



Animal &
Plant Health
Agency

Parasitology group: Vectors and vector borne diseases

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

August 2020



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Year of publication: 2020

www.gov.uk/defra

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Expert Review

The following papers and articles were viewed and collated as those of particular interest to APHA and scanning surveillance.

VECTORS - Ticks and arthropods

Boulanger et al. discuss in a paper titled “Ticks and tick-borne diseases” the significance of ticks due to the diversity of pathogens they transmit, by their impact on human and animal health, and by their socioeconomic implication especially in countries of the Southern Hemisphere. The situation in Europe is also discussed and how it’s changed with major modifications in the biodiversity caused by humans.

Novel tick species UK

The exotic tick, *Hyalomma rufipes*, was found on an untraveled horse in the south west of England. The lack of travel by the horse itself and any other animals on the premises suggested a potential introduction of the tick on a migratory bird. The tick tested positive for *Rickettsia aeschlimannii*, a spotted fever group rickettsia linked to a number of human cases in Africa and Europe. Further surveillance of horses following the spring bird migration is recommended as a result of this case, due to the potential human and animal health risks of such events occurring more regularly (Hansford et al.).

Vector control strategies

A review paper on vector control strategies was written by Benelli which references many other papers, exploring a range of potential methods for the control of mosquitoes and ticks.

The issues and reasons why new vaccines for the control of ectoparasite vectors have not been registered and commercialized is discussed by de la Fuente et al. in a second review paper.

Tick vaccines

Rego et al also published a review article exploring the current knowledge on relationships between ticks, the pathogens they carry and the mammalian hosts, and examining the methods that are being used to identify anti-tick vaccine candidates that can prevent the transmission of tick-borne pathogens.

A study trialed oral vaccination with a formulation combining *Rhipicephalus microplus* subolesin with heat inactivated *Mycobacterium bovis*, which was found to reduce tick infestations in cattle, providing a form of tick vaccination (Contreras et al.).

Resistance to certain vaccines has been associated with the genetic diversity among the *Rhipicephalus microplus* populations in Mexico, with varying haplotypes found in differing areas that could account for the poor vaccine efficacy (Martínez-Arzate et al.).

Research is currently under way into an anti-tick "vaccine" undertaken by the team under the EU project "ANTIDOTE" (Anti-tick Vaccines to Prevent Tick-borne Diseases in Europe). The first results of the research have been reported (Płusa).

Research into new tick treatments

Due to the importance of the tick *Rhipicephalus microplus*, in the tropical and subtropical countries; the costs to control these ticks; damage to the environment caused by the inappropriate use of chemical acaricides and the increased resistance against most of the commonly used chemical acaricides, alternative methods of control have been explored:

Plant oil: Ethanol extracts from three *Leucaena* spp. genotypes: *L. leucocephala* (Lam.) de Wit (Native); *L. leucocephala* (Cunningham); and *L. Leucocephala* x *L. padilla* (KX2). Larval efficacy up to 92% and adult mortality of up to 50% with certain types and concentrations (González-López et al., 2019).

Essential oils of cloves (*Syzygium aromaticum*), bark of cinnamon (*Cinnamomum zeylanicum*) and leaves of lemon grass (*Cymbopogon citratus*) on unfed larvae. Acaricidal effects were found with all, and combinations of the oils had additive or synergistic effects (Jyoti, Singh et al., 2019).

Combinations of individual essential oils were trialed using different proportions of individual essential oils from cinnamon (*Cinnamomum zeylanicum*), cumin (*Cuminum cyminum*) and allspice (*Pimenta dioica*), producing mixtures with potent acaricidal activity. The formulations were found to have the potential to successfully control tick infestations. (Lazcano Díaz et al.).

Brunfelsia uniflora (Brazilian plant) leaf extract is an alternative for the control of the bovine tick cycle, mainly in the free-living stage (non-parasitic stage) under field conditions (Sugauara et al.).

Acaricide resistance research

Synthetic pyrethroid resistance is one of the most prevalent and well-studied forms of resistance in arthropods, being linked with target site alterations in the sodium ion channel gene. Research on *Rhipicephalus microplus* using sequencing, identified gene mutations associated with this resistance in ticks in the US (Klafke et al., 2019).

Acaricide resistance was examined in *Rhipicephalus sanguineus sensu lato*. Cross-tolerance to two sodium channel-inhibiting pesticides, permethrin and etofenprox, was observed in one population. This study has provided a single etofenprox concentration that can be used in larval packet tests to determine resistance status in collected peridomestic brown dog ticks, which is particularly important when a single to a few engorged adult ticks are provided for evaluation. Although metabolic resistance is presumed to be the primary resistance mechanism, a sodium channel mutation also confers tolerance to etofenprox. (Tucker et al.).

Research into new insect treatments

Midges of the genus *Culicoides* (Diptera: Ceratopogonidae) are the principal vectors of several notable viral pathogens infecting animal livestock. Recent studies highlight how, in the near future, the application of "dry" fungal conidia of *Metarhizium anisopliae* in animal shelters and microenvironment (e.g. dung, manure, leaf litter, and livestock surroundings) may be used to control the *Culicoides* vector, thus, reducing the incidence of *Culicoides*-borne diseases (Ansari et al.).

The horn fly, *Haematobia irritans irritans* (L.) (Diptera: Muscidae), is an important and cosmopolitan blood feeding ectoparasite of cattle. Resistance to conventional insecticides is increasingly problematic and alternative pesticides, including natural products, are being investigated. Limonene is a cyclic monoterpene repellent to some insects that occurs in citrus fruit rinds and in other plants. Certain concentrations were ovicidal and caused adult immobilization. Lower concentrations interestingly were potentially acted as attractants (Showler et al.).

VECTOR BORNE DISEASE - Test and surveillance developments

A research team in India developed a disease model for a fatal tick-borne viral haemorrhagic disease, Kyasanur Forest Disease (KFD), an infection that is spreading across degraded forest ecosystems in India. The aim of the model was to improve both control and prevention of the disease (Purse et al.).

A consensus modelling methodology has been designed to estimate the habitat suitability for mosquito species in the UK deemed competent for Rift Valley fever virus (RVF), and it was applied in an assessment of the relative risk of establishing RVF virus in the UK livestock population. The model results suggested there are areas of high suitability for RVF competent mosquito species across the length and breadth of the UK, notable areas of high suitability were the South West of England and coastal areas of Wales. The outputs can be used as part of a multidisciplinary approach to inform risk based disease surveillance activities (Simons et al.).

A deep amplicon sequencing platform was developed in another study to identify proportions of piroplasm species in co-infected communities, to develop the concept of a "haemoprotobiome". The "haemoprotobiome" concept has several potential applications in veterinary and human research, including the understanding of responses to drug treatment; parasite epidemiology and ecology; species interactions during mixed infections; and parasite control strategies (Chaudhry et al.).

Tick pathogen surveys in Europe

Multiple tick borne pathogens, including ones with zoonotic significance, were identified in *Ixodes ricinus* ticks collected in a recreational forest in Denmark, The main agents were *Borrelia spp.*, *Anaplasma phagocytophilum* and *Rickettsia helvetica*, while *Candidatus Neoehrlichia mikurensis* and *Babesia venatorum* with 3 or 4 infectious agents found in some ticks (Klitgaard et al.).

Corsica: Ticks were collected from cattle, sheep and rodents, the tick species collected were identified as *Rhipicephalus bursa*, *Hyalomma marginatum* and *Ixodes ricinus*. Tick borne diseases found were: *Rickettsia Aeschlimannii*, *Candidatus Ri. Barbariae*, *Anaplasma marginale* and *Borrelia burgdorferi sl* (Cicculi et al.).

Tularemia

Francisella tularensis is a Gram-negative, intracellular bacterium causing the zoonosis tularemia. The infection can be spread by tick bites but also by direct contact with infected animals or via mosquitoes. A paper discusses outbreaks associated with water borne infection, and the epidemiological traits of the infection (Hennebique et al.).

Borrelia

Plusa discusses borreliosis due to increasing numbers of human cases in Europe. Recently published new recommendations for the diagnosis and treatment of Lyme disease developed by specialists from the National Institute for Health and Care Excellence (NICE) in the United Kingdom did not bring significant changes to the existing recommendations for the diagnosis and treatment of patients with borreliosis. Research is currently under way into an anti-tick "vaccine" undertaken by the team under the EU project "ANTIDOTE" (Anti-tick Vaccines to Prevent Tick-borne Diseases in Europe). The first results of the research have been reported.

Human babesiosis

Laboratory diagnosis of human babesiosis is described (Kukina et al.).

Livestock vector-borne diseases

Mycoplasma ovis and *Anaplasma ovis* were described in an outbreak of hemolytic anemia in a sheep flock, leading to severe livestock loss on a ranch of Mexico (Martinez-Hernandez et al.).

A study of Tropical theileriosis in cattle, in Turkey, identified a diverse range of parasite genotypes, every *Theileria annulata* positive cattle blood sample harbored multiple parasite genotypes. Vaccine genotypes did not appear to influence the genetic diversity in the cattle. Reduction in vaccine efficacy, changes in parasite behaviour, changes in pathogenicity or in host adaptation can occur when pathogen genotypic variations occur (Bilgin Bilgic et al.).

Water buffalo were identified as reservoirs of multiple and mixed tick borne pathogens, including of *Babesia* and *Anaplasma* species associated with disease in cattle (Obregon et al.).

Multiple pathogens, *Theileria ovis*, *Theileria lestoquardi*, *Anaplasma ovis*, were detected by PCR in goats in Iran including co-infections in some cases (Hakimi et al.).

Anaplasma bovis and *Anaplasma capra* were detected by PCR and sequencing in goats in Korea (Seo et al.).

Babesia

Babesiosis is considered an emerging disease because its incidence has significantly increased in the last 30 years, with an expanding range of this rare but potentially life-threatening zoonotic disease. *Babesia divergens* is a causative agent of babesiosis in humans and cattle in Europe. The genomic structure of *Babesia divergens* was examined using sequencing, as part of a study into the invasion and egress processes of *Babesia divergens* (Gonzalez et al).

Babesia bovis, *Babesia bigemina* and *Babesia divergens* are considered by international health authorities (OIE) as the principal species of *Babesia* that cause bovine babesiosis. Animals that recover infection may remain as persistent carriers with no clinical signs of disease and can be the source of infection for ticks. Several procedures that have been developed for parasite detection and diagnosis of this infectious carrier state constitute the basis for a review by Antonio Alvarez et al.

A review of bovine babesia infections and of the parasite's cellular and molecular pathogenesis was published, with the aim of improving control and preventative strategies (Suarez et al.).

There was also a comprehensive review of the recorded tick vectors of *Babesia* (Gray et al.).

Babesia venatorum (*Babesia* sp. EU1) was described in sheep in Scotland in the UK (Gray et al.) and was described causing fatal babesiosis in a captive reindeer calf in Switzerland (Novacco et al.).

Research into new *Babesia* treatments

There are no effective vaccines against *Babesia* and *Theileria* parasites; therefore, therapy depends heavily on antiprotozoal drugs. The search for new chemotherapy options against *Babesia* and *Theileria* (of low toxicity) has become increasingly urgent due to a limited range of products available and parasite resistance to current drugs.

A study using Ivermectin (IVM) on four *Babesia* species, including *B. bovis*, *B. bigemina*, *B. divergens*, *B. caballi*, and *Theileria equi* suggested Ivermectin has the potential to be an alternative remedy for treating piroplasmosis (Batiha et al.).

Ellagic acid (EA) is a polyphenol found in various plant products and has antioxidant, antibacterial and effective antimalarial activity in vitro and in vivo without toxicity. A study demonstrated the in vitro and in vivo antibabesial action of EA-NPs and thus supports the use of nanoparticles as an alternative antiparasitic agent (Beshbishy et al.).

The antimalarial, anticancer and antioxidant effect of thymoquinone (TQ), a phytochemical compound found in the plant *Nigella sativa*, might be a promising medicinal compound for use in the treatment of animal piroplasmosis (El-Sayed et al.).

Babesia vaccines

Rathinasamy et al. review historic and recent attempts in babesiosis vaccine development to guide the development of next-generation babesiosis vaccines.

Bovine besnoitiosis

Curcumin, a polyphenolic compound from *Curcuma longa* rhizome has anti-protozoan effects. Curcumin treatments were found to reduce tachyzoite viability and induce lethal effects in up to 57% of *Besnoitia besnoiti* tachyzoites. Feeding cattle with *Curcuma longa* rhizomes may provide a new strategy for besnoitiosis treatment (Cervantes-Valencia et al.).

A serological study was carried out in beef, dairy and bullfighting cattle herds in Catalonia, north-eastern Spain, to determine the seroprevalence of *B. besnoiti* in the region. Staged testing was done using an ELISA, followed by an IFAT and final confirmation was with a *B. besnoiti* tachyzoite-based immunoblot. *Besnoitia*-seropositive cases were exclusively beef cattle from the Pyrenees area, resulting in a prevalence of 25.1% at the animal level and of 46% at the herd level (Garrido-Castane et al.).

In a previous study bovine besnoitiosis was established in calves that were intravenously inoculated with *Besnoitia besnoiti* tachyzoites. In the present study, 3-month-old calves were inoculated with *B. besnoiti* tachyzoites via subcutaneous and intradermal routes and successful infection was established. Infected animals developed mild-moderate acute besnoitiosis characterized by lymphadenopathy from four days to 47 days pi, and sporadic fever peaks were observed in one calf. No signs characteristic of chronic besnoitiosis were clinically detected, the calves were euthanased at 70 days post-infection. Respiratory and reproductive tissues were more reliably PCR positive (Diezma-Diaz et al.).

Besnoitia besnoiti was successfully transferred by *Stomoxys calcitrans* flies from a chronically infected cow to a rabbit, after flies were transferred on three occasions following an interrupted bloodmeal from the infected cow. One rabbit showed acute clinical signs, was PCR positive and subsequently seroconverted (Sharif et al.).

Seropositivity to *Besnoitia besnoiti* was confirmed in cattle, at a prevalence of 2.7%, in the Eastern and Southeastern Anatolia region of Turkey; although no typical clinical signs had been seen in these herds (Ozidal et al.).

A study reported on bovine besnoitiosis in a 217 cow dairy farm in Italy. Serological screening was performed on the herd using the recommended approach of ELISA followed by a confirmatory Western Blot. Seropositive animals were clinically examined and risk factors and the effects of infection on reproductive and productive performances were evaluated. Tissue samples that were histologically lesion positive and PCR positive from a slaughtered chronically affected cow were described. Cows with cutaneous cysts were found to have reduced productive parameters (Villa et al.).

A study was carried out to develop in vitro culture systems using target cells favoured by *Besnoitia besnoiti* to aide drug trial studies and to facilitate unravelling host-parasite interactions. The study additionally identified BVDV infection favoured early *Besnoitia* invasion of cells (Jimenez-Melendez et al.).

Trypanosomes

A Case of Bovine Trypanosomiasis Caused by *Trypanosoma Theileri* in Sicily, Italy: Weight loss was described and the spleen was slightly enlarged on post mortem, and there were raised circulating blood lymphocytes and neutrophils. PCR and Giemsa stains are used to detect the organism, in this case PCR detected the organism in bone marrow, brain and testis. Detection in the testis suggest the potential for sexual transmission needs investigating (Amato et al.).

Wildlife vector-borne diseases

Roe deer in Spain were identified with a range of *Babesia* and *Theileria* species, including the first description of *B. venatorum* and *B. bigemina* in roe deer from Spain (Remesar et al.).

Wildlife Anaplasma

A study aimed to identify the *Anaplasma* species carried by captive red deer and swamp deer in a wild fauna reserve in France. *Anaplasma phagocytophilum* was identified in the blood from 23.7% of red deer but not in any of the swamp deer (7 animals). The recently reported *Anaplasma capra* was found in 2 red deer (*Cervus elaphus*) and 1 swamp deer (*Rucervus duvaucelii*). This is the first report of this organism in France, a species otherwise described only in China, Japan, Malaysia and South Korea in goats, sheep, deer, cattle and Japanese serows (*Capricornis crispus*). The organism could have been introduced by infected imported animals with subsequent transmission to locally born deer (Jouglin et al.).

Anaplasma phagocytophilum survey of red and roe deer and moose in Norway identified different ecotypes in the different deer types. Red deer was exclusively linked to circulation of ecotype I, while roe deer was exclusively linked to circulation of ecotype II. Moose were found to carry both ecotypes. Both ecotypes were found in questing ticks in areas with multiple cervid species present, while only ecotype I was found in ticks in a region with only red deer present (Stigum et al.).

Wildlife Babesia

The genetic similarity of *Babesia* spp., forming a group commonly termed as a clade VI, complicates the identification of Babesias in deer. Two independent genetic loci were therefore used in parallel to identify *Babesia* isolates in free-ranging red, sika, and roe deer in the Czech Republic. The findings confirmed *B. divergens*, *Babesia* sp. EU1 and *B. capreoli* in the studied deer species as well as the presence of another unnamed species that matches a taxon previously referred to as *Babesia* cf. *odocoilei* or *Babesia* CH1 group in several other sites throughout Europe. The invasive sika deers enter the life cycle of at least three piroplasmid species detected in native deer fauna. The presence of *B. divergens* in both sika and red deer in an area where bovine babesiosis is apparently absent raises important questions regarding the epidemiology, host specificity and taxonomic status of the parasite (Hrazdilova et al.).

Wildlife Borrelia

A summary of pathogens resulting in Lyme's disease in humans is detailed in one paper. The disease is caused by multiple genospecies of *Borrelia burgdorferi* sensu lato bacteria transmitted by ixodid (hard) ticks, and the major host groups transmit *Borrelia* genospecies with different pathogenicity, causing variable clinical symptoms in humans. *Borrelia afzelii*, with mainly small mammals as reservoirs, is the most common pathogen causing Lyme borreliosis, and it is often responsible for the largest proportion of infected host-seeking tick nymphs in Europe. The bird-borne *Borrelia garinii*, though less prevalent in nymphal ticks, is more likely to cause Lyme neuroborreliosis, but whether *B. garinii* causes disseminated disease more frequently has not been documented. Migratory birds were thought to play a significant role increasing the levels of infected ticks (Mysterud et al.).

Wildlife Theileria

Pathogenic theileriosis in African wild artiodactyls was reviewed including the taxonomy, diagnosis, epidemiology, haematology, pathology, and aspects of control in various species. Molecular studies have identified a diverse range of Theilerias, nontheilerial hemoprotozoa and rickettsia-like bacteria in these animals. Clinical disease signs and pathology associated with *Theileria* infection is described (Cliff et al.).

Bird blood parasites

Migratory Aquatic Warblers in Poland - *Plasmodium*, *Leucocytozoon* and *Trypanosoma* protozoal parasites were detected in sampled birds (Kubacka et al.).

Tick borne encephalitis (TBE) virus

Virus transmission via the alimentary tract in humans was reported, as a result of raw goat milk consumption. TBE can occur following ingestion of unpasteurized milk from viremic animals. Cases were described in four patients in Poland (Krol et al.).

