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Molecular detection of *Babesia canis vogeli* and *Hepatozoon canis* in dogs in the department of Magdalena (Colombia)

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ABSTRACT

The canine population in the cities of Ciénaga and Santa Marta has been estimated at 54,953 based on individual dogs with owners. Due to the role that dogs play in society, either as pets or as transmitters of zoonoses to humans, we conducted a study with 169 blood samples from dogs that visited two veterinary clinics in these locations between March and September of 2017. The objective of the study was to detect species of *Babesia* and *Hepatozoon canis* by amplifying the 18S gene using conventional polymerase chain reaction (PCRc). The presence of *Babesia* sp. and *Hepatozoon canis* was detected in 15 (8.87%) and 12 (7.10%) DNA samples, respectively. In addition, 7 (4.14%) cases of coinfection were recorded. The *Babesia* sp. sequences obtained corresponded to the *B. canis vogeli* subspecies. This both pathogens in the Colombian Caribbean region and cases of coinfection in Colombian dogs. Therefore, the national veterinary community is encouraged to consider the information presented here in their differential diagnoses associated with companion vector-borne diseases (CVBDs). This information will allow veterinary professionals to create control and prevention strategies to prevent the spread of these infections.

Keywords: PCR, Colombia, dogs, *Babesia canis vogeli*, *Hepatozoon canis.*

Detección molecular de *Babesia canis vogeli* y *Hepatozoon canis* en perros en el departamento del Magdalena (Colombia)

RESUMEN

La población canina en las ciudades de Ciénaga y Santa Marta se ha estimado en 54.953 individuos con propietarios. Debido al rol que desempeñan los perros en la sociedad, ya sea como animales de compañía o como transmisores de zoonosis al humano, se realizó un estudio con 169 muestras sanguíneas de perros que visitaron dos clínicas veterinarias en estas localidades entre marzo y septiembre del año 2017. El objetivo del estudió consistió en detectar especies de *Babesia* y *Hepatozoon canis* amplificando el gen 18S mediante reacción en cadena de la polimerasa convencional (PCR-c). La presencia de *Babesia* sp. y *Hepatozoon canis* se detectó en 15 (8,87%) y 12 (7,10%) muestras de ADN, respectivamente. Además, se registraron 7 (4,14%) casos de coinfección. Las secuencias obtenidas de *Babesia* sp. correspondieron a la subespecie *B. canis vogeli*. Se presentan ambos patógenos para la

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región Caribe colombiana y casos de coinfección en perros de Colombia. Por lo tanto, se exhorta a la comunidad veterinaria nacional a considerar la información presentada en sus diagnósticos diferenciales asociados a las enfermedades transmitidas por vectores de compañía (CVBDs). Esta información permitirá a los profesionales veterinarios crear estrategias de control y prevención para mitigar la propagación de estas infecciones.
Palabras clave: PCR, Colombia, perros, *Babesia canis vogeli*, *Hepatozoon canis.*

INTRODUCTION

Nearly half of the world's population is at risk of contracting vector-borne diseases (VBD); after mosquitoes, ticks are of great importance in public and veterinary health as vectors of pathogens (such as bacteria, helminths, protozoans, and viruses, among others) that affect animals and humans (WHO 2017). Pets, especially canines, play an important role in the propagation of VBDs to humans as they are hosts for these arthropods (Dantas-Torres and Otranto 2016).

In Santa Marta and Ciénaga (Magdalena), the canine population was estimated at 54,953 based on individuals with owners (Minsalud 2017). Canines are commonly parasitized by *Rhipicephalus sanguineus* (brown dog tick) (Baneth *et al.* 2001). This species, together with *Rhipicephalus microplus*, *Amblyomma ovale* and *Amblyomma cajennense*, have been reported as a vector species for *Babesia* and *Hepatozoon canis* (de Miranda *et al.* 2011; Ribeiro *et al.* 2017).

