequency of the parasite sharply decreases when anthelmintic baits are regularly distributed to foxes. However, eradication of the parasite is unlikely and long-term baiting campaigns are actually the most effective tool to significantly lower the infection pressure with parasite eggs. Regarding the long latency of 5-15 years of alveolar echinococcosis, however, such measures can only be cost effective if they are pursued for several decades and concentrate on restricted areas which are most relevant for the transmission of alveolar echinococcosis such as highly endemic areas in densely populated zones. Thus, the implementation of this approach strongly depends on factors such as public attitude, available financial resources and priority setting of political decision-makers. (Hegglin and Deplazes, 2013)

Alveolar echinococcosis (AE) is caused by the larval stage (metacestode) of Echinococcus multilocularis. The domestic dog can act as a definitive host and harbor adult cestodes in its small intestine or become an aberrant intermediate host carrying larval stages that may cause severe lesions in the liver, lungs and other organs with clinical signs similar to AE in humans. A case of canine AE, affecting the liver and prostate with development of multilocular hydatid paraprostatic cysts and possible lung involvement is described in an 8-year-old neutered male Labrador retriever dog. Cytological examination of fine-needle aspirates of the liver, prostate and paraprostatic cyst revealed parasitic hyaline membranes typical of an Echinococcus infection and the presence of E. multilocularis-DNA was confirmed by PCR. The dog was treated with albendazole and debulking surgery was considered in case there was a good response to antiparasitic treatment. Constipation and stranguria resolved completely. Six months after the definitive diagnosis, the dog was euthanized due to treatment-resistant ascites and acute anorexia and lethargy. To the authors' knowledge, this is the first publication of an E. multilocularis infection in a dog causing prostatic and paraprostatic cysts. (Geigy et al., 2013).

Exceptionally high proportions of livers affected by encapsulated nodules containing whitish to light yellow, viscous to pasty material (“microabscesses”) were detected by meat inspection of pigs in Germany. The animals had been raised on four different farms, being located in distinct regions of Germany. Macroscopical and histological examination of 77 samples of livers revealed granulomatous to necrotizing hepatitis with attendance of numerous eosinophils. In 61 % (n = 47) of the lesions, eosinophilic, band-like acellular structures resembling the laminated layer of Echinococcus sp. were visible. Moreover, representative samples (n = 11) showed a positive reaction of these structures with Periodic acid-Schiff. Altogether, the findings were consistent with alveolar echinococcosis. Echinococcus multilocularis DNA could be demonstrated in selected samples (n = 7) by polymerase chain reaction. Epidemiological considerations suggest contamination of the forage with fox tapeworm eggs to be the most likely source of infection on two of the farms, as some of the fodder had been stored in the open, being amenable to infected definitive hosts. On the two other farms, mainly straw litter has to be taken into account regarding the transmission route, since carnivores excreting eggs of E. multilocularis could have gained access to the straw storage. The presented cases show that adequate mechanisms of meat inspection may provide important data for the purposes of surveillance and risk assessment of human alveolar echinococcosis. (Boettcher et al., 2013)

E. granulosus

The identity of the causative agent of cystic echinococcosis (CE) in humans from central Poland receiving treatment between 2000 and 2010 was determined. A total of 47 samples obtained after hepatectomy were examined and protoscoleces were identified in wet preparations in 27 cases. Using DNA extracted from the samples, two mitochondrial regions (nad1 and cox1 genes) were amplified and the nad1 fragment was sequenced. This PCR analysis confirmed the presence of Echinococcus species in 30 cases and nad1 sequence alignments showed identity with the G7 (pig) strain, Echinococcus canadensis. This data demonstrates that the pig strain of this parasite is the most frequent causative agent of human cystic echinococcosis in central Poland. (Dy bicz et al., 2013)
Cystic echinococcosis is a serious and neglected parasitic zoonosis that is regarded as an emerging disease world-wide. Effective control of the disease is based on understanding the variability of *Echinococcus granulosus* (sensu lato), as genotypic characteristics may influence lifecycle patterns, development rate, and transmission. No molecular epidemiological research has previously been conducted to shed light on genotypes responsible for the disease in South Africa. To identify strains circulating in the country, parasite material was collected from patients between August 2010 and September 2012 and analyzed by PCR/RFLP methods. A total of 32 samples were characterized as *E. granulosus* sensu stricto (G1-G3) (81%), *E. canadensis* (G6/7) (16%) and *E. ortleppi* (G5) (3%). Furthermore, two co-amplifying G6/7 genotypes were confirmed as G7 by sequencing. This is the first report on genotyping of *Echinococcus* species in South Africa, and, to the best of our knowledge, the first report of the G5 and G7 genotypes from humans in Africa. (Mogoye et al., 2013)

There is substantial evidence suggesting that certain parasites can have antitumor properties. We evaluated mucin peptides derived from the helminth *Echinococcus granulosus* (denominated Egmuc) as potential inducers of antitumor activity. We present data showing that Egmuc peptides were capable of inducing an increase of activated NK cells in the spleen of immunized mice, a fact that was correlated with the capacity of splenocytes to mediate killing of tumor cells. This evidence may contribute to the design of tumor vaccines and open new horizons in the use of parasite-derived molecules in the fight against cancer. (Noya et al., 2013)

Cystic echinococcosis (CE) is an important and widespread zoonotic infection caused by the larval stages of taeniid cestodes of the genus *Echinococcus*. The disease represents a serious animal health concern in many rural areas of the world, causing important economic losses derived from decreased productivity and viscera condemnation in livestock species. In this review a comprehensive overview on recent research progress in the epidemiology of CE in production animals from a global perspective is presented. Particular attention has been paid to the discussion of the extent and significance of recent molecular epidemiologic data. The financial burden associated to CE on the livestock industry has also been addressed. Data presented are expected to improve our current understanding of the parasite's geographical distribution, transmission, host range, immunogenicity, pathogenesis, and genotype frequencies. This information should be also valuable for the design and implementation of more efficient control strategies against CE. (Cardona and Carmena, 2013)


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TRICHINELLA

REVIEWS


Pork is the most consumed meat in the world and is a source of foodborne diseases. To develop effective food safety interventions for pork, it is crucial to understand the nature of the important pathogens affecting the pork industry, their prevalence at different phases of pork production, and interventions against pathogens in pork. The purpose of this study was to outline the significance of Salmonella, Campylobacter, Trichinella spiralis, Toxoplasma gondii, Listeria monocytogenes, and methicillin-resistant Staphylococcus aureus to the pork industry. Trichinella and Toxoplasma are historically relevant pathogens to pork and represent the effectiveness that preharvest intervention strategies can accomplish for the control of toxoplasmosis and trichinellosis. Salmonella and Campylobacter are common inhabitants of swine intestines causing a high prevalence of these pathogens on the farm as well as potential contamination during slaughter. However, both Salmonella and Campylobacter can be reduced through on-farm strategies, hygienic slaughter practices, and processing technologies. Methicillin-resistant S. aureus is an emerging pathogen with increasing focus on the livestock industry and interventions pre and postharvest have been considered for reduction of this microorganism. The greatest challenge for processors is L. monocytogenes as contamination of the further processing environment requires adequate interventions for both pork and the environment. Novel technologies such as use of bacteriophages, feed additives, and high-pressure processing are being explored as interventions against pathogens of pork. Overall, pork does contribute to foodborne diseases and various interventions are now being used against the different pathogens found in pork.


Infectious pathogens from wild animals have become increasingly important throughout the world in recent years, as they have had a substantial impact in livestock and human health. A large number of pathogens (61% of the 1415 currently identified human pathogens within 313 different genera) are zoonotic and can infect multiple animal species. Multi-host pathogens are predominant among animal and human emerging diseases. Multi-host pathogens (including all zoonotic agents, pathogens that can infect more than one taxonomic order and pathogens that can infect wildlife hosts) have a higher relative risk for emergence than species-specific pathogens. Of 800 zoonotic diseases currently identified, 619 (77%) are caused by pathogens that affect wildlife; of 125 emerging zoonotic diseases, 113 (90%) affect wildlife. Of the diseases that have
emerged in the last few decades around 75% are of wildlife origin. Many factors influence changes in disease incidence, including economic, climatic and microbiological effects. Increasingly, close interaction of humans and livestock with wild animals has led to increased frequency of zoonotic infections. Forest clearance and movement of animals or animal products are factors, which pose significant risks of introducing disease into a new region. Changing climate affects disease incidence by changes in land use or animal production practices, as well as by movement or changes in distribution of animal reservoirs or insect vectors. Local increases in biting midges or mosquito numbers, changes in the distribution of known vector species and/or recruitment of novel vector species, have increased the risk of spread or introduction of diseases. Pathogen evolution may occur in response to changes of which humans are not aware. The evolution may be occurring in many hosts, currently poorly monitored. Microbial evolution may affect the extent to which established methods of diagnosis can detect infectious agents. Other endemic diseases may also change in incidence for largely unknown reasons. Increased information on prevalence in a wide range of hosts will increase our understanding of these reasons. Wildlife can play an important role in the epidemiology of small ruminant and human diseases, by representing a source of disease via various transmission routes. Recent studies in infections of wildlife in Europe have highlighted the impact on small ruminant health. In Greece, blood/organ samples were collected from 60 wild deer and 140 wild boars (2006-2011). Serum samples were tested for presence of antibodies against Toxoplasma gondii, Neospora caninum, Mycobacterium avium subsp. paratuberculosis, Chlamydophila, Salmonella and Trichinella spiralis, by using appropriate immunodiagnostic techniques. Tissue samples were examined for Mycobacterium bovis by using PCR. In serum samples from deer, antibodies against T. gondii, N. caninum and Chlamydophila were detected in 15%, 5% and 5% of samples, respectively. In serum samples from wild boars, antibodies against Salmonella, T. gondii and T. spiralis were detected in 15%, 5% and 5% of samples, respectively. No M. bovis was found in tissue samples. In Spain, Bluetongue virus, Brucella spp., Coxiella burnetii and M. avium were detected in many wild cervid species. Spanish wild boars have been found to be greatly exposed to Salmonella spp., an important small ruminant intestinal pathogen. In Austria, Spain and Poland, Anaplasma phagocytophilum has been detected in various cervids. Finally, in Poland and Spain, wild deer and wild boars were found to be exposed to T. gondii and N. caninum. The results indicate that wildlife may be carriers of several pathogens, which can be transmitted to domestic small ruminants and their farmers. It is noteworthy that samples from many European countries will be collected and tested to ensure a broader evaluation of the epidemiological role of wildlife.

The parasitic zoonoses human cysticercosis (Taenia solium), taeniasis (other Taenia species) and trichinellosis (Trichinella species) are endemic in the Lao People's Democratic Republic (Lao PDR). This study was designed to quantify the economic burden pig-associated zoonotic disease pose in Lao PDR. In particular, the analysis included estimation of the losses in the pork industry as well as losses due to human illness and lost productivity. A Markov-probability based decision-tree model was chosen to form the basis of the calculations to estimate the economic and public health impacts of taeniasis, trichinellosis and cysticercosis. Two different decision trees were run simultaneously on the model's human cohort. A third decision tree simulated the potential impacts on pig production. The human capital method was used to estimate productivity loss. The results found varied significantly depending on the rate of hospitalisation due to neurocysticercosis. This study is the first systematic estimate of the economic impact of pig-associated zoonotic diseases in Lao PDR that demonstrates the significance of the diseases in that country.

