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Research paper

Prevalence of vector-borne pathogens in dogs from Haiti

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ABSTRACT

Canine vector-borne pathogens are common on some Caribbean islands, but survey data in Haiti are lacking. To determine the prevalence of selected vector-borne pathogens in dogs from Haiti, we tested blood samples collected from 210 owned dogs, 28 (13.3%) of which were infested with *Rhipicephalus sanguineus* ticks at the time of blood collection. No other tick species were identified on these dogs. A commercially available ELISA identified antibodies to *Ehrlichia* spp. in 69 (32.9%), antibodies to *Anaplasma* spp. in 37 (17.6%), and antigen of *Dirofilaria immitis* in 55 (26.2%); antibodies to *Borrelia burgdorferi* were not detected in any sample. Molecular assays of whole blood from 207 of the dogs confirmed infection with *Ehrlichia canis* (15; 7.2%), *Anaplasma platys* (13; 6.3%), *D. immitis* (46; 22.2%), *Wolbachia* spp. (45; 21.7%), *Babesia vogeli* (16; 7.7%), and *Hepatozoon canis* (40; 19.3%), but *Anaplasma phagocytophilum*, *Babesia canis*, *Babesia rossi*, *Babesia gibsoni*, *Ehrlichia chaffeensis*, *Ehrlichia ewingii*, or *Hepatozoon americanum* were not detected. Co-infection with two or more vector-borne pathogens was detected by serology in 42 (20.0%) dogs and by molecular assays in 22 (10.6%) dogs; one dog was co-infected with *B. vogeli* and *E. canis* as detected by PCR with *D. immitis* detected by serology (antigen). Overall, evidence of past or current infection with at least one vector-borne pathogen was identified in 142/210 (67.6%) dogs in this study, underscoring the common nature of these pathogens, some of which are zoonotic, in Haiti.

1. Introduction

Vector-borne pathogens cause significant morbidity and mortality in dogs and people worldwide. Previously reported canine vector-borne infections in the Caribbean include *Acanthocheilonema reconditum*, *Anaplasma phagocytophilum*, *Anaplasma platys*, *Babesia vogeli*, *Babesia gibsoni*, *Bartonella vinsonii* subsp. *berkhoffii*, *Dirofilaria immitis*, *Ehrlichia canis*, and *Hepatozoon canis* (Bool and Sutmoller, 1957; Huxsoll et al., 1970; Villanueva and Rodriguez-Perez, 1993; Saleh et al., 1988; Duran-Struuck et al., 2005; Georges et al., 2008; Hoff et al., 2008; Yabsley et al., 2008; Asgarali et al., 2012; Kelly et al., 2013; Loftis et al., 2013; Qurollo et al., 2014). Several of these agents are zoonotic (Dashiell, 1961; Roux et al., 2000; Perez et al., 2006; Bakken and Dumler, 2008; Maggi et al., 2013).

The brown dog tick, *Rhipicephalus sanguineus*, is the most common tick infesting dogs in Grenada, Trinidad, St. Kitts, and Martinique and is responsible for transmission of the tick-borne agents commonly seen in the region (Groves et al., 1975; Simpson et al., 1991; L'Hostis et al., 1998; Inokuma et al., 2000; Uilenberg et al., 1989; Anderson et al., 1979; Baneth et al., 2001; Yabsley et al., 2008; Asgarali et al., 2012; Kelly et al., 2013; Loftis et al., 2013). *E. canis* is the most commonly reported tick-borne pathogen, with canine seroprevalence of 24.1%–47.6% and molecular detection in 14.1%–24.7% of dogs (Georges et al., 2008; Hoff et al., 2008; Yabsley et al., 2008; Asgarali et al., 2012; Kelly et al., 2013; Loftis et al., 2013; Qurollo et al., 2014). Other *Ehrlichia* spp. have not been reported from dogs from the Caribbean to date. Similarly, antibodies to *Anaplasma* spp. have been reported from 10.8% to 24.1% of dogs from the region with DNA detected in 2.3%–19.2% of dogs (Georges et al., 2008; Yabsley et al., 2008; Kelly et al., 2013; Loftis et al., 2013; Qurollo et al., 2014).

