

Abstract Booklet



Vector-borne Diseases **2016**

19–20 October 2016

Southern European Veterinarian Conference, Granada, Spain

Introduction

ESCCAP was delighted to be able to hold this conference on emerging vector-borne diseases at the Palacio de Congresos in the beautiful city of Granada in Spain and would like to take this opportunity to thank Professor Guadalupe Miró Corrales for her involvement in the organisation of this important scientific meeting. The directors and members of ESCCAP very much appreciate the contribution she has made.

ESCCAP would also like to thank all of the guest speakers for giving their time to take part in this event.

The directors and members of ESCCAP also extend their sincere appreciation to all their sponsors, without whom these important events would not be possible.



Meeting Programme

ESCCAP Emerging Vector-Borne Diseases 2016: Epidemiology and Clinical Management

DAY 1: Wednesday 19th October (afternoon)



Reinhard K. Straubinger Lyme disease: state of the art



Gad Baneth Canine babesiosis: epidemiology and control of old and new species



Laura Kramer Wolbachia, doxycycline and macrocyclic lactones: new prospects in the treatment of canine heartworm disease



Patrick Bourdeau Fleas and flea-borne diseases: questions and facts

DAY 2: Thursday 20th October (morning)



Barbara Kohn

Canine anaplasmosis and ehrlichiosis –
similar infectious agents, different diseases



Michael Lappin

Feline vector-borne diseases



Maria Grazia Pennisi

Feline leishmaniosis: is the cat a small dog?



Guadalupe Miró

Canine and human leishmaniosis: status quo
in Europe

Guest Speaker Abstracts

ESCCAP Emerging Vector-Borne Diseases 2016: Epidemiology and Clinical Management

Lyme disease: state of the art

Straubinger, Reinhard K.

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Lyme disease is a tick-transmitted inflammatory disease induced by spirochetes of the *Borrelia burgdorferi* sensu lato complex. The infection of susceptible hosts is common in moderate climate regions of the northern hemisphere; specific antibody prevalence against borrelia organisms in dogs is in general up to 10 %, but regional prevalence may exceed this rate.

At least three closely interrelated elements must be present in nature to spread Lyme borreliosis: (i) the Lyme disease-causing bacteria, (ii) *Ixodes* ticks as transmitting vectors for the pathogens, and (iii) mammals, birds and reptiles that provide a blood meal and transportation for the ticks through their various life stages.

Although a high proportion of dogs and other hosts are positive for specific antibodies in endemic areas, only a fraction of the infected animals develop clinical signs. Therefore, serologic testing is absolutely essential to support clinical diagnosis. Nowadays, a selection of assays is available for specific antibody detection. However, only antibody tests (Western blots, line immunoassays, rapid tests), which include VlsE or C6 as capture antigens, are recommended for routine diagnostic procedures.

Therapy consists of antibiotic treatment with doxycycline or amoxicillin for four weeks. Several vaccines for animals, which block the spirochetes' transmission from the tick to the host by the effect of antibodies directed against the bacterial outer surface protein A, are available for several European countries and the USA.

Canine babesiosis: epidemiology and control of old and new species

Gad Baneth

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Babesia are tick-borne protozoan parasites of erythrocytes that infect vertebrates. Canine infections are caused by different *Babesia* species. These include the large form species *Babesia rossi*, *B. canis* and *B. vogeli* which have a similar morphology but differ in the severity of clinical manifestations which they cause, their tick vectors, genetic characteristics and geographic distribution. Another unnamed *Babesia* species most closely related to *B. bigemina* infects immunocompromised dogs in the USA. The small *Babesia* spp. include *B. gibsoni*, *B. conradae* and *B. vulpes* (*Theileria annae*, *B. microti*-like). The geographical distribution of the different *Babesia* species is largely dependent on the habitat of their tick vector species, with the exception of *B. gibsoni* where evidence for dog to dog transmission indicates that infection can be transmitted among dogs independently of the limitations of vector tick infestation. *Babesia vogeli* and *B. gibsoni* are present in Europe, Africa, Asia, America and Australia, whereas *B. rossi* and *B. canis* have been mostly restricted to Africa and Europe, respectively. *Babesia conradae* has been reported only from North America whereas *B. vulpes* was reported in Europe and North America. Large *Babesia* spp. of dogs are commonly treated with imidocarb dipropionate or diminazene aceturate. Small *Babesia* spp. are considered more resistant and treated with the combinations of atovaquone and azithromycin or clindamycin, metronidazole and doxycycline. Prevention of babesiosis relies on acaricides and vaccines available in some European countries.