The term food borne diseases or food borne illnesses or more commonly food poisoning are used to denote gastrointestinal complications that occur following recent consumption of a particular food or drink. Millions of people suffer worldwide every year and the situation is quiet grave in developing nations creating social and economic strain. The food borne pathogens include various bacteria viz., Salmonella, Campylobacter, Escherichia coli, Listeria monocytogenes, Yersinia enterocolitica, Staphylococcus, Arcobacter, Clostridium perfringens, Cl. botulinum and Bacillus cereus and helminths viz., Taenia. They also include protozoa viz., Trichinella, Sarcocystis, Toxoplasma gondii and Cryptosporidium parvum. The zoonotic potential and the ability to elaborate toxins by many of the microbes causing fatal intoxication are sufficient to understand the seriousness of the situation. The viral agents being host specific their transmission to humans through food of animal origin is not yet confirmed although these animal viruses are similar to that of viruses infecting human. Food-borne bacteria; protozoa and helminthes have complex distribution pattern in the environment and inside the host system. This along with complexity of the maintenance chain and life cycle (of parasites) has made it difficult for epidemiologist and diagnostician to undertake any immediate safety measures against them. Serological and molecular diagnostic tests viz. ELISA, Latex agglutination test, Lateral flow assays, Immunomagnetic separation assays, molecular assays viz. Polymerase Chain Reaction (PCR), multiplex PCR, Immuno-PCR, Realtime PCR, Random Amplified Polymorphic DNA (RAPD)-PCR, DNA microarrays and probes are widely used. Along with these LAMP assays, Capillary Electrophoresis-Single Strand Confirmation polymorphism (CE-SSCP); Flow cytometry, FISH, Biosensors, Direct epifluorescent filter technique, nanotechnology based methods and sophisticated tools (ultrasonography, magnetic resonance imaging and chlonangio-pancreatography) have aided in the diagnosis greatly. Most of the food-borne illnesses are self-limiting but in many instances antibiotics are recommended. With the increased drug resistance however use of chicken immunoglobulin, bacteriophage therapy, probiotics and herbs are gaining much importance these days. Adoption of proper prevention and control measures (including cooking procedures; hygiene, strict adherence to HACCP principles, public awareness and disease surveillance and monitoring) are the need of hour. All these have been discussed vividly in this review to help epidemiologists, diagnosticians, clinicians and above all common people so as to enable them avoid negligence regarding such serious issue.

**Technical specifications on harmonised epidemiological indicators for biological hazards to be covered by meat inspection of domestic solipeds.**
In this report, harmonised epidemiological indicators are proposed for food-borne
biological hazards to public health that are related to domestic solipeds and meat thereof and that can be addressed within meat inspection. These hazards include only Trichinella. An epidemiological indicator is defined as the prevalence or concentration of the hazard at a certain stage of the food chain or an indirect measure of the hazard that correlates with the human health risk caused by the hazard. The indicators can be used by the European Commission and the Member States to consider when adaptations to meat inspection methods may be relevant and to carry out risk analysis to support such decisions. It is foreseen that the indicators are used in a risk-based system for domestic soliped meat as proposed in the EFSA Scientific Opinion on the public health hazards to be covered by inspection of meat from domestic solipeds, particularly to help categorise countries/regions and animals according to the risk related to Trichinella. Depending on the purpose and the epidemiological situation, risk managers should decide on the most appropriate indicator(s) to use, either alone or in combination, at national, regional or slaughterhouse level. It is recommended that risk managers should define legal requirements for improving traceability of horses, recording information on all animal movements. Member States are invited to report data generated by the implementation of the indicators in accordance with Directive 2003/99/EC. The proposed indicators should be regularly reviewed in the light of new information and the data generated by their implementation.

Hathaway, S. C. (2013). Food control from farm to fork: implementing the standards of Codex and the OIE. Revue Scientifique et Technique - Office International des Epizooties 32(2): 479-485. The Codex Alimentarius (Codex) international food standards help to ensure food safety and promote fair practices in the international food trade. Implementing these standards using a risk management framework (RMF) approach to decision-making is an increasingly common aspect of the food control programmes of national governments. The Codex Alimentarius Commission (CAC) provides guidance at both the system and food commodity levels. In the case of zoonoses, similarities in the risk analysis methodologies used to underpin standard setting by the CAC and the World Organisation for Animal Health (OIE) are highly enabling of integrated food control systems. The CAC and the OIE are increasingly working together to develop their respective standards for foodborne zoonoses and other hazards so that they are non-duplicative, cohesive and utilise the whole food chain. There is a clear need for effective integration of food safety and animal health monitoring and surveillance information to better control foodborne zoonoses. This is increasingly supported by Codex and OIE standards working together in a variety of ways and realisation of benefits is highly dependent on coordination and sharing of information between Competent Authorities and other food safety stakeholders at the national level.

Hazards, E. P. o. B. (2013). Scientific opinion on the public health hazards to be covered by inspection of meat (solipeds). EFSA Journal 11(6): 3263-3263. A risk ranking process identified Trichinella spp. as the most relevant biological hazard in the context of meat inspection of domestic solipeds. Without a full and reliable soliped traceability system, it is considered that either testing all slaughtered solipeds for Trichinella spp., or inactivation meat treatments (heat or irradiation) should be used to maintain the current level of safety. With regard to general aspects of current meat inspection practices, the use of manual techniques during current post-mortem soliped meat inspection may increase microbial cross-contamination, and is considered to have a detrimental effect on the microbiological status of soliped carcass meat. Therefore, the use of visual-only inspection is suggested for -non-suspect-solipeds. For chemical hazards, phenylbutazone and cadmium were ranked as being of high potential concern. Monitoring programmes for chemical hazards should be more flexible and based on the risk of occurrence, taking into account Food Chain Information (FCI), covering the specific on-farm environmental conditions and individual animal treatments, and the ranking of chemical substances, which should be regularly updated and include new hazards. Sampling, testing and intervention protocols for chemical hazards should be better integrated and should focus particularly on cadmium, phenylbutazone and priority -essential substances- approved for treatment of equine
animals. Implementation and enforcement of a more robust and reliable identification system throughout the European Union is needed to improve traceability of domestic solipeds. Meat inspection is recognised as a valuable tool for surveillance and monitoring of animal health and welfare conditions. If visual only post-mortem inspection is implemented for routine slaughter, a reduction in the detection of strangles and mild cases of rhodococcosis would occur. However, this was considered unlikely to affect the overall surveillance of both diseases. Improvement of FCI and traceability were considered as not having a negative effect on animal health and welfare surveillance.

**Scientific opinion on the public health hazards to be covered by inspection of meat from farmed game.** 

Salmonella spp. in farmed wild boar and Toxoplasma gondii in farmed deer and farmed wild boar were ranked as a high priority for meat inspection. Trichinella spp. in wild boar was ranked as low priority due to current controls, which should be continued. For chemical hazards, all substances were ranked as medium or lower potential concern. More effective control of biological hazards could be achieved using an integrated farm to chilled carcass approach, including improved food chain information (FCI) and risk-based controls. Further studies are required on Salmonella spp. in farmed wild boar and T. gondii in farmed wild boar and farmed deer. If new information confirms a high risk to public health from meat from these species, setting targets at carcass level should be considered. Palpation and incision should be omitted, as it will not detect biological hazards considered to be a high priority for meat inspection while increasing the potential spread and cross-contamination of the carcasses with Salmonella. Palpation and/or incision may be applied where abnormalities have been detected but away from the slaughter line. However, the elimination of routine palpation and incision would be detrimental for detecting tuberculosis. As farmed deer and farmed wild boar can act as tuberculosis reservoirs, any reduction in the detection, due to changes in the post-mortem inspection procedures, will have consequences for the overall surveillance of tuberculosis. Monitoring programmes for chemical hazards should be more flexible and based on the risk of occurrence, taking into account FCI, which should be expanded to reflect the specific environmental conditions of the farms where the animals are reared, and the ranking of chemical substances, which should be regularly updated and include new hazards. Control programmes across the food chain, national residue control programmes, feed control and monitoring of environmental contaminants should be better integrated.

**Hygiene in wild meat.** 

This article presents some examples of zoonoses from the ingestion of wild pig and other game meat in Germany and the importance of meat hygiene procedures to avoid infections.

**Integrating animal health and food safety surveillance data from slaughterhouse control.** 

Surveillance at the slaughterhouse level for animal health and food safety purposes encompasses examination for the presence of pathology, pathogens, drug residues, chemical contaminants and antimicrobial resistance. Government, industry and academia are the primary proponents of such surveillance. A variety of policies and policy instruments from voluntary to legislative may be applied to promote or obligate participation. Efforts to integrate data across such diverse organisations encounter significant legal, logistical and financial challenges. Enhancement of policies to encourage effective integration of animal health and food safety surveillance data from slaughterhouse control should promote: a long-term approach; collaboration among government, industry and academia; application of a risk-based scheme; and transparent
public access to data, with generation of consumer-oriented communications derived from the data. A strong case can be made that the complementary pursuit of both sustainable animal health and food safety can continue to be aided by surveillance at the slaughterhouse level.

The aim of this review is to provide information on Trichinella infection in humans, livestock and wildlife in sub-Saharan Africa mainly focusing on geographical distribution of species/genotypes, biology, host range, life cycles and to identify research gaps. *Trichinella britovi, Trichinella nelsoni* and *Trichinella zimbabwensis* and one genotype (*Trichinella T8*) are known to occur in sub-Saharan Africa. Distinct geographic ranges with overlapping of some taxa in some areas have been observed. Genetic variants of *T. nelsoni* has been reported to occur among parasites originating from Eastern and Southern Africa and sequence heterogeneity also occurs among *T. zimbabwensis* isolates originating from different regions of Zimbabwe and South Africa. Field observations so far indicate that sylvatic *Trichinella* infections in the region are common in carnivores (mammals and reptiles) and to a lesser extent in omnivores. Cannibalism, scavenging and predation appear to be the most important routes of transmission and maintenance of the sylvatic cycles of the *Trichinella* taxa. To date, human trichinellosis has been documented in only four sub-Saharan countries (8.7%, 4/46). Bushpigs and warthogs have been the source of human infection with *T. britovi* and *T. nelsoni* being the aetiological agents. An increase in bushmeat trade and the creation of Transfrontier Conservation Areas (TFCAs) may have increased the risk of human trichinellosis in the region. With the creation of TFCAs in the region, sampling of wildlife hosts from protected areas of most sub-Sahara African countries is required to fully map the distribution of *Trichinella* species/genotypes in this region. More structured field surveys are still needed to determine the sylvatic host distribution of the different *Trichinella* taxa. Biological data of the *Trichinella* taxa in both wild and domestic animals of sub-Saharan Africa is very limited and further research is required. (C) 2012 Elsevier B.V. All rights reserved.