Bluetongue virus

A study indicated there was no evidence that red deer had played a role in the spread or maintenance of Bluetongue virus (BTV) in France. Surveillance in red deer for both BTV1 and BTV8 over an 8 year period indicated they did not contribute to the 2015 BTV8 re-emergence in France (that occurred 5 years after the last domestic case from the first outbreak), and there was no evidence of spread of the virus along green corridors, away from the domestic outbreak range (Rossi et al.).

Surveillance data has estimated Bluetongue serotype 8 vertical transmission in French cattle has been potentially at a level of 56% in unvaccinated heifers that had been infected late in gestation. The potential impact on BTV-8 spread and persistence due to a high probability of vertical transmission should be further investigated (Courtejoie et al.).

Crimean-Congo Haemorrhagic Fever Virus (CCHFV)

CCHFV genome was detected in a migratory bird in Italy, sequencing suggested the virus had originated in Africa (Toma et al.).

CCHFV is a tick-borne pathogen causing severe infections in many parts of Africa, Asia and in Europe. The virus is primarily transmitted by ticks. The spectrum of natural hosts for CCHFV includes a wide variety of domestic and wild animals. The presence of CCHFV was suspected in Hungary therefore 2085 of four different species of wild rodents were serologically screened to check for exposure, 0.96% were positive for CCHFV antibodies (Foldes et al.).

CCHF is the most widespread, tick-borne viral disease affecting humans and a review was done exploring CCHF seroprevalence in a global context by assessing 206 papers representing areas throughout the world. The total means of CCHF seroprevalence were, respectively 4.7 and 24.6% for humans and animals; and were 17.1, 18.9, 24.3, 29.3 and 27.1% for camels, cattle, goats, sheep and livestock. Globally, CCHF seroprevalence in at-risk professionals was 7.5 fold greater than in other human populations. Animal contact, animal husbandry, farming, tick bite history and secretion exposure were the most frequently reported CCHF seropositivity risk factors (Nasirian).

Livestock serve as a transient reservoir for CCHF, but do not show clinical signs. The seroprevalence in sheep and goats in an endemic area in Pakistan were found to be for sheep 19% and goats 5%. Viraemia was indicated in 5% of sheep with a positive PCR result. Keeping free-ranging chickens appeared to reduce the risk in sheep and goats on farms; it was speculated the birds may eat the ticks (Khan Kasi et al.).

Rift valley fever virus and phleboviruses

A paper reviewed the molecular aspects of Rift Valley fever phlebovirus (RVFV) including the genetic diversity of strains, and the virus's susceptibility to reassortment. Reassortment has the potential to produce viruses that are more pathogenic, more easily transmissible, and that can have a wider vector or host range, with implications therefore for introductions to new areas and for increased pathogenicity (Gaudreault et al.).

A review of phleboviruses circulating in Portugal (both the continental territory and the islands of São Miguel, in the Azores, and Madeira), as well as in the Spanish western regions of Extremadura and Castilla and León. Phlebovirus sequences were frequently detected (L-segment) from both questing and feeding ticks, but especially in *Rhipicephalus sanguineus* sensu lato (s.l.) specimens. These sequences were detected in adult ticks, as well as nymphs and eggs, supporting the hypothesis of viral maintenance by vertical transmission. This viral group are important due to the large diversity of new tick-borne phleboviruses, and the potential impact of the virulent virus strains on human/animal health (Pimentel et al.).

Other arboviruses

Severe fever with thrombocytopenia syndrome (SFTS) is an infection in humans caused by a tick-borne bunyavirus in China with the virus detected in *Haemaphysalis longicornis* ticks. Seropositivity to the virus was detected in local livestock, poultry and small wild animals (Hu et al.).

A newly identified tick-borne Alongshan virus (ALSV) was detected by PCR in *Ixodes ricinus* ticks in south-eastern Finland. The virus is a segmented Jingmen virus group flavivirus that had been recently associated with human disease in China (Kuivanen et al.).

Ticks and mosquitoes, as well *Culicoides*, can transmit viruses in the *Reoviridae* family and with the help of next-generation sequencing (NGS), previously unreported reoviruses such as equine encephalosis virus, Wad Medani virus (WMV), Kammavanpettai virus (KVPTV) have been characterized; and now Karyana virus and Kundal virus have been discovered in India (from *Hyalomma anatolicum*.ticks). Other vector borne viruses are described in the paper (Yadav et al.).

Filarial worms

Filarial parasites were detected in mosquitoes in Spain. An *Aedes caspius* pool was positive for *Setaria equina* and an unidentified worm related to *Onchocerca* was detected in a *Culex pipiens* pool (Martinez-de la Puente et al.).

Equine piroplasms

A published review of Equine Piroplasmiasis (EP) (*Babesia caballi* and *Theileria equi*) focused on all aspects of the disease from the historical overview, biology of the parasite, epidemiology of the disease (specifically highlighting other non-equine hosts, such as dogs and camels), vector, clinical manifestations, risk factors, immunology, genetic diversity, diagnosis, treatment, and prevention (Onyiche et al.).

Camino et al. described serological, molecular and hematological diagnosis in horses with clinical suspicion of equine piroplasmiasis caused by *Theileria equi* and/or *Babesia caballi*. PCR would be the test of choice.

Montes Cortés et al found a relatively high prevalence of *Theileria equi* and *Babesia caballi* in Castilla-León and Extremadura, Spain using PCR testing on horse blood samples. Serology on the same samples returned a lower rate of positivity.

Equine Trypanosomiasis

Equine trypanosomiasis is a complex of infectious diseases called dourine, nagana and surra. It is caused by several species of the genus *Trypanosoma* that are transmitted cyclically by tsetse flies, mechanically by other haematophagous flies, or sexually. *Trypanosoma congolense* (subgenus *Nannomonas*) and *T. vivax* (subgenus *Dutonella*) are genetically and morphologically distinct from *T. brucei*, *T. equiperdum* and *T. evansi* (subgenus *Trypanozoon*). Recent outbreaks of surra and dourine in Europe illustrate the risk and consequences of importing equine trypanosomiasis. Unfortunately, clinical signs are not pathognomonic, and serological tests can lack in sensitivity or specificity; and molecular tests cannot distinguish the taxa within the *Trypanozoon* subgenus, therefore further work on test improvement is suggested in the review (Büscher et al.).

Domestic pet vector-borne diseases

A study assessed the prevalence of *Rickettsia* spp., *Babesia* spp., *Cytauxzoon* spp. and *Leishmania infantum* infections in cat populations living in central Italy, by molecular and serological tools. A total of 286 healthy cats were randomly selected from catteries and colonies in central Italy. Cats were found to be positive for *R. felis* and *R. conorii* and *Leishmania*. Tests for *Cytauxzoon* spp. were negative despite previous evidence in wild cats in the same area (Morganti et al.).

Abdullah et al. investigated pathogens in fleas collected from cats and dogs in the UK. 11.3% of pooled samples of were positive for *Bartonella* sp., (*Bartonella henselae*, *Bartonella clarridgeiae*, *Bartonella alsatica* and *Bartonella grahamii*) and 3% were positive for the tapeworm *Dipylidium caninum*. Three flea samples were positive for *Mycoplasma haemofelis* / *Mycoplasma haemocanis*. *Bartonella* has zoonotic potential with the potential for more severe disease in immunocompromised individuals. *Bartonella* sp. is potentially transmitted via skin scratches, fleas, lice, biting flies and ticks.

Tahir et al. discuss the vector-borne nematode diseases of dogs and cats that are found in the mediterranean basin, such as dirofilariosis, onchocercosis, thelaziosis, Cercopithifilaria, and Acanthocheilonema infections, are some of these vectorized diseases, several of which are zoonoses. They are all caused by parasitic nematodes transmitted by arthropods, including mosquitoes (*Dirofilaria* spp.), black flies (*Onchocerca lupi*), drosophilids (*Thelazia callipaeda*), ticks (*Acanthocheilonema dracunculoides* and *Cercopithifilaria binae*), and fleas and lice (*Acanthocheilonema reconditum*).

Hepatozoon

Three different *Hepatozoon* (Apicomplexa, Hepatozoidae) species have been described infecting domestic cats in Europe (i.e. *H. felis*, *H. canis* and *H. silvestris*), however, reports on clinical hepatozoonosis are uncommon and treatment protocols are not clearly defined. A cat from Austria was confirmed with clinical infection and presented with poor general condition, lethargy, anorexia, icterus, a painful abdomen, fever, ruffled hair and a tick infestation. The cat had never left Austria. (Basso et al.).

Canine Babesia

Canine babesiosis is a severe disease caused by several *Babesia* spp. A canine-infecting piroplasmid pathogen that is closely related to the *Babesia microti* group has been classified as *Babesia vulpes*, the morphology and phylogenetics are described. The parasite was previously referred to as *Theileria annae* Zahler, *Babesia annae*, *Babesia vulpes* Baneth, "*Babesia microti*-like piroplasm", "*Babesia* Spanish dog isolate" or *Babesia* cf. *microti*. The parasite is distinct from *Babesia gibsoni* and *Babesia conradae* Kjemtrup (Baneth et al.).

Leishmania

A study determined that dogs that have clinical leishmaniasis, caused by the parasite *Leishmania infantum*, are commonly co-infected with other pathogens, especially vector-borne pathogens (VBP). Concurrent pathogens such as *E. canis/Ehrlichia ewingii*, *Anaplasma phagocytophilum/Anaplasma platys* and *Dirofilaria immitis* were found (Attipa et al.).

Dirofilaria (Heart worm)

Carretón examined and confirmed the efficacy of a shorter treatment regime for *Dirofilaria immitis* in dogs.

Selamectin (6 mg/kg) plus sarolaner (1 mg/kg) topical treatment was 100% effective in preventing heartworm development in cats when administered as one dose 30 days after inoculation or as three consecutive monthly doses starting 30 days post-inoculation (McTier et al.).

New edition of the book:

“Pests and vector-borne diseases in the livestock industry” edited by Claire Garros, Jérémy Bouyer, Willem Takken and Renate C. Smallegange

Schistosomes

Since 2013, human urogenital schistosomiasis has become established on the island of Corsica (France). The *Schistosoma* involved in the Corsican outbreak is a hybrid form between *Schistosoma haematobium*, a human parasite, and *Schistosoma bovis*, a livestock parasite. *S. bovis* had been detected in Corsican livestock a few decades ago raising the question whether hybridization occurred in Corsica and if animals could behave as a reservoir for the recently established parasite lineage. The latter hypothesis has huge epidemiological implications as the emergence of a zoonotic form of schistosome would be much more difficult to control and eradicate (Oleaga et al.).

Mini-FLOTAC as a Diagnostic Tool for *Schistosoma Mansoni* and Other Trematode Infections in Wildlife Reservoirs: Qualitative and quantitative data derived from post-mortem examinations, coprological analyses using the Mini-FLOTAC technique, and molecular tools were used to assess parasite community composition in mice from northern Senegal. Parasites isolated at post-mortem examination were identified as *Plagiorchis* sp., *Anchitrema* sp., *Echinostoma caproni*, *Schistosoma mansoni*, and a hybrid between *Schistosoma haematobium* and *Schistosoma bovis*. Detection using Mini-FLOTAC technique on faeces correlated very closely with parasite detection during post mortem, therefore providing an alternative non-lethal method of detection for use on wildlife species (Catalano et al.).

Abstracts

VECTORS - ticks and arthropods

Author: Boulanger, N., Boyer, P., Talagrand-Reboul, E. and Hansmann, Y.

Title: Ticks and tick-borne diseases

DOI: 10.1016/j.medmal.2019.01.007

Abstract: Ticks are a major group of arthropod vectors, characterized by the diversity of pathogens they transmit, by their impact on human and animal health, and by their socioeconomic implication especially in countries of the Southern Hemisphere. In Europe, Ixodes is the most important tick due to its wide distribution in the ecosystems and the variety of transmitted pathogens, in particular Borrelia (responsible for Lyme borreliosis), but also the tick-borne encephalitis virus. Their increased presence in the environment since the beginning of the 20th century is undeniable, because of major modifications in the biodiversity caused by humans. Increasing the awareness of health professionals and the general population is required to achieve better control of these infections. Thus, “a better understanding of these tick-borne diseases for a better control” is a simple but effective approach, considering their ubiquity in the environment and their particular mode of pathogen transmission (long-lasting blood meal for hard ticks and delayed transmission for bacteria and parasites). Finally, these ectoparasites are problematic due to the potential allergic reactions and other damages caused by their saliva, in humans and animals.

Author: Kayleigh M Hansford ¹, Daniel Carter ², Emma L Gillingham ³, Luis M Hernandez-Triana ⁴, John Chamberlain ⁵, Benjamin Cull ⁶, Liz McGinley ⁶, L Paul Phipps ⁴, Jolyon M Medlock ⁷

Title: Hyalomma rufipes on an untraveled horse: Is this the first evidence of Hyalomma nymphs successfully moulting in the United Kingdom?

Ticks Tick Borne Dis . 2019 Apr;10(3):704-708. doi: 10.1016/j.ttbdis.2019.03.003. Epub 2019 Mar 9.

Abstract: During September 2018, a tick was submitted to Public Health England's Tick Surveillance Scheme for identification. The tick was sent from a veterinarian who removed it from a horse in Dorset, England, with no history of overseas travel. The tick was identified as a male Hyalomma rufipes using morphological and molecular methods and then tested for a range of tick-borne pathogens including; Alkhurma virus, Anaplasma, Babesia, Bhanja virus, Crimean-Congo Haemorrhagic fever virus, Rickettsia and Theileria. The tick tested positive for Rickettsia aeschlimanii, a spotted fever group rickettsia linked to a number of human cases in Africa and Europe. This is the first time H. rufipes has been reported in the United Kingdom (UK), and the lack of travel by the horse (or any in-contact

horses) suggests that this could also be the first evidence of successful moulting of a *Hyalomma* nymph in the UK. It is postulated that the tick was imported into the UK on a migratory bird as an engorged nymph which was able to complete its moult to the adult stage and find a host. This highlights that passive tick surveillance remains an important method for the detection of unusual species that may present a threat to public health in the UK. Horses are important hosts of *Hyalomma* sp. adults in their native range, therefore, further surveillance studies should be conducted to check horses for ticks in the months following spring bird migration; when imported nymphs may have had time to drop off their avian host and moult to adults. The potential human and animal health risks of such events occurring more regularly are discussed.

Vector control strategies

Benelli G^{1,2}.

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

Saudi J Biol Sci. 2019 Jul;26(5):921-929.

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

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

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

DOI: 10.3390/vaccines7030075

Abstract: The prevention and control of vector-borne diseases is a priority for improving global health. Despite recent advances in the characterization of ectoparasite-host-pathogen molecular interactions, vaccines are not available for most ectoparasites and

vector-borne diseases that cause millions of deaths yearly. In this paper, in response to the question of why new vaccines for the control of ectoparasite vectors have not been registered and commercialized, and to contribute developing new effective vaccines against ectoparasite vectors, we propose challenges and approaches to be addressed.

Tick vaccines

Rego ROM¹, Trentelman JJA², Anquita J^{3,4}, Nijhof AM⁵, Sprong H⁶, Klempa B⁷, Hajdusek O⁸, Tomás-Cortázar J³, Azagi T⁶, Strnad M^{8,9}, Knorr S⁵, Sima R⁸, Jalovecka M^{8,9}, Fumačová Havlíková S⁷, Ličková M⁷, Sláviková M⁷, Kopacek P^{8,9}, Grubhoffer L^{8,9}, Hovius JW².

Counterattacking the tick bite: towards a rational design of anti-tick vaccines targeting pathogen transmission

[Parasit Vectors](#). 2019 May 14;12(1):229.

Author: Marinela Contreras ¹, Paul D Kasaija ^{1 2}, Octavio Merino ³, Ned I de la Cruz-Hernandez ³, Christian Gortazar ¹, José de la Fuente ^{1 4}

Title: Oral Vaccination With a Formulation Combining *Rhipicephalus microplus* Subolesin With Heat Inactivated *Mycobacterium bovis* Reduces Tick Infestations in Cattle

Front Cell Infect Microbiol . 2019 Mar 1;9:45.

Author: Martínez-Arzate, S. G., Sánchez-Bermúdez, J. C., Sotelo-Gómez, S., Diaz-Albiter, H. M., Hegazy-Hassan, W., Tenorio-Borroto, E., Barbabosa-Pliego, A. and Vázquez-Chagoyán, J. C.

Title: Genetic diversity of Bm86 sequences in *Rhipicephalus* (*Boophilus*) *microplus* ticks from Mexico: Analysis of haplotype distribution patterns

DOI: 10.1186/s12863-019-0754-8

Author: Płusa, T.

Title: A "new" look at "old" borreliosis

Abstract: The threat of borreliosis is a growing medical problem. The number of cases is constantly increasing, and the diagnostic methods and recommended treatment methods do not bring new values in comparison to the findings from over 30 years ago. Among the pathogens transmitted by ticks, apart from *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, *Babesia* species, *Neoehrlichia mikurensis*, as well as fever caused by rickettsiae and others, are referred to as "emerging" or "rediscovered". Recently published new recommendations for the diagnosis and treatment of Lyme disease developed by specialists from the National Institute for Health and Care Excellence (NICE) in the United

Kingdom did not bring significant changes to the existing recommendations for the diagnosis and treatment of patients with borreliosis. This study does not provide information about vaccine research, which, it turned out, was introduced in the 1990s and withdrawn from the market. Research is currently under way into an anti-tick "vaccine" undertaken by the team under the EU project "ANTIDOTE" (Anti-tick Vaccines to Prevent Tick-borne Diseases in Europe). The first effects of the research are already presented, but we still have to wait for the final product.

Research into new tick treatments

Author: González-López, G., Ojeda-Chi, M. M., Casanova-Lugo, F., Oros-Ortega, I., Hernández-Chávez, L. I., Piñeiro-Vázquez, Á T. and Rodríguez-Vivas, R. I.

Title: In vitro acaricide activity of extracts from three *Leucaena* spp. Genotypes versus *Rhipicephalus microplus*

DOI: 10.22319/rmcp.v10i3.4822

Author: Jyoti, Singh, N. K., Singh, H., Mehta, N. and Rath, S. S.

Title: In vitro assessment of synergistic combinations of essential oils against *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae)

DOI: 10.1016/j.exppara.2019.04.007

Author: Lazcano Díaz, E., Padilla Camberos, E., Castillo Herrera, G. A., Estarrón Espinosa, M., Espinosa Andrews, H., Paniagua Buelnas, N. A., Gutiérrez Ortega, A. and Martínez Velázquez, M.

Title: Development of essential oil-based phyto-formulations to control the cattle tick *Rhipicephalus microplus* using a mixture design approach

DOI: 10.1016/j.exppara.2019.04.008

Author: Sugauara, E. Y. Y., Sugauara, E., Sugauara, R. R., Bortolucci, W. C., de Oliveira, H. L. M., Silva, E. S., Campos, C. F. A. A., Gonçalves, J. E., Colauto, N. B., Gazim, Z. C. and Linde, G. A.

Title: Control of bovine tick [*Rhipicephalus (Boophilus) microplus*] with *Brunfelsia uniflora* leaf extract

DOI: 10.21475/ajcs.19.13.06.p1653

Acaricide resistance research

Author: Klafke, G. M., Miller, R. J., Tidwell, J. P., Thomas, D. B., Sanchez, D., Feria Arroyo, T. P. and Pérez de León, A. A.