Wolbachia, doxycycline and macrocyclic lactones: new prospects in the treatment of canine heartworm disease

Laura Kramer

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Melarsomine dihydrochloride is the only approved adulticidal drug for treatment of heartworm disease. However, according to the American Heartworm Society (AHS) guidelines, in cases where arsenical therapy is not possible or is contraindicated, a monthly heartworm preventive along with doxycycline for a 4-week period, which targets the bacterial endosymbiont *Wolbachia*, might be considered. There are published reports on the efficacy of ivermectin and doxycycline in both experimentally and naturally infected dogs, but no data on the use of moxidectin with a similar treatment regime. Preliminary results of an on-going study show that moxidectin, the only macrocyclic lactone (ML) registered as a microfilaricide, is also adulticidal when combined with doxycycline. It is not yet known if the efficacy of these combination therapies is due to pharmacokinetic synergism. A recent study showed that serum levels of doxycycline in dogs treated with the combination protocol were not statistically different, however, compared to dogs treated with doxycycline alone.

Fleas and flea borne diseases – questions and facts

Patrick Bourdeau

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The fleas form an original group of haematophagous insects, ectoparasites at adult stage, associated with an important risk of transmission of pathogens to domestic animals or humans. Amongst the numerous agents suggested to be transmitted, not all of them have really been demonstrated. Sometimes their role is only anecdotic without epidemiological significance. The most historically famous flea-transmitted bacteria is *Yersinia pestis* (“plague”), still present in many countries. *Ctenocephalides* spp. are considered a poor vector for plague. Amongst other species, *Bartonella henselae*, responsible for human “cat scratch disease”, is frequently transmitted among cats by *Ctenocephalides felis* (or flea faeces). *Rickettsia felis* infects fleas with a transovarial and transstadial transmission. It is an agent in humans of the likely misdiagnosed “flea-borne spotted fever”. Feline hemotropic mycoplasmas (prev. *Haemobartonella*), initially thought to be flea transmitted, have other more important modes of transmission. Fleas are vectors of protozoa like *Trypanosoma* in rodents but not *Leishmania* as recurrently suggested. *Dipylidium caninum* is highly prevalent in companion animals and contracted from ingestion of cysticercoids-containing fleas, infested at larval stage. A mildly pathogenic filaria *Acanthocheilonema reconditum* uses fleas as an intermediate host. Fleas (*Spilopsyllus*) play an important role in the transmission of myxoma virus in rabbits. Although suspected to transmit Feline Leukaemia virus, *Ctenocephalides* has only a transient, non-significant, role and the risk is no more than a short residual syringe effect.

Canine anaplasmosis and ehrlichiosis – similar infectious agents, different diseases

Barbara Kohn

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Ehrlichia canis causes canine ehrlichiosis (CE), *Anaplasma phagocytophilum* granulocytic anaplasmosis (CGA); both infectious agents are tick-transmitted (*Rhipicephalus sanguineus* and *Ixodes* ticks, resp.) and belong to the family *Anaplasmataceae*.

Monocytic ehrlichiosis

After an incubation period of 8 - 20 days, fever, depression, anorexia, lymphaden- and splenomegaly, pale mucous membranes, ocular abnormalities, surface bleeding and rarely polyarthritis and CNS signs may occur. Laboratory abnormalities include thrombocytopenia, anaemia, leucopenia, neutropenia, lymphopenia, rarely granular lymphocytosis. Pancytopenia typifies the chronic CE. Hypergammaglobulinemia (poly-, rarely monoclonal), hypoalbuminaemia and mildly elevated liver enzymes are common. Diagnosis is based on (travel) history, clinical signs, laboratory abnormalities, and *Ehrlichia*-specific testing (serology, PCR). Doxycycline is effective in acute cases, but inconsistent in eradicating the infection in chronic forms. Supportive treatment (e.g. infusions, blood transfusions, short-term glucocorticoids) is often needed. Prognosis is good in acute but may be grave in chronic CE.

