

Prevalence of select vector-borne disease agents in owned dogs of Ghana

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Ticks, sera and ethylenediaminetetraacetic acid (EDTA) blood were collected from dogs evaluated at the Amakom Veterinary Clinic in Kumasi, Ghana. Sera were evaluated for *Dirofilaria immitis* antigen and antibodies against *Borrelia burgdorferi*, *Anaplasma phagocytophilum* and *Ehrlichia canis*. Conventional polymerase chain reaction assays designed to amplify the deoxyribonucleic acid (DNA) of *Ehrlichia* spp. or *Anaplasma* spp. or *Neorickettsia* spp. or *Wolbachia* spp., *Babesia* spp., *Rickettsia* spp., *Hepatozoon* spp., *Bartonella* spp. and the haemoplasmas were performed on DNA extracted from EDTA blood and all positive amplicons were sequenced. This small survey shows that the following vector-borne pathogens are present in urban Ghanaian dogs: *Ehrlichia canis*, *Hepatozoon canis*, *Dirofilaria immitis* and *Anaplasma platys*. *Bartonella henselae* was isolated from ticks but not from the dogs.

Introduction

Previous studies in southern and eastern Africa show relatively high rates of canine vector-borne infections (10% – 49%) (Matjila *et al.* 2008; Sasaki *et al.* 2008). In addition, domestic dogs can be used as sentinels for human diseases caused by potentially zoonotic agents. The objective of this study was to screen for the presence of selected vector-borne agents in Kumasi, Ghana by analysing a limited sample set from domestic dogs.

One of the investigators collected blood samples and ticks from dogs presented to the Amakom Veterinary Clinic in Kumasi, Ghana, during a 28 day period between December 2010 and January 2011. Blood was collected from those dogs whose owners gave consent for venopuncture. This was the only selection criterion. No travel history was documented. Sampled dogs were presented for a variety of complaints, including lethargy, inappetance and vomiting, or for vaccination. Blood was obtained and 1 mL was placed into a 1.5 mL ethylenediaminetetraacetic acid (EDTA) tube and allowed to clot for serum separation. Ticks, if present, were removed from the dogs that were sampled and placed in isopropanol. Ticks, sera and blood were refrigerated until transport to the United States of America and then stored at -80 °C until assayed.

Sera were evaluated for *Dirofilaria immitis* antigen and antibodies against *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, and *Ehrlichia canis* (SNAP® 4DX, IDEXX Laboratories, Portland ME). Ticks were identified by microscopy and total deoxyribonucleic acid (DNA) was extracted from the ticks and blood using QIAGEN® DNeasy Blood and Tissue Kits. Polymerase chain reaction assays designed to amplify the DNA of *Ehrlichia* spp. or *Anaplasma* spp. or *Neorickettsia* spp. or *Wolbachia* (multi-species primers), *Babesia* spp., *Bartonella* spp., *Rickettsia* spp., *Hepatozoon* spp. and the haemoplasmas were performed on total DNA extracted from EDTA blood and the ticks as previously described (Allen *et al.* 2008; Birkenheuer, Levy & Breitschwerdt 2003; Jensen *et al.* 2000, 2001; Lappin *et al.* 2004). Positive amplicons were sequenced (Macromolecular Resources, Colorado State University, Fort Collins, Colorado) and compared with sequences reported in the National Center for Biotechnology Information database (GenBank).

Ticks from individual dogs ($n = 8$; all *Rhipicephalus* spp.) as well as sera and blood ($n = 17$) were collected. Ten dogs (58.8%) and three (38.0%) ticks showed evidence of carrying blood-borne organisms. Positive Polymerase chain reaction (PCR) and 4DX results are presented in Table 1.