Title: High-resolution melt (HRM) analysis for detection of SNPs associated with pyrethroid resistance in the southern cattle fever tick, *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae)

DOI: 10.1016/j.ijpddr.2019.03.001

Author: Tucker, N. S. G., Kaufman, P. E. and Weeks, E. N. I.

Title: Identification of permethrin and etofenprox cross-tolerance in *Rhipicephalus sanguineus sensu lato* (Acari: Ixodidae)

DOI: 10.1002/ps.5391

Research into new insect treatments

Minshad Ansari¹, Miranda Walker¹, Paul Dyson¹

Fungi as Biocontrol Agents of *Culicoides* Biting Midges, the Putative Vectors of Bluetongue Disease *Vector Borne Zoonotic Dis*. 2019 Jun;19(6):395-399.

Biting midges of the genus *Culicoides* (Diptera: Ceratopogonidae) are the principal vectors of several notable viral pathogens infecting animal livestock. Sickness and animal deaths caused by the *Culicoides*-transmitted bluetongue virus, as well as the recent Schmallenberg virus outbreak, have threatened the livestock industry in Europe. Recent studies highlight how, in the near future, the application of "dry" fungal conidia of *Metarhizium anisopliae* in animal shelters and microenvironment (*e.g.*, dung, manure, leaf litter, and livestock surroundings) may be used to control the *Culicoides* vector, thus, reducing the incidence of *Culicoides*-borne diseases.

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

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

DOI: 10.1093/jme/tjz020

Abstract: The horn fly, *Haematobia irritans irritans* (L.) (Diptera: Muscidae), is an important and cosmopolitan blood feeding ectoparasite of cattle. Resistance to conventional insecticides is increasingly problematic and alternative pesticides, including natural products, are being investigated. Limonene is a cyclic monoterpene repellent to some insects that occurs in citrus fruit rinds and in other plants. We assessed laboratory

grade limonene and a commercial product, Orange Guard (5.8% AI limonene), against *H. irritans irritans* in terms of their contact effects upon contact on egg mortality, adults, and larval and pupal development; adult repellency as well as sublethal and fumigation effects. Egg viability declined when they were exposed to Orange Guard at concentrations of 1.45%, 2.9%, and 5.8% whereas laboratory grade limonene at 5.8% and 11.6% was ovicidal. Contact exposure of adult *H. irritans irritans* to 5.8% laboratory grade limonene and 2.9% Orange Guard caused up to 100 and 88% knockdown (immobilization), respectively. At higher concentrations, laboratory grade limonene and Orange Guard resulted in less, and often shorter periods of knockdown. Although direct contact of 2.9 and 5.8% laboratory grade limonene caused mortality it was negligible when flies were sprayed directly with undiluted Orange Guard. Female *H. irritans irritans* exposed to sublethal concentrations of Orange Guard did not reduce the numbers of eggs produced, but the undiluted product reduced egg hatchability. Interestingly, limonene and Orange Guard attracted adult *H. irritans irritans* at concentrations <0.1%. We suggest that the attractancy of unformulated pure limonene might be useful for trapping *H. irritans irritans* adults.

VECTOR BORNE DISEASE - Test and surveillance developments

Bethan V Purse¹, Narayanaswamy Darshan^{2 3}, Gudadappa S Kasabi², France Gerard¹, Abhishek Samrat⁴, Charles George¹, Abi T Vanak^{4 5 6}, Meera Oommen^{4 7}, Mujeeb Rahman⁴, Sarah J Burthe⁸, Juliette C Young^{8 9}, Prashanth N Srinivas¹⁰, Stefanie M Schäfer¹, Peter A Henrys¹¹, Vijay K Sandhya², M Mudassar Chanda¹², Manoj V Murhekar¹³, Subhash L Hoti³, Shivani K Kiran²

Predicting Disease Risk Areas Through Co-Production of Spatial Models: The Example of Kyasanur Forest Disease in India's Forest Landscapes

PLoS Negl Trop Dis. 2020 Apr 7;14(4)

Zoonotic diseases affect resource-poor tropical communities disproportionately, and are linked to human use and modification of ecosystems. Disentangling the socio-ecological mechanisms by which ecosystem change precipitates impacts of pathogens is critical for predicting disease risk and designing effective intervention strategies. Despite the global "One Health" initiative, predictive models for tropical zoonotic diseases often focus on narrow ranges of risk factors and are rarely scaled to intervention programs and ecosystem use. This study uses a participatory, co-production approach to address this disconnect between science, policy and implementation, by developing more informative disease models for a fatal tick-borne viral haemorrhagic disease, Kyasanur Forest Disease (KFD), that is spreading across degraded forest ecosystems in India. We integrated knowledge across disciplines to identify key risk factors and needs with actors and beneficiaries across the relevant policy sectors, to understand disease patterns and develop decision support tools. Human case locations (2014-2018) and spatial machine

learning quantified the relative role of risk factors, including forest cover and loss, host densities and public health access, in driving landscape-scale disease patterns in a long-affected district (Shivamogga, Karnataka State). Models combining forest metrics, livestock densities and elevation accurately predicted spatial patterns in human KFD cases (2014-2018). Consistent with suggestions that KFD is an "ecotonal" disease, landscapes at higher risk for human KFD contained diverse forest-plantation mosaics with high coverage of moist evergreen forest and plantation, high indigenous cattle density, and low coverage of dry deciduous forest. Models predicted new hotspots of outbreaks in 2019, indicating their value for spatial targeting of intervention. Co-production was vital for: gathering outbreak data that reflected locations of exposure in the landscape; better understanding contextual socio-ecological risk factors; and tailoring the spatial grain and outputs to the scale of forest use, and public health interventions. We argue this interdisciplinary approach to risk prediction is applicable across zoonotic diseases in tropical settings.

Robin R L Simons¹, Simon Croft², Eleanor Rees¹, Oliver Tearne¹, Mark E Arnold¹, Nicholas Johnson¹

Using Species Distribution Models to Predict Potential Hot-Spots for Rift Valley Fever Establishment in the United Kingdom

PLoS One . 2019 Dec 23;14(12):e0225250.

Vector borne diseases are a continuing global threat to both human and animal health. The ability of vectors such as mosquitos to cover large distances and cross country borders undetected provide an ever-present threat of pathogen spread. Many diseases can infect multiple vector species, such that even if the climate is not hospitable for an invasive species, indigenous species may be susceptible and capable of transmission such that one incursion event could lead to disease establishment in these species. Here we present a consensus modelling methodology to estimate the habitat suitability for presence of mosquito species in the UK deemed competent for Rift Valley fever virus (RVF) and demonstrate its application in an assessment of the relative risk of establishment of RVF virus in the UK livestock population. The consensus model utilises observed UK mosquito surveillance data, along with climatic and geographic prediction variables, to inform six independent species distribution models; the results of which are combined to produce a single prediction map. As a livestock host is needed to transmit RVF, we then combine the consensus model output with existing maps of sheep and cattle density to predict the areas of the UK where disease is most likely to establish in local mosquito populations. The model results suggest areas of high suitability for RVF competent mosquito species across the length and breadth of the UK. Notable areas of high suitability were the South West of England and coastal areas of Wales, the latter of which was subsequently predicted to be at higher risk for establishment of RVF due to higher livestock densities. This study demonstrates the applicability of outputs of species distribution models to help predict hot-spots for risk of disease establishment. While there is still uncertainty associated with the outputs we believe that the predictions are an improvement on just using the raw presence points from a database alone. The outputs

can also be used as part of a multidisciplinary approach to inform risk based disease surveillance activities.

Umer Chaudhry¹, Qasim Ali², Imran Rashid², Muhammad Zubair Shabbir², Muhammad Ijaz², Muhammad Abbas³, Mike Evans⁴, Kamran Ashraf², Ivan Morrison⁴, Liam Morrison⁴, Neil D Sargison⁴

Development of a Deep Amplicon Sequencing Method to Determine the Species Composition of Piroplasm Haemoprotozoa

Ticks Tick Borne Dis . 2019 Oct;10(6):101276.

Piroplasmosis is caused by tick-borne haemoprotozoa of the genera *Theileria* and *Babesia*. These parasitic infections can seriously impact on the health of livestock and production. Piroplasms of multiple species can be present in a single host, but reliable molecular diagnostic tools are needed in order to understand the composition of these complex parasite communities. *Theileria* and *Babesia* vary in their epidemiology, drug sensitivity, pathogenicity and interaction with co-infecting species, but are similar in that infected animals become persistent carriers after recovery from primary infection, acting as reservoir hosts. Here, we describe for the first time the use of a deep amplicon sequencing platform to identify proportions of piroplasm species in co-infecting communities and develop the concept of a "haemoprotobiome". First, four phenotypically-verified species of *Theileria* and *Babesia* were used to prepare mock DNA pools with random numbers of the parasites amplified by four different numbers of PCR cycles to assess sequence representation for each species. Second, we evaluated the detection threshold of the deep amplicon sequencing assay for each of the four species and to assess the accuracy of proportional quantification of all four species. Finally, we applied the assay to the field samples to afford insight of the species composition of piroplasm communities in small and large ruminants in the Punjab province of Pakistan. The "haemoprotobiome" concept has several potential applications in veterinary and human research, including understanding of responses to drug treatment; parasite epidemiology and ecology; species interactions during mixed infections; and parasite control strategies.

Tick pathogen surveys in Europe

Klitgaard K¹, Kjær LJ², Isbrand A², Hansen MF², Bødker R².

Multiple infections in questing nymphs and adult female *Ixodes ricinus* ticks collected in a recreational forest in Denmark.

Ticks Tick Borne Dis 2019 Aug;10(5):1060-1065.

During its lifecycle, the generalist *Ixodes ricinus* takes up three blood meals from a wide selection of vertebrate hosts, some of which are reservoirs for multiple vector-associated pathogens. Since *I. ricinus* also readily bites humans, pets, and livestock, these hosts are at risk of becoming infected with more than one tick-borne pathogen. Multiple tick-borne

infections are a public health concern, since they may increase diversity and duration of symptoms and complicate differential diagnosis and therapy. We used an existing Fluidigm real-time PCR chip to identify the minimum risk of exposure to infected/co-infected ticks in Denmark. We screened 509 nymphs and 504 adult female *I. ricinus* ticks for 17 different vector-associated pathogenic agents. The questing ticks were collected by flagging during the same season in two consecutive years in Grib forest in the capital region of Copenhagen. Overall, 19.1% of the nymphs and 52.2% of the adult female ticks harbored at least one zoonotic pathogen. The main agents were *Borrelia* spp., *Anaplasma phagocytophilum* and *Rickettsia helvetica*, while *Candidatus Neoehrlichia mikurensis* and *Babesia venatorum* both were present in less than 1% of the ticks. In 3.5% of the nymphs and 12.3% of adults we found more than one tick-borne pathogen. Of these, 15% were potentially triple or quadruple infections. Whereas mixed infections with *Borrelia* were equally distributed among both life stages, the adult ticks hosted 84.5% of the co-infections with different species of tick-borne pathogens, chiefly involving *Borrelia* species in combination with either *R. helvetica* or *A. phagocytophilum*. Statistical analyses indicated non-random co-occurrence of *Borrelia spielmanii*/*Borrelia garinii* in both life stages and *B. garinii*/*Borrelia afzelii* and *B. garinii*/*Borrelia valaisiana* in the nymphs. Although the overall prevalence of ticks hosting more than one infection only constituted 7.9% at the particular site investigated in this study, our results still underline that co-infections should be considered in diagnosis and treatment of tick-borne diseases in northern Europe.

V Cicculi¹, L Capai², Y Quilichini³, S Masse⁴, A Fernández-Alvarez⁵, L Minodier⁶, P Bompard⁷, R Charrel⁸, A Falchi⁹

Molecular Investigation of Tick-Borne Pathogens in Ixodid Ticks Infesting Domestic Animals (Cattle and Sheep) and Small Rodents (Black Rats) of Corsica, France

Ticks Tick Borne Dis . 2019 Apr;10(3):606-613.

Although livestock farming (sheep, goats, pigs, and cattle) is an important economic activity in Corsica, a French Mediterranean island, knowledge about the tick fauna and microorganisms carried by them remains scarce. This study aimed to investigate the presence and perform molecular characterization of Anaplasmataceae, *Rickettsia* spp., and *Borrelia burgdorferi sensu lato* (sl) in tick species collected in Corsica. Ticks from cattle (*Bos taurus*), sheep (*Ovis aries*), and rodents (*Rattus rattus*) were collected from May to September 2016. DNA was purified from ticks, submitted to quantitative real-time polymerase chain reaction (qPCR) and sequenced for phylogenetic analysis. In total, 660 ticks were collected from 111 animals during the study. The most abundant collected tick species from cattle was *Rhipicephalus bursa* (n = 495; 84.5%), followed by *Hyalomma marginatum* (n = 91; 15.5%). *Rhipicephalus bursa* and *Ixodes ricinus* were the only tick species collected from sheep and rodents, respectively. Overall, *Rickettsia* was the most common pathogen group (n = 48; 24%) detected in ticks. Sequence analysis of partial *gltA* and *ompA* genes revealed the presence of *Ri. aeschlimannii* and *Candidatus Ri. barbariae*. Anaplasmataceae DNA was detected in eight (6%) of the 127 cattle pools and in one (2%) of the 61 *R. bursa* specimens collected from sheep. Sequence analysis of the *rpoB* gene revealed the presence of one *Anaplasma* species, *A. marginale*. *Borrelia*

burgdorferi sI DNA was detected in one pool of *H. marginatum* collected from cattle and in two (15%) of the 13 *I. ricinus* pools collected from nine black rats. To our knowledge, this is the first report of the occurrence and molecular characterization of *Candidatus Ri. barbariae*, an emerging member of the *Rickettsia* group causing spotted fever, in Corsica. The detection of *B. burgdorferi* sI DNA, which was previously believed to be rare in Corsica, confirms the presence of this agent on the island.

Tularemia

Hennebique A^{1,2}, Boisset S^{1,2}, Maurin M^{1,2}.

Tularemia as a waterborne disease: a review.

Emerg Microbes Infect. 2019;8(1):1027-1042. doi: 10.1080/22221751.2019.1638734.

Francisella tularensis is a Gram-negative, intracellular bacterium causing the zoonosis tularemia. This highly infectious microorganism is considered a potential biological threat agent. Humans are usually infected through direct contact with the animal reservoir and tick bites. However, tularemia cases also occur after contact with a contaminated hydro-telluric environment. Water-borne tularemia outbreaks and sporadic cases have occurred worldwide in the last decades, with specific clinical and epidemiological traits. These infections represent a major public health and military challenge. Human contaminations have occurred through consumption or use of *F. tularensis*-contaminated water, and various aquatic activities such as swimming, canyoning and fishing. In addition, in Sweden and Finland, mosquitoes are primary vectors of tularemia due to infection of mosquito larvae in contaminated aquatic environments. The mechanisms of *F. tularensis* survival in water may include the formation of biofilms, interactions with free-living amoebae, and the transition to a 'viable but nonculturable' state, but the relative contribution of these possible mechanisms remains unknown. Many new aquatic species of *Francisella* have been characterized in recent years. *F. tularensis* likely shares with these species an ability of long-term survival in the aquatic environment, which has to be considered in terms of tularemia surveillance and control.

Borrelia

Author: Plusa, T.

Title: A "new" look at "old" borreliosis

Abstract: The threat of borreliosis is a growing medical problem. The number of cases is constantly increasing, and the diagnostic methods and recommended treatment methods do not bring new values in comparison to the findings from over 30 years ago. Among the pathogens transmitted by ticks, apart from *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, *Babesia* species, *Neoehrlichia mikurensis*, as well as fever caused by *rickettsiae* and others, are referred to as "emerging" or "rediscovered". Recently published

new recommendations for the diagnosis and treatment of Lyme disease developed by specialists from the National Institute for Health and Care Excellence (NICE) in the United Kingdom did not bring significant changes to the existing recommendations for the diagnosis and treatment of patients with borreliosis. This study does not provide information about vaccine research, which, it turned out, was introduced in the 1990s and withdrawn from the market. Research is currently under way into an anti-tick "vaccine" undertaken by the team under the EU project "ANTIDOTE" (Anti-tick Vaccines to Prevent Tick-borne Diseases in Europe). The first effects of the research are already presented, but we still have to wait for the final product.

Human Babesiosis

Author: Kukina, I. V., Zelya, O. P. and Karan, L. S.

Title: Laboratory diagnostic of human babesiosis

DOI: 10.18821/0869-2084-2019-64-9-560-564

Abstract: Human babesiosis caused by parasitic protozoan *Babesia* spp. is sporadic zoonotic vector-borne infection. The course of babesiosis and prognosis depend on the type of pathogen and on the patient's immunological status. Significance this disease is a severe, often fatal course with immunocompromised patients resembling complicated falciparum malaria. In Europe to date, more than 50 cases of confirmed human babesiosis have been reported in most cases caused by *Babesia divergens*. Possible there are unrecognized cases. Pathogen is an obligate intraerythrocyte parasite of vertebrate animals. The organism is transmitted from animal to man through bite of Ixodidae tick. Asexual reproduction of the parasite occurs in a vertebrate host. The pathogenesis of babesiosis is caused by the destruction of host cells. Intensive haemolysis of red blood cells leads to the development of haemolytic anemia, haematuria, jaundice, and polyorgan failure may develop. The clinical manifestations of the disease are nonspecific. Detection of intraerythrocyte parasites in blood smears stained Gimsa-Romanovsky confirms the proposed diagnosis. Blood smears and some laboratory signs from fatal cases were analyzed in the Reference-centre of E. I. Martsinovskii Institute. Original microphotographs *B. divergens* are shown. The main morphological forms of the parasite are shown. In addition to the well-known tetrads of parasites «Maltese Cross», for the first time, the parasites dividing into 6 interconnected trophozoites - "sextet" - were found. Originally, the invasion of *Babesia* in a normoblast is shown. An unusually high multiple invasion (14 parasites) of erythrocytes is noted. Because the patients, initially, were incorrectly diagnosed with malaria, the differential diagnosis of *Babesia* with *Plasmodium* is described step-by-step. It is important, since the treatment with antimalarial drugs is ineffective. Deviation laboratory signs are discussed. Complex morphological characteristics allowed us to speciated the parasites as *B. divergens*. DNA was detected in the sample with specific primers Bab di hsp70F/Bab di hsp70R and the probe Bab di hsp70P. The sequence demonstrated 99-100% and 98% similarity to the 18S rRNA gene fragment of *B.*

divergence and *Babesia venatorum*, respectively. Molecular biological and serological methods of laboratory diagnosis of babesiosis are considered.

Livestock vector-borne diseases

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Molecular Detection of *Mycoplasma Ovis* in an Outbreak of Hemolytic Anemia in Sheep From Veracruz, Mexico

Trop Anim Health Prod . 2019 Jan;51(1):243-248.