Humans suffer from several foodborne helminth zoonotic diseases, some of which can be deadly (e.g. trichinellosis, cerebral cysticercosis) while others are chronic and cause only mild illness (e.g. intestinal taeniosis). The route of infection is normally consumption of the parasite's natural host as a human food item (e.g. meat). The risk for infection with these parasites is highest wherever people have an inadequate knowledge of infection and hygiene, poor animal husbandry practices, and unsafe management and disposal of human and animal waste products. The design of surveillance and control strategies for the various foodborne parasite species, and the involvement of veterinary and public health agencies, vary considerably because of the different life cycles of these parasites, and epidemiological features. *Trichinella spiralis,* which causes most human trichinellosis, is acquired from the consumption of pork, although increasingly cases occur from eating wild game. For cysticercosis, however, the only sources for human infection are pork (*Taenia solium*) or beef (*T. saginata*). The chief risk factor for infection of humans with these parasites is the consumption of meat that has been inadequately prepared. For the pig or cow, however, the risk factors are quite different between *Trichinella* and *Taenia.* For *T. spiralis* the major source of infection of pigs is exposure to infected animal meat (which carries the infective larval stage), while for both *Taenia* species it is human faecal material contaminated with parasite eggs shed by the adult intestinal stage of the tapeworm. Consequently, the means for preventing exposure of pigs and cattle to infective stages of *T. spiralis,* *T. solium,* and *T. saginata* vary markedly, especially the requirements for ensuring the biosecurity of these animals at the farm. The surveillance strategies and methods required for these parasites in livestock are discussed, including the required policy-level actions and the necessary collaborations between the veterinary and medical sectors to achieve a national reporting and control programme.
Veterinary Parasitology 194(2-4): 128-132.
For more than 100 years, Trichinella spiralis (former Trichina spiralis) was considered a zoonotic parasite of the domestic habitat involving pigs, synanthetic rats and humans. In the last 70 years, there has been increasing evidence that the biomass of nematodes of the genus Trichinella is greater in wild animals than in domestic animals. Omnvores and carnivores (mammals, birds and some reptiles), mainly those with cannibalistic and scavenger behaviour, act primarily as reservoirs for the 12 Trichinella taxa recognized to date. The distribution areas of Trichinella spp. hosts can help to identify the environmental suitability where the different Trichinella taxa can be detected. Both the survival of larvae in decaying muscles of their hosts, which is favoured by high humidity and low temperatures, and human behaviour in the domestic and wild habitats play roles in the transmission patterns of these nematodes. Although Trichinella taxa develop in different host species circulating in different geographical regions, there is a common denominator among the hosts, namely their scavenging behaviour.

Since the discovery of hybridoma cells by Kohler and Milstein, the uses of monoclonal antibody (mAb), the protein produced by such cells are in vogue. Such antibodies with single isotype have higher specificity, and the serological tests employed in show higher reproducibility compared to those with use of polyclonal antisera. There are several procedures of mAb production which vary considerably but the principle remains the same which states that antigens introduced into animals generally result in the stimulation of lymphocytes, some of which produce antibody of only one type, although the isotype may change. The developments in the field of cell culture and transfection technology have lead to the production of improved qualities of mAbs. In general, monoclonal antibodies are important reagents used in biomedical research, such as, in the field of diagnostics and therapeutics as well as targeted drug delivery systems. They have got importance not only for infectious diseases caused by microbes and parasites, but also for cancer, metabolic and hormonal disorders, in the diagnosis of lymphoid and myeloid malignancies and tissue typing, enzyme linked immunosorbent assay (ELISA) (especially blocking ELISA), radio immunoassay (RIA), serotyping of pathogens and their immunological intervention with passive antibody, anti-idiotyp inhibition or magic bullet therapy with cytotoxic agents coupled with antimouse specific antibody. The application of mAbs in diagnosis of various livestock diseases is an important area of concern as these diseases are a major and increasingly important factor reducing livestock productivity in various parts of the world. In this context, the application of mAbs for diagnosis of important bacterial diseases viz., Anthrax, Brucellosis, Paratuberculosis, Leptospirosis, Listeriosis, Clostridial infections and Mycoplasmosis (CBPP), fungal diseases viz., Zygomycosis, Cryptococcosis, Histoplasmosis and Paracoccidioidomycosis, viral diseases viz., Foot-and-mouth disease (FMD), Infectious bovine rhinotachetitis/Infecctious pestular vulvovaginitis (IBR/IPV), Rota viral diarrhoea, Blue tongue, Rabies, Classical swine fever and re-emerging viral diseases like Hendra and Nipah viral infections and parasitic diseases viz., dirofilariosis, and Trichinellosis and haemportozoan diseases (including Trypanosomiasis, Leishmaniasis, Anaplasmiosis, infections caused by Plasmodium spp. as well as tick borne diseases) have been discussed thoroughly along with the specifications of the diagnostic assays for each disease for the convenience of the diagnosticians, researchers, scientists and students to employ such assays, both in field and laboratories to strengthen the disease control programme.

In the National Institute for Public Health and the Environment (Rijksinstituut voor Volksgezondheid en Milieu, RIVM), veterinarians have always played a role in the field of
public health. In 1921 the implementation of the law on meat hygiene was assigned to the Institute and in 1922 a separate veterinary division was created. In 1948 the name of this division was changed to Laboratory for Zoonoses and Pathology. Research comprised subjects such as salmonellosis and the bacteriology of eggs, egg products, and canned meat products. In 1960 the name was changed to the Laboratory for Zoonoses. The number of subjects expanded: salmonellosis, listeriosis, E. coli, resistance to antibiotics, food of animal origin, water and the environment, anisakiasis, trichinellosis, and studies on the (forbidden) administration of estrogens to calves. Veterinary Public Health continued to require the attention and input of RIVM veterinarians on problems such as avian influenza (H7N7) in 2003. In the compilation of the 2004 report on Emerging Zoonoses by The Health Council of The Netherlands, the expertise of the RIVM veterinarians played an important role. The recommendations led to the Emzoo (Emerging zoonoses) program, a system for early warning and surveillance. Recent problems such as the relation of antibiotics in animal husbandry to resistant bacteria in humans, as well as - since the outbreak of Q-fever - the health risks for living in the vicinity of farms, justify the conclusion that Veterinarians, the RIVM, and Veterinary Public Health are and will be a fruitful combination.


In Serbia, infection with Trichinella spp. has been recognized as a human health and animal husbandry problem for almost a century. The rate of swine infection gradually decreased from 0.14% to 0.02% between 2001 and 2010. For the past 5 years, Trichinella infections among swine were detected at levels higher than 0.05% in 3 districts of Serbia while prevalence persisted at lower levels for the rest of the country. During this 10-year period, there were 2257 cases of human trichinellosis, including 3 deaths; however, a significant decrease in the number of cases was reported during the last 5 years (fewer than 200 cases per year). The fact that prevalence data presented here are similar to prevalence data from 1990 indicates that this period of 10 years was needed to overcome the re-emergence of Trichinella infection in swine and humans that occurred during the last decade of the previous century. (C) 2013 Elsevier B.V. All rights reserved.


The occurrence of trichinellosis in a resident of the Netherlands prompted us to examine the likelihood of this originating from infected rats in spite of prevailing biosecurity and testing procedures. In so doing, we sought to calculate the possible risks for trichinellosis in countries deemed non-endemic. The infection risk was determined by simulating a scenario from a reservoir of minimally contaminated wildlife to pigs to humans. Results indicate that humans might become infected even in the event that artificial digestion had been performed on individually tested pig carcasses. Our conclusions justify reconsidering Trichinella control strategies based on the current testing protocol, and emphasize the importance of proper cooking as further insurance against human infection.


Recent molecular studies have revealed that many genes are mobilized during nurse cell formation, including those involved in activation and proliferation of satellite cells, dedifferentiation, cell cycle re-entry and arrest, apoptosis and transformation. This article reviews the kinetics of gene expression from a cellular biology point-of-view in an effort to dissect the complex events that lead to unusual pathological changes after a Trichinella infection.
In Germany and Poland, the high population density of the red fox (Vulpes vulpes) is considered a public health risk since this wild canid is one of the main reservoirs of Trichinella spp. In 2010 in Poland, a program to monitor the prevalence of Trichinella spp. in the red fox population was launched. After two years, Trichinella spp. larvae were detected in 44 (2.7%) out of 1634 foxes tested. In Germany in the period 2002-2011, Trichinella spp. larvae were in 27 foxes. The Trichinella species detected were: T. spiralis in 15 foxes from Germany (one co-infection with Trichinella britovi and one with Trichinella pseudospiralis) and in 9 foxes from Poland; T. britovi in 8 and 32 foxes from Germany and Poland, respectively; and T. pseudospiralis in 1 fox from Germany. The arctic species Trichinella nativa was detected in 3 foxes from Germany (one co-infection with Trichinella spiralis) and in 1 fox from Poland. The detection of T. nativa outside its known distribution area opens new questions on the ability of this Trichinella species to colonize temperate regions.

For several years, the demand for pork has been on the rise in Nepal. To assess the importance of pork as a carrier of zoonotic agents, we performed a cross-sectional study in the Kathmandu Valley of Nepal, in which we serologically determined the infection status of slaughtered pigs with regard to three of the most important parasites transmitted through pork consumption: Trichinella spp., Taenia solium cysticerci, and Toxoplasma gondii. From 2007 to 2010, 742 pigs were sampled at slaughter, of which 0.1% (95% confidence interval [CI] 0.0-0.7%) were found positive for Trichinella infection, 13.8% (95% credibility interval [CrI] 0.8-28.5%) for T. solium cysticercosis, and 11.7% (95% CI 5.2-17.5%) for Toxoplasma infection. Further monitoring of the related animal and human disease burden and strengthening of food safety protocols throughout the pork production chain are strongly recommended.

Black bears (Ursus americanus) are hosts for two important zoonotic parasites, Toxoplasma gondii and Trichinella spp. and bears are hunted for human consumption in the USA. Little is known of the genetic diversity of T. gondii circulating in wildlife. In the present study, antibodies to T. gondii were found in juice-from-tongues of 17 (25.7%) of 66 wild black bear from Maryland during the hunting season of 2010 and 2011. Antibodies to T. gondii were assessed by the modified agglutination test. Tongues of 17 seropositive bears were bioassayed in mice and viable T. gondii was isolated from three samples. These three T. gondii isolates (TgBbMd1-3) were further propagated in cell culture and DNA isolated from culture-derived tachyzoites was characterized using 11 PCR-RFLP markers (SAG1, 5' and 3'-SAG2, alt.SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1 and Apico). Results revealed three genotypes. TgBbMd1 is a Type 12 strain (ToxoDB PCR-RFLP genotype #4) and TgBbMd2 is ToxoDB PCR-RFLP genotype #216, and TgBbMd3 is a Type II clonal strain (ToxoDB PCR-RFLP genotype #1). The isolate TgBbMd2 was highly virulent for outbred Swiss Webster mice; all infected mice died of acute toxoplasmosis. Results indicate that mouse virulent strains of T. gondii are circulating in wildlife in the USA. These 66 tongues in addition to tongues collected during hunts in previous years were further investigated for the presence of muscle larvae of Trichinella spp. Tongues from 40 bears in 2005,41 in 2006, 51 in 2007, 56 in 2008, 68 in 2009, 67 in 2010, and 66 in 2011 were subjected to digestion with pepsin/HCl and microscopic examination. Two bears were infected with Trichinella spp.; one in 2008 and one in 2009. Genotyping of collected muscle larvae revealed that the infecting species in
both cases was Trichinella murrelli.