Babesia and *Hepatozoon* are two genera of hemoparasites protozoans transmitted by ticks (Homer *et al.* 2000; Baneth *et al.* 2003), high parasitemia of theses parasites in an individual produces babesiosis and hepatozoonosis, respectively (Homer *et al.* 2000; Baneth *et al.* 2001). Babesiosis is an emerging zoonosis affecting humans and animals, while hepatozoonosis is classified as an emerging infection only affecting animals (Florez *et al.* 2018; González-Ascanio and Vásquez-Franco 2018).

These hemoparasites have been reported in Africa (Lorusso *et al.* 2016; Harris *et al.* 2017), Asia (Adao *et al.* 2017), Australia (Greay *et al.* 2018), Europe (Ebani *et al.* 2015), North America (Birkenheuer *et al.* 2005; Little *et al.* 2009), Central America (Rojas *et al.* 2014) and South America (Eiras *et al.* 2007, 2008; Rey-Valeirón *et al.* 2007, 2012; de Almeida *et al.* 2010; Otranto *et al.* 2011 Da Silva *et al.* 2016; Vezzani *et al.* 2017). In Colombia, Vargas-Hernández *et al.* (2012a, 2012b) reported findings of 5/91 dogs positive for *Babesia* sp. and 29/91 individuals positive for *H. canis* using conventional polymerase chain reaction (PCRc). Additionally, Galván *et al.* (2018) recently reported 11/42 dogs positive for *Babesia canis vogeli* at the Colombian Caribbean region and Cala *et al.* (2018) recently found one Siberian Husky positive for *H. canis* at the city Cúcuta, using the same technique. These pathogens have also been reported in different locations of the country (Santander and Cauca) using blood smear techniques and antibody titers (Guerra *et al.* 2012; Florez *et al.* 2018).

Despite their importance in public (babesiosis) and veterinary health (babesiosis and hepatozoonosis) (Vargas-Hernandez *et al.* 2012b; Dantas-Torres and Otranto 2016), few studies in Colombia have been conducted on human babesiosis, with research being restricted mainly to livestock production systems (babesiosis) because of the economic importance of

this industry (Zapata 2012; Cortés *et al.* 2016). However, there have been reports of *Babesia* sp. infecting humans (a case coinfection with *Ehrlichia* sp.) in the Colombian Caribbean (Montes-Farah *et al.* 2012), in addition to one report in the department of Cordoba with serum IgG antibodies of *Babesia microti* through direct immunofluorescence (Buelvas *et al.* 2008).

Additionally, the department of Magdalena has climatic conditions that favor the proliferation of diseases transmitted by ticks (TBD) (Mutz 2009). Moreover, the recent report by Galván *et al.* (2018) on the presence *B. canis vogeli* in Caribbean region domestics dogs (in the department of Córdoba) and the fact that most of the ticks usually associated to dogs lack host specificity (Dick and Patterson 2007), denotes the necessity and relevance to perform research on these hemoparasites in domestic canines in order to understand their epidemiological roles and associated risks in the transmission and propagation of tick-borne diseases (Dantas-Torres and Otranto 2016). In the present study, we sought to detect *Babesia* and *H. canis* species by PCRc in blood samples from dogs that visited two veterinary clinics in two locations in the department of Magdalena (Colombia).

MATERIALS AND METHODS

All the procedures performed in this study were approved by the research ethics committee of the University of Magdalena. The animals in this research were treated with prior authorization from their owners.

Study area

For this work, samples were collected from urban areas in two localities from the department of Magdalena (Colombia), in Ciénaga (veterinary clinic Fincas y Mascotas) and Santa Marta (veterinary clinic Origen Animal), north of Colombia (Figure 1). These localities are characterized by a tropical dry forest, with a semi-arid climate and marked water deficit between December and March (dry season); for Santa Marta the precipitation regime is unimodal-bi-seasonal with an annual average rainfall of 608.8 mm, moreover, Ciénaga presents a bimodal-tetra-seasonal precipitation regime with an average rainfall of 716.8 mm per year (Rangel and Carvajal-Cogollo 2012).