Granulocytic anaplasmosis

Most dogs are diagnosed during the acute stage after an incubation period of 1–2 weeks. Clinical and laboratory abnormalities resemble the ones in acute CE. Many naturally infected dogs probably remain healthy (subclinical disease / silent elimination). Diagnosis is based on 1) morulae in granulocytes; 2) a positive PCR test; 3) a four-fold increase / decrease in the antibody titre within 4 weeks. Treatment of choice is doxycycline combined with supportive treatment. Prognosis is good; if the pathogen induces chronic infection is unknown.

Vector borne diseases in cats

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There are multiple vector-borne diseases in cats, the most prominent being those transmitted by mosquitoes, sand flies, fleas and ticks. Many of the agents vectored by fleas or ticks have been grown or amplified from blood or have induced serum antibodies in the serum of normal cats or those with clinical signs like fever. As many as 80% of fleas collected from cats contain at least one organism that could induce illness in cats or people.

Anaplasma phagocytophilum, *Bartonella* spp., *Borrelia* spp., *Ehrlichia* spp., haemoplasmas, and *Rickettsia* spp. infect cats and can be associated with clinical illness. *Anaplasma phagocytophilum* and *B. burgdorferi* are transmitted by *Ixodes* spp., *Ehrlichia* spp. and *A. platys* are transmitted by *Rhipicephalus sanguineus*. Fleas vector *Bartonella* spp., haemoplasmas, and *R. felis*. Recently, *R. typhus* was detected in cats in Spain.

Lethargy and fever are common findings in cats with flea and tick-borne diseases. haemoplasmas are associated with hemolytic anemia. Moderate thrombocytopenia is associated with the tick-vectored agents. Polymerase chain reaction assays performed on blood collected in the acute phase of infection can be used to prove presence of the organisms. Doxycycline at 5 mg/kg, PO, twice daily or 10 mg/kg, PO, daily can be effective for resolving the clinical signs. If doxycycline is ineffective or not tolerated, fluoroquinolones can be effective for treatment of clinical illness associated with *Bartonella* spp., haemoplasmas, and *Rickettsia* spp. Use of flea and tick control products is effective for blocking transmission of many of the agents.

Feline leishmaniosis: is the cat a small dog?

Maria Grazia Pennisi

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Leishmaniosis is caused in Europe by *Leishmania infantum*, agent of visceral leishmaniosis, a potentially fatal disease for humans and dogs transmitted by sand flies.

This *Leishmania* species is also isolated from cats in endemic areas. Pathogenesis of leishmaniosis in cats is not known but some experimental data supported the hypothesis that cats are less susceptible than dogs to *Leishmania* infection.

Information on feline leishmaniosis is derived from single case reports or case series in which diagnosis was confirmed by serological, parasitological and/or molecular methods.

About half of cats developing the disease could have had an impaired immune competence because of retroviral infection or immunosuppressive therapies. On the other hand, FIV infection was found to be associated to *Leishmania* in some epidemiological studies.

The most common clinical findings included skin or mucosal lesions (ulcerative or nodular), lymph node enlargement, ocular (uveitis) or oral lesions, weight loss. Anaemia, hyperglobulinemia, proteinuria and chronic renal failure occurred. *Leishmania* amastigotes were found by cytological or histological examination of lesions or tissues. Diagnosis was also confirmed by detection of anti-*Leishmania* antibodies or parasite DNA.

Therapy was empirically carried out in cats with drugs conventionally used for canine leishmaniosis, mainly allopurinol or meglumine antimoniate, and clinical cure was usually obtained.

In conclusion, clinical features of feline leishmaniosis show strong similarities with the canine disease but there are main issues that need to be addressed by means of prospective controlled trials.

Canine and human leishmaniosis: status quo in Europe

Prof. Guadalupe Miró Corrales

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Leishmaniosis, caused by *Leishmania infantum* in dogs and humans, is currently a well-known disease in Europe, dogs being considered to be the major reservoir of the parasite for humans and other mammalian hosts.

Reported human leishmaniosis (HL) case figures are widely acknowledged to represent gross underestimates of the true burden, but active searching promoted by WHO provides yearly reported visceral and cutaneous leishmaniosis incidence worldwide. Specifically, the European countries with high incidence for HL in the last decade were: Spain, Italy, Albania, Greece and Portugal.