Mycoplasma ovis is a small, pleiotropic bacterium, which parasitizes the external surface of erythrocytes of several species of artiodactyl mammals, especially sheep and goats. We here report an outbreak of ovine mycoplasmosis in a sheep flock of a private ranch (Universidad Veracruzana) in Veracruz, Mexico. For the identification of *Mycoplasma* and other hemoparasitic bacterial agents, we stained blood smears with the DiffQuick® technique and additionally amplified several fragments of 16S rDNA gene. We detected the presence of morulas in erythrocytes from 30 sick female adult sheep, and found *Mycoplasma ovis* DNA in all of them. Furthermore, three of these animals also tested positive for *Anaplasma ovis*. Our findings represent the first record of *M. ovis* and *A. ovis* in an outbreak of hemolytic anemia in a sheep flock, leading to severe livestock loss in a ranch of Mexico. This study highlights the importance of establishing an active surveillance of both pathogens in the country.

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Infection Dynamics of *Theileria Annulata* Over a Disease Season Following Cell Line Vaccination

Vet Parasitol . 2019 Jan;265:63-73.

Tropical theileriosis is a tick-borne haemoparasitic disease of cattle caused by the protozoan parasite *Theileria annulata*. Globally, the economic impact of the disease is immense and enhanced control measures would improve livestock production in endemic regions. Immunisation with a live attenuated vaccine is an effective and widely used control method, however, the repeated use of live vaccines may have an impact on the field parasite population at a genetic level. Additionally, there has been an increasing number of reports of vaccine breakthrough cases in recent years. Thus, the present study was designed to evaluate the genetic composition of a parasite population over a disease season in a locality where live cell line vaccination is practised. A diverse range of parasite genotypes was identified and every *T. annulata* positive cattle blood sample harboured multiple parasite genotypes. An alteration in the major genotype and an increasing

multiplicity of infection in individual animals was observed over the course of the disease season. Vaccination status was found not to effect within-host multiplicity of infection, while a significantly higher number of genotypes was detected in grazed cattle compared to non-grazed ones. A degree of genetic isolation was evident between parasite populations on a micro-geographic scale, which has not been reported previously for *T. annulata*. Analysis of parasite genotypes in vaccinated animals suggested only a transient effect of the vaccine genotype on the genetic diversity of the *T. annulata* population. The vaccine genotype was not detected among clones of two vaccine 'breakthrough' isolates and there is no suggestion that it was responsible for disease. The obtained data indicated that in the system studied there is no apparent risk of introducing the vaccine genotype into the population with only a transient effect on the genetic diversity of the parasite population during the disease season.

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High Co-Infection Rates of Babesia Bovis, Babesia Bigemina, and Anaplasma Marginale in Water Buffalo in Western Cuba

Parasitol Res. 2019 Mar;118(3):955-967.

Water buffalo is important livestock in several countries in the Latin American and Caribbean regions. This buffalo species can be infected by tick-borne hemoparasites and remains a carrier of these pathogens which represent a risk of infection for more susceptible species like cattle. Therefore, studies on the epidemiology of tick-borne hemoparasites in buffaloes are required. In this study, the prevalence of Babesia bovis, Babesia bigemina, and Anaplasma marginale were determined in water buffalo herds of western Cuba. To this aim, a cross-sectional study covering farms with large buffalo populations in the region was performed. Eight buffalo herds were randomly selected, and blood samples were collected from 328 animals, including 63 calves (3-14 months), 75 young animals (3-5 years), and 190 adult animals (> 5 years). Species-specific nested PCR and indirect ELISA assays were used to determine the molecular and serological prevalences of each hemoparasite, respectively. The molecular and serological prevalence was greater than 50% for the three hemoparasites. Differences were found in infection prevalence among buffalo herds, suggesting that local epidemiological factors may influence infection risk. Animals of all age groups were infected, with a higher molecular prevalence of *B. bigemina* and *A. marginale* in young buffalo and calves, respectively, while a stepwise increase in seroprevalence of *B. bovis* and *B. bigemina* from calves to adult buffaloes was found. The co-infection by the three pathogens was found in 12% of animals, and when analyzed by pair, the co-infections of *B. bovis* and *B. bigemina*, *B. bigemina* and *A. marginale*, and *B. bovis* and *A. marginale* were found in 20%, 24%, and 26%, respectively, underlying the positive interaction between these pathogens infecting buffaloes. These results provide evidence that tick-borne pathogen infections can be widespread among water buffalo populations in tropical livestock ecosystems. Further studies should evaluate whether these pathogens affect the health status and productive

performance of water buffalo and infection risk of these pathogens in cattle cohabiting with buffalo.

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Epidemiology, Risk Factors, and Co-Infection of Vector-Borne Pathogens in Goats From Sistan and Baluchestan Province, Iran

PLoS One . 2019 Jun 20;14(6):e0218609.

Several vector-borne pathogens restrict livestock farming and have significant economic impact worldwide. In endemic areas livestock are exposed to different tick species carrying various pathogens which could result in co-infection with several tick-borne pathogens in a single host. Although the co-infection of and the interaction among pathogens are critical factors to determine the disease outcome, pathogen interactions in the vector and the host are poorly understood. In this study, we surveyed the presence of *Babesia ovis*, *Theileria ovis*, *Theileria lestoquardi*, *Anaplasma ovis*, *Anaplasma phagocytophilum*, and *Anaplasma marginale* in 200 goats from 3 different districts in Sistan and Baluchestan province, Iran. Species-specific diagnostic PCRs and sequence analysis revealed that 1.5%, 12.5%, and 80% of samples were positive for *T. lestoquardi*, *T. ovis*, and *A. ovis*, respectively. Co-infections of goats with up to 3 pathogens were seen in 22% of the samples. We detected a significant association between *T. ovis* infection and age, *T. ovis* infection and location (Zabol), and *A. ovis* infection and location (Sarbaz) by multivariate logistic regression analysis. In addition, by analyzing the data with respect to *Plasmodium caprae* infection in these goats, a negative correlation was found between *P. caprae* and *A. ovis* infection. This study contributes to understanding the epidemiology of vector-borne pathogens and their interplay in goats.

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Molecular Detection and Phylogenetic Analysis of *Anaplasma* Spp. In Korean Native Goats From Ulsan Metropolitan City, Korea

Vector Borne Zoonotic Dis . 2019 Oct;19(10):773-776.

Tick-borne diseases (TBDs) can be fatal to humans as well as to animals causing severe economic losses globally to livestock industries. Many countries conduct regular surveillance of TBDs in livestock. Serological and molecular surveillance of TBDs in livestock and humans was carried out in the Republic of Korea. However, there are not a lot of data on analyses of anaplasmosis in Korean native goats and the correlation with rearing methods and seasons. In this study, goats in Ulsan city were tested for anaplasmosis by PCR and 16S rRNA sequencing. A total of 452 goat blood samples were collected from 20 farms in 2016. The goat farms in Ulsan city had three different types of rearing methods: conventional, confined, and mixed grazing-confined. Forty-nine of the 452 goats (10.8%) were anaplasmosis positive. Sequence analysis of the PCR products from these 49 goats revealed that 39 of 452 goats (8.6%) were *Anaplasma bovis* positive,

and 10 of 452 goats (2.2%) were infected with *Anaplasma capra*. The highest outbreaks of anaplasmosis occurred in mixed grazing-confined type of farms (27.1%, 33/122) ($\chi^2 = 60.72$, $df = 2$, $p < 0.05$), but there was no significant difference in the occurrence of anaplasmosis between spring, summer, and fall seasons. This study was the first detection of *A. bovis* in Korean native goats and its relationship with rearing methods and seasons. These findings suggested that Korean native goats were highly exposed to *Anaplasma* spp. during summers when the tick population is the highest and in farms employing mixed grazing-confined rearing methods.

Babesia

Author: González, L. M., Estrada, K., Grande, R., Jiménez-Jacinto, V., Vega-Alvarado, L., Sevilla, E., De La Barrera, J., Cuesta, I., Zaballos, Á, Bautista, J. M., Lobo, C. A., Sánchez-Flores, A. and Montero, E.

Title: Comparative and functional genomics of the protozoan parasite *Babesia divergens* highlighting the invasion and egress processes

DOI: 10.1371/journal.pntd.0007680

Abstract: Babesiosis is considered an emerging disease because its incidence has significantly increased in the last 30 years, providing evidence of the expanding range of this rare but potentially life-threatening zoonotic disease. *Babesia divergens* is a causative agent of babesiosis in humans and cattle in Europe. The recently sequenced genome of *B. divergens* revealed over 3,741 protein coding-genes and the 10.7-Mb high-quality draft become the first reference tool to study the genome structure of *B. divergens*. Now, by exploiting this sequence data and using new computational tools and assembly strategies, we have significantly improved the quality of the *B. divergens* genome. The new assembly shows better continuity and has a higher correspondence to *B. bovis* chromosomes. Moreover, we present a differential expression analysis using RNA sequencing of the two different stages of the asexual lifecycle of *B. divergens*: The free merozoite capable of invading erythrocytes and the intraerythrocytic parasite stage that remains within the erythrocyte until egress. Comparison of mRNA levels of both stages identified 1,441 differentially expressed genes. From these, around half were upregulated and the other half downregulated in the intraerythrocytic stage. Orthogonal validation by real-time quantitative reverse transcription PCR confirmed the differential expression. A moderately increased expression level of genes, putatively involved in the invasion and egress processes, were revealed in the intraerythrocytic stage compared with the free merozoite. On the basis of these results and in the absence of molecular models of invasion and egress for *B. divergens*, we have proposed the identified genes as putative molecular players in the invasion and egress processes. Our results contribute to an understanding of key parasitic strategies and pathogenesis and could be a valuable genomic resource to exploit for the design of diagnostic methods, drugs and vaccines to improve the control of babesiosis.

Author: Antonio Alvarez, J., Rojas, C. and Figueroa, J. V.

Title: Diagnostic tools for the identification of Babesia sp. In persistently infected cattle

DOI: 10.3390/pathogens8030143

Abstract: Bovine babesiosis is a tick-borne disease of cattle caused by the protozoan parasites of the genus Babesia. Babesia bovis, Babesia bigemina and Babesia divergens are considered by International health authorities (OIE) as the principal species of Babesia that cause bovine babesiosis. Animals that recover from a babesial primo infection may remain as persistent carriers with no clinical signs of disease and can be the source of infection for ticks that are able to acquire Babesia parasites from infected cattle and to transmit Babesia parasites to susceptible cattle. Several procedures that have been developed for parasite detection and diagnosis of this infectious carrier state constitute the basis for this review: A brief description of the direct microscopic detection of Babesia-infected erythrocytes; PCR-based diagnostic assays, which are very sensitive particularly in detecting Babesia in carrier cattle; in-vitro culture methods, used to demonstrate presence of carrier infections of Babesia sp.; animal inoculation, particularly for B. divergens isolation are discussed. Alternatively, persistently infected animals can be tested for specific antibabesial antibodies by using indirect serological assays. Serological procedures are not necessarily consistent in identifying persistently infected animals and have the disadvantage of presenting with cross reactions between antibodies to Babesia sp.

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Unravelling the Cellular and Molecular Pathogenesis of Bovine Babesiosis: Is the Sky the Limit?

Int J Parasitol . 2019 Feb;49(2):183-197.

The global impact of bovine babesiosis caused by the tick-borne apicomplexan parasites Babesia bovis, Babesia bigemina and Babesia divergens is vastly underappreciated. These parasites invade and multiply asexually in bovine red blood cells (RBCs), undergo sexual reproduction in their tick vectors (Rhipicephalus spp. for B. bovis and B. bigemina, and Ixodes ricinus for B. divergens) and have a trans-ovarial mode of transmission. Babesia parasites can cause acute and persistent infections to adult naïve cattle that can occur without evident clinical signs, but infections caused by B. bovis are associated with more severe disease and increased mortality, and are considered to be the most virulent agent of bovine babesiosis. In addition, babesiosis caused by B. divergens has an important zoonotic potential. The disease caused by B. bovis and B. bigemina can be controlled, at least in part, using therapeutic agents or vaccines comprising live-attenuated parasites, but these methods are limited in terms of their safety, ease of deployability and long-term efficacy, and improved control measures are urgently needed. In addition, expansion of tick habitats due to climate change and other rapidly changing environmental factors complicate efficient control of these parasites. While the ability to cause persistent

infections facilitates transmission and persistence of the parasite in endemic regions, it also highlights their capacity to evade the host immune responses. Currently, the mechanisms of immune responses used by infected bovines to survive acute and chronic infections remain poorly understood, warranting further research. Similarly, molecular details on the processes leading to sexual reproduction and the development of tick-stage parasites are lacking, and such tick-specific molecules can be targets for control using alternative transmission blocking vaccines. In this review, we identify and examine key phases in the life-cycle of Babesia parasites, including dependence on a tick vector for transmission, sexual reproduction of the parasite in the midgut of the tick, parasite-dependent invasion and egression of bovine RBCs, the role of the spleen in the clearance of infected RBCs (IRBCs), and age-related disease resistance in cattle, as opportunities for developing improved control measures. The availability of integrated novel research approaches including "omics" (such as genomics, transcriptomics, and proteomics), gene modification, cytoadhesion assays, RBC invasion assays and methods for in vitro induction of sexual-stage parasites will accelerate our understanding of parasite vulnerabilities. Further, producing new knowledge on these vulnerabilities, as well as taking full advantage of existing knowledge, by filling important research gaps should result in the development of next-generation vaccines to control acute disease and parasite transmission. Creative and effective use of current and future technical and computational resources are needed, in the face of the numerous challenges imposed by these highly evolved parasites, for improving the control of this disease. Overall, bovine babesiosis is recognised as a global disease that imposes a serious burden on livestock production and human livelihood, but it largely remains a poorly controlled disease in many areas of the world. Recently, important progress has been made in our understanding of the basic biology and host-parasite interactions of Babesia parasites, yet a good deal of basic and translational research is still needed to achieve effective control of this important disease and to improve animal and human health.

Author: Gray, A., Capewell, P., Loney, C., Katzer, F., Shiels, B. R. and Weir, W.

Title: Sheep as host species for zoonotic Babesia venatorum, United Kingdom

DOI: 10.3201/eid2512.190459

Abstract: Babesia venatorum is an increasingly prominent zoonotic parasite that predominantly infects wild deer. Our molecular examination of Babesia infecting mammals in the United Kingdom identified 18S sequences in domestic sheep isolates identical to zoonotic B. venatorum. Identification of this parasite in livestock raises concerns for public health and farming policy in Europe.

Author: Novacco, M., Hofmann-Lehmann, R., Grimm, F., Meli, M. L. and Stirn, M.

Title: Fatal acute babesiosis associated with Babesia venatorum infection (Babesia sp. EU1) in a captive reindeer calf in Switzerland

DOI: 10.1016/j.vprsr.2019.100336

Abstract: *Babesia venatorum* was isolated from a captive reindeer calf in Switzerland. The clinical signs consistent with acute babesiosis included hemolytic anemia and hemoglobinuria. The diagnosis was made based on visualization of intraerythrocytic parasites in the stained blood smears and confirmed by PCR analysis of the 18S rRNA gene, with subsequent species identification within *Babesia* confirmed by sequencing. The reindeer calf was initially treated with supportive care and an antiprotozoal drug (imidocarb dipropionate) but died a few days after hospitalization. *Babesia venatorum* is also known as *Babesia* sp. EU1 and can infect different mammalian species, including humans. The current case report aims to increase awareness among veterinarians and reindeer owners about the presence and the associated risk of this zoonotic pathogen. Considering the high morbidity and possible mortality associated with acute babesiosis, captive reindeer should receive tick prevention measures and be tested for subclinical infections in endemic area.

Vectors of Babesiosis

Gray JS, Estrada-Peña A, Zintl A. Gray JS, et al.

Vectors of Babesiosis.

Annu Rev Entomol. 2019 Jan 7;64:149-165. doi: 10.1146/annurev-ento-011118-111932. Epub 2018 Oct 1.

Research into new Babesia treatments

Author: Batiha, G. E. S., Beshbishy, A. M., Tayebwa, D. S., Adeyemi, O. S., Yokoyama, N. and Igarashi, I.

Title: Evaluation of the inhibitory effect of ivermectin on the growth of *Babesia* and *Theileria* parasites in vitro and in vivo

DOI: 10.1186/s41182-019-0171-8

Abstract: Background: Treatment is the principle way to control and eliminate piroplasmiasis. The search for new chemotherapy against *Babesia* and *Theileria* has become increasingly urgent due to parasite resistance to current drugs. Ivermectin (IVM) was the world's first endectocide, capable of killing a wide variety of parasites and vectors, both inside and outside the body. It is currently authorized to treat onchocerciasis, lymphatic filariasis, strongyloidiasis, and scabies. The current study documented the efficacy of IVM on the growth of *Babesia* and *Theileria* in vitro and in vivo. Methods: The fluorescence-based assay was used for evaluating the inhibitory effect of IVM on four *Babesia* species, including *B. bovis*, *B. bigemina*, *B. divergens*, *B. caballi*, and *Theileria equi*, the combination with diminazene aceturate (DA), clofazimine (CF), and atovaquone (AQ) on in vitro cultures, and on the multiplication of a *B. microti*-infected mouse model. The cytotoxicity of compounds was tested on Madin-Darby bovine kidney (MDBK), mouse

embryonic fibroblast (NIH/3 T3), and human foreskin fibroblast (HFF) cell lines. Results: The half-maximal inhibitory concentration (IC₅₀) values determined for IVM against *B. bovis*, *B. bigemina*, *B. divergens*, *B. caballi*, and *T. equi* were 53.3 ± 4.8 , 98.6 ± 5.7 , 30.1 ± 2.2 , 43.7 ± 3.7 , and 90.1 ± 8.1 μM , respectively. Toxicity assays on MDBK, NIH/3 T3, and HFF cell lines showed that IVM affected the viability of cells with a half-maximal effective concentration (EC₅₀) of 138.9 ± 4.9 , 283.8 ± 3.6 , and 287.5 ± 7.6 μM , respectively. In the in vivo experiment, IVM, when administered intraperitoneally at 4 mg/kg, significantly ($p < 0.05$) inhibited the growth of *B. microti* in mice by 63%. Furthermore, combination therapies of IVM-DA, IVM-AQ, and IVM-CF at a half dose reduced the peak parasitemia of *B. microti* by 83.7%, 76.5%, and 74.4%, respectively. Moreover, this study confirmed the absence of *B. microti* DNA in groups treated with combination chemotherapy of IVM + DA and IVM + AQ 49 days after infection. Conclusions: These findings suggest that IVM has the potential to be an alternative remedy for treating piroplasmosis.

Author: Beshbishy, A. M., Batiha, G. E. S., Yokoyama, N. and Igarashi, I.

