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Molecular detection of vector-borne bacteria and protozoa in healthy hunting dogs from Central Italy

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PEER REVIEW

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Comments

This is an interesting and well-written paper. The authors investigated the occurrence of some vector-borne pathogens in a category of dogs (hunting dogs) which were few investigated in Italy, even though they were frequently exposed to ticks as a result of the environment.
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ABSTRACT

Objective: To determine the prevalence of vector-borne bacteria and protozoa in hunting dogs living in Central Italy.

Methods: Molecular testing was executed on DNA which was extracted from blood specimens collected from 117 asymptomatic dogs to detect *Anaplasma phagocytophilum, Babesia canis* (*B. canis*), *Bartonella* spp., *Coxiella burnetii* (*C. burnetii*), *Ehrlichia canis, Hepatozoon canis,* and *Leishmania infantum*.

Results: A total of 48 dogs (41.0%) were infested by *Ixodes ricinus* and *Rhipicephalus sanguineus* ticks. Tick-borne infections were observed in 64 (54.7%) animals. More in detail, 38 dogs (32.5%) screened positive for *Hepatozoon canis*, 24 (20.5%) for *Bartonella vinsonii* subsp. *berkhoffii*, 20 (17.1%) for *Leishmania infantum*, 6 (5.1%) for *C. burnetii*, 5 (4.3%) for *B. canis* (3 *B. canis vogeli* and 2 *B. canis canis*), 3 (2.5%) for *Anaplasma phagocytophilum*, and 2 (1.7%) for *Ehrlichia canis*. Mixed infection by 2 agents occurred in 17 (14.5%) subjects, by 3 agents in 7 (6.0%) dogs, and by 4 agents in 1 (0.9%) animal.

Conclusions: The results demonstrated that several vector-borne pathogens were circulating in this region and dogs infected by these agents were usually asymptomatic. A relevant finding was the presence of DNA of *C. burnetii*, a severe zoonotic agent, in the 5.1% of tested dogs, which can be source of infection for their owners not only through tick bites, but also directly with urine, feces and birth products.

KEYWORDS
Arthropod-borne infection, Bacteria, Dog, PCR, Protozoa

1. Introduction

Vector-borne diseases are caused by parasites, bacteria or viruses which are transmitted by hematophagous arthropods. The past few years have seen the emergence of new diseases, or re-emergence of existing ones. These epidemiological changes are supposed to be due to human factors and climatic changes that can influence arthropod distribution and activity[1].

Dogs, in particular hunting ones, are frequently exposed to ticks. Evidence of current or past infections in these animals can be used to determine whether there is a risk of infection by tick-borne pathogens in a given geographic area.

Humans are susceptible to many tick-borne bacteria and protozoa which usually infect animals. Among them, *Coxiella burnetii* (*C.*

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burnetii) may be transmitted by different hematophagous arthropods, even if infection is usually acquired by humans and animals through inhalation of contaminated aerosol or ingestion of contaminated food, which are mainly raw milk and dairy products[2].

Anaplasma phagocytophilum (*A. phagocytophilum*) is a tick-borne bacterium mainly transmitted by *Ixodes ricinus* and a variety of wild animals, including rodents and deer, which act as reservoir hosts. Dogs are accidental hosts, which may develop acute or sub-clinical infections[3].

Several *Bartonella* species are zoonotic with their main natural hosts that exist in felids, canids, rodents and lagomorphs. Canids have been reported as the main reservoirs for *Bartonella vinsonii* subsp. *berkhoffii* (*B. vinsonii* subsp. *berkhoffii*), but other *Bartonella* species have been detected in domestic dogs, including *Bartonella henselae*, which is traditionally associated to cats[4].

Ehrlichia canis (*E. canis*) causes the canine monocytic ehrlichiosis and it is mainly transmitted by the brown dog tick *Rhipicephalus sanguineus* (*R. sanguineus*). Dogs and other canids are the natural hosts of *E. canis*, which has a worldwide distribution. *E. canis* is generally not considered as a zoonotic agent, but some cases of human infection have been reported in Venezuela[5].