New distribution of canine leishmaniosis (CanL) in northern areas of Europe due to the large numbers of re-homed pets from southern endemic countries to non-endemic areas is a fact. Most of all, new alternative routes of transmission in the absence of competent phlebotomine vectors may be the cause of the spread of this important zoonosis.

Prevention of CanL leishmaniosis requires an integrated approach to reduce the risk of infection, disease development, transmission and geographic spread, using insecticides with rapid and residual activity to protect dogs against sand-fly bites and vaccines to reduce the odds of evolution of infection to clinical disease.

In conclusion, changes to the social role of dogs in the last decades because of the importance of the human-animal bond brings about the need to recognise new concepts on the epidemiology, diagnosis and control of canine and human zoonotic leishmaniosis from a “One Health” approach.

Poster Abstracts

Ticks infesting domestic dogs in the UK

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Recent changes in the distribution of ticks and tick-borne disease highlight the importance of ongoing surveillance. Here are the results of a large-scale survey of tick abundance on dogs presented to veterinary practices in the UK over a period of 16 weeks (April - July 2015). 1,094 veterinary practices were recruited. Participating practices were asked to randomly select and examine five dogs each week for ticks and any ticks were then sent to the investigators for identification. 12,096 dogs were examined and 6,555 tick samples from infested dogs were received. *Ixodes ricinus* (Linnaeus) was identified on 5,265 dogs (89%), *Ixodes hexagonus* Leach on 577 (9.8%) and *Ixodes canisuga* Johnston on 46 (0.8%). Ten dogs had *Dermacentor reticulatus* (Fabricius), one had *Dermacentor variabilis* (Say), 3 had *Haemaphysalis punctata* Canestini and Fanzago and 13 had *Rhipicephalus sanguineus* Latreille. 640 ticks were too damaged for identification. All the *R. sanguineus* were on dogs with a recent history of travel outside the UK. The overall prevalence of tick attachment was 30% (range 28-32%). The data presented provide a comprehensive spatial understanding of tick distribution and species abundance in the UK against which future changes can be compared. Relative prevalence maps show the highest rates in Scotland and south-west England providing a valuable guide to tick-bite risk in the UK.

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Funding

Abdullah S. was supported by a University of Bristol Zutshi-Smith PhD scholarship and his travel grant is being provided by the University of Bristol Alumni Foundation. MSD Animal Health contributed to consumables costs for this survey.

Contact is required between dogs treated with Vectra® 3D and *Aedes aegypti* mosquitoes for insecticidal efficacy

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Introduction

This study was designed to determine whether contact between the ectoparasite and the treated dog was required for insecticidal efficacy of dinotefuran-permethrin-pyriproxyfen (Vectra® 3D, DPP) against mosquitoes.

Material and methods

Six dogs were allocated to an untreated control (9.8-11.5 kg BW, n=3) or to a DPP group (9.4-12.9 kg BW, n=3). The dogs in the treated group were administered topically 3.6 mL of DPP on day 0. Under sedation, each dog was exposed to ~40 free adult female *Aedes aegypti* mosquitoes on day 7. Three screened cartons containing ~40 sentinel mosquitoes each (n=120) were also placed inside each mosquito-proof exposure container, close to but without possible contact with the dog. After 1h of exposure to the animals, the free mosquitoes were counted and categorised as live/moribund/dead and fed/unfed. All the live/moribund mosquitoes were incubated and assessed 1, 2, 3 and 7 days after exposure. Insecticidal efficacy was calculated.

Results and conclusion

The product was well tolerated. Only the free mosquitoes released into the exposure containers with the DPP treated dogs died (100% in 24h). Mosquitoes from the control group and from the no-contact containers survived for 7 days after the exposure. Contact is required between treated dogs and the mosquitoes for insecticidal efficacy of DPP.

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This study was funded by Ceva Santé Animale.