*Toxoplasma gondii* is among the most studied parasites worldwide but there is not much information about it published in Ireland. The objectives of this study were to determine the seroprevalence of *T. gondii* in sheep, pigs, deer and chickens and the molecular detection of *T. gondii* DNA in muscle tissue. Serum samples were collected from these species at the time of slaughter at Irish abattoirs during 2007 and tested for anti-*T. gondii* antibodies using a commercial semi-quantitative latex agglutination test. Antibodies (titre 1:64) were found in 36% (105/292) sheep, 4.7% (15/317) pigs and 6.6% (23/348) deer. In chickens, 18% (65/364) had antibody titres, ranging between 1:5 and 1:1024. Significant (P<0.05) age-related differences in seroprevalence were found in adult sheep (58.1%) and pigs (23.1%). Significant gender differences in seroprevalence was also found in sheep with more females (43%) than males (22.4%) being positive. However, when adjusted for age through logistic regression gender was no longer significant. Seroprevalence was also evaluated on farm locations grouped to NUTS level 3, but the prevalence was too low to draw any statistical conclusions. Using a nested PCR, the presence of *T. gondii* DNA was detected in diaphragm samples from 3.6% (3/83) sheep, 13.0% (3/23) pig and 4.2% (3/71) deer. Meat digestion liquids from a Trichinella spp. survey in pigs were also used for the first time to detect *T. gondii*. *Toxoplasma gondii* DNA was detected in 50% (10/20) of pooled samples. This is the first in depth study of *T. gondii* seroprevalence in animals in Ireland and a novel method, using digestion liquid from pooled diaphragm samples, for PCR detection in pigs is described.


*Trichinella zimbabwensis* has been found naturally infecting crocodiles (Crocodylus niloticus) in Zimbabwe, Mozambique, Ethiopia and South Africa, as well as monitor lizards (Varanus niloticus) in Zimbabwe. The reports on natural infections were mostly accidental rather than structured surveys and involved very few animals. Previous surveillance studies in South Africa reported a 38.5% prevalence of *T. zimbabwensis* among wild crocodiles tested from the Mpumalanga province and Kruger National Park (KNP). No studies have been conducted to date on the geographical distribution and occurrence of *T. zimbabwensis* in wild crocodiles and varans in countries in southern Africa. Recent outbreaks of pansteatitis in crocodile populations of the KNP, South Africa, provided an opportunity to conduct a more structured survey aimed at elucidating the occurrence and distribution of *T. zimbabwensis* in culled wild crocodile populations within the KNP. Results from this study showed that *T. zimbabwensis* occurred in 10 out of 12 culled crocodiles from the KNP. The results also showed that the natural distribution of *T. zimbabwensis* in crocodiles includes all the major river systems in the KNP. The predilection sites of larvae in muscles followed a different pattern in naturally infected crocodiles compared to observations in experimentally infected mammalian hosts.


This report compiles the available information on unwanted horses in Ireland for 2011 and 2012 and builds upon the previous report for the period 2005 to 2010. Similar trends are present in the high value responsible ownership category and the practicing veterinary profession although extensively involved in horse welfare, euthanises a small proportion of Ireland’s unwanted horses. Welfare groups have limited resources and a limited ability to deal with such an extensive problem, which has involved very large numbers of horses. Local authorities continue to have to devote significant efforts and calls on public finances to deal with unwanted horses. Those that they have to deal with are, in the main, not
identifiable by either passports or microchips. Category 2 plants and abattoirs continue to provide the principal means of disposal of unwanted horses. The need for abattoirs continues to increase and it is essential that these facilities remain in operation. They processed more than 49,000 horses between 2010 and 2012. The samples they have to submit for Trichinella testing are the most sensitive indicator of the extent of the unwanted horse problem and the most immediate source of information on when it may begin to abate. Trichinella sample numbers and this by inference, horses ponies and donkeys sent to slaughter have fallen by some 35% from 2012 numbers, in the year to date (2013). This may reflect the commercial decision to cease horse slaughter by two slaughterhouses that had hitherto provided this service. Their commercial decision was not in any way related to the identification of fraudulent mislabeled beef in other plants.

Ludovisi, A., et al. (2013). Development of an ELISA to detect the humoral immune response to Trichinella zimbabwensis in Nile crocodiles (Crocodylus niloticus). Veterinary Parasitology 194(2-4): 189-192. Crocodiles are known reservoir hosts of Trichinella papuae and Trichinella zimbabwensis, two zoonotic parasites that also infect mammals. Since commercial crocodile farming represents a key source of income in several countries, it is important to monitor this nematode infection in both farmed crocodiles and in breeding stocks which are frequently introduced from the wild. For this purpose, an indirect ELISA was developed to detect the anti-Trichinella immune response in crocodile sera. New Zealand rabbits were immunized with pooled sera from non-infected farmed crocodiles in the presence of Freund's complete adjuvant. The anti-crocodile serum was then conjugated with horseradish peroxidase. Serum samples from four Nile crocodiles (Crocodylus niloticus) experimentally infected with T. zimbabwensis and from four uninfected crocodiles were used to set up the ELISA. The larval burden per gram of muscle tissue was determined by muscle biopsy. The test was performed on serum samples from an additional 15 experimentally infected crocodiles as well as eight wild Nile crocodiles. Among the 19 experimentally infected crocodiles, sero-conversion was observed in 11 animals. The highest antibody response was observed six weeks post infection (p.i.), but in most of these animals, antibodies were not detectable after six weeks p.i. even though live larvae were present in the muscles up to six months p.i. (C) 2013 Elsevier B.V. All rights reserved.

Mirilovic, M., et al. (2013). Distribution and transmission tendency of trichinellosis in wild boars (Sus scrofa) at the territory of Serbia. Veterinarski Glasnik 67(3/4): 187-199. Trichinellosis (trichinellosis) is a disease common for both people and animals, which was mentioned in even some several centuries old notes. As well as domestic pigs, wild boars, being omnivores and the animals that broadly cover the territory of Serbia, could definitely be considered as one of possible trichinellosis indicators, and that is the main reason for starting this investigation with the objective to perceive the level of infection of wild boars in different hunting areas at the territory of the Republic of Serbia. In this work it is presented the distribution of T. spiralis in wild boars at the territory of Serbia in the period from 2006 to 2010. Besides the distribution of trichinellosis in wild boars, we have also calculated the tendency of changing in the number of infected wild boars at the territories of different hunting associations, in the period from 2006. to 2010. The distribution of T. spiralis in shot wild boars showed that trichinellosis appeared at the total of 24 hunting associations territories during the investigated period. Only at the territories of Pirot and Dimitrovgrad hunting associations, there was diagnosed at least one case of trichinellosis in shot wild boars in all five years. Out of the total of shot and inspected wild boars (20.250) in 123 of them, there was diagnosed the presence of T. spiralis infectious forms (0,61%). The greatest number (29) of the infected wild boars was shot in 2007. By analysing the change in number of positive wild boars in the interval from 2006. to 2010. It was found out that there is a constant tendency of growth, namely 1.1 head of boar per year. Change in number of trichinellosis wild boars by years of observation is a second degree polynomial (Y=0,6+17,81x-2,79x^2) with correlation coefficient of 0,69. On the basis of the obtained analyses that were carried out it can be concluded that the
occurrence of trichinellosis is most frequent in border areas towards Bulgaria, Romania, Hungary, Croatia and Bosnia and Herzegovina.


The aim of this study was to determine the presence of trichinellosis in backyard-farmed pigs and the risk factors associated with the infection in Zaria, Kaduna State. Serum samples were collected from 120 pigs selected at random from 50 small backyard farms, and the presence of Trichinella spp. antibodies was determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit. Data on farm management practices from the farms were obtained through the use of a structured questionnaire. The overall seroprevalence of Trichinella spp.-specific antibodies was 40 % (48/120) by ELISA. All the extensive farms sampled had at least one Trichinella-positive animal. The age and sex of the animals were not significantly (p > 0.05) associated with the infection; however, the management systems, presence of rodents, rodent control, and access to dead pigs showed significant (p < 0.05) association with Trichinella spp.-infected pigs on the farm. In conclusion, there was a high prevalence of antibodies to trichinellosis in backyard raised pigs in Zaria, and intensive pig farming with the adoption of proper biosecurity measures is advocated to prevent the transmission and spread of trichinellosis.


Trichinellosis is an important emerging or re-emerging zoonotic disease in Southeast Asia. In Vietnam, data on trichinellosis are scarce. Therefore, the present study was designed to determine the seroprevalence of trichinellosis in the domestic lifecycle in two provinces of northwestern Vietnam, where recently isolated outbreaks of human trichinellosis occurred. Serum samples were obtained from 558 pigs, 125 dogs and 98 cats, transported on filter paper, and tested for Trichinella antibodies by ELISA and Western blot, using larval excretory-secretory (E/S) antigens. The overall seroprevalence of antibodies to Trichinella was 5.6%, 4% and 0% in pigs, dogs and cats, respectively. In pigs, positive cases were distributed in 8/20 districts of the two provinces. This study suggests that Trichinella spp. is circulating in the domestic life cycle in northwestern Vietnam. Further study is recommended to investigate the presence of Trichinella in a sylvatic cycle, and to identify the occurring Trichinella species. (C) 2012 Elsevier B.V. All rights reserved.


Background: In many parts of the developing world, pigs are kept under low-input systems where they roam freely to scavenge food. These systems allow poor farmers the opportunity to enter into livestock keeping without large capital investments. This, combined with a growing demand for pork, especially in urban areas, has led to an increase in the number of small-holder farmers keeping free range pigs as a commercial enterprise. Despite the benefits which pig production can bring to a household, keeping pigs under a free range system increases the risk of the pig acquiring diseases, either production-limiting or zoonotic in nature. This study used Global Positioning System (GPS) technology to track free range domestic pigs in rural western Kenya, in order to understand their movement patterns and interactions with elements of the peri-domestic environment. Results: We found that these pigs travel an average of 4,340 m in a 12 hr period and had a mean home range of 10,343 m2 (range 2,937-32,759 m2) within which the core utilisation distribution was found to be 964 m2 (range 246-3,289 m2) with pigs spending on average 47% of their time outside their homestead of origin. Conclusion: These are the first data available on the home range of domestic pigs kept under a free range system: the data show that pigs in these systems spend much of their time scavenging outside their homesteads, suggesting that these pigs may be exposed to
infectious agents over a wide area. Control policies for diseases such as Taenia solium, Trypanosomiasis, Trichinellosis, Toxoplasmosis or African Swine Fever therefore require a community-wide focus and pig farmers require education on the inherent risks of keeping pigs under a free range system. The work presented here will enable future research to incorporate movement data into studies of disease transmission, for example for the understanding of transmission of African Swine Fever between individuals, or in relation to the life-cycle of parasites including Taenia solium.