Among protozoan parasites, *Babesia canis* (*B. canis*) and *Babesia gibsoni* cause significant disease in dogs. *B. canis* includes three subspecies: *Babesia canis rossi*, usually transmitted by *Haemaphysalis* spp., *Babesia canis canis* (*B. canis canis*) by *Dermacentor* spp., and *Babesia canis vogeli* (*B. canis vogeli*) by *R. sanguineus* ticks[6].

Hepatozoon canis (*H. canis*) which is transmitted by *R. sanguineus* is distributed throughout the old world. Disease associated with the infection is usually asymptomatic, while disease, when present, may range from subclinical and chronic, especially in the absence of concurrent infections, to severe and life-threatening[7].

Canine leishmaniosis due to *Leishmania infantum* (*L. infantum*) is enzootic in Mediterranean countries[1] and it can be responsible for asymptomatic to patient clinical forms. The parasite is injected into the skin of the host by biting of female sandflies, and it is an emerging zoonosis in canine endemic foci.

Wild boar hunting in Central Italy is by largely practiced dogs. These animals spend most of their life outdoor and are frequently exposed to tick infestations. Although the arthropod-borne infections in dogs are well known, data about the prevalence of bacteria and protozoa which were transmitted by haematophagous vectors in canine population living in this region are scant.

The aim of the present study was to determine, by molecular testing, the prevalence of tick-borne bacteria and protozoa, such as *A. phagocytophilum*, *Bartonella* spp., *B. canis*, *E. canis* and *Hepatozoon* spp., in dogs living in Central Italy that were employed for wild boar hunting in the Maremma Region, an area traditionally endemic for canine leishmaniosis due to *L. infantum*.

2. Material and methods

The Maremma Region is a very extensive area, which comprises part of Southwestern Tuscany and part of Northern Lazio. It is characterized by a rich vegetation which varies base on the territory, in particular where sandy coast, palus and forest vegetation are present. Several wild animal species live in the forest area such as wild boars (*Sus scrofa*), foxes (*Vulpes vulpes*), roe deers (*Capreolus capreolus*), fallow deers (*Dama dama*), hares (*Lepus europaeus*), hedgehogs (*Erinaceus europaeus*), badgers (*Meles meles*), porcupines (*Hystrix cristata*), and a wide range of birds.

A PCR survey was conducted in the hunting season (November 2012 to January 2013) to investigate the prevalence of vector-borne agents which infected hunting dogs with no history of recent tick treatments and no overt clinical manifestations. A total of 117 animals, of both genders (69 males and 48 females), aged from 8 to 132 months [(54.0±31.5) months], were randomly selected among dogs which were brought to the veterinary physician because of wild boar attacks during hunting activity.

Ticks occurring on the canine hosts were removed into tubes with 75% ethanol and stored at 20 ˚C . Later, the ticks were morphologically identified[8]. A blood specimen was drawn from the cephalic vein of each animal and DNA was extracted using a Qiagen DNeasy Blood & Tissue Kit (Qiagen, Milano, Italy) and used for PCR purposes.

2.1. A. phagocytophilum

A primary amplification was carried out to amplify a 932 bp fragment of the 16S rRNA gene of *A. phagocytophilum*, using the primers GE 3a and GE 10r. A nested PCR, with the primers GE 9F and GE 2, amplified a 546 bp fragment of the same gene. Primary and secondary amplifications were performed with the same cycling conditions[9].

2.2. B. canis

B. canis was detected by using primer PIRO-A and antisense oligonucleotide primer PIRO-B that amplify an approximatively 400 bp portion of the small subunit ribosomal DNA of most *Babesia* species. A PCR-restriction fragment length polymorphism analysis of amplification products was carried out using HinfI and TaqI restriction enzymes to discriminate among *B. canis* subspecies[10].

2.3. Bartonella spp.

DNA samples were employed in a PCR assay to identify *Bartonella* genus. The primers p24E and p12B were used to amplify a 296 bp fragment of the *Bartonella* 16S rRNA gene[11].

2.4. C. burnetii

C. burnetii was identified by amplifying a 687 bp fragment of the IS1111a gene using primers Trans-1 and Trans-2[2].

2.5. E. canis

An initial reaction amplified a 478 bp fragment common among all known *Ehrlichia* species employing the primers ECB and ECC[12]. In the nested reaction, a 389 bp of 16S rRNA gene was amplified