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Vector-borne pathogens in dogs from Costa Rica: First molecular description of *Babesia vogeli* and *Hepatozoon canis* infections with a high prevalence of monocytic ehrlichiosis and the manifestations of co-infection

Alicia Rojas[a,*], Diana Rojas[a], Víctor Montenegro[b], Ricardo Gutiérrez[c], Daniel Yasur-Landau[c], Gad Baneth[c]

[a] *Departamento de Parasitología, Centro de Investigación en Enfermedades Tropicales, Facultad de Microbiología, Universidad de Costa Rica, P.O. Box 11501-2060, San José, Costa Rica*
[b] *Departamento de Parasitología, Escuela de Medicina Veterinaria, Universidad Nacional, P.O. Box 86-3000, Heredia, Costa Rica*
[c] *Koret School of Veterinary Medicine, Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76100, Israel*

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ABSTRACT

Infection with canine vector-borne pathogens was evaluated in dogs from four different regions of Costa Rica by PCR. Demographic data, clinical signs, packed cell volume values, and the presence of tick infestation were recorded for each dog. Forty seven percent (69/146) of the dogs were infected with at least one pathogen and 12% were co-infected with two pathogens. *Ehrlichia canis* was detected in 34%, *Anaplasma platys* in 10%, *Babesia vogeli* in 8%, and *Hepatozoon canis* in 7.5% of the blood samples. No infection was detected with *Leishmania* spp. in blood, skin scrapings or conjunctival swabs. Thirty percent of the dogs presented at least one clinical sign compatible with vector-borne disease, and of those, 66% were infected with a pathogen. Subclinical infections were determined in 58% of the infected dogs including 82% (9/11), 58% (29/50), 42% (5/12) and 36% (5/14) of the dogs with *H. canis*, *E. canis*, *B. vogeli* and *A. platys* infections, respectively. A distinct relationship was found between infection and anemia. The mean PCV values were 34.4% in dogs with no infection, 31.5% in those who had a single infection and 23% in those with co-infection. Co-infected dogs had significantly lower PCV values compared to non-infected and single-infected dogs ($p < 0.0001$). Thirty five percent (51/146) of the dogs were infested with ticks, 82% of them were infested with *Rhipicephalus sanguineus* sensu lato and 18% with *Amblyomma ovale*. Dogs infected with *A. platys*, *B. vogeli*, or *E. canis* were significantly associated with *R. sanguineus* s.l. infestation ($p < 0.029$).

This is the first description of infections with *B. vogeli* and *H. canis* in Costa Rica as well as in Central America. The results of this study indicate that multiple vector-borne pathogens responsible for severe diseases infect dogs in Costa Rica and therefore, increased owner and veterinarian awareness are needed. Moreover, prevention of tick infestation is recommended to decrease the threat of these diseases to the canine population.

1. Introduction

Vector-borne diseases are infections caused by viruses, bacteria, protozoa and helminths transmitted by arthropods such as mosquitoes, sandflies, ticks and fleas (Otranto and Dantas-Torres, 2010). Some of the vector-borne diseases are considered as emergent or re-emergent, having

* Corresponding author at: Departamento de Parasitología, Centro de Investigación en Enfermedades Tropicales, Facultad de Microbiología, Universidad de Costa Rica, P.O. Box 11501-2060, San José, Costa Rica. Tel.: +506 25118645.
E-mail addresses: anaalicia.rojas@ucr.ac.cr, alira.2212@gmail.com (A. Rojas).

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a major impact on animal and human health, as well as an economic burden (Harrus and Baneth, 2005; Yuasa et al., 2012). Dogs are affected by several of these pathogens, causing diseases such as leishmaniasis, monocytic ehrlichiosis, anaplasmosis, babesiosis, and hepatozoonosis (Otranto et al., 2009).

Vector-borne infections represent a diagnostic challenge as they usually cause non-specific signs and clinical manifestations which may vary according to the pathogen involved, parasitemia or bacteremia level, immune status and age of the dog (Baneth et al., 2012). The most common clinical findings are fever, lymphadenomegaly, pale mucous membranes, lethargy, scleral injection, anemia and petechiae. However, many affected dogs remain subclinically infected, complicating the therapeutic intervention and the control of the disease (Otranto et al., 2009). Moreover, subclinically-infected animals can play a role as reservoirs of pathogens.