Title: Ellagic acid microspheres restrict the growth of Babesia and Theileria in vitro and Babesia microti in vivo

DOI: 10.1186/s13071-019-3520-x

Abstract: Background: There are no effective vaccines against Babesia and Theileria parasites; therefore, therapy depends heavily on antiprotozoal drugs. Treatment options for piroplasmosis are limited; thus, the need for new antiprotozoal agents is becoming increasingly urgent. Ellagic acid (EA) is a polyphenol found in various plant products and has antioxidant, antibacterial and effective antimalarial activity in vitro and in vivo without toxicity. The present study documents the efficacy of EA and EA-loaded nanoparticles (EA-NPs) on the growth of Babesia and Theileria. Methods: In this study, the inhibitory effect of EA, β -cyclodextrin ellagic acid (β -CD EA) and antisolvent precipitation with a syringe pump prepared ellagic acid (APSP EA) was evaluated on four Babesia species and Theileria equi in vitro, and on the multiplication of *B. microti* in mice. The cytotoxicity assay was tested on Madin-Darby bovine kidney (MDBK), mouse embryonic fibroblast (NIH/3T3) and human foreskin fibroblast (HFF) cell lines. Results: The half-maximal inhibitory concentration (IC₅₀) values of EA and β -CD EA on *B. bovis*, *B. bigemina*, *B. divergens*, *B. caballi* and *T. equi* were 9.58 ± 1.47 , 7.87 ± 5.8 , 5.41 ± 2.8 , 3.29 ± 0.42 and 7.46 ± 0.6 μM and 8.8 ± 0.53 , 18.9 ± 0.025 , 11 ± 0.37 , 4.4 ± 0.6 and 9.1 ± 1.72 μM , respectively. The IC₅₀ values of APSP EA on *B. bovis*, *B. bigemina*, *B. divergens*, *B. caballi* and *T. equi* were 4.2 ± 0.42 , 9.6 ± 0.6 , 2.6 ± 1.47 , 0.92 ± 5.8 and 7.3 ± 0.54 μM , respectively. A toxicity assay showed that EA, β -CD EA and APSP EA affected the viability of cells with a half-maximal effective concentration (EC₅₀) higher than 800 μM . In the experiments on mice, APSP EA at a concentration of 70 mg/kg reduced the peak parasitemia of *B. microti* by 68.1%. Furthermore, the APSP EA-atovaquone (AQ) combination showed a higher chemotherapeutic effect than that of APSP EA monotherapy. Conclusions: To our knowledge, this is the first study to demonstrate the in vitro and in vivo antibabesial action of EA-NPs and thus supports the use of nanoparticles as an alternative antiparasitic agent.

Author: El-Sayed, S. A. E. S., Rizk, M. A., Yokoyama, N. and Igarashi, I.

Title: Evaluation of the in vitro and in vivo inhibitory effect of thymoquinone on piroplasm parasites

DOI: 10.1186/s13071-019-3296-z

Abstract: Background: Developing new antibabesial drugs with a low toxic effect to the animal and with no resistance from Babesia parasites is in urgent demand. In this concern, the antimalarial, anticancer and antioxidant effect of thymoquinone (TQ), a phytochemical compound found in the plant *Nigella sativa*, has been reported. Therefore, in the present study, the antibabesial effect of this compound was evaluated on the growth of piroplasm parasites. Results: Significant inhibition ($P < 0.05$) of the in vitro growth of piroplasm parasites were observed after treatment by TQ with IC 50 values of 35.41 ± 3.60 , 7.35 ± 0.17 , 0.28 ± 0.016 , 74.05 ± 4.55 and 67.33 ± 0.94 μM for *Babesia bovis*, *Babesia bigemina*, *Babesia divergens*, *Theileria equi* and *Babesia caballi*, respectively. The in vitro inhibitory effect of TQ was significantly enhanced ($P < 0.05$) when used in combination with either diminazene aceturate on bovine Babesia and equine Babesia and *Theileria* cultures. In *B. microti*-infected mice, oral and intraperitoneal administrations of TQ showed significant ($P < 0.05$) inhibition of parasite growth at a dose of 70 mg/kg and 50 mg/kg, respectively, compared to the control group. Conclusions: The obtained results indicate that thymoquinone might be a promising medicinal compound for use in the treatment of animal piroplasmosis.

Babesia vaccines

Author: Rathinasamy, V., Poole, W. A., Bastos, R. G., Suarez, C. E. and Cooke, B. M.

Title: Babesiosis Vaccines: Lessons Learned, Challenges Ahead, and Future Glimpses

DOI: 10.1016/j.pt.2019.06.002

Abstract: The incidence and prevalence of babesiosis in animals and humans is increasing, yet prevention, control, or treatment measures remain limited and ineffective. Despite a growing body of new knowledge of the biology, pathogenicity, and virulence of *Babesia* parasites, there is still no well-defined, adequately effective and easily deployable vaccine. While numerous published studies suggest that the development of such anti-*Babesia* vaccines should be feasible, many others identify significant challenges that need to be overcome in order to succeed. Here, we review historic and recent attempts in babesiosis vaccine discovery to avoid past pitfalls, learn new lessons, and provide a roadmap to guide the development of next-generation babesiosis vaccines.

Bovine besnoitiosis

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Antiparasitic Efficacy of Curcumin Against *Besnoitia besnoiti* Tachyzoites *in vitro*

Front Vet Sci . 2019 Jan 11;5:333.

Curcumin, a polyphenolic compound from *Curcuma longa* rhizome is well-known for its antioxidant, anti-inflammatory, immunomodulatory and also anti-protozoan effects. Hence, the objective of this study was to evaluate the effects of curcumin on viability, motility, invasive capacity, and proliferation of *B. besnoiti* tachyzoites replicating in primary bovine umbilical vein endothelial cells (BUVEC) *in vitro*. Functional inhibition assays revealed that curcumin treatments reduce tachyzoite viability and induce lethal effects in up to 57% of tachyzoites (IC₅₀ in 5.93 µM). Referring to general motility, significant dose-dependent effects of curcumin treatments were observed. Interestingly, curcumin treatments only dampened helical gliding and twirling activities whilst longitudinal gliding motility was not significantly affected. In addition, curcumin pretreatments of tachyzoites resulted in a dose-dependent reduction of host cell invasion as detected by infections rates at 1 day p. i. These findings demonstrate feeding cattle with *Curcuma longa* rhizomes may represent a new strategy for besnoitiosis treatment.

Author: Garrido-Castañé, I., Romero, A. O., Espuny, J. C., Hentrich, B. and Basso, W.

Title: *Besnoitia besnoiti* seroprevalence in beef, dairy and bullfighting cattle in Catalonia (north-eastern Spain): A cross-sectional study

DOI: 10.1016/j.parint.2018.12.001

Besnoitia besnoiti is the causative agent of bovine besnoitiosis, a chronic and debilitating disease of cattle that recently re-emerged and seems to be spreading in Europe. A cross-sectional serological study was carried out in different cattle herds in Catalonia, north-eastern Spain, to determine the seroprevalence of *B. besnoiti* in the region. A total of 791 serum samples (beef cattle n = 338, dairy cattle n = 291; bullfighting cattle n = 162) were tested. Sera were first screened for antibodies against *Besnoitia* using a commercial Enzyme-Linked Immunosorbent Assay (ELISA) applying a cut-off that was lower than that recommended by the manufacturer in order to reach highest sensitivity. Sera above the chosen cut-off of 15% positivity (PP) were further tested by the Indirect Fluorescent Antibody Test (IFAT) and respectively positive results were confirmed by a *B. besnoiti* tachyzoite-based immunoblot. A total of 504/791 (63.7%) sera showed ELISA values above the selected cut-off, and 91 of these samples also yielded positive results in IFAT (cut-off titre 1:200). By immunoblot, a positive result was obtained in 93.4% (85 out of the 91) of the IFAT-positive samples. Interestingly, all confirmed *Besnoitia*-seropositive cases corresponded exclusively to beef cattle from the Pyrenees area, resulting in a prevalence of 25.1% (85/338) at the animal level and of 46% (36/78) at the herd level in this cattle

group. No specific antibodies against *Besnoitia* could be detected in dairy and bullfighting cattle. The obtained results suggested that *Besnoitia* infections are present in Catalonia, consequently, diagnosis of this parasitic infection should be included in the sanitary control and before trading and movement of animals.

Author: Diezma-Díaz, C., Ferre, I., Re, M., Jiménez-Meléndez, A., Tabanera, E., González-Huecas, M., Pizarro-Díaz, M., Yanguas-Pérez, D., Brum, P. L., Blanco-Murcia, J., Ortega-Mora, L. M. and Álvarez-García, G.

Title: The route of *Besnoitia besnoiti* tachyzoites inoculation does not influence the clinical outcome of the infection in calves

DOI: 10.1016/j.vetpar.2019.02.001

In a previous attempt, an experimental model of bovine besnoitiosis was established in calves that were intravenously inoculated with different doses of *Besnoitia besnoiti* tachyzoites. Despite the fact that all infected calves developed the acute stage of disease, only microscopic findings characteristic of chronic besnoitiosis were reported. In the present study, calves were inoculated by subcutaneous and intradermal routes with *B. besnoiti* tachyzoites with the aim of developing clinical signs and macroscopic lesions characteristic of chronic besnoitiosis. Nine 3-month-old male calves were randomly distributed into three groups of three animals each. Next, 10^6 tachyzoites were inoculated by either the subcutaneous (G1) or intradermal route (G2). The negative control group (G3) was inoculated with PBS. Daily clinical monitoring and regular blood collection were performed. At 70 days post-infection (pi), animals were euthanized, and tissues were collected to investigate lesions and parasites. Infected animals developed mild-moderate acute besnoitiosis characterized by lymphadenopathy from four days to 47 days pi, and sporadic fever peaks were only observed in one calf from G2. However, other clinical signs and macroscopic lesions characteristic of chronic besnoitiosis were not detected. Only nine tissue samples were *B. besnoiti*-DNA-positive, eight of which belonged to reproductive and respiratory tracts tissues from G1. Finally, the kinetics of the immune responses were similar in both infected groups. However, delayed and lower cellular and humoral immune responses were observed in G1 followed by G2 and were compared with intravenously inoculated calves. The differences observed among the three inoculation routes could be due to different effector mechanisms of the host early innate immune response against *B. besnoiti*. Accordingly, the inoculation route of *B. besnoiti* tachyzoites does not significantly influence the clinical outcome of the infection in calves. Thus, a further refinement of this experimental model of bovine besnoitiosis is needed to reproduce macroscopic lesions characteristic of chronic stage disease.

Author: Sharif, S., Jacquiet, P., Prevot, F., Grisez, C., Raymond-Letron, I., Semin, M. O., Geffré, A., Trumel, C., Franc, M., Bouhsira, É and Liénard, E.

Title: *Stomoxys calcitrans*, mechanical vector of virulent *Besnoitia besnoiti* from chronically infected cattle to susceptible rabbit

DOI: 10.1111/mve.12356

Cattle besnoitiosis caused by *Besnoitia besnoiti* (Eucoccidiorida: Sarcocystidae) is a re-emerging disease in Europe. Its mechanical transmission by biting flies has not been investigated since the 1960s. The aim of this study was to re-examine the ability of *Stomoxys calcitrans* (Diptera: Muscidae) to transmit virulent *B. besnoiti* bradyzoites from chronically infected cows to susceptible rabbits. Three batches of 300 stable flies were allowed to take an interrupted bloodmeal on chronically infected cows, followed by an immediate bloodmeal on three rabbits (Group B). A control group of rabbits and a group exposed to the bites of non-infected *S. calcitrans* were included in the study. Blood quantitative polymerase chain reaction (qPCR) analyses, and clinical, serological and haematological surveys were performed in the three groups over 152 days until the rabbits were killed. Quantitative PCR analyses and histological examinations were performed in 24 tissue samples per rabbit. Only one rabbit in Group B exhibited clinical signs of the acute phase of besnoitiosis (hyperthermia, weight loss, regenerative anaemia and transient positive qPCR in blood) and was seroconverted. Parasite DNA was detected in four tissue samples from this rabbit, but no cysts were observed on histological examination. These findings indicate that *S. calcitrans* may act as a mechanical vector of *B. besnoiti* more efficiently than was previously considered.

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Prevalence of ELISA-detected Specific Antibodies Against *Besnoitia besnoiti* in Cattle of the Eastern and Southeastern Anatolian Regions, Turkey

Iran J Vet Res . Spring 2019;20(2):143-146.

Besnoitiosis caused by *Besnoitia besnoiti* is regarded as a re-emerging disease in cattle because of the increased number of cases and geographical distribution in many European countries. The present study was conducted to determine the presence of *B. besnoiti* in cattle in the Eastern and Southeastern Anatolia of Turkey. Blood samples were collected from 450 cattle in the provinces of Mus, Van, Siirt, and Diyarbakir. PrioCHECK[®] *Besnoitia* Ab 2.0 *enzyme-linked immunosorbent assay* (ELISA) kit was used to detect specific anti-*B. besnoiti* antibodies in the serum samples. Twelve (2.7%) of the 450 asymptomatic cattle were seropositive against *B. besnoiti*. In cattle, the prevalence rates were 0%, 3.7%, 3.4%, and 1.1% in Mus, Siirt, Diyarbakir, and Van provinces ($P>0.05$), respectively. This study is the first to investigate the presence of *B. besnoiti* in cattle raised in the Eastern and Southeastern Anatolia of Turkey. Although the ELISA test revealed some positive cases, concrete evidence for the establishment of clinical *B. besnoiti* infection could not be verified. More comprehensive analysis would be necessary to determine the significance of the present observations.

Author: Villa, L., Gazzonis, A. L., Zanzani, S. A., Perlotti, C., Sironi, G. and Manfredi, M. T.

Title: Bovine besnoitiosis in an endemically infected dairy cattle herd in Italy: serological and clinical observations, risk factors, and effects on reproductive and productive performances

DOI: 10.1007/s00436-019-06501-9

Bovine besnoitiosis (*Besnoitia besnoiti*) is an emerging parasitic disease of cattle in Europe. This study reports a case of bovine besnoitiosis in a dairy farm housing 217 cattle in Italy. A serological screening was performed on the whole herd using the recommended approach of ELISA and confirmatory Western Blot. Seropositive animals were clinically examined to reveal symptoms and lesions of besnoitiosis. Risk factors and the effects of the parasite infection on reproductive and productive performances were evaluated. Histopathology and molecular analyses on tissues from a slaughtered cow affected by the chronic phase of the disease were carried out. An overall seroprevalence of 23.5%, which increased up to 43.5% considering only cows, was recorded. Clinical examination of 33 of the seropositive cows evidenced the presence of tissue cysts in at least one of the typical localizations (sclera, vulva, or skin) in 25 animals. Statistical analysis did not evidence any significant impact of the parasite infection on herd efficiency; however, a decrease of productive parameters was recorded in cows showing cutaneous cysts. Concerning the chronically affected cow, histopathology revealed *B. besnoiti* tissue cysts in the skin of the neck, rump, hind legs, eyelid and vulva, in the muzzle, in mucosal membranes of the upper respiratory tract, and in the lungs. Parasite DNA was detected also in masseter muscles, tonsils, mediastinal lymph nodes, liver, cardiac muscle, aorta wall, ovaries, uterus, and vulva. Bovine besnoitiosis continues to spread in the Italian cattle population. Breeders and veterinarians should be aware of this parasitic disease, and control programs should be developed based on surveillance through a diagnostic procedure including both clinical examination and laboratory tests.

Author: Jiménez-Meléndez, A., Fernández-Álvarez, M., Calle, A., Ramírez, M. A., Diezma-Díaz, C., Vázquez-Arbaizar, P., Ortega-Mora, L. M. and Álvarez-García, G.

Title: Lytic cycle of *Besnoitia besnoiti* tachyzoites displays similar features in primary bovine endothelial cells and fibroblasts

DOI: 10.1186/s13071-019-3777-0

Bovine besnoitiosis, caused by the cyst-forming apicomplexan parasite *Besnoitia besnoiti*, is a chronic and debilitating cattle disease that continues to spread in Europe in the absence of control tools. In this scenario, in vitro culture systems are valuable tools to carry out drug screenings and to unravel host-parasite interactions. However, studies performed in bovine target cells are scarce. The objective of the present study was to obtain primary bovine aortic endothelial cells (BAECs) and fibroblast cell cultures, target cells during the acute and the chronic stage of the disease, respectively, from healthy

bovine donors. Afterwards, expression of surface (CD31, CD34 and CD44) and intracellular markers (vimentin and cytokeratin) was studied to characterize cell populations by flow cytometry. Next, the lytic cycle of *B. besnoiti* tachyzoites was studied in both target cells. Invasion rates (IRs) were determined by immunofluorescence at several time points post-infection, and proliferation kinetics were studied by quantitative PCR (qPCR). Finally, the influence of bovine viral diarrhoea virus (BVDV) co-infection on the host cell machinery, and consequently on *B. besnoiti* invasion and proliferation, was investigated in BAECs. Morphology and cytometry results confirmed the endothelial and fibroblast origins. CD31 was the surface marker that best discriminated between BAECs and fibroblasts, since fibroblasts lacked CD31 labelling. Expression of CD34 was weak in low-passage BAECs and absent in high-passage BAECs and fibroblasts. Positive labelling for CD44, vimentin and cytokeratin was observed in both BAECs and fibroblasts. Regarding the lytic cycle of the parasite, although low invasion rates (approximately 3-4%) were found in both cell culture systems, more invasion was observed in BAECs at 24 and 72 hpi. The proliferation kinetics did not differ between BAECs and fibroblasts. BVDV infection favoured early *Besnoitia* invasion but there was no difference in tachyzoite yields observed in BVDV-BAECs compared to BAECs. We have generated and characterized two novel standardized in vitro models for *Besnoitia besnoiti* infection based on bovine primary target BAECs and fibroblasts, and have shown the relevance of BVDV coinfections, which should be considered in further studies with other cattle pathogens.

Trypanosomes

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A Case of Bovine Trypanosomiasis Caused by *Trypanosoma theileri* in Sicily, Italy

Parasitol Res . 2019 Sep;118(9):2723-2727.

Despite some researchers reporting clinical signs in cattle associated with *Trypanosoma theileri*, its role as a pathogen is still unclear. We describe here the isolation of *Trypanosoma theileri* during a routine laboratory investigation. Mature and immature vital parasitic forms were observed within hematopoietic cell cultures from the bone marrow of one cow for monocyte isolation. The animal was submitted to clinical examination and blood sample counting (CBC). Postmortem analysis included gross and histological examination and PCR in the liver, spleen, brain, lymph nodes, and lungs. PCR and Giemsa staining were used for parasite identification. A second cow belonging to the same farm was positive for *Trypanosoma theileri* by PCR performed on blood sample. In this case, the postmortem analysis included also testis. Clinical examination showed only a reduction in body weight in both cases. The CBC revealed an increase of lymphocytes and neutrophils while red blood cells were within the normal range. Spleen was slightly increased in volume and the histology revealed a proliferative activity of the white and red pulp. The biomolecular analysis identified the parasite as *Trypanosoma theileri* and its

DNA was detected in the bone marrow, testis, and brain. The unusual finding of parasite in the brain, testis, and bone marrow raises new clinical implication on disease course and also possible sexual transmission.

Wildlife vector-borne diseases

Remesar S¹, Díaz P¹, Prieto A¹, Markina F², Díaz Cao JM¹, López-Lorenzo G¹, Fernández G¹, López CM¹, Panadero R¹, Díez-Baños P¹, Morrondo P¹.

Prevalence and distribution of *Babesia* and *Theileria* species in roe deer from Spain.

Int J Parasitol Parasites Wildl. 2019 May 21;9:195-201.

