Ph.D. THESIS

Vector borne diseases in domestic and wild felids in Europe

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ABSTRACT

In recent decades, the incidence and diversity of tick-borne diseases in both humans and animals have increased due to several factors, including the existence of advances diagnostic methods, changes in the environment (e.g. climate change), increased awareness, and increased contact of humans with wildlife and vectors (ESTRADA-PEÑA ET AL., 2012; JAENSON ET AL., 2012; ALVARADO-RYBAK ET AL., 2016). Despite that in the recent years there has been renewed interest in investigating the canine vector-borne pathogens, there is limited knowledge about their prevalence and nature in felids. Cats are ubiquitous in both developed and developing societies and equally share the environment with canine counterparts (CHOMEL AND SUN, 2011).

Three species of wild felids are present in Europe: the Iberian lynx (*Lynx pardinus)*, the Eurasian lynx (*Lynx lynx*), and the European wild cat (*Felis silvestris silvestris)* (SUNQUIST AND SUNQUIST, 2009). The Eurasian lynx and the European wild cat are also present in Romania (MURARIU, 2010).

Multiple ways of pathogen transmission from domestic to wild animals, and vice versa, are known, and they are represented by complex webs within the ecosystems (OTRANTO ET AL., 2015). As shown in the present paper, there is abundant evidence of vector-borne infections in domestic and wild felids in Europe, in some circumstances displaying high infection rates. Moreover, there are species that serve as reservoirs, and a variety of potential vectors that may facilitate the maintenance of the parasites sylvatic life cycles in their geographical area of distribution (ALVARADO-RYBAK ET AL., 2016). However, important data on the presence and distribution of vector-borne pathogens in felids is lacking. Therefore, the aims of the present work are the following:

- To describe the clinical and laboratory findings of the infection with European *Cytauxzoon* isolates in association with feline immunodeficiency virus infection in a domestic cat and to molecularly confirm the presence of the piroplasm.
- To assess the genetic diversity of European *Cytauxzoon* isolates in wild felids in Europe by analyzing 18S rDNA, mitochondrial genes and complete mitochondrial genomes of representative isolates.
- To determine the diversity of feline *Babesia* spp. in wild felids in Romania and to molecularly characterize the species by using the 18S rDNA and mitochondrial markers.
- To evaluate the occurrence of *Babesia* spp., *Cytauxzoon* spp., and *Hepatozoon* spp. in domestic cats in Romania by means of PCR and to identify the potential risk factors associated with these pathogens.

- To investigate the presence and distribution of spotted fever group rickettsiae, *Bartonella* spp., *Francisella tularensis*, *Borrelia burgdorferi* sensu lato, *Ehrlichia* spp., *Anaplasma phagocytophilum*, *Dirofilaria immitis* and *D. repens* in domestic and wild cats in Romania.

The first part of the thesis (1. Introduction), presents summarized literature data regarding the ecology of domestic and wild felids, the main parasitic arthropods and the parasitic and bacterial vector-borne pathogens identified in domestic and wild felids in Europe to date. The second part (2. Original research) consists in 5 original manuscripts focused on the presence and diversity of apicomplexan hemoparasites and non-apicomplexan vector-borne agents, in both wild and domestic felids in Europe.

The first chapter of the second part (2.1) presents the clinical picture and laboratory findings of the infection with European *Cytauxzoon* isolates in association with feline immunodeficiency virus infection in a domestic cat. Cytauxzoonosis is described as an emerging tick-borne disease of domestic and wild felids caused by protozoans of the genus *Cytauxzoon*. This study describes the first case of *Cytauxzoon* sp. infection in Germany, in 6-year-old male domestic cat presented with anorexia, lethargy and weight loss. Serum clinical chemistry analysis revealed azotaemia with markedly increased symmetric dimethylarginine, hypercreatinemia, hyperphosphatemia and hypoalbuminemia. Moreover, a mild non-regenerative anaemia was present. Approximately one year prior to these findings, the domestic cat was diagnosed with a feline immunodeficiency virus infection. These results pointed toward a decreased glomerular filtration rate, presumably as a result of kidney dysfunction. Round to oval signet-ring shaped intraerythrocytic organisms, morphologically suggestive for a piroplasm, were revealed during blood smear evaluation. PCR analyses and sequencing of a 18S rDNA fragment confirmed the presence of *Cytauxzoon* sp. infection, with 99-100% nucleotide sequence identity with previously published *Cytauxzoon* isolates. As this is the first molecular confirmation of *Cytauxzoon* sp. infection in a domestic cat in Germany, these findings suggest that cytauxzoonosis should be considered as a differential diagnosis in cases of anaemia in outdoor domestic cats.

The second chapter on of the second part (2.2) provides data on *Cytauxzoon* spp. diversity in wild felids from Europe. Protists of the genus *Cytauxzoon* infect a wide variety of wild and domestic felids worldwide. While the American *Cytauxzoon felis* has been well described, data on the European isolates of *Cytauxzoon* are still scant. The aim of the current study was to determine the genetic diversity of European *Cytauxzoon* spp. in wild felids by analysing one nuclear and two mitochondrial genes, along with representative complete mitochondrial genomes.