Splenectomised Estonian dog with a fatal *Babesia canis canis* infection

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We describe a case of fatal babesiosis in a previously splenectomised dog from Estonia. The 4.5-year-old dog was presented with a sudden lack of appetite and pigmenturia. Its anamnesis included recent travel in the United Kingdom, Poland, Germany, and France, without prophylactic treatment against ticks. Anaemia was not evident but severe thrombocytopenia and numerous protozoan haemoparasites were seen in blood smears. During the following hours, the dog's condition deteriorated, it went into unresponsive seizures, and was euthanized. The causative parasite species was afterwards confirmed as *Babesia canis canis* by sequencing. The sequence was identical to other European *B. canis canis* isolates with AG genotype at positions 610/611 of the whole-length 18S rRNA gene. The described infection was likely travel-related, but could have been autochthonous. Splenectomy was an obvious, iatrogenic contributor for the peracute course of babesiosis in this dog.

The poster has also been presented at EMOP XII. The case report is published in *Acta Vet Scand* 2016 (58:7).

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Detection of *Dirofilaria* spp. and *Angiostrongylus vasorum* in canids from Portugal

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Dirofilariosis and angiostrongylosis are major and potentially fatal canine heartworm diseases increasingly reported worldwide. Despite their importance, few studies have been performed in Portugal. For this reason, a large epidemiological survey was conducted involving 944 shelter dogs between 2011-2015. In total, 11.9% dogs were positive to *Dirofilaria immitis* antigen, 1.3% had antibodies to *Angiostrongylus vasorum* and 0.7% both *A. vasorum* antigens and antibodies. A complementary epidemiological survey was conducted to investigate the prevalence of *D. immitis* and *A. vasorum* in 119 wild red foxes (*Vulpes vulpes*) shot or accidentally killed in Portugal between 2008-2010. In total, 8.4% were found infected with *D. immitis*.

Additionally, geospatial tools were used to assess the transmission risk of *Dirofilaria* spp. in Portugal, using a degree-day model and daily temperatures registered between 2003-2013 in five meteorological stations. To characterize deworming practices implemented in companion animals in Portugal, 312 owners were surveyed at the Small Animal Hospital, ULisboa. Only 11.8% of dogs were internally dewormed at the recommended ESCCAP regimen and 28.4% uninterruptedly protected throughout the year against arthropods.

This set of results highlights the need of an effective prophylaxis in pets and of a robust One Health integrated surveillance approach.

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The most recent prevalence of *Babesia vulpes* infection in red foxes (*Vulpes vulpes*), from Galicia (north-west Spain)

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Currently, vector-borne diseases have become of great importance, piroplasmiasis being no exception. In this large group of diseases, we include *Babesia vulpes*, a haematic protozoa, first reported in Spain (Zahler *et al.*, 2000). Its great prevalence in European and American red foxes (*Vulpes vulpes*) has been proven in recent studies. The objective of this project was to study this parasite infection at its different levels, unknown to date. Spleen, ticks (if present), and blood samples were taken from hunted red foxes from the Galician Community (North-West Spain). Blood smears were performed for direct parasite observation, as well as nested PCR in spleen samples. These tests were assessed in order to study the prevalence of infection in the Galician foxes, since older studies recorded high prevalence in other European countries. Prevalence of *B. vulpes* infection in Galician red foxes was 72,5%, *Ixodes hexagonus* being the most predominant tick, categorising it as a potential vector to foxes and other carnivores, including dogs. We may conclude that piroplasmiasis prevalence is very high in this area, considering red foxes as the main reservoir of *Babesia vulpes*. Further studies are needed to better understand the connection between wild and domestic life cycle of this piroplasma.

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Prevalence of main parasitosis and retrovirosis in feline communities from Madrid area (Spain)

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There are groups of unowned, stray and/or feral cats roaming free in most big cities around the world, commonly known as “feline communities”. This reflects the poor control of feline overpopulation in these areas.

In the present study, we collected blood, faecal and skin samples from 217 cats, belonging to three feline colonies in Madrid, coinciding with a Trap-Neutered-Return (TNR) control program. The aim of this study was to evaluate the prevalence of parasitic diseases and retroviruses in this kind of population. The whole prevalence of endoparasites obtained was 27.2%. Cestodes were the best represented parasitic group with a prevalence of 11.5%, followed by *Toxocara cati* (10.6%). Related ectoparasites, flea and tick infestations prevalence was 7% on sampled cats and 14.3% were positive to an *Otodectes cynotis* infestation. Of the cats tested, 2.9% were positive for feline immunodeficiency virus and 2.9% were positive for leukaemia virus. However, none of the cats tested positive for both. This study provides further information about clinical status of feline communities related to some important zoonotic parasites and retrovirus infections. These preliminary results highlight the need for developing new strategies – including TNR programs that incorporate health-monitoring schemes – to increase understanding and control of these feline populations with an aim to improving their health status and reducing their impact on public health.