Trichinella infections are endemic in the Balkan region of Europe. Though trichinellosis and agents thereof are serious problems for human health and animal husbandry, only a limited number of Trichinella isolates from Serbia have been identified at the species level so far. The aim of the present study was the surveillance and monitoring of Trichinella in domestic pigs and wild animals from the endemic district of Braničevo. Investigations performed during the 2009-2010 period revealed Trichinella infections in 344 out of 282,960 (0.12%) domestic pigs. Among wildlife, Trichinella infections were detected in 11 out of 94 (11.7%) wild boars (Sus scrofa), 7 out of 57 (12.3%) red foxes (Vulpes vulpes), 7 out of 13 (53.8%) golden jackals (Canis aureus), and in all three examined wolves (Canis lupus). Trichinella spiralis and Trichinella britovi were the only two species identified. T. britovi was identified in 31% of isolates from wildlife of the Braničevo district and T. spiralis was found in 53% of wild animals; mixed infections were observed in 16% of the animals examined. Findings form the basis of an information campaign for veterinary services, pig owners and the hunter’s associations about the risk of the transmission of these zoonotic agents. The application of control programs as established at the Veterinary Specialist Institute of Pozarevac resulted in a decline in Trichinella infections among domestic pigs and the absence of human trichinellosis in the last three years in the Braničevo district.

OUTBREAKS


The presence of the parasite Trichinella spiralis in humans does not always manifest itself with obvious clinical symptoms; the clinical manifestations of trichinellosis are polymorphic and can cause diagnostic difficulties. Our aim was to identify risk factors that can be linked to the severity of the disease. We conducted a retrospective analysis of 143 cases of trichinellosis admitted to the Infectious Disease Hospital in Brasov, Romania during 2001-2008. We found that children 10 and younger were less prone to exhibit medium or severe symptoms. Patients with leukocytosis had a 1.75 times higher risk of developing medium to severe symptoms relative to those with normal white blood cell counts. Patients with high eosinophil counts had a 2.05 times higher risk of exhibiting average or serious symptoms relative to those with low or normal eosinophil counts. Repeated-consumption of contaminated meat increased the chances of developing discernible forms of the disease by 5.25 fold when compared to those who only occasionally ate meat contaminated with Trichinella. Regular consumption of raw or undercooked meat increased the chances of developing medium or severe forms of this disease by 1.67 times when compared to those who consume meat that had been thoroughly cooked.


Currently, Trichinella spiralis is the only species that has been identified in Chile as an agent of trichinellosis in both domestic and wild hosts. Preliminary studies have not identified the infection in animals native to Chile. We report the first finding of Trichinella sp. isolated in diaphragmatic and intercostal muscle of a puma (Puma concolor puma)
found dead by telemetry tracking and subjected to necropsy on the mountain range of Region de La Araucania, Chile. The diagnosis was made by trichinoscopy and artificial digestion. This finding demonstrates the presence of Trichinella sp. in chilean wild carnivorous animals.

Nematode worms of the genus Trichinella are one of the most widespread zoonotic pathogens. Natural transmission between hosts can only occur through the ingestion of infected meat. To date, two Trichinella species are known to be etiological agents of disease among domestic animals and wildlife in Poland: T. spiralis and T. britovi. In the last decades, since the administration of an oral vaccination against rabies, the red fox population in Poland has increased exponentially. The study area covers the Nowy Targ region: a mountainous area (585–1138 m above the sea) in southern Poland. Of 24 red foxes examined in the study, four were infected with Trichinella isolates: three were identified as T. britovi and one as T. pseudospiralis. The muscle of red foxes infected with T. britovi harboured 2.75, 3.11, 4.4 LPG and with T. pseudospiralis 0.36 LPG. Trichinella larvae were identified at species level by genomic and mitochondrial multiplex PCR, the products of which were sequenced for comparison with other sequences available in GenBank. The sequences obtained from the Polish T. pseudospiralis isolate, deposited in GenBank under the accession numbers JQ809660.1 and JQ809661.1, matched sequences already published in GenBank. Sequence comparison showed a 100% match with the large subunit ribosomal RNA gene of T. pseudospiralis isolate ISS 013, and a 96-95% match with those of T. pseudospiralis isolates ISS 141 and ISS 470. This is the first report of the identification of T. pseudospiralis larvae from red fox in Poland.

Infective muscle larvae (ML), adults (Ad) and new born larvae (NBL) of Trichinella spiralis express many immunogenic proteins which can elicit a host protective response, and may be useful in the diagnosis of Trichinella infected humans and animals. The present study was carried out to identify T. spiralis antigens recognized by antibodies from pigs infected with T. spiralis. To that end, the crude extracts of ML, Ad, NBL and ML excretory-secretory (E-S) and Ad E-S proteins were analyzed by sodium dodecyl sulfate polycrystalline gel electrophoresis (SOS-PAGE). To identify antigens of T. spiralis that are recognized by host antibodies, crude extracts and E-S proteins were subjected to immunoblot with antisera derived from pigs experimentally infected with 200 or 20,000 T. spiralis ML. Searching for T. spiralis antigens with diagnostic potential, immunoblots showed that all T. spiralis antisera, regardless of the infective dose, recognized common proteins in each examined life stage with molecular weights around 20-27 kDa, 41 kDa and 197-105 kDa. Interestingly, all the common proteins were detected by T. spiralis sera throughout the infection, from 5 days post infection (dpi) to 60 dpi. These results extend our knowledge of specific antigenic components of T. spiralis. The finding of common components among all T. spiralis life stages may be useful in the preparation of parasite antigens for diagnostic use, as these antigens are relevant regardless of infection phase.

Background: Trichinella spiralis is a zoonotic tissue-dwelling parasitic nematode that infects humans and other mammals. Its surface proteins are recognized as antigenic in many infected hosts, being directly exposed to the host's immune system and are the main target antigens that induce the immune responses. The larval surface proteins may also interact with intestinal epithelial cells and may play an important role in the invasion and development
process of T. spiralis. The purpose of this study was to analyze and characterize the surface proteins of T. spiralis muscle larvae by two-dimensional gel electrophoresis (2-DE) and mass spectrometry. Methods: The surface proteins of T. spiralis muscle larvae were stripped from the cuticle of live larvae by the cetyltrimethylammonium bromide (CTAB) and sodium deoxycholate. The surface protein stripping was examined by an immunofluorescent test (IFT). The surface proteins were analyzed by SDS-PAGE and Western blotting, and then identified by 2-DE and MALDI-TOF/TOF mass spectrometry analysis. Results: The IFT results showed that the surface proteins-stripped larvae were not recognized by sera of mice immunized with surface antigens. Western blotting showed 7 of 12 protein bands of the surface proteins were recognized by mouse infection sera at 18 dpi and at 42 dpi. The 2-DE results showed that a total of approximately 33 proteins spots were detected with molecular weights varying from 10 to 66 kDa and isoelectric point (pi) from 4 to 7. Twenty-seven of 33 protein spots were identified and characterized to correlate with 15 different proteins. Out of the 14 proteins identified as T. spiralis proteins, 5 proteins (partial P49 antigen, deoxyribonuclease II family protein, two serine proteases, and serine proteinase) had catalytic and hydrolase activity. All of these 5 proteins were also associated with metabolic processes and 2 of the five proteins were associated with cellular processes. Conclusions: In this study, T. spiralis muscle larval surface proteins have been identified, which will provide useful information to elucidate the host-parasite interaction, identify the invasion-related proteins, early diagnostic antigens and the targets for a vaccine.


An immunochromatographic strip method, developed with the excretory-secretory antigens from muscle larvae (ML) of Trichinella spiralis labeled with colloidal gold, was used for the detection of anti-Trichinella antibodies in serum of experimentally-infected swine. Sera from swine infected with 200, 2000 and 20,000 infective ML were collected at different days post infection (dpi) and used to evaluate the method. The strip method was shown able to detect anti-Trichinella antibodies by 35 dpi, 28 dpi and 21 dpi for the three different infection doses, respectively, and closely correlated with the results of an ELISA test. The strip method is rapid and easy to perform and is suggested as an acceptable alternative for clinical laboratories lacking specialized equipment, and for field diagnosis of trichinellosis.


An antigen detection kit (Trichin-L), based on latex agglutination and developed by the Bio-Rad company was validated at five European laboratories. The validation parameters included specificity, sensitivity, robustness and reproducibility. Specificity was evaluated by testing parasite antigens from five non-Trichinella parasites in addition to the Trichinella genus. To evaluate sensitivity, 10 pork samples spiked with 1, 3, 6 or 15 Trichinella larvae were tested in each laboratory. To evaluate the robustness of the test, the solubilized antigens were maintained at room temperature and tested at different times. Reproducibility was assessed in each laboratory using 40, 100 g minced pork samples, each spiked with Trichinella spiralis. The use of larval homogenates obtained from the Trichin-L kit as a template for parasite identification at the species level by a multiplex PCR, was also evaluated. The results showed a high specificity and sensitivity where solubilized antigens maintained their stability and reactivity for up to three days. Reproducibility was high, as similar results were obtained in the five laboratories. The larval homogenates obtained using the Trichin-L kit were successfully used in multiplex PCRs to identify Trichinella species.


Freeze-tolerance of encapsulated Trichinella muscle larvae (ML) is mainly determined by Trichinella species, but is also influenced by host species, the age of the infection and the storage time and temperature of the infected meat. Moreover, the freeze-tolerance of the encapsulated species appears to be correlated to the development of thick capsule walls.
which increases with age. An extended infection period and the muscle composition in some hosts (e.g. herbivores) may provide freeze-avoiding matrices due to high carbohydrate contents. The present experiment compares freeze-tolerance of Trichinella spiralis and Trichinella britovi ML in wild boar meat 24 weeks post inoculation (wpi). Three groups of four wild boars were infected with 200, 2000 or 20,000 ML of T. britovi (ISS 1575), respectively. Additionally, three wild boars were inoculated with 20,000 ML of T. spiralis (ISS 004) and two animals served as negative controls. All wild boars were sacrificed 24 wpi. Muscle samples of 70 g were stored at -21 degrees C for 19,30 and 56 h, and for 1-8 weeks. Larvae were recovered by artificial digestion. Their mobilities were recorded using Saisam (R) image analysis software and their infectivities were evaluated using mouse bioassays. Samples frozen for 19, 30 and 56 h allowed recovery of mobile ML, but samples frozen for 1 or 2 weeks did not. Correspondingly, only T. spiralis and T. britovi larvae isolated from wild boar meat frozen for 19,30 and 56 h established in mice. This study showed that freezing at -21 degrees C for 1 week inactivated T. spiralis and T. britovi ML encapsulated in wild boar meat for 24 weeks.

Comparison of three molecular detection methods for detection of Trichinella in infected pigs. 
Parasitology Research 112(5): 2087-2093.

Different molecular detection methods require diverse molecular platforms, but there is no uniform standard for people to reference in the detection of Trichinella. In this study, real-time PCR, loop-mediated isothermal amplification (LAMP), and conventional PCR were developed for the detection of Trichinella by targeting mitochondrial large subunit ribosomal DNA (mt-lsrdNA). We compared the performance of the three newly developed assays. The results revealed that the detection limits of the real-time PCR, LAMP, and conventional PCR assays were 10 and 100 fg/μL and 1 pg/μL of Trichinella spiralis genomic DNA, respectively. The assays were used in the detection of Trichinella in the field. A total of 192 samples were obtained from pigs: 75 samples from free range farming and 117 from intensive feeding factory. The infection rate was 8/192 (4.2 %), 7/192 (3.6 %), and 1/192 (1.0 %) through the real-time PCR, LAMP, and conventional PCR assays, respectively. These data indicate that Taqman real-time PCR was a rapid, specific, and sensitive tool as a preferred option for investigation of valuable samples, but that LAMP assay was closed tube, highly sensitive, cost-effective, rapid, easy-to-perform, and was the optimal choice for detection of Trichinella in the field. The results of a model of experimental infection in mice indicated that spleen can be used as sampling site for the detection of early T. spiralis infection. However, the diaphragm and myocardium were the most suitable sampling sites for the detection of T. spiralis.