Sample collection

Between March and September 2017, 191 blood samples were collected from dogs that visited the *Fincas y Mascotas* veterinary clinic in Ciénaga (34 samples) and the *Origen Animal* clinic in Santa Marta (157 samples) within the department of Magdalena (Colombian Caribbean). Both are in the urban area of each locality. Samples were taken by convenience, as patients arrived, with prior authorization of their owners, and without discriminating clinical status. One cubic centimeter (cc) of blood was drawn for each individual and stored in tubes with 500 µL ethylenediaminetetraacetic acid (EDTA). Tubes were labeled R (samples from Ciénaga) and RC (samples from Santa Marta). Data on origin, breed, sex and age were recorded. The samples were stored at -20 degrees Celsius (°C) for acid desoxiribonucleic (DNA) extraction (< 72 hours).

DNA extraction and gene amplification

One hundred microliters of blood were used for DNA extraction following the protocol of the MasterPure ™ extraction

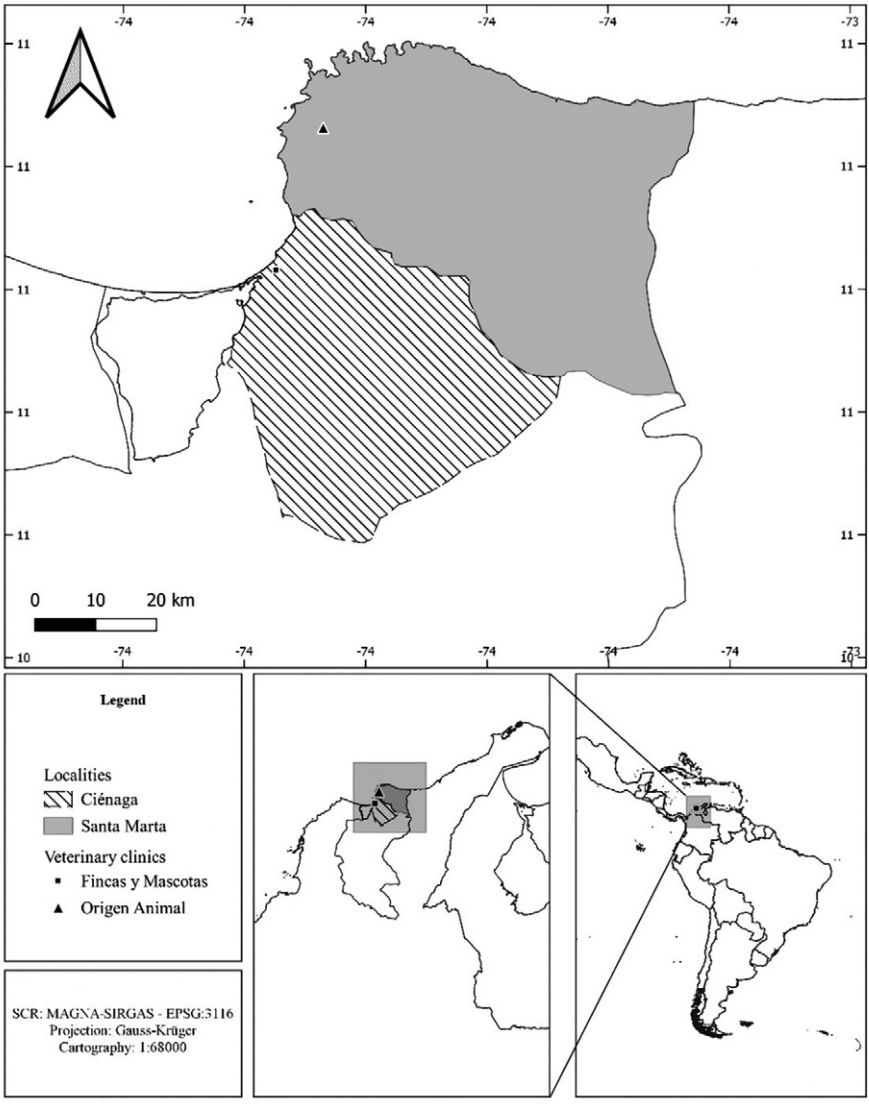

FIGURE 1. Study area: department of Magdalena (Colombia), urban area from the municipalities of Ciénaga and Santa Marta, veterinary clinics Fincas y Mascotas (Ciénaga) and Origen Animal (Santa Marta).

kit from Epicenter (USA); the resulting pellet was resuspended in 40 µL of double-distilled water (ddH_2O). The quality of the extraction was verified by electrophoresis in agarose gel with GelRed (Biotum) stain and HyperLadder™ 50 base pairs (bp) (5 X) was used as a molecular weight marker.