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Canine leishmaniosis control and monitoring with quantitative serology in an animal shelter from Madrid (Spain)

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Canine leishmaniosis (CanL) is an important zoonotic disease endemic all over Southern Europe, including Spain. Imported cases are increasing in Central and Northern European countries where a competent vector for the transmission of *Leishmania infantum* is absent. This parasitic disease is considered a Public Health concern and animal shelters have an important role in its control. The objective of this project was to present an effective method of handling the diagnosis of the infection and disease management in such a scenario. During 2014, we monitored and clinically evaluated 474 stray dogs allocated to the dog shelter ALBA. Immunofluorescence antibody test (IFAT) was used as a diagnosis test. The overall seroprevalence obtained was 6.8%, quite similar to previous studies. According to data from Community of Madrid, an 8.2% of sheltered dogs were seropositive to *L. infantum* infection in 2014. Of these seropositive stray dogs, 84.37% had been adopted during the same year of the study. To control CanL in dog shelters it should be mandatory to perform a physical exam and a quantitative serology to increase sensitivity and avoid false negative results. Moreover, specific treatment should be applied to sick dogs before commencing adoption procedures. A thorough and specific diagnosis is essential before re-homing stray dogs from endemic areas of CanL to non-endemic areas.

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Prevalence of intestinal and pulmonary parasite infections in red foxes (*Vulpes vulpes*) in the north-west and central regions of Spain

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During the last decades, populations of red foxes (*Vulpes vulpes*) have displayed changes on behaviour and habitat due to urbanisation. This may have had an impact on disease transmission to both domestic animals and humans. The objectives of this project were to evaluate the prevalence of main intestinal and pulmonary parasites affecting red foxes, epidemiological factors which predispose their occurrence and to determine the epidemiological role of red foxes as reservoirs. One hundred and five faecal samples from red foxes were collected from different geographic areas (north-west and central Spain), and analysed by conventional coprological and molecular techniques. Statistical analyses were performed in order to associate prevalence of parasitic diseases with three variables: geographic origin, sex and age. Different parasite species were reported: protozoa (*Cryptosporidium* spp., *Cystoisospora* spp. and *Giardia duodenalis*), cestodes (Class Cestoidea) and nematodes (Fam. Ancylostomatidae, *Angiostrongylus vasorum*, *Capillaria* spp., *Crenosoma vulpis*, *Toxascaris leonina*, *Toxocara canis* and *Trichuris vulpis*), revealing the importance of red foxes as reservoirs of parasitic diseases for both other carnivores and human beings. Prevalence of Fam. Ancylostomadiae, *T. leonina* and *T. vulpis* infection was statistically significant according to the foxes' geographical origin, and for *Cryptosporidium* spp. and *T. vulpis* infection was related to sex variable.

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Controlling and monitoring the spread of zoonotic visceral leishmaniasis in Europe: A survey-based study among Spanish and French veterinarians