Detection of circulating antigen in serum of mice infected with Trichinella spiralis by an IgY-IgM mAb sandwich ELISA.
Experimental Parasitology 133(2): 150-155.

In this study, a sandwich ELISA based on IgY (egg yolk immunoglobulin) and IgM monoclonal antibody (mAb) against excretory-secretory (ES) antigens of Trichinella spiralis muscle larvae was developed for detection of circulating antigens (CAg) in serum from mice infected with T. spiralis. The IgY-IgM sandwich ELISA involved the use of chicken antibody IgY as a capture antibody and mouse IgM mAb 5A7G4 as a detecting antibody. This assay was able to detect as little as 1 ng/ml of ES antigens added to normal mouse serum. Two groups of BALB/c mice infected with T. spiralis larvae were used: heavily infected mice (20 mice infected with 300 larvae) and lightly infected mice (20 mice infected with 100 larvae) and 10 normal mice as control. The CAg was detectable as early as 4 days post infection (dpi) in the sera from both groups of infected mice, then increased rapidly, reached a peak with detection rate of 100% in heavily infected mice at 10 dpi and 80% in lightly infected mice at 22 dpi, respectively. The anti-Trichinella IgM antibodies was first detected in 40% of heavily infected mice and 20% of lightly infected mice at 8 dpi, and reached a peak positive rate of 100% in heavily infected mice at 16 dpi and in lightly infected mice at 26 dpi, respectively. The novel assay appears to be sensitive for detection of antigens of T. spiralis and valuable to the early diagnosis of trichinellosis.

Although larvae of the genus Trichinella are the most common parasite species detected in vertebrate muscles using artificial digestion, nematode larvae belonging to other genera are sometimes detected and incorrectly identified as Trichinella. However, it is often very difficult to identify these larvae at the species, genus or family level using microscopy because of the absence of specific morphological characters or cuticle damage, and the only means of identification is PCR and sequencing of specific molecular markers (12S mtDNA; COI; 18S rDNA; and ITS1). From 2008 to 2011, 18 nematode isolates not belonging to the genus Trichinella were collected from different host species. Eleven of these isolates were successfully identified at the species, genus or superfamily level: larvae from two common kestrels, three hooded crows, a hen harrier and a domestic pig were identified as Toxocara cati; larvae from a badger were identified as Toxocara canis; larvae from a domestic pig were identified as a free-living nematode of the genus Panagrolaimus; larvae from a wild boar were identified as belonging to the Metastrongylus genus; and larvae from a rough-legged buzzard were identified as belonging to the superfamily Filarioidea. The recovery of nematodes belonging to genera other than Trichinella during routine meat inspection suggests that the persons performing the analyses need to be informed of the possibility of false positives and that a molecular-based identification system that allows for a rapid and reliable response must be adopted (i.e., a DNA barcoding-like system).


The consumption of raw or undercooked Trichinella infected meat, especially pork and horse meat, can have important implications for public health. Therefore each animal carcass from a Trichinella susceptible species intended for human consumption must be examined for Trichinella. Laboratories carrying out testing of official control samples must undergo a quality assurance program and should regularly participate in proficiency testing schemes. To date, Trichinella proficiency samples are prepared with live larvae, which, as a level 2 pathogen, require specific shipping and disinfection procedures. Therefore, the suitability of using inactivated Trichinella larvae as proficiency samples was tested. We found that Trichinella larvae treated with 2% formaldehyde for 24h had lost their infectivity and showed a comparable recovery rate to naive larvae after artificial digestion, albeit with a prolonged sedimentation time.


Emerging and re-emerging zoonotic diseases affect both public and animal health and require the development and contemporary implementation of suitable detection methods. A growing number of findings of the mesocercarial stage of the digenean trematode Alaria alata in game inhabiting wetlands have necessitated the development of a specific detection method. With the Alaria spp. mesocercariae migration technique (AMT), a specific and sensitive detection method is now available. To make the method accessible to the official controls, method validation is necessary. In this context, interlaboratory tests (IT) are a key factor to demonstrate both (1) the suitability of the respective method and (2) the reference materials. In the first IT performed on this issue, 15 laboratories from nine German federal states took part. Every lab received two negative and four positive standard samples each as well as a standardized examination device for AMT, and a standard operating procedure. All participating laboratories showed very good results in terms of qualitative analysis: 96.7 % of the samples were assessed correctly positive or negative. An analysis of the qualitative performance shows that 263 (58.4 %) of 450 mesocercariae that were inserted in the meatballs were identified by the participants, and 5 (33.3 %) out of 15 labs were able to count at least 70 % of the Alaria spp. mesocercariae. A direct comparison with the results of the
German Trichinella IT, which were conducted since 2004, shows that the overall sensitivity of the AMT is even higher than that registered for the reference method for Trichinella detection (e.g., 93 % in 2010). Also, in terms of quantitative analysis, AMT stands up to the comparison with the results from the German Trichinella IT. The refinement of the implementation protocol of this innovative, easy-to-use and cost-effective method harbours great potential for further optimization and successful implementation in the official controls.


Proficiency testing (PT) is the use of inter-laboratory comparisons to determine the performance of individual laboratories for specific tests or measurements, and to monitor a laboratory's performance. Participation in proficiency testing provides laboratories with an objective means of assessing and demonstrating the reliability of the data they are producing. To ensure the reliability of Trichinella detection and meat hygiene within the European Union and afford optimal protection to the consumer, PT is conducted under the direction of the European National Reference Laboratories for Trichinella. Evaluation of data from the national PT showed that lab-internal shortcomings are frequent. These shortcomings are specifically related to: (1) improper sample collection and preparation; (2) incorrect transposition and application of the protocol as laid down in Annex I, Chapter I, Nr. 3 (a-g) of the Commission Regulation (EC) No. 2075/2005; (3) insufficient sedimentation times; and (4) improper equipment (e.g., Prost and Nowakowski, 1990; Rossi and Pozio, 2008; Forbes and Gajadhar, 1999, Rossi and Pozio, 2008). To test the hypothesis that both method based errors as well as internal lab errors can influence the accuracy and precision of the magnetic stirrer method for pooled sample digestion (MSM), we initiated a study to evaluate the analytical uncertainty of the MSM. Results presented here are based on: (i) data from PT in Germany (2008, 2009, and 2010); (ii) within-lab performance conducting high volumes of MSM; (iii) larval recovery experiments; and (iv) statistical evaluation of data resulting from these procedures. Quantitative data from the PT show that on average only 60% of Trichinella larvae were detected. Even laboratories that showed relatively good performance (>80% larva recovery, no false negative or false positive results), frequently reported samples with an unexpectedly low larval count (loss of >2 larvae). In our own laboratory, high numbers of repeated analyses of standards and re-analyses of residual fluids indicated that these outliers could be described by a binomial distribution based on a laboratory-specific Trichinella-detection probability. Results of recovery experiments indicate that only a part of the total larval losses can be attributed to lab-internal shortcomings inasmuch as a significant number of L1 could be isolated from the residual and washing fluids. (C) 2013 Elsevier B.V. All rights reserved.


Real-time fluorescence resonance energy transfer (FRET) PCR and melting curve analysis using newly developed fluorophore-labeled hybridization probes were applied for the detection of Trichinella spiralis DNA in muscle of mice following oral inoculation with 300 T. spiralis larvae. The developed assay could detect and differentiate T. spiralis, Trichinella papuae, and Trichinella pseudospiralis DNAs by the different melting temperatures (Tm). The assay had a detection limit of 5x10^2 positive control plasmid copies, which was equivalent to 1ng of T. spiralis DNA spiked into 250mg of muscle sample. No fluorescence signal was detected when the technique was applied to the DNA of 27 parasites other than Trichinella spp. The assay could detect T. spiralis DNA in muscle at 7, 14, and 21 days postinoculation. The range, mean +/- standard deviation, and median of the Tm values of all positive muscle tissue samples were 60.4-60.8, 60.6 +/- 0.2, and 60.5, respectively. This assay provides an effective tool for the specific, sensitive, and high-throughput detection of T. spiralis DNA in muscle during the early stage of infection. In addition, the technique can be useful for epidemiologic surveillance in naturally infected wildlife.
**A bead-based suspension array for the serological detection of Trichinella in pigs.**  
The feasibility of using bead-based suspension arrays to detect serological evidence of Trichinella in pigs was assessed. Trichinella spiralis excretory-secretory antigen was covalently coupled to paramagnetic beads and used to bind serum antibodies, which were subsequently detected using anti-swine antibody. The assay was evaluated by testing pig sera from farms where trichinellosis was endemic and comparing the results with those obtained using two commercially available ELISAs. With cut-offs established by receiver operating characteristic (ROC) analysis, digestion-negative sera from a Trichinella-free population of pigs were deemed seronegative. When anti-swine antibody was replaced with protein A/G, higher test sensitivity (94% vs. 88%) at similar test specificity (95%), was achieved. The potential use of this assay in species other than swine was also demonstrated by testing human sera. (C) 2012 Elsevier Ltd. All rights reserved.

**IMMUNOLOGY**  
**A prime-boost vaccination of mice with attenuated Salmonella expressing a 30-mer peptide from the Trichinella spiralis gp43 antigen.**  
Veterinary Parasitology 194(2-4): 202-206.  
Protection against Trichinella infections has been achieved using various parasite antigens and adjuvants. Recently, we reported that immunization of mice with an attenuated Salmonella strain displaying a 30-mer peptide (residues 210-239) from the Trichinella spiralis gp43 antigen using the ShdA autotransporter induced partial protection against T. spiralis infection. To improve the efficacy of vaccination, we used the MisL autotransporter system to display the Ts30mer peptide on the surface of Salmonella enterica ser. Typhimurium in combination with a prime-boost vaccination strategy. This vector and immunization regimen induced superior protection against T. spiralis when compared to our previously reported approach. Data presented herein showed a significant reduction in adult worm and muscle larvae burdens, high IgG titers, and increased production of intestinal mucus with entrapped adult worms. This prime-boost vaccination scheme is a suitable strategy to elicit enhanced protective immunity against T. spiralis. (C) 2013 Elsevier B.V. All rights reserved.