PCR was performed in an Eppendorf Mastercycler® Pro thermocycler using primers for *Babesia* species Ba103, 5´-CCA ATC

CTG ACA CAG GGA GGT AGT GAC A-3′ and Ba721, 5′-CCC CAG AAC CCA AAG ACT TTG ATT TCT CTC AAG-3′ (619 bp) and for *Hepatozoon canis* Hep001, 5′-CCT GGC TAT ACA TGA GCA AAA TCT CAA CTT-3′ and Hep737, 5′-CCAACTGTCCCTATCAATCAT-TAAAGC-3′ (737 bp) (Kledmanee *et al.* 2009) in a final volume of 25 microliter (µL) consisting of 2 µL of DNA, 5 µL of dNTP's [10 milimolar (mM)], 1 µL of MgCl (50 mM), 2.5 µL of PCR buffer (10 X), 1 µL of each primer [10 micromolar (µM)], 17.75 µL of ddH_2O and 0.25 µL of Taq polymerase (BIOLASE™ from Bioline; 5 U/µL). The amplification conditions were taken from Kledmanee *et al.* (2009) with an initial denaturation of 95°C for 15 minute (min) followed by 30 cycles of 94°C for 45 seconds (s), annealing at 65°C for 45 s, extension at 72°C for 1 min, and a final phase of 72°C for 7 min (final extension).

Sequence analyses and phylogenetics analyses

The sequences were verified using the BLASTn® (Basic Local Alignment Search Tool: https://blast.ncbi.nlm.nih.gov/Blast.cgi) and were edited with ProSeq® Version 3 (Filatov 2009). The resulting sequences in this study and a set of sequences downloaded from GenBank were aligned using the ClustalW algorithm (Thompson *et al.* 1994) in MEGA 7.0 (Kumar *et al.* 2018).

The best substitution models and partition schemes were established for each data set using Partition Finder (Lanfear *et al.* 2012) in accordance with the Bayesian information criterion (BIC) (Schwarz 1978). The GTR + G model was selected both for the 18-subunit ribosomal acid ribonucleic (18S rRNA) gene of *Babesia* sp. and *H. canis*. For phylogenetic reconstruction, the Bayesian inference (BI) and maximum likelihood (ML) methods were used, using MrBayes 3.2.2 (Ronquist *et al.* 2012) for the first and RAxML 8.0.24 (Stamatakis 2006) for the second method.

For the BI, two independent runs of 10^7 generations were performed, sampling the trees every 1000 generations and excluding the initial 25% of trees as burnin. Tracer v1.7.1 was used to validate the convergence of the MCMC chain (Rambaut *et al.* 2018). Bayesian posterior probabilities (BPPs) were used to estimate the statistical support on internal nodes; considering values ≥0.70 as strong statistical support (Huelsenbeck and Ronquist 2001). The ML analyses were performed using heuristic search, with evaluation the robustness of the inferred tree using the bootstrap (BP) algorithm of RAxML with 1000 replicates. Values of BP > 70% were considered statistically supported (Hillis and Bull 1993).

Statistical analyses

To determine the infection frequency (FI) of *Babesia* sp. and *H. canis* in the samples examined, we used the Epidat V 4.2 software, using a 95% confidence interval (CI). The Pearson Chi-squared test was used to establish statistically significant differences between the positive cases and the datasets (origin, bread, sex and age) compiled during sampling using SPSS software V 20.0. P-values < 0.05 represented statistically significant differences. Age was organized in three age groups: young dogs (< 2 years), adult dogs (2-5 years) and older dogs (> 5 years), according to the vet recommendations.