Overall, 106 biological samples from wild felids (92 from *Felis silvestris* and 14 from *Lynx lynx*) from Germany, Romania, Czech Republic, and Luxembourg were screened for the presence of *Cytauxzoon* spp. using nested PCR protocols, targeting the 18S rDNA, mitochondrial cytochrome *b* and cytochrome *c* oxidase subunit I genes. Furthermore, 18 previously confirmed wild felid biological samples from Europe, and comparative material from USA positive for *C. felis*, were included in the study. In 18S rDNA sequences analyses, *Cytauxzoon* spp. from felids formed two separate clades of New World and Old World isolates, with a low inner diversity of the European clade. In contrast to 18S rDNA, the phylogenetic analyses of mitochondrial genes revealed three highly supported clades, resulting in three defined genotypes. Similar intra- and interspecific variability of mitochondrial genes was observed in the case of different *Babesia* spp. Considering geography, host species and analyses of three genes, we conclude that the three detected genotypes of *Cytauxzoon* in European wild felids represent three new species, which we herein describe.

The third chapter of the second part (2.3) evaluates the diversity of *Babesia* spp. in wild felids in Romania. Haemoparasites of the genus *Babesia* infect a wide range of domestic and wild animals. Feline babesiosis is considered endemic in South Africa, while data on *Babesia* spp. infection in felids in Europe is scarce. Using samples from 51 wild felids, 44 *Felis silvestris* and 7 *Lynx lynx*, the study aimed to determine the presence and genetic diversity of *Babesia* spp. in wild felids in Romania by analysing the 18S rDNA and two mitochondrial markers, cytochrome *b* and cytochrome *c* oxidase subunit I genes. By 18S rDNA analyses, *Babesi*a spp. DNA was detected in 20 European wild felids. All sequences showed 100% similarity to *B. canis* by BLAST analysis. Conversely, mitochondrial genes analyses revealed the presence of two *Babesia* spp., *B. pisicii* n. sp., which we herein describe, and *B. canis*. The pairwise comparison of both mitochondrial genes of *B. pisicii* n. sp. showed a genetic distance of at least 10.3% from the most closely related species, *B. rossi*. Phylogenetic analyses of mitochondrial genes revealed that *B. pisicii* n. sp. is related to the so-called "large" canid-associated *Babesia* species forming a separate subclade in a sister position to *B. rossi*.

The forth chapter of the second part (2.4) reports the presence of hemoparasites in domestic cats in Romania. Apicomplexan hemoparasites are protozoans that infect a variety of domestic and wild animal species, as well as humans. The aim of the study was to assess the occurrence of *Babesia* spp., *Cytauxzoon* spp., and *Hepatozoon* spp. in domestic cats in Romania by using molecular tools. Blood samples from 371 domestic cats were initially screened for the presence of piroplasmids. All samples that yielded a visible band in agarose gels were subsequently tested by specific assays targeting the 18S rDNA of *Babesia* spp., *Cytauxzoon* spp., and *Hepatozoon* spp. Moreover, nested PCR assays targeting mitochondrial genes of *Babesia* spp. were used for screening of all *Babesia* spp. 18S rDNA positive samples. From the total number of

sampled cats, 19.4% were positive in the PCR assay targeting Piroplasmids. Furthermore, *Babesia* spp. were identified in 15.1% of cats, while 0.5% were positive for *Hepatozoon* spp. Molecular analyses confirmed the presence of *B. canis*. No samples were positive for *Cytauxzoon* spp. The high infection rates of domestic cats with *Babesia* spp. and the need of species differentiation, highlights the importance of mitochondrial genes as targets for molecular protocols.

The last chapter of the second part (2.5) provides data on vector-borne pathogens in domestic and wild cats from Romania. The aim of the study was to evaluate the presence and distribution of spotted fever group rickettsiae, *Bartonella* spp., *Francisella tularensis*, *Borrelia burgdorferi* sensu lato, *Ehrlichia* spp., *Anaplasma phagocytophilum*, *Dirofilaria immitis* and *D. repens* in domestic and wild felids. A total of 421 blood and tissue samples from domestic cats (n=371), wild cats (n=34) and Eurasian lynx (n=6) were screened by using molecular methods. From the total number of domestic cats, 21.3% were PCR positive for vector borne pathogens, mainly 0.8% were infected with *Bartonella* spp. (*B. henselae* in two cats and *B. clarridgeiae* in one cat), 1.1% with *E. canis*, and 19.4% with *A. phagocytophilum*. *A. phagocytophilum* was the only pathogen detected in wild felids with an infection rate of 50%. Consequently, the presence of vector-borne bacteria is described among domestic and wild felids by means of PCR, with varying prevalence of infection.

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