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Protozoal tick-borne infections are commonly detected in dogs from Caribbean islands as well. *B. vogeli* has been reported in 3.2%–11.8% dogs from Aruba, Grenada, St. Kitts, Trinidad, and the Virgin Islands while *B. gibsoni* has been documented in 9.7%–9.9% dogs from St. Kitts (Bool and Sutmoller, 1957; Huxsoll et al., 1970; Georges et al., 2008; Yabsley et al., 2008; Kelly et al., 2013; Loftis et al., 2013). In addition, *H. canis* has been reported in 3.0% – 6.8% of dogs from various Caribbean islands (Bool and Sutmoller, 1957; Huxsoll et al., 1970; Yabsley et al., 2008; Kelly et al., 2013; Loftis et al., 2013).

Both *D. immitis* and *A. reconditum* have also been reported from dogs in the Caribbean. Microfilaria of both species have been reported from the blood of dogs in Cuba, Curacao, and Puerto Rico, with *A. reconditium* found in 2.1%–27.8% and *D. immitis* found in 6.7%–63.2% of dogs (Duménigo et al., 1988; Saleh et al., 1988; Kozek et al., 1995). Antigen of adult *D. immitis* has been reported from 17.3% to 35.8% of dogs in the Turks and Caicos and the Dominican Republic, and 53.4% of stray dogs from the Bahamas had adult *D. immitis* at necropsy (Grieve et al., 1986; Menda, 1989; Duran-Struuck et al., 2005; Hoff et al., 2008).

Here we report the results of a survey conducted to understand the prevalence of vector-borne agents infecting dogs in Haiti.

2. Materials and methods

2.1. Study participants

Stored anticoagulated (EDTA) whole blood samples and ethanol preserved ticks were available from 210 dogs that had been examined at government sponsored vaccination clinics throughout Haiti from February to April, 2013; locations included Artibonite Valley, Cap Haitien, Croix de Bouquets, Fon de Blan, Jacmel, Jacsonville, Leogane, Les Cayes, Marie Charles, Melon, Pignon, and Port au Prince. Blood samples were frozen after collection and initial testing (see Section 2.2) until used in this study. When samples were available from multiple dogs per household, a sample from only one dog from each household was included in the study. Age of each dog and presence of ticks, when noted, was obtained from the medical records. Ticks were identified to species by morphological characteristics (Keirans and Litwak, 1989).

2.2. Serologic testing

Whole blood samples (n = 210) had been screened at time of sample collection with a commercial lateral flow assay (SNAP® 4Dx® Plus, IDEXX Laboratories, Inc., Westbrook, Maine) and results were provided; the assay is designed to detect antigen of *D. immitis* and antibodies against *A. phagocytophilum*/*A. platys*, *Borrelia burgdorferi*, and *E. canis*/*E. ewingii* (Stillman et al., 2014).

2.3. DNA extraction, PCR testing, and sequencing

Of the 210 samples, 207 had sufficient volume to pursue further analysis via polymerase chain reaction (PCR) assays. Extraction of DNA from whole blood was completed using the Illustra™ blood genomic Prep Mini Spin Kit (GE Healthcare UK Limited, Buckinghamshire, United Kingdom) according to manufacturer's instructions using 200 μL anticoagulated blood and a final elution volume of 200 μL. Extractions of DNA and negative water controls were performed in a dedicated area of the lab. Water controls were included in each PCR described below.

