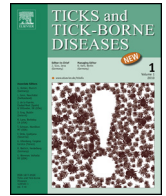




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Molecular detection of vector-borne pathogens in wild and domestic carnivores and their ticks at the human–wildlife interface

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ABSTRACT

Urbanization of natural areas is considered one of the causes of the current apparent emergence of infectious diseases. Carnivores are among the species that adapt well to urban and periurban environments, facilitating cross-species disease transmission with domestic dogs and cats, and potentially with their owners. The prevalence of vector-borne pathogens (VBP) of zoonotic and veterinary interest was studied in sympatric wild and domestic carnivores into Barcelona Metropolitan Area (NE Spain). Blood or spleen samples from 130 animals, including 34 common genet (*Genetta genetta*), 12 red foxes (*Vulpes vulpes*), 10 stone martens (*Martes foina*), three Eurasian badgers (*Meles meles*), 34 free-roaming domestic cats and 37 dogs with outdoor access, were collected either in protected or adjacent residential areas. A total of 309 ticks (chiefly *Rhipicephalus turanicus*) were collected on these animals. The samples were analyzed with a battery of PCR assays targeting the DNA of *Rickettsia* spp., Anaplasmataceae, *Coxiella burnetii*, *Bartonella* spp., and Piroplasmida, and the amplicons were sequenced. The fox showed the highest prevalence (58%) and diversity of VBP (four pathogens), whereas none of the dogs were infected. *Bartonella* spp. (including *B. clarridgeiae*, *B. henselae*, and *B. rochalimae*) was the most prevalent pathogen. Infection of wild carnivores with *Ehrlichia canis*, *C. burnetii*, *Theileria annae* and *Babesia vogeli* was also confirmed, with some cases of coinfection observed. The presence of DNA of *T. annae* and *B. vogeli* was also confirmed in tick pools from four species of wild carnivores, supporting their role in piroplasmid life-cycle. By the sequencing of several target genes, DNA of *Rickettsia massiliae* was confirmed in 17 pools of *Rh. turanicus*, *Rh. sanguineus*, and *Rh. pusillus* from five different species, and *Rickettsia conorii* in one pool of *Rh. sanguineus* from a dog. None of the hosts from which these ticks were collected was infected by *Rickettsia*. Although carnivores may not be reservoir hosts for zoonotic *Rickettsia*, they can have an important role as mechanical dispersers of infected ticks.

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1. Introduction

The majority of pathogen species causing disease in humans is zoonotic (Taylor et al., 2001) and the urbanization of natural areas is proposed as a cause of the current apparent emergence of infectious diseases (Bradley and Altizer, 2007). One result of human encroachment on natural areas, with subsequent habitat degradation and fragmentation, is more opportunity for contact between

humans, domestic animals and wildlife (Deem et al., 2001). Human-induced landscape change selects for synanthropic wild mammals and increases our exposure to their pathogens (McFarlane et al., 2012). Different species of carnivores can adapt well to urban and periurban environments (Gehrt et al., 2010), and there is increasing evidence of a role for carnivores in the life cycle of pathogens of public health and veterinary interest. For example, different species of wild canids have been recently confirmed as definitive hosts for *Neospora caninum* (Dubey et al., 2007). Less is known about their participation in the cycle of vector-borne diseases. Recently, a high proportion of red foxes (*Vulpes vulpes*) was found to be infected by the piroplasm *Theileria annae* (syn. *Babesia microti*-like, syn.