**Interleukin-25 (IL-25) Promotes Efficient Protective Immunity against Trichinella spiralis Infection by Enhancing the Antigen-Specific IL-9 Response.**  
Infection and Immunity 81(10): 3731-3741.  
Mammalian hosts often develop distinct immune response against the diverse parasitic helminths that have evolved for immune evasion. Interleukin-25 (IL-25), an IL-17 cytokine family member, plays a key role in initiating the protective immunity against several parasitic helminths; however, the involvement and underlying mechanisms by which IL-25 mediates immune response against Trichinella spiralis infection have not been investigated. Here we showed that IL-25 functions in promoting protective immunity against T. spiralis infection. Mice treated with IL-25 exhibited a lower worm burden and fewer muscle larvae in the later stage of T. spiralis infection. In contrast, mice treated with neutralizing antibody against IL-25 failed to expel T. spiralis effectively. During T. spiralis infection, intestinal IL-25 expression was rapidly elevated before the onset of IL-4 and IL-9 induction. While antigen-specific Th2 and Th9 immune responses were both developed during T. spiralis infection, an antigen-specific Th9 response appeared to be transiently induced in the early stage of infection. Mice into which antigen-specific T cells deficient in IL-9 were transferred were less effective in worm clearance than those given wild-type T cells. The strength of the antigen-specific Th9 immune response against T. spiralis could be enhanced or attenuated after treatment with IL-25 or neutralizing antibody against IL-25, respectively, correlating positively with the levels of intestinal mastocytosis and the expression of IL-9-regulated genes, including mast cell- and Paneth cell-specific genes. Thus, our study demonstrates that intestinal IL-25 promotes protective immunity against T. spiralis infection by inducing antigen-specific Th9 immune response.

The aim of this study was to evaluate differences between the small and large intestines (SI and LI) with regard to colonization and immunity during infection with *Trichinella spiralis*. In orally infected C57BL/6 mice, the gender ratios of worms differed among the SI, cecum, and LI. Mucosal mastocytosis developed in the SI but not in the LI, consistent with reduced IL-9 and IL-13 production by explants from the LI. Despite these differences, worms were cleared at the same rate from both sites. Furthermore, IL-10 production was reduced in the LI, yet it was instrumental in limiting local inflammation. Finally, passive immunization of rat pups with tyvelose-specific antibodies effectively cleared first-stage larvae from all intestinal regions. We conclude that despite regional differences in immune responsiveness and colonization, immune mechanisms that clear *T. spiralis* operate effectively throughout the intestinal tract.

Chen, Y., et al. (2013). **Coinfection with Clonorchis sinensis modulates murine host response against Trichinella spiralis infection.** *Parasitology Research* 112(9): 3167-3179.

Concomitant infections of different species of parasites are common in the field. Infection with one parasite species likely triggers host responses that may influence the subsequent infection of another species and alter disease outcomes. So far, the majority of studies have focused on single species parasite infection, and the mechanisms of protection induced by the first parasite infection against the secondary infection remain poorly defined. In this study, we assess the impact of trematode *Clonorchis sinensis* infection on the course of another tissue nematode *Trichinella spiralis* challenge. We observed that mice with preexisting *C. sinensis* infection had lower worm burden of intestinal *T. spiralis* than those infected with *T. spiralis* alone; mice with preexisting *C. sinensis* also had severe enteric histopathological changes and higher counts of intestinal Paneth cells in responses to *T. spiralis* challenge. The mRNA levels of interleukin (IL)-4, IL-10, IL-13, and tumor necrosis factor (TNF)-alpha from the small intestine and spleen of the different groups were analyzed using quantitative real-time polymerase chain reaction. Compared with that in mice infected with *T. spiralis* alone, the mRNA expression of IL-13 was significantly increased in the small intestine tissues and IL-4, IL-13, and TNF-alpha were significantly increased in the spleen tissues in the dually infected mice. Our findings suggest that a "preexisting" trematode infection of *C. sinensis* is a factor which contributes to reducing the establishment of *T. spiralis* adult worms in the small intestine.


The nurse cell (NC), formed from muscle cells upon infection with the parasitic nematode *Trichinella* spp. constitutes a confined habitat for muscle larvae of encapsulating species. Signaling pathway-directed analysis of microarray data allowed identification of the stage of NC cell cycle arrest as being of G1-like type, accompanied by cellular senescence. In accord with the specificity of senescent cellular systems, up-regulation of pro-inflammatory molecules was also found within the NC preparations. Potential immune-related activities associated with NCs as inferred from the aforementioned analysis, are reviewed herein. Transcriptional data suggest that the NC which harbors the larvae may exhibit the following immune-related functions: (i) production of complement components, (ii) antigen presentation and phagocytosis, (iii) pro-inflammatory cytokine secretion, (iv) oxidative stress generation and (v) eicosanoid synthesis.


Trichinellosis has major economic impacts on animal husbandry and food safety, and the control and elimination of trichinellosis is a major objective of veterinary medicine. A gene encoding serine protease of *Trichinella spiralis* (Ts-Adsp) was identified by immunoscreening
an adult T. spiralis cDNA library. In this study, the recombinant Ts-Adsp protein (rTs-Adsp) was cloned and expressed in a prokaryotic expression system and purified by Ni-affinity chromatography. To determine whether the purified rTs-Adsp is a potential vaccine candidate for the control of T. spiralis infection, we immunized BALB/c mice with this protein in combination with an alum adjuvant and subsequently challenged with T. spiralis larvae. The results showed that mice vaccinated with rTs-Adsp exhibited an average reduction in the muscle larvae burden of 46.5% relative to the control group. Immunization with the rTs-Adsp antigen induced both humoral and cellular immune responses, which manifested as elevated specific anti rTs-Adsp IgG and IgE antibodies and a mixed Th1-Th2 response, as determined by Th1 (IFN-gamma and IL-2) and Th2 (IL-4, IL-10, and IL-13) cytokine profiling, with the Th2 predominant. Thus, purified rTs-Adsp is able to limit the invasion of T. spiralis, and this protein could be an effective vaccine candidate for trichinellosis.


In this study, we report the cloning and characterization of a cDNA encoding a Trichinella serine protease gene (TspSP-1.3) from GenBank. The recombinant TspSP-1.3 protein (rTspSP-1.3) was expressed in an Escherichia coli expression system and purified with Ni-affinity chromatography. Real-time quantitative PCR analysis revealed that TspSP-1.3 was expressed at significantly higher levels in muscle larvae and adult worms than in newborn larvae. TspSP-1.3 was detected in excretory-secretory proteins of Trichinella spiralis with western blotting. Immunization with the rTspSP-1.3 antigen induced humoral immune responses, which manifested as elevated specific anti-rTspSP-1.3 IgG and IgE antibodies and a mixed Th1/Th2 response. To determine whether purified rTspSP-1.3 had good antigenicity and could be a vaccine candidate for the control of T. spiralis infection, we immunized BALB/c mice with rTspSP-1.3 and subsequently challenged the mice with T. spiralis larvae. The results showed that mice vaccinated with rTspSP-1.3 exhibited an average reduction in the muscle larvae burden of 39% relative to the control group. These results suggest that TspSP-1.3 could be a novel vaccine candidate for controlling Trichinella infection.


The goal of this work was to identify novel, early antigens present in Trichinella spiralis. To this end, a cDNA library generated from 3-day old adult worms (Ad3) was immunologically screened using serum from a pig infected with 20,000 muscle larvae. The serum was obtained from multiple, time course bleeds coinciding with early worm development: Seventeen positive clones were isolated using serum obtained at 20 days post infection (dpi). All clones corresponded to one gene that exhibited high sequence identity with the T. spiralis ATP-dependent RNA helicase DDX19B which is involved in parasite growth and development. In addition, nine additional positive clones representing 5 unique genes were identified when the library was screened with 30 dpi serum; four of these five genes displayed high similarity with members of a putative T. spiralis serine protease family known to be involved in host invasion and host-parasite interactions. The remaining gene aligned with the T. spiralis hypothetical ORF 11.30. The identification of these antigens provides potential candidates for the early diagnosis of trichinellosis and for the development of a vaccine against this parasite. (C) 2013 Elsevier B.V. All rights reserved.


Trichinella spiralis actively passes through the epithelial cells of the intestinal mucosa but morphologically, these cells do not manifest apparent damage. The possible activation of apoptotic mechanisms in the small intestine mucosa after infection with larvae and adults of
Trichinella spiralis was explored by immunohistochemistry. Sporadic individual cells of normal intestinal epithelium showed activation of caspase-3, increased expression AIF, or Bax. The larval stage of intestinal trichinellosis was characterized by distortion of cells on the villus tips that were strongly reactive to caspase-3, Bax, and survivin antibodies. There was a transient loss of the survivin expression on the brush border of the epithelial cells at 15-h post infection, which reappeared on the fifth day. Bcl-2 changed its normal apical distribution and re-localized to the basal part of the epithelial cells. No significant changes of expression of the selected apoptosis-related proteins were observed in the intestinal epithelial cells immediately surrounding the worms. The presence of Trichinella affects intestinal epithelial cells, but unlike in muscle cells, invading them does not initiate apoptotic factors activation.

Wu, X. P., et al. (2013). Unique antigenic gene expression at different developmental stages of Trichinella pseudospiralis. Veterinary Parasitology 194(2-4): 198-201. Parasite-induced and parasite-regulated larval capsule formation and host immunosuppression are two major characteristics that are unique in Trichinella spp. infections, but the molecule(s) and mechanism(s) that mediate these processes remain largely unknown. Trichinella pseudospiralis and Trichinella spiralis, are obviously different with respect to these two characteristics. A comparative study of these two species, in particular their antigen expression profiles at different developmental stages (the main molecules involved in the cross-talk or interaction between each parasite and its host), may help us better understand the parasite molecules and mechanisms involved. Here, we constructed cDNA libraries from T. pseudospiralis adults (Ad), newborn larvae (NBL) and muscle larvae (ML) mRNA and screened them with pig anti-T. pseudospiralis serum collected 26, 32 and 60 days post-infection (p.i.). The most abundant antigens were found to vary among life-cycle stages. Pyroglutamy peptidase 1-like and 6-phosphogluconolactonase-like genes predominated in the Ad stage and a serine protease (SS2-1-like gene) predominated in NBL similar to that observed in T. spiralis. Muscle larvae expressed proteasome activator complex subunit 3-like and 21 kDa excretory/secretory protein-like genes. This study indicated that parasites of two species may utilise different molecules and mechanisms for larvae capsule formation and host immunosuppression during their infections. Proteins of antigenic genes identified in this study may be also good candidates for diagnosis, treatment or vaccination for T. pseudospiralis infection, and also for the differential diagnosis of two species' infections.