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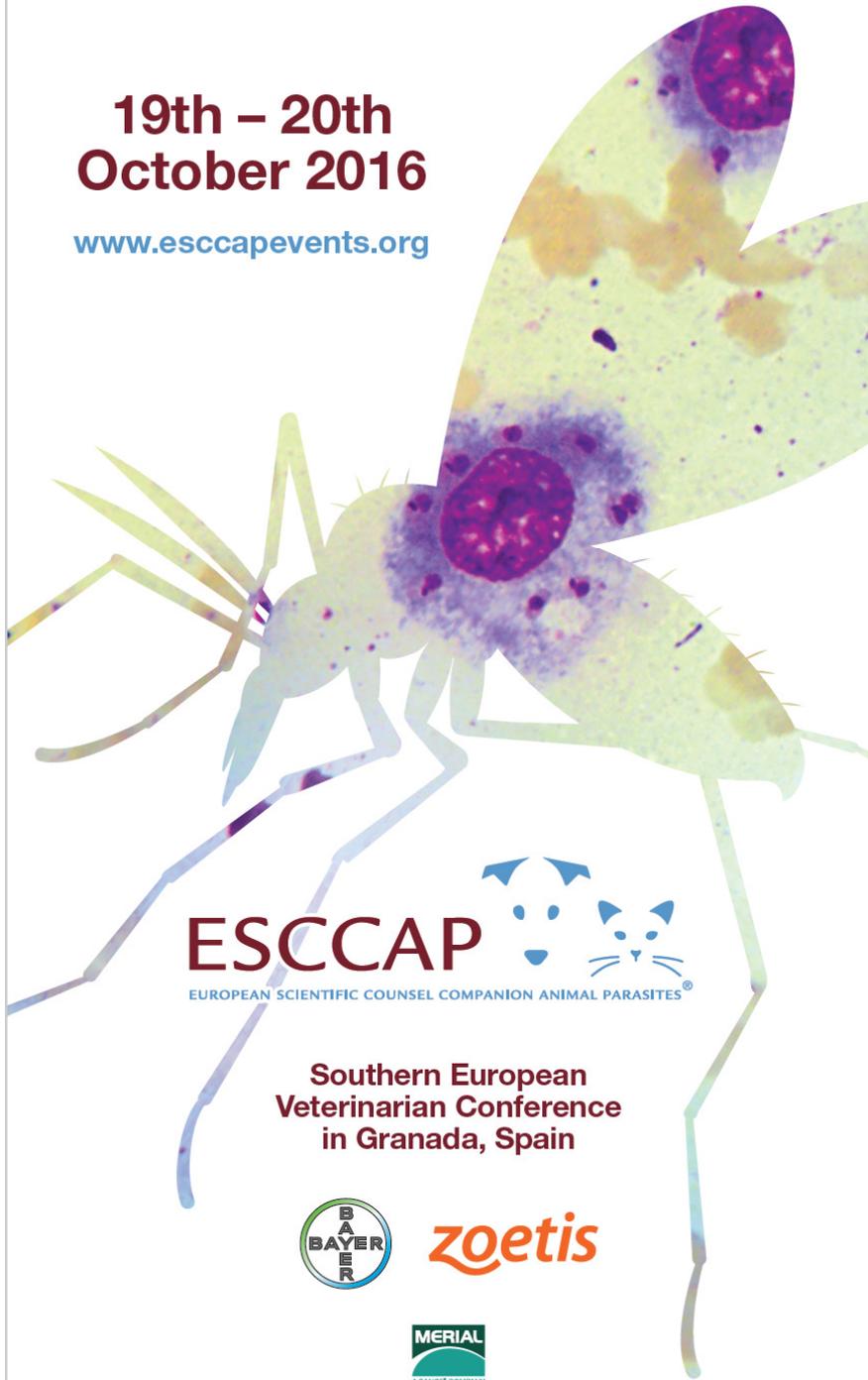
Zoonotic visceral leishmaniasis (ZVL) is transmitted by female sand flies infecting dogs, humans, and wildlife. In the last decade, the disease prevalence has increased by an alarming fivefold in several parts of southern Europe, where the main drivers of disease transmission are contributed to the increase in sand fly distribution due to climate change and the traveling and migration of dogs. The EU, WHO, EFSA, and ESCCAP among others have created international guidelines, providing protocols describing intervention strategies to control the spread of ZVL in Europe. In this study an online survey was developed and distributed among veterinarians in Spain and France to question their 1) awareness of the spread and public health risks of ZVL in Europe, 2) awareness of the guidelines, 3) type of protocol used when suspecting and confirming a ZVL case, and 4) their reporting of confirmed cases. 451 veterinarians completed the survey and even though only 30% were aware of any guidelines, most of their preventive and treatment actions were in line with the intervention strategies as suggested in the guidelines' protocols. However, only 12% of veterinarians would report a confirmed case, including notifying colleagues within the same practice. We therefore suggest that an online network where both veterinarians and general practitioners report the presence of confirmed cases seems crucial in order to be able to monitor, control and prevent the further spread of zoonotic visceral leishmaniasis in Europe at the regional, national and international level.



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