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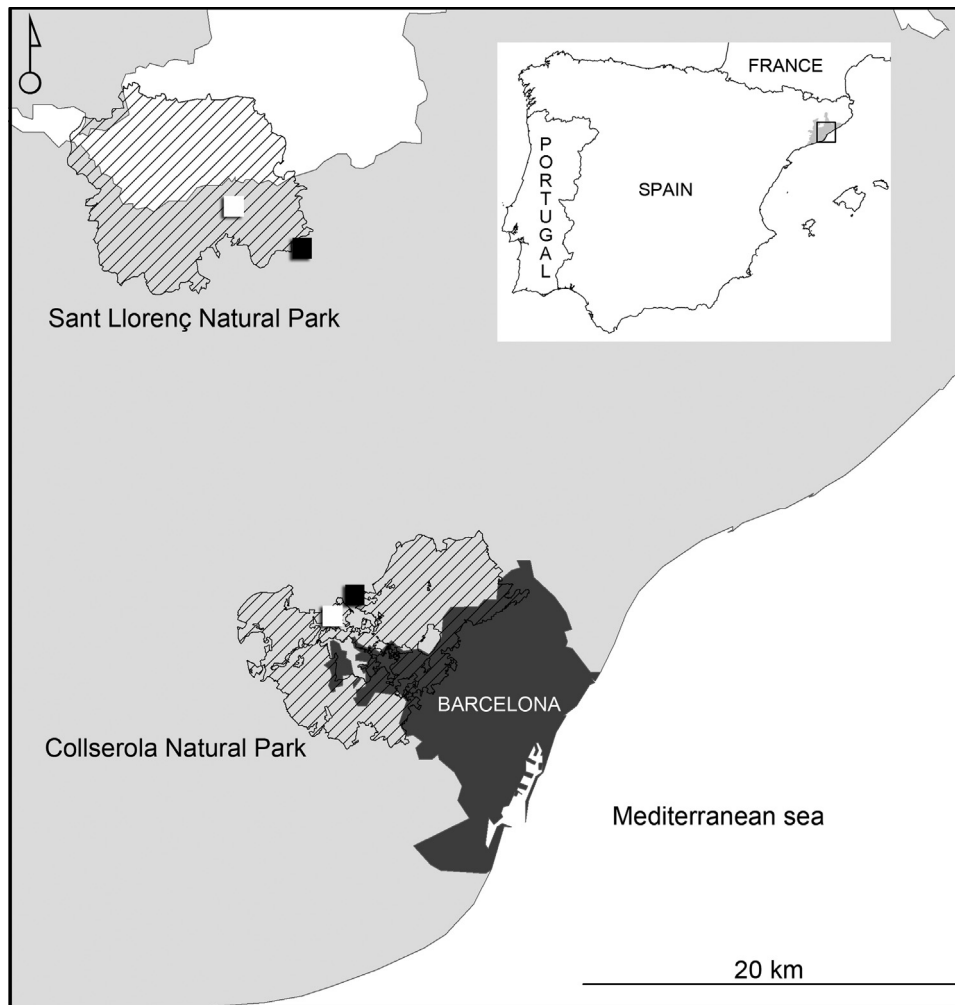


Fig. 1. Map of the study areas. Dark gray: Barcelona city; light gray: Barcelona metropolitan area; striped: the studied Natural Parks, encroaching on the metropolitan area. Black squares: domestic carnivores sampling areas; white squares: wild carnivores trapping areas.

B. vulpes) in Portugal, suggesting that foxes may be a source of infection for dogs (Cardoso et al., 2013). Similarly, a high prevalence of *Bartonella clarridgeiae*-like infection was recorded in gray foxes (*Urocyon cinereoargenteus*) in California (Henn et al., 2009), and *B. henselae* DNA isolated in California mountain lions (*Puma concolor*) was similar to organisms isolated in domestic cats and humans, supporting the hypothesis that mountain lions serve as enzootic reservoirs of cat-scratch disease (Girard et al., 2012). Other vector-borne pathogens (VBP) recently detected in wild carnivores worldwide include *Anaplasma phagocytophilum*, *Hepatozoon canis*, or *Ehrlichia canis* (e.g. Mascarelli et al., 2015; Tolnai et al., 2015).

According to the United Nations, the Barcelona Metropolitan Area (BMA, NE Spain, Fig. 1.) has about 5.5 million habitants (United Nations, 2012) and is the most populous area on the Mediterranean coast. This conurbation contains a number of protected areas, where hundreds of thousands of people enjoy outdoor activities every weekend. In addition, on the edges of these natural areas, some of which are now completely surrounded by developed land, there is an increasing number of residential areas where the possession of cats allowed to roam free or dogs with outdoor access, is frequent. Therefore, hikers, nature lovers and local residents, along with their dogs and cats, can easily encounter arthropods, wildlife, and the pathogens they host. The aim of the present study was to determine the prevalence of locally important VBP in wild carnivores captured in the BMA and domestic dogs and cats sampled in residential areas adjacent to periurban protected areas.

2. Materials and methods

2.1. Study areas

Animals were sampled in two natural parks and two adjacent residential areas in the BMA (Fig. 1). In each one of the four study areas, a 1-km² zone was selected for the study. The studied natural parks were Collserola (Co; 41°26'N, 2°08'E) and Sant Llorenç del Munt (SL; 41°38'N, 2°01'E). Co (ca. 8000 ha), a fairly mountainous area with altitudes ranging between 60 and 512 meter above sea level, is completely surrounded by the metropolitan area and annually receives about 2 million visitors. SL (13,694 ha) is located to the north of the metropolitan area and annually receives about 250,000 visitors. Its altitudinal range is between 280 and 1100 masl. Residential areas are characterized by the presence of detached houses in a density of about 100 houses per km², scattered among habitats that are not completely developed (i.e., roads and tracks cross the area but houses are surrounded by tracts of scrub and forest) and similar to natural areas. In Co, the residential study area (La Floresta) is only 600 m from the selected natural study area, whereas in SL, an area with less pressure from construction, the residential area (Sant Feliú del Racó) is about 10 km from the corresponding natural study area (Fig. 1).

The minimum density of common genet (*Genetta genetta*) was estimated at 0.98 ind/km² in Co (Camps y Llimona 2004), with mean densities of genets and stone martens (*Martes foina*) in SL at about

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