Epidemiological studies are essential to describe the trends of infection with a particular pathogen and its vectors locally and regionally. Knowledge on vector-borne infections at the local level allows veterinary practitioners to recognize the pathogens that can affect their patients facilitating a prompt diagnosis and treatment (Baneth et al., 2012). Serological techniques have limited sensitivity and specificity which decrease their usefulness, however, molecular techniques allow the detection of pathogen DNA with high accuracy. Hence, molecular methods have become an excellent tool for epidemiological surveys worldwide (Tavares et al., 2011). Despite that, in Costa Rica, as well as in other countries of Central America, the status of dog infection with many canine pathogens is still vague. To date, no information has been available on infection with *Babesia*, *Hepatozoon* or *Leishmania* spp. in dogs in this country; and partial data on *Ehrlichia canis* and *Anaplasma platys* has been obtained only from a limited area of Costa Rica (Abrego et al., 2009; Romero et al., 2011). Furthermore, it is important to study the relationship between those infections, the dog's clinical status and presence of ectoparasite vectors, in order to have a wider epidemiological perspective.

The purpose of this study was to evaluate infection with vector-borne pathogens of dogs in four different regions from Costa Rica, and to associate the potential infection-status with other co-infecting pathogens, tick infestation, clinical manifestations and hematological findings.

2. Materials and methods

2.1. Animals and samples

Sampling of dogs was carried out in the regions of San Ramón, Alajuela (10°05′14.28″N 84°28′13.25″O), Kéköldi, Limón (9°38′17.62″N 82°47′48.53″O), Liberia, Guanacaste (10°38′57.56″N 85°26′00.19″O) and Chomes, Puntarenas (10°3′56.16″N 84°57′47.38″O) (Fig. 1), in Costa Rica during the rainy season (July, August and September) of 2012. A questionnaire was filled for each dog with information on sex, age, and results of physical examination. Blood was drawn by puncture of the cephalic vein and collected in EDTA tubes. A conjunctival sterile-swab was taken from each eye and placed in a sterile microcentrifuge tube. A skin scraping of the dog's left ear was taken with a sterile blade and kept in a sterile tube. The samples were transported at 4 °C to the laboratory and stored at −20 °C until further analysis. Additionally, presence of tick infestation on dogs was assessed by visual inspection and combing, and tick specimens were collected and kept in sterile tubes. Taxonomic identification of ticks was determined by observation of morphological characteristics according to the guide of Barros-Battesti et al. (2006).

The packed cell volume (PCV) was measured from the EDTA blood samples by glass capillary centrifugation. The dogs sampled were divided into three groups according to PCV values: group 1 (7–24%), group 2 (25–34%) and group 3 (35–50%). Dogs in group 3 were the only ones with a normal PCV which ranges between 35 and 55%.

The project was approved by the Inter-Institutional Committee for the Care and Use of Animals (CICUA), Universidad de Costa Rica.

2.2. DNA extraction

DNA from EDTA blood samples and skin scrapes was extracted with commercial kits (Illustra Blood Genomic Prep Mini Spin Kit and Illustra Tissue and Cells MiniSpin Prep, GE Healthcare, Buckinghamshire, United Kingdom), following the manufacturer recommendations. DNA from conjunctival swabs was extracted with a phenol:chloroform:isoamylic alcohol protocol as described previously (Strauss-Ayali et al., 2004).

2.3. Conventional and real-time PCR for Ehrlichia, Anaplasma, Hepatozoon, Babesia, and Leishmania spp.

Blood samples were screened for the presence of *Ehrlichia*, *Anaplasma*, *Babesia*, and *Hepatozoon* spp. by conventional PCR. PCR was performed using the Syntezza PCR-Ready™ High Specificity (Syntezza Bioscience, Israel) kit tubes with 20 μl of PCR grade water (Sigma, St. Louis, United States), 1 μl of 500 nM of each primer, and 3 μl of the respective DNA. For *Hepatozoon* spp. detection, primers HEP-F and HEP-R (Inokuma et al., 2002) which amplify a fragment of approximately 666 bp of the *18S* gene of ribosomal DNA were employed, following conditions described by Otranto et al. (2011). The primers Piroplasmid-F and Piroplasmid-R were employed to detect a 400 bp fragment of the *18S* ribosomal DNA of piroplasmid such as *Babesia* and *Theileria* spp. as described previously (Tabar et al., 2008). Finally, primers EHR-16SD and EHR-16SR (Parola et al., 2000) were used to amplify a 345 bp fragment of the *16S* gene of *Ehrlichia* and *Anaplasma* spp. and the reaction protocol was modified from Parola et al. (2000) as follows: 95 °C for 5 min, 45 cycles of 94 °C for 30 s, 59 °C for 30 s and 72 °C for 60 s; and a final elongation at 72 °C for 5 min. All amplicons were visualized in ethidium-bromide stained 2% agarose gels.

Specific-pathogen real-time assays were performed for the detection of *E. canis*, *Babesia vogeli*, and *Leishmania* spp. in blood samples. In addition, *Leishmania* spp. DNA was further screened in the conjunctival swabs and skin scrapes. Samples for real time PCRs were prepared in a final