Babesiosis and Theileriosis are important worldwide-distributed tick-borne diseases for human and animals. Their presence in a particular area depends on the presence of suitable tick-vector and host species as well as competent reservoirs such as roe deer, one of the most abundant wild cervids in Spain. Spleen samples from 174 roe deer hunted in Spain were analysed to determine the prevalence of *Babesia* and *Theileria* species. DNA of both piroplasms was firstly detected using a commercial qPCR. Then, positive samples were molecularly characterized at the 18S rRNA and ITS1 genes of *Babesia* spp. and *Theileria* spp. The possible influence of some factors such as ecological area, age and sex was also assessed. Overall, 89.7% of roe deer were positive to any of the two piroplasms. *Theileria* spp. was more prevalent (60.9%) than *Babesia* spp. (19.0%); species identification could not be achieved in 17.3% of positive samples. *Babesia* prevalence was significantly higher in young animals and in roe deer from Oceanic regions, in contrast to *Theileria* spp. Five species were identified: *Theileria* sp. OT3 (60.3%), *Babesia capreoli* (15.5%), *Babesia venatorum* (2.9%), *Theileria* sp. 3185/02 (0.6%) and *Babesia bigemina* (0.6%). The coinfection *B. capreoli*/T. sp. OT3 was the most common (4.6%) followed by *B. venatorum*/T. sp. OT3 (0.6%) and *B. bigemina*/T. sp. OT3 (0.6%). Our results reveal that *Theileria* spp. and *Babesia* spp. are prevalent piroplasms in roe deer from Spain. These cervids can act as reservoirs for several *Babesia* and *Theileria* species, including the zoonotic *B. venatorum*. This study represents the first description of *B. venatorum* and *B. bigemina* in roe deer from Spain.

Wildlife Anaplasma

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First detection and molecular identification of the zoonotic *Anaplasma capra* in deer in France.

PLoS One. 2019 Jul 5;14(7):e0219184. doi: 10.1371/journal.pone.0219184. eCollection 2019.

Cervids are known to be reservoirs of zoonotic bacteria transmitted by ticks. This study aimed to identify the *Anaplasma* species carried by captive red deer and swamp deer in a wild fauna reserve in France. Blood from 59 red deer and 7 swamp deer was collected and analyzed over a period of two years. A semi-nested PCR targeting the 23S rRNA was performed to detect and characterize *Anaplasma* spp. and determine the presence of zoonotic species. *Anaplasma phagocytophilum* was identified in 14/59 red deer (23.7%) but it was not identified in any of the swamp deer (7 animals). Three sequences could not be assigned to any particular species based on the 23S rRNA sequences. Complementary nested PCR targeting 16S rRNA, *gltA* and *groEL* genes and sequencing analysis then identified these sequences as a recently reported zoonotic species, *Anaplasma capra*; this species was found in 2 red deer (*Cervus elaphus*) and 1 swamp deer (*Rucervus duvaucelii*). This is the first report of the tick-borne zoonotic bacterium *A. capra* in France, a species otherwise described only in China, Japan, Malaysia and South Korea in goats, sheep, deer, cattle and Japanese serows (*Capricornis crispus*). While this bacterium may have been introduced into the reserve by infected imported animals, its local epidemiological cycle via tick transmission seems possible as locally born deer were found infected. Diagnostic methods, especially molecular ones, should take into account the potential infection of animals and humans with this species.

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Infection Prevalence and Ecotypes of *Anaplasma Phagocytophilum* in Moose *Alces Alces*, Red Deer *Cervus Elaphus*, Roe Deer *Capreolus Capreolus* and *Ixodes Ricinus* Ticks From Norway

Parasit Vectors. 2019 Jan 3;12(1):1.

The geographical expansion of the tick *Ixodes ricinus* in northern Europe is a serious concern for animal and human health. The pathogen *Anaplasma phagocytophilum* is transmitted by ticks and causes emergences of tick-borne fever (anaplasmosis) in livestock. The transmission dynamics of the different ecotypes of *A. phagocytophilum* in the ecosystems is only partly determined. Red deer and roe deer contribute to circulation of different ecotypes of *A. phagocytophilum* in continental Europe, while the role of moose for circulation of different ecotypes is not fully established but an important issue in northern Europe. We determined infection prevalence and ecotypes of *A. phagocytophilum* in moose (n = 111), red deer (n = 141), roe deer (n = 28) and questing ticks (n = 9241) in Norway. As previously described, red deer was exclusively linked to circulation of ecotype I, while roe deer was exclusively linked to circulation of ecotype II. Surprisingly, we found 58% ecotype I (n = 19) and 42% of ecotype II (n = 14) in moose. Both ecotypes were found in questing ticks in areas with multiple cervid species present, while only ecotype I was found in ticks in a region with only red deer present. Hence, the geographical distribution of ecotypes in ticks followed the distribution of cervid species present in a given region and their link to ecotype I and II. Moose probably function as reservoirs for both ecotype I and II, indicating that the ecotypes of *A. phagocytophilum* are not entirely host-specific and have overlapping niches. The disease hazard depends also on both host

abundance and the number of immature ticks fed by each host. Our study provides novel insights in the northern distribution and expansion of tick-borne fever.

Wildlife Babesia

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Diversity of Babesia Spp. In Cervid Ungulates Based on the 18S rDNA and Cytochrome C Oxidase Subunit I Phylogenies

Infect Genet Evol . 2020 Jan;77:104060.

Free ranging ungulates, represented in Europe mostly by several deer species, are important hosts for ticks and reservoirs of tick-borne infections. A number of studies have focused on the prevalence of tick borne pathogens in deer chiefly with the aim to determine their potential role as reservoir hosts for important human and livestock pathogens. However, genetic similarity of Babesia spp. forming a group commonly termed as a clade VI that accommodates the deer piroplasms, complicates this task and has led to the description of a bewildering array of poorly characterised strains. This study aims to resolve this issue by using two independent genetic loci, nuclear 18S rRNA and mitochondrial cytochrome c oxidase subunit I genes, used in parallel to identify Babesia isolates in free-ranging red, sika, and roe deer in two areas of their co-occurrence in the Czech Republic. The COX1 loci, in contrast to 18S rRNA gene, shows a clear difference between interspecific and intraspecific variation at the nucleotide level. The findings confirm *B. divergens*, *Babesia* sp. EU1 and *B. capreoli* in studied deer species as well as common presence of another unnamed species that matches a taxon previously referred to as *Babesia* sp. or *Babesia* cf. *odocoilei* or *Babesia* CH1 group in several other sites throughout Europe. The invasive sika deers enter the life cycle of at least three piroplasmid species detected in native deer fauna. The presence of *B. divergens* in both sika and red deer in an area where bovine babesiosis is apparently absent raises important questions regarding the epidemiology, host specificity and taxonomic status of the parasite.

Wildlife Borrelia

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Lyme neuroborreliosis and bird populations in northern Europe

Proc Biol Sci. 2019 May 29;286

Many vector-borne diseases are transmitted through complex pathogen-vector-host networks, which makes it challenging to identify the role of specific host groups in disease emergence. Lyme borreliosis in humans is now the most common vector-borne zoonosis

in the Northern Hemisphere. The disease is caused by multiple genospecies of *Borrelia burgdorferi sensu lato* bacteria transmitted by ixodid (hard) ticks, and the major host groups transmit *Borrelia* genospecies with different pathogenicity, causing variable clinical symptoms in humans. The health impact of a given host group is a function of the number of ticks it infects as well as the pathogenicity of the genospecies it carries. *Borrelia afzelii*, with mainly small mammals as reservoirs, is the most common pathogen causing Lyme borreliosis, and it is often responsible for the largest proportion of infected host-seeking tick nymphs in Europe. The bird-borne *Borrelia garinii*, though less prevalent in nymphal ticks, is more likely to cause Lyme neuroborreliosis, but whether *B. garinii* causes disseminated disease more frequently has not been documented. Based on extensive data of annual disease incidence across Norway from 1995 to 2017, we show here that 69% of disseminated Lyme borreliosis cases were neuroborreliosis, which is three times higher than predicted from the infection prevalence of *B. garinii* in host-seeking ticks (21%). The population estimate of migratory birds, mainly of thrushes, explained part of the annual variation in cases of neuroborreliosis, with a one-year time lag. We highlight the important role of the genospecies' pathogenicity and the host associations for understanding the epidemiology of disseminated Lyme borreliosis.

Wildlife Theileria

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The Pathology of Pathogenic Theileriosis in African Wild Artiodactyls

Vet Pathol . 2020 Jan;57(1):24-48.

The published literature on schizont-"transforming," or pathogenic theileriosis, in African wild artiodactyls is dated and based on limited information. Here the authors review the taxonomy, diagnosis, epidemiology, hematology, pathology, and aspects of control in various species. Molecular studies based on 18S and 16S rRNA gene sequences have shown that African wild artiodactyls are commonly infected with diverse *Theileria spp.*, as well as nontheilerial hemoprotozoa and rickettsia-like bacteria, and coinfections with pathogenic and nonpathogenic *Theileria* species are often recorded. Although theileriosis is still confusingly referred to as cytauxzoonosis in many species, the validity of a separate *Cytauxzoon* genus in artiodactyls is debated. The epidemiology of theileriosis is complex; the likelihood of fatal disease depends on the interplay of parasite, vertebrate host, tick vector, and environmental factors. Roan calves (*Hippotragus equinus*) and stressed animals of all host species are more susceptible to fatal theileriosis. Even though regenerative anemia is common, peripheral blood piroplasm parasitemia does not correlate with disease severity. Other than anemia, common macroscopic lesions include icterus, hemorrhages (mucosal, serosal, and tissue), fluid effusions into body cavities, lung edema, and variably sized raised cream-colored foci of leukocyte infiltration in multiple organs. Histopathologic findings include vasocentric hyperproliferation and lysis of atypical leukocytes with associated intracellular schizonts, parenchymal necrosis, hemorrhage,

thromboembolism, and edema. Immunophenotyping is required to establish the identity of the schizont-transformed leukocytes in wild ungulates. Throughout the review, we propose avenues for future research by comparing existing knowledge on selected aspects of theileriosis in domestic livestock with that in African wild artiodactyls.

Bird blood parasites

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Correlates of Blood Parasitism in a Threatened Marshland Passerine: Infection by Kinetoplastids of the Genus *Trypanosoma* Is Related to Landscape Metrics of Habitat Edge

Parasitology . 2019 Jul;146(8):1036-1046

In birds, vector-borne parasites invading the bloodstream are important agents of disease, affect fitness and shape population viability, thus being of conservation interest. Here, we molecularly identified protozoan blood parasites in two populations of the threatened Aquatic Warbler *Acrocephalus paludicola*, a migratory passerine nesting in open marsh. We explored whether prevalence and lineage diversity of the parasites vary by population and whether infection status is explained by landscape metrics of habitat edge and individual traits (body mass, fat score, wing length and sex). Aquatic Warblers were infected by genera *Plasmodium*, *Leucocytozoon* and *Trypanosoma*, with seven, one and four lineages, and 29.9, 0.7 and 12.5% prevalence, respectively. No *Haemoproteus* infections were detected. Prevalence did not vary between the populations, but lineage diversity was higher in Polesie than in Biebrza for all the lineages pooled and for *Plasmodium*. Infection by *Trypanosoma* decreased with patch core area and increased with density of habitat edge. Infection status was not predicted by the individual traits. Our study is the first to show an association between edge-related landscape features and blood parasitism in an open habitat bird. This finding will support informed conservation measures for avian species of the globally shrinking marshland and other treeless habitats.

Arboviruses (viruses transmitted by mosquitoes, ticks, or other arthropods)

Tick borne encephalitis (TBE) virus

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Outbreak of Alimentary Tick-Borne Encephalitis in Podlaskie Voivodeship, Poland
Przegł Epidemiol . 2019;73(2):239-248.

Tick-borne encephalitis (TBE) virus remains one of the main etiological agents of central nervous system infections in Europe. The disease occurs endemically in Central and Eastern Europe, Southern part of Russia and Scandinavia. Between 2000 and 2015 there were 3 662 registered TBE cases in Poland and approximately 45% of them were reported in Podlaskie voivodeship. TBE typically develops as a result of being bitten by infected tick, however, it can also be a consequence of ingestion of unpasteurized milk from viremic animal. The aim of the article was to underline to the importance of TBE virus transmission via alimentary route and clinical description of four patients who developed TBE as a result of raw goat milk consumption. A retrospective analysis of documentation of four patients hospitalized in Observational-Infectious Department of Independent Public Healthcare Centre in Hajnówka and Department of Infectious Diseases and Neuroinfections at the Medical University of Białystok from June 10th 2017 to July 11th 2017 due to alimentary TBE. Patients were between 24 and 36 years of age. They have consumed goat milk from the same source. None of the patients have been vaccinated against TBE virus. In all patients typical biphasic disease course with the presence of prodromal and neurological phase was observed. TBE was confirmed by demonstration of anti-TBE antibodies in serum and/or cerebrospinal fluid of patients. Diagnosis of TBE should be considered in every case of encephalitis in endemic areas. Due to the risk of TBE infection after consumption of unpasteurized milk from livestock, it is crucial to raise awareness of general population and farm owners about the possibility of TBE infection via alimentary route.

Bluetongue Virus

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Red Deer (*Cervus elaphus*) Did Not Play the Role of Maintenance Host for Bluetongue Virus in France: The Burden of Proof by Long-Term Wildlife Monitoring and *Culicoides* Snapshots

Viruses . 2019 Sep 27;11(10):903.

Bluetongue virus (BTV) is a *Culicoides*-borne pathogen infecting both domestic and wild ruminants. In Europe, the Red Deer (*Cervus elaphus*) (RD) is considered a potential BTV reservoir, but persistent sylvatic cycle has not yet been demonstrated. In this paper, we explored the dynamics of BTV1 and BTV8 serotypes in the RD in France, and the potential role of that species in the re-emergence of BTV8 in livestock by 2015 (i.e., 5 years after the former last domestic cases). We performed 8 years of longitudinal monitoring (2008-2015) among 15 RD populations and 3065 individuals. We compared *Culicoides* communities and feeding habits within domestic and wild animal environments (51,380

samples). *Culicoides* diversity (>30 species) varied between them, but bridge-species able to feed on both wild and domestic hosts were abundant in both situations. Despite the presence of competent vectors in natural environments, BTV1 and BTV8 strains never spread in RD along the green corridors out of the domestic outbreak range. Decreasing antibody trends with no PCR results two years after the last domestic outbreak suggests that seropositive young RD were not recently infected but carried maternal antibodies. We conclude that RD did not play a role in spreading or maintaining BTV in France.

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Quantifying Bluetongue Vertical Transmission in French Cattle From Surveillance Data

Vet Res . 2019 May 14;50(1):34.

Bluetongue is a vector-borne disease of ruminants with economic consequences for the livestock industry. Bluetongue virus serotype 8 (BTV-8) caused a massive outbreak in Europe in 2006/2009 and re-emerged in France in 2015. Given the unprecedented epidemiological features of this serotype in cattle, the importance of secondary routes of transmission was reconsidered and transplacental transmission of BTV-8 was demonstrated in naturally and experimentally infected cattle. Here we used surveillance data from the on-going outbreak to quantify BTV-8 vertical transmission in French cattle. We used RT-PCR pre-export tests collected from June to December 2016 on the French territory and developed a catalytic model to disentangle vertical and vector-borne transmission. A series of in silico experiments validated the ability of our framework to quantify vertical transmission provided sufficient prevalence levels. By applying our model to an area selected accordingly, we estimated a probability of vertical transmission of 56% (55.8%, 95% credible interval 41.7-70.6) in unvaccinated heifers infected late in gestation. The influence of this high probability of vertical transmission on BTV-8 spread and persistence should be further investigated.

Crimean-Congo Haemorrhagic Fever Virus (CCHFV)

Mancuso E, Toma L, Polci A, d'Alessio SG, Di Luca M, Orsini M, Di Domenico M, Marcacci M, Mancini G, Spina F, Goffredo M, Monaco F.

Crimean-Congo Hemorrhagic Fever Virus Genome in Tick from Migratory Bird, Italy.

Emerg Infect Dis. 2019 Jul;25(7):1418-1420.

We detected Crimean-Congo hemorrhagic fever virus in a *Hyalomma rufipes* nymph collected from a whinchat (*Saxicola rubetra*) on the island of Ventotene in April 2017. Partial genome sequences suggest the virus originated in Africa. Detection of the genome of this virus in Italy confirms its potential dispersion through migratory birds.

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Serologic survey of the Crimean-Congo haemorrhagic fever virus infection among wild rodents in Hungary.

Ticks Tick Borne Dis. 2019 Jul 8. pii: S1877-959X(19)30089-5. doi: 10.1016/j.ttbdis.2019.07.002. [Epub ahead of print]

Crimean-Congo haemorrhagic fever virus (CCHFV) is a tick-borne pathogen, which causes an increasing number of severe infections in many parts of Africa, Asia and in Europe. The virus is primarily transmitted by ticks, however, the spectrum of natural hosts regarding CCHFV includes a wide variety of domestic and wild animals. Although the presence of CCHFV was hypothesized in Hungary, data in support of CCHFV prevalence has thus far, proven insufficient. In the present study, rodents belonging to four species, the yellow-necked mouse (*Apodemus flavicollis*), the striped field mouse (*A. agrarius*), the wood mouse (*A. sylvaticus*) and the bank vole (*Myodes glareolus*), were all systematically trapped in the Mecsek Mountain region (Southwest Hungary), from 2011 through 2013. Rodent sera were collected and screened for CCHFV antibodies with dot-blot pre-screening and immunofluorescence assay. Among the 2085 tested rodents, 20 (0.96%) were positive for IgG antibody against CCHFV. Seroprevalence was the highest (1.25%) in *A. flavicollis* serum samples. Distinctly, we now provide the first data regarding CCHFV occurrence and seroprevalence among wild rodents in Hungary. This observation represents a need for large-scale surveillance to effectively assess the enzootic background and the potential public health risk of CCHFV in Hungary.

Hassan Nasirian ¹

Crimean-Congo Hemorrhagic Fever (CCHF) Seroprevalence: A Systematic Review and Meta-Analysis

Acta Trop. 2019 Aug;196:102-120.

Crimean-Congo haemorrhagic fever (CCHF) is the most widespread, tick-borne viral disease affecting humans and therefore this paper performed a meta-analysis to highlight seroprevalence features of CCHF in a global context. After a preliminary review of the 396 papers representing areas throughout the world, 206 were selected for detailed meta-analysis. In general the total means of CCHF seroprevalence were, respectively 4.7 and 24.6% for humans and animals; and 17.1, 18.9, 24.3, 29.3 and 27.1% for camels, cattle, goats, sheep and livestock. Statistical analysis revealed a significant difference in seroprevalence between humans and camels ($P = 0.043$), cattle ($P = 0.010$), goats ($P = 0.015$), sheep ($P = 0.005$) and livestock ($P = 0.017$). Regionally, there also was a difference between humans, and goats ($P = 0.0001$), sheep ($P = 0.007$) and livestock ($P = 0.002$). Globally, CCHF seroprevalence in at-risk professionals was 7.5 fold greater than in normal humans, while CCHF seroprevalence was 5 fold greater in animals, camels, cattle, goats, sheep and livestock than normal humans. Animal contact, animal husbandry,

farming, tick bite history and secretion exposure were the most frequently reported CCHF seropositivity risk factors. This study serves as an important resource for epidemiological discussions related to CCHF and CCHF seroprevalence features, providing specific information in understanding human and animal mean and trend CCHF seroprevalence for different regions of the world and on an aggregate global scale; seroprevalence in at-risk professionals; and total mean and trend CCHF seropositivity involving risk factors.