A portion of the 18S rRNA gene of *Babesia* spp. and *Hepatozoon* spp. was amplified by nested PCR using external primers 3.1 and 5.1 and internal primers RLBH-F and RLBH-R (Gubbels et al., 1999; Yabsley et al., 2005). *Ehrlichia* spp., *Anaplasma* spp., and *Wolbachia* spp. 16S rDNA fragments were amplified by nested PCR using external primers (ECC/ECB) and then internal primers designed for detection of *E. canis* (HE3/ECA), *E. chaffeensis* (HE3/HE1), *E. ewingii* (HE3/EE72), and *A. phagocytophilum*/*A. platys*/*Wolbachia* spp. (GE9F/GA1UR) (Chen et al., 1994; Little et al., 1997). A subset of resultant amplicons were sequenced as described below. In addition, to confirm identity of *A. phagocytophilum*/*A. platys*/*Wolbachia* spp. (GE9F/GA1UR) amplicons, nucleic acid extracts from samples positive for that PCR were subjected to an additional PCR for a groESL heat-shock protein gene fragment (PLA-HS475F/PLA-HS1198R) (Chae et al., 2000; Inokuma et al., 2002; Cicuttin et al., 2014). A real-time PCR for detection of *Wolbachia* spp. was also performed as previously described (Turba et al., 2012).

A single-step PCR using DID-F and DID-R primers was used for detection of *D. immitis* and *A. reconditum* (Rishniw et al., 2006). All amplicons were visualized using standard agarose gel electrophoresis with ethidium bromide, purified using a commercial kit (Wizard PCR Preps, Promega Corporation, Madison, Wisconsin), and 10 amplicons (including all 4 amplicons with discordant *D. immitis* serologic or *Wolbachia* spp. real-time PCR results) were submitted for commercial sequencing (SimpleSeq™, Eurofins MWG Operon Inc., Huntsville, Alabama; and Molecular Core Facility at Oklahoma State University, Stillwater, Oklahoma). Resultant sequences were analyzed using the Basic Local Alignment Search Tool (BLAST) and compared with existing sequence data listed in the National Center for Biotechnology Information database.

2.4. Statistical analyses

Reported age groups (<2, 2–3.9, 4–6.9, and 7+ years) were compared to infection detected by serology with *Anaplasma* spp. antibodies, *Ehrlichia* spp. antibodies, and *D. immitis* antigen as well as infection detected by PCR for *A. platys, A. reconditum, B. vogeli, D. immitis, E. canis*, and *H. canis* using Chi-square tests with significance assigned at $p < 0.05$ (Daniel and Cross, 2013). Reported age was also compared to *Ehrlichia* spp. antibody status, *D. immitis* antigen and PCR status, and *A. reconditum* PCR status using a Mann-Whitney rank sum test with significance assigned at $p < 0.05$ (Daniel and Cross, 2013).

Infection with any tick-borne agent as detected by serology or PCR was compared to the presence of ticks at time of sample collection using Chi-square tests with significance assigned at $p < 0.05$ (Daniel and Cross, 2013).

3. Results

3.1. Study participants

Ages of dogs included in this study ranged from 6 months to 13 years (average age = 3.8 ± 2.6 years). Tick infestation was identified on 28/210 (13.3%) dogs. All ticks submitted for morphological identification (20 nymphs, 9 male, and 15 female) were *R. sanguineus* sensu lato.

3.2. Serologic results

In total, 109/210 (51.9%) dogs had detectable antibody and/or antigen for at least one vector-borne agent (Table 1). Antibodies to *Ehrlichia* spp. and *Anaplasma* spp. were detected in 69/210 (32.9%) and 37/210 (17.6%) of dogs, respectively, while antigen of *D. immitis* was detected in 55/210 (26.2%) of dogs; antibodies to *B. burgdorferi* were not detected in any dog. Past or current co-infections based on serologic results alone were detected in 42/210 (20.0%) of dogs. Sixteen dogs (7.6%) had evidence of *D. immitis* and *Ehrlichia* spp., 3 (1.4%) had evidence of *D. immitis* and *Anaplasma* spp., 13 (6.2%) had