RESULTS

From a total of 191 samples collected, satisfactory DNA extractions were achieved for

TABLE 1. Demographic data between breed and sex.

BREED	N	SEX (N *F/*M)	POSITIVE		
			BABESIA SP. (N= 15/169)	H. CANIS (N= 12/169)	CO-INFECTION (N= 7/169)
Basset Hound	1	M	-	-	-
Beagle	4	2F/2M	1	-	-
Boxer	2	H	-	-	-
French Bulldog	2	H	-	-	-
English Bulldog	2	1F/1M	-	-	-
Bullterrier	1	M	-	-	-
Chow-Chow	1	H	1	-	-
Cocker Spaniel	4	2F/2M	1	-	-
Doberman	2	M	-	-	1
French Poodle	21	10F/11M	2	5	2
Golden Retriever	6	3F/3M	-	-	1
Great Dane	1	M	-	1	-
Jack Russell Terrier	3	1F/2M	-	-	-
Labrador	9	3F/6M	1	-	-
Maltese	1	H	-	-	-
Mixed breed	59	26F/33M	3	3	2
German Shepard	2	1F/1M	2	-	-
Pinscher	11	7F/4M	-	-	1
Pit Bull	6	3F/3M	2	-	-
Pug	5	3F/2M	-	-	-
Rottweiler	1	H	-	-	-
Schnauzer	13	6F/7M	1	3	-
Shih Tzu	8	2F/6M	1	-	-
Siberian Husky	2	1F/1M	-	-	-
Yorkshire Terrier	2	M	-	-	-
Sex					
Female	78		7	4	1
Male	91		8	8	6

TABLE 2. Chi-squared tests of breed, sex, origin and age versus positive sample for *Babesia* sp. [B (+)], *H. canis* [H (+)] and coinfected (*Babesia* sp. and *H. canis*) [BH (+)] individuals. * *p*-value < 0.05.

		H (+)		B (+)		BH (+)	
	Degrees of freedom	**Value**	***p*-value**	**Value**	***p*-value**	**Value**	***p*-value**
Breed	24	35	0.068	27.378	0.287	23.333	0.5
sex	1	3.39	0.066	0.975	0.323	2.984	0.084
Origin	1	5.391	0.02*	4.153	0.042*	1.839	0.175
Age	2	3.932	0.14	0.961	0.619	0.879	0.644

169 samples (87.56%), with 34 samples corresponding to the *Fincas y Mascotas* veterinary clinic in Ciénaga and 135 samples from the *Origen Animal* clinic in Santa Marta. 15/169 samples were found to be positive for *Babesia* sp. (8.87%; IC = 5,053-14,217) and 12/169 (7.10%; IC = 3,723-12,075) samples were found to be positive for *H. canis*.

In addition, 7/169 (4.14%; IC = 1,681-8,348) individuals were found to be coinfected with *Babesia* sp. and *H. canis* (Table 1). Individuals positive for *Babesia* sp. were aged between 1-8 years, while the age range for *H. canis* was between 0.4-9 years and the age, range for coinfected animals was between 0.9-10 years.

The FI of *Babesia* species in samples from Santa Marta was estimated at FI= 5.15% (7/135) of dogs aged between 1 and 8 years. For *H. canis,* the FI was 8.82% (12/135) of individuals between 0.4 and 9 years. For samples collected in Ciénaga, the FI of *Babesia* species was 23.53% (8/34) of dogs aged between 2-8 years, while the FI of *H. canis* was zero.

The Pearson Chi-squared tests showed no significant differences between the variables of breed, sex and age for any positive cases of *Babesia* sp., *H. canis* or coinfections. However, significant differences were found between the origin and positive cases for both pathogens (Table 2).