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Epidemiological Investigations of Crimean-Congo Haemorrhagic Fever Virus Infection in Sheep and Goats in Balochistan, Pakistan

Ticks Tick Borne Dis . 2020 Mar;11(2):101324.

Crimean-Congo haemorrhagic fever (CCHF) is a tick-borne zoonotic disease caused by the arbovirus Crimean-Congo haemorrhagic fever virus (CCHFV). Livestock serve as a transient reservoir for CCHFV, but do not show clinical signs. In this cross-sectional study, sheep and goats in Balochistan, Pakistan, were examined to determine the CCHFV seroprevalence, spatial distribution of seropositive sheep and goats, and to identify potential risk factors for seropositivity to CCHFV in these animals. To this end, farms and animals were selected by systematic sampling, blood samples from 800 sheep and 800 goats were collected and information regarding farm management and the kept animals were retrieved using a standard questionnaire. Sera were tested for antibodies against CCHFV in two independent ELISA formats and an immunofluorescence assay (IFA) following a hierarchical diagnostic decision tree. By these assays 149 (19 %, 95 %-CI: 16-21 %) out of 800 sheep serum samples and 37 (5 %, 95 %-CI: 3-6 %) out of 800 goat serum samples were positive for CCHFV-specific IgG antibodies. Interestingly, at least 8 (5 %, 95 %-CI: 2-10 %) out of 160 sera pools were from CCHFV viraemic sheep, as sera (in pools of 5) tested positive for CCHFV genome by real-time PCR (RT-qPCR). Risk factor analysis revealed that the open type of housing (OR = 3.76, 95 %-CI: 1.57-9.56, p-value = 0.003), grazing (OR = 4.18, 95 %-CI: 1.79-10.37, p-value = 0.001), presence of vegetation in or around the farm (OR = 3.13, 95 %-CI: 1.07-10.15, p-value = 0.043), lack of treatment against ticks (OR = 3.31, 95 %-CI: 1.16-10.21, p-value = 0.029), absence of rural poultry (OR = 2.93, 95 %-CI: 1.41-6.29, p-value = 0.004), animals with age \geq 2 years (OR = 4.15, 95 %-CI: 2.84-6.19, p-value < 0.001), animals infested with ticks (OR = 2.35, 95 %-CI: 1.59-3.52, p-value < 0.001), and sheep species (OR = 4.72, 95 %-CI: 3.24-6.86, p-value < 0.001) represented statistically significant risk factors associated with seropositivity to CCHFV. Taken together this study confirms the circulation of CCHFV in livestock in Balochistan, Pakistan. The identification of risk factors might help to reduce the risk of infection in sheep and goats, which may also mitigate the risk for human infection. An interesting option for reducing the risk of CCHFV infection in small ruminants is keeping also chickens, since they pick ticks that transmit CCHFV.

Rift valley fever virus and phleboviruses

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Molecular Aspects of Rift Valley Fever Virus and the Emergence of Reassortants

Virus Genes . 2019 Feb;55(1):1-11.

Rift Valley fever phlebovirus (RVFV) is a mosquito-transmitted pathogen endemic to sub-Saharan Africa and the Arabian Peninsula. RVFV is a threat to both animal and human health and has costly economic consequences mainly related to livestock production and trade. Competent hosts and vectors for RVFV are widespread, existing outside of endemic countries including the USA. Thus, the possibility of RVFV spreading to the USA or other countries worldwide is of significant concern. RVFV (genus Phlebovirus) is comprised of an enveloped virion containing a three-segmented, negative-stranded RNA genome that is able to undergo genetic reassortment. Reassortment has the potential to produce viruses that are more pathogenic, easily transmissible, and that have wider vector or host range. This is especially concerning because of the wide use of live attenuated vaccine strains throughout endemic countries. This review focuses on the molecular aspects of RVFV, genetic diversity of RVFV strains, and RVFV reassortment.

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Geographic dispersal and genetic diversity of tick-borne phleboviruses (Phenuiviridae, Phlebovirus) as revealed by the analysis of L segment sequences.

Ticks Tick Borne Dis. 2019 Jun;10(4):942-948. doi: 10.1016/j.ttbdis.2019.05.001. Epub 2019 May 3.

The large diversity of new tick-borne phleboviruses, and the negative impacts of the virulent viruses on human/animal health have led to a growing interest in their analysis. In this report, new insights are brought out into the diversity of putative phleboviruses circulating in Portugal (both the continental territory and the islands of São Miguel, in the Azores, and Madeira), as well as in the Spanish western regions of Extremadura and Castilla and León. Phlebovirus sequences were frequently detected (L-segment) from both questing and feeding ticks, but especially in *Rhipicephalus sanguineus sensu lato* (s.l.) specimens. These sequences were detected in adult ticks, as well as nymphs and eggs, supporting the hypothesis of viral maintenance by vertical transmission. Though multiple genetic groups could be identified in phylogenetic trees (AnLuc, KarMa, RiPar virus 1, and Spanish group 1 and 2), all the sequences from Portugal and Spain shared common ancestry with other viral sequence obtained from samples collected over a large geographic coverage. Spatiotemporal analysis placed Middle-East as the geographic origin of the most recent common ancestor (MRCA) of all phleboviruses analysed in the present study. More recent viral transitions might include migrations from Spain to

continental Portugal, and from there to the Portuguese Islands. Our findings suggest that the time of the MRCA of phleboviruses was dated around 225 years ago [95% HPD: 124-387 year before the last sampling date].

Other arboviruses

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A Cluster of Bunyavirus-Associated Severe Fever With Thrombocytopenia Syndrome Cases in a Coastal Plain Area in China, 2015: Identification of a Previously Unidentified Endemic Region for Severe Fever With Thrombocytopenia Bunyavirus.

Open Forum Infect Dis. 2019 May 13;6(6):ofz209. doi: 10.1093/ofid/ofz209. eCollection 2019 Jun.

Severe fever with thrombocytopenia syndrome (SFTS) is a typical tick-borne, natural focal disease. The natural foci of SFTS were considered to exist in hilly and mountainous areas before 2015. A cluster of 3 patients exposed to a patient with a fulminant disease consistent with SFTS occurred from July to August 2015 in Dongtai County, which is characterized by alluvial plains; this prompted investigation. The epidemiological, clinical, and laboratory features of 4 patients in the cluster were analyzed. Serum samples from the indigenous healthy population and native domesticated animals were collected to conduct laboratory tests, along with small wild animals and ticks. In 3 secondary case patients, high fever, thrombocytopenia and leukopenia developed within 8-13 days after contact with blood or bloody secretions from the index patient; SFTS was then diagnosed by means of reverse-transcription polymerase chain reaction. Genomic sequencing and analysis of S and L segments of 2 viral strains isolated from 2 secondary case patients showed that they shared 99.8%-99.9% homology in nucleotide sequence. The seroprevalences among indigenous healthy population, native livestock, native poultry, and small wild animals was 0.74%, 17.54%, 6.67%, and 1.12%, respectively. Three questing ticks, 61 feeding ticks, and 178 small wild animals were collected in August 2015. Survey on tick density and seasonal fluctuation in 2016 showed that ticks were active from March to October. All ticks were identified as *Haemaphysalis longicornis*. Severe fever with thrombocytopenia bunyavirus (SFTSV)-specific RNA was detected in the ticks collected in 2016, and the minimum SFTSV infection rate in these ticks was 0.54% (1 of 185). Wild mammals and ticks collected in August 2015 tested negative for SFTSV-specific RNA. Aside from hilly or mountainous area, a coastal plain was identified as the natural foci of SFTSV in Dongtai County, China. The involvement of migration in the evolution of SFTSV might lead to a transregional transmission event of SFTSV.

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Detection of novel tick-borne pathogen, Alongshan virus, in *Ixodes ricinus* ticks, south-eastern Finland, 2019.

Euro Surveill. 2019 Jul;24(27).

The newly identified tick-borne Alongshan virus (ALSV), a segmented Jingmen virus group flavivirus, was recently associated with human disease in China. We report the detection of ALSV RNA in *Ixodes ricinus* ticks in south-eastern Finland. Screening of sera from patients suspected for tick-borne encephalitis for Jingmen tick virus-like virus RNA and antibodies revealed no human cases. The presence of ALSV in common European ticks warrants further investigations on its role as a human pathogen.

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Characterization of Novel Reoviruses Wad Medani Virus (Orbivirus) and Kundal Virus (Coltivirus) Collected From *Hyalomma anatolicum* Ticks in India During Surveillance for Crimean Congo Hemorrhagic Fever

J Virol. 2019 Jun 14;93(13):e00106-19.

In 2011, ticks were collected from livestock following an outbreak of Crimean Congo hemorrhagic fever (CCHF) in Gujarat state, India. CCHF-negative *Hyalomma anatolicum* tick pools were passaged for virus isolation, and two virus isolates were obtained, designated Karyana virus (KARYV) and Kundal virus (KUNDV), respectively. Traditional reverse transcription-PCR (RT-PCR) identification of known viruses was unsuccessful, but a next-generation sequencing (NGS) approach identified KARYV and KUNDV as viruses in the *Reoviridae* family, *Orbivirus* and *Coltivirus* genera, respectively. Viral genomes were *de novo* assembled, yielding 10 complete segments of KARYV and 12 nearly complete segments of KUNDV. The VP1 gene of KARYV shared a most recent common ancestor with Wad Medani virus (WMV), strain Ar495, and based on nucleotide identity we demonstrate that it is a novel WMV strain. The VP1 segment of KUNDV shares a common ancestor with Colorado tick fever virus, Eyach virus, Tai Forest reovirus, and Tarumizu tick virus from the *Coltivirus* genus. Based on VP1, VP6, VP7, and VP12 nucleotide and amino acid identities, KUNDV is proposed to be a new species of *Coltivirus*. Electron microscopy supported the classification of KARYV and KUNDV as reoviruses and identified replication morphology consistent with other orbi- and coltiviruses. The identification of novel tick-borne viruses carried by the CCHF vector is an important step in the characterization of their potential role in human and animal pathogenesis. **IMPORTANCE** Ticks and mosquitoes, as well *Culicoides*, can transmit viruses in the *Reoviridae* family. With the help

of next-generation sequencing (NGS), previously unreported reoviruses such as equine encephalosis virus, Wad Medani virus (WMV), Kammavanpettai virus (KVPTV), and, with this report, KARYV and KUNDV have been discovered and characterized in India. The isolation of KUNDV and KARYV from *Hyalomma anatolicum*, which is a known vector for zoonotic pathogens, such as Crimean Congo hemorrhagic fever virus, *Babesia*, *Theileria*, and *Anaplasma* species, identifies arboviruses with the potential to transmit to humans. Characterization of KUNDV and KARYV isolated from *Hyalomma* ticks is critical for the development of specific serological and molecular assays that can be used to determine the association of these viruses with disease in humans and livestock.

Filarial worms

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Filarial Worm Circulation by Mosquitoes Along an Urbanization Gradient in Southern Spain *Transbound Emerg Dis* . 2019 Jul;66(4):1752-1757.

Mosquitoes are the main vectors of pathogens affecting wild animals, livestock and humans. Here, we used molecular tools to assess the local circulation of filarial parasites in mosquitoes collected during 2013 from natural, rural and urban habitats from southern Spain. We screened parasites in 22,791 female mosquitoes of the genera *Aedes*, *Culex* and *Culiseta*. Filarial worms were only detected in two mosquito pools. An *Ae. caspius* pool was positive for *Setaria equina* and an unidentified worm related to *Onchocerca* was detected in a *Cx. pipiens* pool. None of the mosquito pools were positive for *Dirofilaria*. These results underlay the role of *Ae. caspius* in the transmission of *Setaria* parasites among livestock and/or wildlife to humans in southern Spain.

Equine piroplasmas

Author: Onyiche, T. E., Suganuma, K., Igarashi, I., Yokoyama, N., Xuan, X. and Thekisoe, O.

Title: A review on equine piroplasmosis: Epidemiology, vector ecology, risk factors, host immunity, diagnosis and control

DOI: 10.3390/ijerph16101736

Abstract: Equine Piroplasmosis (EP) is a tick-borne disease caused by apicomplexan protozoan parasites, *Babesia caballi* and *Theileria equi*. The disease is responsible for serious economic losses to the equine industry. It principally affects donkeys, horses, mules, and zebra but DNA of the parasites has also been detected in dogs and camels raising doubt about their host specificity. The disease is endemic in tropical and temperate regions of the world where the competent tick vectors are prevalent. Infected equids

remain carrier for life with *T. equi* infection, whilst, infection with *B. caballi* is cleared within a few years. This review focuses on all aspects of the disease from the historical overview, biology of the parasite, epidemiology of the disease (specifically highlighting other non-equine hosts, such as dogs and camels), vector, clinical manifestations, risk factors, immunology, genetic diversity, diagnosis, treatment, and prevention.

Author: Camino, E., Dorrego, A., Carvajal, K. A., Buendia-Andres, A., de Juan, L., Dominguez, L. and Cruz-Lopez, F.

Title: Serological, molecular and hematological diagnosis in horses with clinical suspicion of equine piroplasmosis: Pooling strengths

DOI: 10.1016/j.vetpar.2019.108928

Abstract: Equine piroplasmosis (EP) is a tick-borne protozoan disease caused by *Theileria equi* and/or *Babesia caballi*. Clinical signs (fever, pale mucosal membranes, jaundice), anemia and hyperbilirubinemia have been associated with the disease. EP is widespread, has a significant economic impact on the equine industry and remains endemic in Spain. This study was carried out with samples belonging to 140 horses residing in Spain and showing common clinical signs of EP. A blood smear microscopic examination and a comparison between the different results obtained by competitive Enzyme-Linked Immunosorbent Assay (cELISA), real-time Polymerase Chain Reaction (PCR) and hematological and biochemical (direct and total bilirubin) screening were conducted. EP positivity rates by cELISA and PCR were 50.7% and 42.9%, respectively, whereas only 9% of the horses were positive in the microscopic analysis. A significantly higher number of *B. caballi*-positive horses were detected by cELISA than PCR, and Kappa value was higher for *T. equi* ($k = 0.575$) than for *B. caballi* ($k = 0.401$). For the first time, an association between a high ELISA inhibition percentage (IP) and a positive PCR result for *B. caballi* was determined. Although most authors have described *T. equi* as more pathogenic than *B. caballi*, we found that horses parasitized by *B. caballi* showed a more severe hemolytic anemia, whereas *T. equi* infections were mostly associated with leukocytosis. The hemogram and clinical chemistry could guide the veterinary surgeon towards the diagnosis of *T. equi* or *B. caballi* since horses showed a significant leukocytosis or anemia and hyperbilirubinemia, respectively; however PCR would be the test of choice in order to confirm the diagnosis. Information about the importance of a correct diagnosis of EP using a combination of techniques is essential in order to allow the early detection of cases and prevent the spread of the disease, as well as to avoid the common practice of treating horses without a laboratory diagnosis.

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Title: A multinested PCR for detection of the equine piroplasmids *Babesia caballi* and *Theileria equi*

DOI: 10.1016/j.ttbdis.2018.11.008

Abstract: Two haemoparasites, *Theileria equi* and *Babesia caballi*, cause equine piroplasmiasis (EP), one of the most prevalent tick-borne diseases in horses. The main aim of the present study was to develop and evaluate a multinested PCR (mn-PCR) for simultaneous detection of the equine piroplasmids *T. equi* and *B. caballi*, by amplification of five genetic markers (18S rRNA, β -tubulin, *cytB*, *EMA-1* and *RAP-1*). This novel assay detected a high prevalence of equine piroplasmids in 235 horse blood samples collected in Castilla-León and Extremadura, Spain. The overall prevalence of infection with equine piroplasmids by mn-PCR was 72.8% (171/235), with 66.0% (155/235) of the animals positive for *T. equi* and 29.4% (69/235) positive for *B. caballi*. The seroprevalence obtained by cELISA for the same set of samples was lower than the infection prevalence recorded by mn-PCR, for either of the two equine piroplasmids (62.6%) as well as for *T. equi* alone (61.7%) or *B. caballi* alone (3.8%). There was high agreement among the mn-PCR and cELISA assays for diagnosis of EP caused by *T. equi* ($\kappa = 0.83$) but not for *B. caballi* ($\kappa = 0.06$). A phylogenetic analysis based on the *RAP-1* gene of *B. caballi* showed that the strains from Spain clustered with those from Israel.

Equine Trypanosomiasis

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Equine Trypanosomiasis: Enigmas and Diagnostic Challenges

Parasit Vectors . 2019 May 15;12(1):234.

Equine trypanosomiasis is a complex of infectious diseases called dourine, nagana and surra. It is caused by several species of the genus *Trypanosoma* that are transmitted cyclically by tsetse flies, mechanically by other haematophagous flies, or sexually. *Trypanosoma congolense* (subgenus *Nannomonas*) and *T. vivax* (subgenus *Dutonella*) are genetically and morphologically distinct from *T. brucei*, *T. equiperdum* and *T. evansi* (subgenus *Trypanozoon*). It remains controversial whether the three latter taxa should be considered distinct species. Recent outbreaks of surra and dourine in Europe illustrate the risk and consequences of importation of equine trypanosomiasis with infected animals into non-endemic countries. Knowledge on the epidemiological situation is fragmentary since many endemic countries do not report the diseases to the World Organisation for Animal Health, OIE. Other major obstacles to the control of equine trypanosomiasis are the lack of vaccines, the inability of drugs to cure the neurological stage of the disease, the inconsistent case definition and the limitations of current diagnostics. Especially in view of the ever-increasing movement of horses around the globe, there is not only the obvious need for reliable curative and prophylactic drugs but also for accurate diagnostic tests and algorithms. Unfortunately, clinical signs are not pathognomonic, parasitological tests are not sufficiently sensitive, serological tests miss sensitivity or specificity, and molecular tests cannot distinguish the taxa within the *Trypanozoon* subgenus. To address the limitations of the current diagnostics for equine trypanosomiasis, we recommend studies into improved molecular and serological tests with the highest possible sensitivity and

specificity. We realise that this is an ambitious goal, but it is dictated by needs at the point of care. However, depending on available treatment options, it may not always be necessary to identify which trypanosome taxon is responsible for a given infection.

Domestic pet vector-borne disease

Author: Morganti, G., Veronesi, F., Stefanetti, V., Di Muccio, T., Fiorentino, E., Diaferia, M., Santoro, A., Passamonti, F. and Gramiccia, M.