The commercial laboratory kit used to detect *A. phagocytophilum* antibodies is known to cross-react with *Anaplasma platys* (IDEXX Laboratories 2008). *Anaplasma phagocytophilum* is commonly transmitted by *Ixodes* spp. ticks, which were not detected in this study, whilst *A. platys* is suspected to most likely be carried by *Rhipicephalus sanguineus*. The presence of *E. canis*, also vectored by *R. sanguineus*, was documented by PCR and serology. Thus, the two dogs that were seropositive for *A. phagocytophilum* antibodies but PCR negative may have been infected with alternative *Anaplasma* species such as *A. platys*, which was isolated by PCR and confirmed by sequencing

TABLE 1: Polymerase chain reaction and 4DX positive result for collected blood, serum and tick samples.

Specimens	PCR positives	4DX positives
Dog 1	<i>Wolbachia</i>	<i>Dirofilaria immitis</i> , <i>Ehrlichia</i> spp.
Dog 2	NPF	<i>Anaplasma</i> spp.
Dog 3	<i>Hepatozoon canis</i>	<i>Ehrlichia</i> spp.
Dog 4	<i>Ehrlichia canis</i>	<i>Ehrlichia</i> spp.
Dog 5	<i>Anaplasma platys</i>	NPF
Dog 6	<i>Ehrlichia canis</i>	NPF
Dog 7	<i>Hepatozoon canis</i>	NPF
Dog 8	<i>Hepatozoon canis</i>	<i>Anaplasma</i> spp.
Dog 9	NPF	<i>Anaplasma</i> spp.
Dog 10	<i>Hepatozoon canis</i>	NPF
Tick 1	<i>Bartonella henselae</i>	NPF
Tick 2	<i>Hepatozoon canis</i>	NPF
Tick 3	<i>Bartonella henselae</i>	NPF

PCR, Polymerase chain reaction; NPF, no positives found.

in a different animal. Discrepancies between serology and PCR results could potentially be attributed to an acute infection or effects of storage.

Hepatozoon canis is also thought to be carried predominantly by *Rhipicephalus* spp. ticks. In the present study, the identity amongst the *H. canis* sequences from the dogs and reference sequences from GenBank ranged from 99.2% to 100% related to *H. canis* sequences. Sequences were 91.7%–92.5% identical to *Hepatozoon americanum* sequences, but it is more likely that the Ghanaian isolates are *H. canis* given that *H. americanum* has never been detected outside of the USA, has a low level of parasitaemia (making it difficult to amplify by PCR) and is vectored by *Amblyomma maculatum*, which has not been documented in this region of Africa.

DNA of *Babesia* spp., *Rickettsia* spp., the haemoplasmas, *Neorickettsia* spp. and *Bartonella* spp. were not amplified from the blood of the dogs tested in this study and no dogs were seropositive for *B. burgdorferi*. Since *Babesia canis vogeli*, *Mycoplasma haemocanis* and possibly *Babesia gibsoni* are associated with *Rhipicephalus* spp. ticks and have all been documented in Africa, the negative findings for these organisms probably relates to the sample size. These organisms are not always associated with clinical disease, but could pose a threat in immunocompromised or young animals.

Overall, the present study provides evidence that a diverse array of vector-borne agents is present in client-owned dogs of Kumasi, Ghana. Also, the evidence of *Bartonella henselae* being carried in ticks indicates a potential zoonotic risk in the region. Further studies are needed to sample larger canine populations and also to compare regions of western Africa. Documentation of clinical disease correlating to detection of

infection will also be important to determine the impact these agents have on dog populations in the region.

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Competing interests

The authors declare that they have no financial or personal relationship(s) which may have inappropriately influenced them in writing this article.

Authors' contributions

L.L.C. (University of Georgia) conceived the study, collected the samples, carried out the immunoassays and the majority of the polymerase chain reactions, and drafted the manuscript. L.R.B. (Colorado State University) identified the ticks. K.A. (Oklahoma State University) and S.E.L. (Oklahoma State University) carried out *Hepatozoon* spp. polymerase chain reactions and sequence alignment. M.R.L. (Colorado State University) helped conceive the study, coordinated laboratory and collaboration efforts and helped draft the manuscript. All authors read and approved the final manuscript.

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