Yu, Y.-R., et al. (2013). Taurine drinking attenuates the burden of intestinal adult worms and muscle larvae in mice with Trichinella spiralis infection. Parasitology Research 112(10): 3457-3463. The parasitic nematode Trichinella spiralis can cause trichinellosis, which leads to pathological processes in the intestine and muscle. The intestinal invasion determines the development, subsequent course, and consequences of the disease. Gastrointestinal nematode infection, including with T. spiralis, is accompanied by a rapid and reversible expansion of mucosal mast cell and goblet cell in the intestinal epithelium, which play important roles in the host immune response to parasite and worm expulsion from the intestine. Taurine and its derivatives have anti-infection and anti-inflammatory properties. We investigated whether taurine supplementation in mice could influence the development and pathological processes of infection with T. spiralis. Supplementing 1 % taurine in drinking water in mice infected with T. spiralis could alleviate the burden of intestinal adult worms on days 7 and 10 postinfection (all p < 0.01) and the formation of infective muscle larvae in striated muscle during T. spiralis infection (p < 0.01). As compared with T. spiralis infection alone, taurine treatment increased the number of goblet cells on days 7, 10, and 15 (p < 0.01 and p < 0.05) and alleviated intestinal mucosal mast cell hyperplasia on days 10 and 15 (all p < 0.01). So taurine supplementation in drinking water increased infection-induced intestinal goblet cell hyperplasia and ameliorated mucosal mastocytosis. Thus, taurine can ameliorate the pathological processes of trichinellosis and may be of great value for the treatment and prevention of infection with T. spiralis and other gastrointestinal nematodes.
Trichinellosis is a zoonosis caused by nematode parasites from the genus Trichinella. It is a disease spread all over the world and endemic in most countries of the European Union, where it is considered a re-emerging food-borne disease, with 1073 human cases notified in 2009. This disease is strongly associated with domestic pig (Sus domestic) and wild boar (Sus scrofa), but it can affect a wide range of domestic and wild animals (hosts). Domestic pigs can be infected by eating food scraps or meat mainly provided by man, or through ingestion of dead animals (mostly rats) infested with Trichinella. Men can also be affected through the consumption of meat from infected animals. In Western Europe, this disease is caused mainly by the consumption of raw or undercooked meat of pork, wild boar and horse. More than 50% of human trichinellosis in Spain are associated with wild boar meat that isn't inspected. The other 50% are related to pork meat that is slaughtered at home and also not subjected to inspection. We believe that in Portugal the situation is the same, but local alimentary habits as well as production systems, will define the importance that different kind of meat have as a potential source of Trichinellosis in humans. Trichinellosis prevention is based on three fundamental pillars: correct management practices of pigs, with thermal treatment of food from animal origin before administering it to domestic pigs, preventing the access of birds and rodents to facilities and the correct disposal of animal carcasses; Trichinella research in muscle tissues as a routine food inspection before the consumption of the meat and application of appropriate heat treatments of the meat before human consumption (cooking and freezing in correct time/temperature are highly effective). In conclusion we can say that Trichinellosis is a disease that can be easily controlled, once preventive measures are taken to protect both animal and public health.

Progress in intervention programs to eradicate foodborne helminth infections.
This chapter discusses the intervention methods used to control two helminthic diseases of public health importance. Trichinella and Taenia are transmitted by undercooked contaminated meat. Important changes made to animal husbandry and animal feeding have led to a decrease in these diseases in parts of the world. Specific information is provided on disease transmission coupled with the success achieved by intervention programs.

Standards for reporting surveillance information in freedom from infection models by example of Trichinella in Canadian market hogs.
Freedom from infection modeling, using scenario trees, has become an established methodology and is well described in the literature. However, standards for organizing and reporting the surveillance information incorporated into such models are less developed. Canada has been routinely testing for Trichinella spiralis in market hogs in federally inspected slaughter plants since the late 1990s. By way of presenting our work on T. spiralis in Canadian hogs, we propose that information in surveillance models be organized in distinct categories, each with specific parameters and values that are thoroughly described and justified. The proposed categories are: (1) definitions for the objectives, (2) initial time period, (3) inputs, (4) data, (5) model settings, (6) outputs, and (7) validation. Having a standardized manner of reporting such studies will facilitate their validation and expedite their evaluation by experts in the field and their use in trade negotiations.

EPIDEMIOLOGY
Occurrence of pathogens in wild rodents caught on Swedish pig and chicken farms.
Epidemiology and Infection 141(9): 1885-1891.
A total of 207 wild rodents were caught on nine pig farms, five chicken farms and five non-farm locations in Sweden and surveyed for a selection of bacteria, parasites and viruses. Lawsonia intracellularia and pathogenic Yersinia enterocolitica were only detected in rodents on pig farms (9% and 8% prevalence, respectively) which indicate that these agents are more likely to be transmitted to rodents from pigs or the environment on infected farms. Brachyspira
hyodysenteriae (1%), Brachyspira intermedia (2%), Campylobacter jejuni (4%),
Campylobacter upsaliensis (2%), leptospires (7%) and encephalomyocarditis virus (9%) were
also detected from rodents not in contact with farm animals. Giardia and Cryptosporidium
spp. were common, although no zoonotic types were verified, and Salmonella enterica was
isolated from 1/11 mice on one farm but not detected by PCR from any of the rodents.
Trichinella spp. and Toxoplasma gondii were not detected.


In this report, harmonised epidemiological indicators are proposed for foodborne biological hazards to public health that are related to farmed game and meat thereof and that can be addressed within meat inspection. These hazards include Salmonella, Toxoplasma, Trichinella and Mycobacterium in farmed wild boar and deer. An epidemiological indicator is defined as the prevalence or concentration of the hazard at a certain stage of the food chain or an indirect measure of the hazard that correlates to the human health risk caused by the hazard. The indicators can be used by the European Commission and Member States to consider when adaptations in meat inspection methods may be relevant and to carry out risk analysis to support such decisions. It is foreseen that the indicators will be used in the revised meat inspection system for farmed game meat outlined in the European Food Safety Authority scientific opinion, particularly to help categorise slaughter batches, animals and slaughterhouses according to the risk related to the hazards and process hygiene or to enable surveillance for the possible emergence of the hazard. Depending on the purpose and the epidemiological situation, risk managers should decide on the most appropriate indicator(s) to use, either alone or in combination, at national, regional, slaughterhouse or farm/herd level. Member States are invited to report data generated by the implementation of the indicators in accordance with Directive 2003/99/EC. The proposed indicators should be regularly reviewed in light of new information and the data generated by their implementation.

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OTHER ZOONOTIC PARASITES

This Reporting Manual provides guidance for reporting on zoonoses, zoonotic agents and antimicrobial resistance in animals, food and feed under the framework of Directive 2003/99/EC. Some advice is also given on reporting on other pathogenic microbiological agents in food. The objective is to harmonise and streamline the reporting made by the Member States in a way that the data collected would be relevant and easy to be analysed at the European Union level. The manual covers all the agents and items included by the current data collection run by the European Food Safety Authority. Detailed guidelines are provided for reporting of the data in the tables and text forms. This guidance typically applies to the agents, animal species and food categories to be reported on. Instructions are given on description of the sampling and monitoring schemes as well as analyses of the results in the national reports. Special reference is made to fields where following of trends would be desirable. (European Food Safety, 2013)

Cerebral involvement in parasitoses is an important clinical manifestation of most of the human parasitoses. Parasites that have been described to affect the central nervous system (CNS), either as the dominant or as a collateral feature, include cestodes (Taenia solium (neurocysticerciasis), Echinococcus granulosus (cerebral cystic echinococcosis), E. multilocularis (cerebral alveolar echinococcosis), Spirometra mansoni (neurospligananos)), nematodes (Toxocara canis and T. cati (neurotoxocariasis), Trichinella spiralis (neurotrichinelliasis), Angiostrongylus cantonensis and A. costaricensis (neuroangiostrongyliasis), Schistosoma mansoni (cerebral bilharziosis), Paragonimus westermani (neuroparagonimiasis)), or protozoa (Toxoplasma gondii (neurotoxoplasmosis), Acanthamoeba spp. or Balamuthia mandrillaris (granulomatous amoebic encephalitis), Naegleria (primary amoebic meningo-encephalitis), Entamoeba histolytica (brain abscess),
Plasmodium falciparum (cerebral malaria), Trypanosoma brucei gambiense/rhodesiense (sleeping sickness) or Trypanosoma cruzi (cerebral Chagas disease)). Adults or larvae of helminths or protozoa enter the CNS and cause meningitis, encephalitis, ventriculitis, myelitis, ischaemic stroke, bleeding, venous thrombosis or cerebral abscess, clinically manifesting as headache, epilepsy, weakness, cognitive decline, impaired consciousness, confusion, coma or focal neurological deficits. Diagnosis of cerebral parasitoses is dependent on the causative agent. Available diagnostic tools include clinical presentation, blood tests (eosinophilia, plasmodia in blood smear, antibodies against the parasite), cerebrospinal fluid (CSF) investigations, imaging findings and occasionally cerebral biopsy. Treatment relies on drugs and sometimes surgery. Outcome of cerebral parasitoses is highly variable, depending on the effect of drugs, whether they are self-limiting (e.g. Angiostrongylus costaricensis) or whether they remain undetected or asymptomatic, like 25% of neurocysticerciasis cases. (Finsterer and Auer, 2013)

Anisakis is a parasitic nematode which infects fish and marine invertebrates, including crustaceans and molluscs. Ingestion of contaminated seafood can cause acute gastrointestinal diseases. Infection can be accompanied by severe allergic reactions such as urticaria, angioedema and anaphylaxis. Diagnosis of allergy due to Anisakis currently relies on the detection of serum IgE antibodies to allergenic proteins and a history of reactions upon exposure to fish. Anisakis proteins demonstrate considerable immunological cross-reactivity to proteins of related nematodes and other invertebrates such as crustaceans and house dust-mites. In contrast, very limited molecular associations with other parasite groups are observed, including trematodes and cestodes. This review outlines current knowledge on Anisakis as a food-borne parasite, with special focus on the underlying immunological mechanisms resulting in allergic host defence responses. (Nieuwenhuizen and Lopata, 2013)

Dipylidiasis is a zoonotic parasitic infestation caused by the dog tapeworm Dipylidium caninum. Human dipylidiasis has been rarely reported in English literature. Young children are mostly at risk of acquiring the infection due to their close association with dogs and cats. We report a rare case of Dipylidium caninum infection in a 4 year old male child in India. The diagnosis was based on microscopic examination of stool. Confirmation of the proglottid segments was done by histopathological examination. (Narasimham et al., 2013)

There have been few studies on human trichostrongyliasis in Southeast Asia; information on its clinical manifestations is also sparse. Trichostrongyliasis occurs predominantly in areas where poor hygiene is common especially where human/animal feces are used as a fertilizer, thereby contaminating vegetables and stream water. One study demonstrated the clinical characteristics of trichostrongyliasis patients include history of loose feces, rashes, and abdominal pain, as well the fact they are likely to regularly consume fresh vegetables, have poor history of hand washing, and close contact with cattle. (Watthanakulpanich et al., 2013)

Human trichostrongylosis has been reported in Thailand. Eight human cases of trichostrongylosis in Thailand and Lao People's Democratic Republic were found to be infected by Trichostrongylus colubriformis and T. axei (identified and confirmed by molecular techniques). This evidence is the first molecular evidence of human T. colubriformis and T. axei infection in Thailand. Infection by these two species was apparently epidemic in some areas (Phosuk et al., 2013).

In endemic countries with soil-transmitted helminths mass drug administration with albendazole or mebendazole are being implemented as a control strategy. However, it is well known in veterinary helminths that the use of the same benzimidazole drugs can place selection on the beta-tubulin gene, leading to resistance. Given the concern that resistance could arise in human soil-transmitted helminths, there is an urgent need to develop accurate diagnostic tools for monitoring resistance. In this study, we developed molecular assays to detect putative resistance genetic changes in Ascaris lumbricoides, Trichuris trichiura, and hookworms, and we optimized an egg hatch assay for the canine hookworm Ancylostoma caninum and applied it to Necator americanus. Both assays were tested on field samples. The molecular assays demonstrated their reproducibility and capacity to detect the presence of worms carrying putative resistance-associated genetic changes. However, further investigations are needed to validate our molecular and biological tests on additional field isolates. (Diawara et al., 2013)


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