Title: Emerging feline vector-borne pathogens in Italy

DOI: 10.1186/s13071-019-3409-8

Abstract: Background: The epidemiology of feline vector-borne pathogens (FeVBPs) has been less investigated in cats than in dogs. The present study assessed the prevalence of *Rickettsia* spp., *Babesia* spp., *Cytauxzoon* spp. and *Leishmania infantum* infections in cat populations living in central Italy, by molecular and serological tools. Results: A total of 286 healthy cats were randomly selected from catteries and colonies in central Italy. Peripheral blood and conjunctival swab (CS) samples were collected during surgical procedures for regional neutering projects. Sera were analysed by IFAT to detect anti-*Rickettsia felis*, *R. conorii*, *Babesia microti* and *Leishmania* IgG antibodies using commercial and home-made antigens. DNA extracted from buffy coats (BCs) was tested for *Rickettsia* spp., and Piroplasmida species, including *Cytauxzoon* spp. and *Babesia* spp. by PCR. Buffy coats and CS samples were assayed by a nested (n)-PCR for *Leishmania* spp. Sixty-two cats (21.67%) were seropositive to at least one of the tested pathogens. The serological assay revealed 23 (8.04%) and 18 (6.29%) positive cats for *R. felis* and *R. conorii*, respectively, with low titers (1/64-1/128). No antibodies against *B. microti* were detected. Neither *Rickettsia* nor Piroplasmida DNA were amplified using the specific PCR assays. Thirty-one cats (10.83%) tested positive to anti-*Leishmania* IgG, with titers ranging from 1:40 to 1:160 and 45 animals (15.73%) tested positive to *Leishmania* CS n-PCR, whereas none of the animals tested positive to BC n-PCR. Considering the results obtained by IFAT and CS n-PCR, a moderate agreement between the two tests was detected ($\kappa = 0.27$). Conclusions: The results of the serological and molecular surveys showed a moderate exposure to *Leishmania* in the investigated cats and highlighted the limited molecular diagnostic value of BC versus CS samples for this pathogen. Conversely no evidence supported the circulation of *Cytauxzoon* spp. in domestic cats, in contrast with previous detections in European wild cats in the same areas monitored. The low positive titres for *R. felis* in association with no DNA BC amplification prevent speculation on the exposure of feline populations to this FeVBP due to the cross-reactivity existing within spotted fever group rickettsiosis (SFGR).

Author: Abdullah, S., Helps, C., Tasker, S., Newbury, H. and Wall, R.

Title: Pathogens in fleas collected from cats and dogs: Distribution and prevalence in the UK

DOI: 10.1186/s13071-019-3326-x

Abstract: Background: Fleas (Siphonaptera) are the most clinically important ectoparasites of dogs and cats worldwide. Rising levels of pet ownership, climate change and globalisation are increasing the importance of a detailed understanding of the endemicity and prevalence of flea-borne pathogens. This requires continued surveillance to detect change. This study reports a large-scale survey of pathogens in fleas collected from client-owned cats and dogs in the UK. Methods: Recruited veterinary practices were asked to follow a standardised flea inspection protocol on a randomised selection of cats and dogs brought into the practice in April and June 2018. A total of 326 practices participated and 812 cats and 662 dogs were examined. Fleas were collected, identified to species and pooled flea samples from each host were analysed for the presence of pathogens using PCR and sequence analysis. Results: Overall, 28.1% of cats and 14.4% of dogs were flea infested. More than 90% of the fleas on both cats and dogs were cat fleas, *Ctenocephalides felis felis*. Fleas of the same species from each infested host were pooled. DNA was amplified from 470 of the pooled flea samples using conventional PCR, 66 of which (14% \pm 95% CI 3.14%) were positive for at least one pathogen. Fifty-three (11.3% \pm 95% CI 2.85%) of the pooled flea DNA samples were positive for *Bartonella* spp., 35 were from cats and 4 from dogs, the remainder had no host record. Seventeen of the *Bartonella* spp. samples were found to be *Bartonella henselae*, 27 were *Bartonella clarridgeiae* (of two different strains), 4 samples were *Bartonella alsatica* and one was *Bartonella grahamii*; 4 samples could not be identified. Fourteen (3% \pm 95% CI 1.53%) of the flea DNA samples were found to be positive for *Dipylidium caninum*, 10 of the *D. caninum*-infected samples were collected from cats and one from a dog, the other 3 positive flea samples had no host species record. Only 3 flea samples were positive for *Mycoplasma haemofelis* or *Mycoplasma haemocanis*; 2 were collected from cats and one had no host species record. Three fleas were positive for both *D. caninum* and *Bartonella* spp. One flea was positive for both *Bartonella* spp. and *M. haemofelis* or *M. haemocanis*. Conclusions: This study highlights the need for ongoing flea control, particularly given the relatively high prevalence of *Bartonella* spp., which is of concern for both animal welfare and human health. The study demonstrates the ongoing need to educate pet owners about the effects of both flea infestation and also the pathogen risks these fleas present.

Author: Tahir, D., Davoust, B. and Parola, P.

Title: Vector-borne nematode diseases in pets and humans in the Mediterranean Basin: An update

DOI: 10.14202/vetworld.2019.1630-1643

Abstract: Vector-borne diseases (VBDs) are among the leading causes of morbidity and mortality in humans and animals. The scale of VBDs is increasing worldwide, including in the Mediterranean Basin, a region exposed to climate changes. Indeed, weather conditions may influence the abundance and distribution of vectors. The vector-borne nematode diseases of dogs and cats, such as dirofilariosis, onchocercosis, thelaziosis, *Cercopithifilaria*, and *Acanthocheilonema* infections, are some of these vectorized diseases, several of which are zoonoses. They are all caused by parasitic nematodes transmitted by arthropods, including mosquitoes (*Dirofilaria* spp.), black flies (*Onchocerca lupi*), drosophilids (*Thelazia callipaeda*), ticks (*Acanthocheilonema dracunculoides* and *Cercopithifilaria baina*), and fleas and lice (*Acanthocheilonema reconditum*). The control and prevention of these infections and diseases require a multidisciplinary approach based on strengthening collaboration between the different actors in the fields of health, research, sociology, economics, governments and citizens, to improve human, animal, and ecosystem health. This is the concept of “one health.” The review aimed to provide a general update on the spatial and temporal distribution of vector-borne nematode diseases affecting companion animals and humans, as well as the vectors involved in the Mediterranean area. Simultaneously, certain epidemiological parameters, diagnosis, treatment, and control of these diseases based on the “one health” concept will also be discussed. Copyright: Tahir, et al. Open Access. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License.

Hepatozoon

Author: Basso, W., Görner, D., Globokar, M., Keidel, A. and Pantchev, N.

Title: First autochthonous case of clinical *Hepatozoon felis* infection in a domestic cat in Central Europe

DOI: 10.1016/j.parint.2019.101945

Abstract: Three different *Hepatozoon* (Apicomplexa, Hepatozoidae) species have been described infecting domestic cats in Europe (i.e. *H. felis*, *H. canis* and *H. silvestris*), however, reports on clinical hepatozoonosis are uncommon and treatment protocols are not clearly defined. A six-year-old male European short-hair cat from Austria presented poor general condition, lethargy, anorexia, icterus, a painful abdomen, fever, ruffled hair and a tick infestation, and it had never left Austria. Laboratory tests revealed leukopenia, thrombocytopenia and increased serum levels of symmetric dimethylarginine (SDMA) and bilirubin. In May Grünwald-Giemsa-stained blood smears, structures resembling *Hepatozoon* gamonts were observed inside neutrophil granulocytes. A PCR targeting a fragment of the 18S rRNA gene of *Hepatozoon* spp. and DNA sequencing allowed the diagnosis of *H. felis*-DNA in blood samples. The cat was treated with imidocarb dipropionate (6 mg/kg body weight, repeated after 14 days) and doxycycline monohydrate (5 mg/kg body weight twice a day, p.o., for four weeks) and recovered completely. A broad haematological and biochemical laboratory control after six months showed all evaluated parameters under normal ranges. Coinfection with other feline pathogens (i.e. feline

leukaemia virus, feline immunodeficiency virus, feline Coronavirus, Leishmania and *Dirofilaria immitis*) could not be detected. This study reveals the presence of *H. felis* in Austria and provides more evidence on the geographical distribution and pathogenicity of this parasite for domestic cats. To the authors' knowledge, this is the first autochthonous case of feline hepatozoonosis in Central Europe.

Babesia

Author: Baneth, G., Cardoso, L., Brilhante-Simões, P. and Schnittger, L.

Title: Establishment of *Babesia vulpes* n. sp. (Apicomplexa: Babesiidae), a piroplasmid species pathogenic for domestic dogs

DOI: 10.1186/s13071-019-3385-z

Abstract: Background: Canine babesiosis is a severe disease caused by several *Babesia* spp. A number of names have been proposed for the canine-infecting piroplasmid pathogen initially named *Theileria annae* Zahler, Rinder, Schein & Gothe, 2000. It was shown to be a member of the *Babesia* (sensu lato) group infecting carnivores and is also closely related to the *Babesia microti* group. Subsequently, the same parasite species was reclassified as a member of the genus *Babesia* and the name *Babesia vulpes* Baneth, Florin-Christensen, Cardoso & Schnittger, 2015 was proposed for it. However, both names do not meet the requirements of the International Code of Zoological Nomenclature (no accompanying descriptions, no deposition of type-specimens) and cannot be recognized as available names from the nomenclatural point of view. The purpose of this study was to further characterize this parasite in order to confirm its validity, to provide its description and to introduce zoological nomenclature for it with the name *Babesia vulpes* n. sp. Results: Morphological description of the parasite in canine erythrocytes demonstrated that it takes the shape of small ($1.33 \times 0.98 \mu\text{m}$), round to oval forms reminiscent of the pyriform and ring shapes of other small canine *Babesia* spp., such as *Babesia gibsoni* Patton, 1910 and *Babesia conradae* Kjemtrup, Wainwright, Miller, Penzhorn & Carreno, 2006. However, these parasite forms were overall smaller than those measured for the latter two species and no tetrad (Maltese cross) form was reported. Furthermore, phylogenetic analysis using the cytochrome c oxidase subunit 1 (COX1) amino acid sequences substantiates the species identity of this parasite as previously demonstrated based on phylogenetic analysis of the 18S rRNA and β -tubulin genes. The holotype of the parasite species was designated and deposited in an accessible public collection. Conclusions: This study ratifies the name *Babesia vulpes* n. sp. proposed for the parasite previously referred to as *Theileria annae* Zahler, Rinder, Schein & Gothe, 2000, *Babesia annae* (Zahler, Rinder, Schein & Gothe, 2000) or *Babesia vulpes* Baneth, Florin-Christensen, Cardoso & Schnittger, 2015, or mentioned as "Babesia microti-like piroplasm", "Babesia Spanish dog isolate" and *Babesia* cf. *microti*.

Leishmania

Author: Attipa, C., Solano-Gallego, L., Leutenegger, C. M., Papasouliotis, K., Soutter, F., Balzer, J., Carver, S., Buch, J. S. and Tasker, S.

Title: Associations between clinical canine leishmaniosis and multiple vector-borne co-infections: A case-control serological study

DOI: 10.1186/s12917-019-2083-6

Abstract: Background: Dogs that have clinical leishmaniosis (ClinL), caused by the parasite *Leishmania infantum*, are commonly co-infected with other pathogens, especially vector-borne pathogens (VBP). A recent PCR-based study found that ClinL dogs are more likely to be additionally infected with the rickettsial bacteria *Ehrlichia canis*. Further information on co-infections in ClinL cases with VBP, as assessed by serology, is required. The research described in this report determined if dogs with ClinL are at higher risk of exposure to VBP than healthy control dogs using a case-control serology study. Results: Of the 47 dogs with ClinL, anti-*E. canis*/*Ehrlichia ewingii* antibodies were detected in 17 (36.2%), anti-*Anaplasma phagocytophilum*/*Anaplasma platys* antibodies in 5 (10.6%) and antigen for *Dirofilaria immitis* in 2 (4.3%). Of the 87 control dogs, anti-*E. canis*/*E. ewingii* antibodies were detected in 14 (16.1%) and anti-*A. phagocytophilum*/*A. platys* antibodies in 2 (2.3%). No anti-*Borrelia burgdorferi* antibody tests were positive. No statistical differences between the ClinL dogs and control dogs regarding lifestyle or use of ectoparasitic prevention, were identified. The ClinL was significantly associated with anti-*E. canis*/*E. ewingii* antibodies (odds ratio = 2.9, 95% confidence interval: 1.3-6.7, $P = 0.010$) compared to controls by both multivariable logistic regression and structural equation modelling. Conclusions: It was demonstrated that an increased risk for *E. canis*/*E. ewingii* seropositivity is present in dogs with ClinL compared to clinically healthy control dogs, despite similar ectoparasitic prevention use and lifestyle. Based on these findings it is suggested that dogs with ClinL should not only be tested for *E. canis* co-infection using PCR but also serologically for *E. canis*/*E. ewingii*.

Dirofilaria (Heartworm)

Author: Carretón, E., Falcón-Cordón, Y., Falcón-Cordón, S., Morchón, R., Matos, J. I. and Montoya-Alonso, J. A.

Title: Variation of the adulticide protocol for the treatment of canine heartworm infection: Can it be shorter?

DOI: 10.1016/j.vetpar.2019.06.006

Abstract: The treatment of canine heartworm has been modified over the years, adding improvements for greater efficacy, safeness and better prognosis. Currently, the recommended adulticidal protocol consists of the administration of three doses of

melarsomine dihydrochloride, preceded by the administration of macrocyclic lactones over two to three months. The objective of this study was to evaluate a variation of the adulticide protocol of heartworm in 76 dogs infected by *Dirofilaria immitis*, which consists of the pre-administration of macrocyclic lactones (ivermectin) during a single month. On the day of diagnosis, presence of circulating microfilariae was determined and an echocardiography was performed to assess the parasite burden. Treatment began on day 0, with doxycycline for 30 days (10 mg/kg BID) and monthly ivermectin (6mcg/kg). On day 30, the first dose of melarsomine dihydrochloride was administered, followed by a second and third dose on days 60 and 61, respectively. On day 90, the dogs were examined and discharged. Six months after the last dose, all dogs were negative to the presence of antigens and amicrofilaremic. Also, 38.1% of animals were evaluated by echocardiography, showing absence of adult parasites. It is considered that the ineffectiveness of melarsomine against worms <4 months should be avoided by the previous administration of macrocyclic lactones for two to three months, killing larvae <2 months while older filariae are allowed to mature to be susceptible to melarsomine dihydrochloride. With this protocol, this gap would be covered for the 2nd and 3rd injections, when worms would be four months and older. In addition, there is evidence that melarsomine is effective against worms under four months and macrocyclic lactones have some efficacy against heartworms older than two months. This modification allows a faster elimination of heartworms and a better compliance from the owners of the infected dogs.

Author: McTier, T. L., Pullins, A., Chapin, S., Rugg, J., von Reitzenstein, M., McCall, J. W., King, V. L. and Vatta, A. F.

Title: The efficacy of a novel topical formulation of selamectin plus sarolaner (Revolution® Plus/Stronghold® Plus) in preventing the development of *Dirofilaria immitis* in cats

DOI: 10.1016/j.vetpar.2018.10.010

Abstract: Three controlled studies were conducted to investigate the efficacy of selamectin plus sarolaner (Revolution® Plus/Stronghold® Plus) in preventing feline heartworm disease in cats. In all studies, cats were inoculated with 100 *Dirofilaria immitis* third stage larvae on Day -30. In the first study, cats were treated with selamectin plus sarolaner as a single dose on Day 0 or as three consecutive monthly doses on Days 0, 28 and 56. In the second and third studies, cats were treated with either sarolaner alone on Day 0, selamectin plus sarolaner on Day 0 or selamectin plus sarolaner as three consecutive monthly doses on Days 0, 28 and 56. In all three studies, dosages were 6 mg/kg selamectin plus 1 mg/kg sarolaner or 1 mg/kg sarolaner alone. Control cats were given a placebo containing inert formulation ingredients (vehicle). All treatments were administered at a single site topically to the skin cranial to the scapulae. Cats were humanely euthanized on Day 145/146 (i.e., 175/176 post-inoculation), and adult *D. immitis* worms were recovered and enumerated. Across the three studies, adult heartworms were recovered from 87 to 100% of control cats, with geometric mean worm counts ranging from 2.1 to 5.4. No adult *D. immitis* worms were recovered from cats treated with selamectin plus sarolaner. Cats treated with sarolaner alone were not protected against *D. immitis* infection, showing geometric mean worm counts of 1.9 to 2.4. In these studies,

selamectin (6 mg/kg) plus sarolaner (1 mg/kg) was 100% effective in preventing heartworm development in cats when administered topically as one dose 30 days after inoculation or as three consecutive monthly doses starting 30 days post-inoculation. These studies demonstrated that a single topical administration of selamectin plus sarolaner at the recommended dosage was completely effective in preventing the development of *D. immitis* in cats.

Schistosomes

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Epidemiological Surveillance of Schistosomiasis Outbreak in Corsica (France): Are Animal Reservoir Hosts Implicated in Local Transmission?

PLoS Negl Trop Dis . 2019 Jun 24;13(6):e0007543.

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

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Mini-FLOTAC as an Alternative, Non-Invasive Diagnostic Tool for *Schistosoma* *Mansoni* and Other Trematode Infections in Wildlife Reservoirs

Parasit Vectors . 2019 Sep 16;12(1):439.

Schistosomiasis and food-borne trematodiasis are not only of major public health concern, but can also have profound implications for livestock production and wildlife conservation. The zoonotic, multi-host nature of many digenetic trematodes is a significant challenge for disease control programmes in endemic areas. However, our understanding of the epidemiological role that animal reservoirs, particularly wild hosts, may play in the transmission of zoonotic trematodiasis suffers a dearth of information, with few, if any, standardised, reliable diagnostic tests available. We combined qualitative and quantitative data derived from post-mortem examinations, coprological analyses using the Mini-FLOTAC technique, and molecular tools to assess parasite community composition and the validity of non-invasive methods to detect trematode infections in 89 wild Hubert's multimammate mice (*Mastomys huberti*) from northern Senegal. Parasites isolated at post-mortem examination were identified as *Plagiorchis* sp., *Anchitrema* sp., *Echinostoma caproni*, *Schistosoma mansoni*, and a hybrid between *Schistosoma haematobium* and *Schistosoma bovis*. The reports of *E. caproni* and *Anchitrema* sp. represent the first molecularly confirmed identifications for these trematodes in definitive hosts of sub-Saharan Africa. Comparison of prevalence estimates derived from parasitological analysis at post-mortem examination and Mini-FLOTAC analysis showed non-significant differences indicating comparable results between the two techniques ($P = 1.00$ for *S. mansoni*; $P = 0.85$ for *E. caproni*; $P = 0.83$ for *Plagiorchis* sp.). A Bayesian model, applied to estimate the sensitivities of the two tests for the diagnosis of *Schistosoma* infections, indicated similar median posterior probabilities of 83.1% for Mini-FLOTAC technique and 82.9% for post-mortem examination (95% Bayesian credible intervals of 64.0-94.6% and 63.7-94.7%, respectively). Our results showed that the Mini-FLOTAC could be applied as an alternative diagnostic technique for the detection of the zoonotic *S. mansoni* and other trematodes in rodent reservoirs. The implementation of non-invasive diagnostics in wildlife would offer numerous advantages over lethal sampling methodologies, with potential impact on control strategies of zoonotic helminthiasis in endemic areas of sub-Saharan Africa.