Molecular characterization

Four (4) positive samples were sequenced for each pathogen analyzed. The 18S rRNA sequences obtained from dogs infected with *Babesia* species showed 99% and 100% identity with *B. canis vogeli* (LC331058.1 and MG586234.1 respectively). For *H. canis*, the samples showed 99% identity with *H. canis* clone 9618 (KC138532.2) and *H. canis* strain SK-144 (JX112783.1). Only one sequence showed a similarity of 97% with *H. canis* isolate M2 (MF588668.1). The analysis of BI and ML with the 18S gene suggests a close relationship between the sequenced samples of *Babesia* sp. and those of *B. canis vogeli* downloaded from GenBank (Figure 2). On the other hand, the phylogenetic analyses for *H. canis* confirm the specificity of the primers used, as our samples grouped with other *H. canis* sequences (Figure 3).

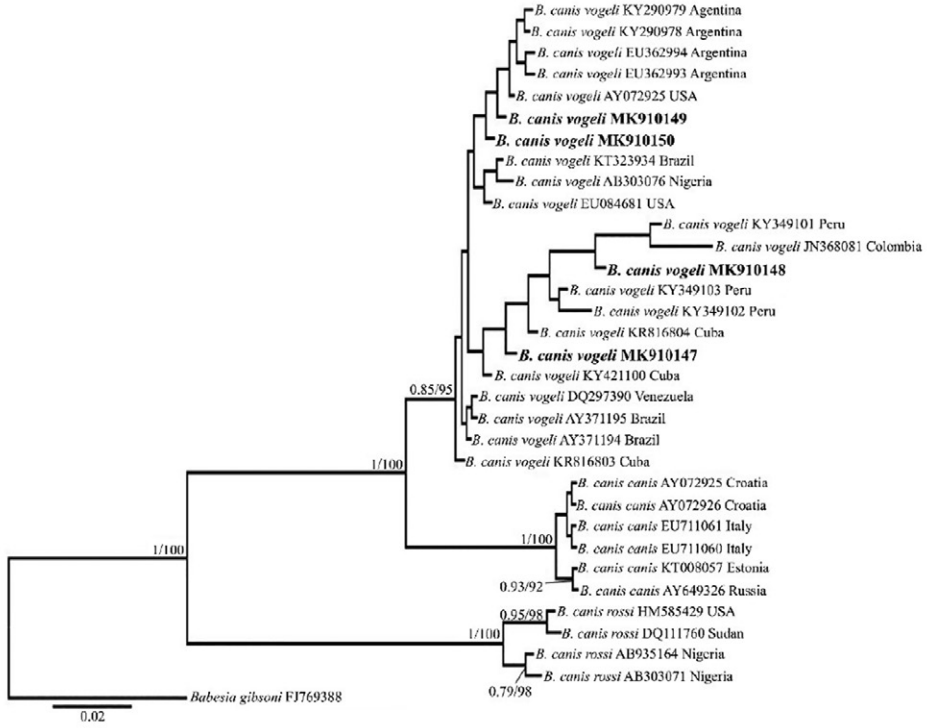

FIGURE 2. Topology of the tree obtained in the Bayesian and max likelihood phylogenetic analyses including the sequences obtained in this study and the *Babesia canis* 18S gene sequence downloaded from GenBank. The numbers correspond to the posterior probability/bootstrap values.

Nucleotide sequence data reported in this paper are available in the GenBank™ database under the accession numbers: MK910141, MK910142, MK910143, MK910144, MK910147, MK910148, MK910149 and MK910150.

DISCUSSION

This is the first report, to our knowledge, of cases of infection and coinfection by the pathogens *Babesia* sp. and *H. canis* in blood samples from dogs in the department of Magdalena. The first report by *H. canis* and second report of *B. canis vogeli* on domestic dogs at the Colombian Caribbean region, using conventional PCR. Also, this is the first report, to our knowledge, of co-infection of *Babesia* sp. and *H. canis* in domestic dogs from Colombia. This type of dual infection has been reported previously in domestics dogs of city Cuiabá and Minas Gerais (Brazil) (Mundim *et al.* 2008; Spolidorio *et al.* 2011).

B. canis and *H. canis* have been reported in dogs from almost all sites with records of *R. sanguineus* (Rojas *et al.* 2014). Although *A. ovale* has also been documented as a vector of *H. canis* (Forlano *et al.* 2005; Rubini *et al.* 2009), and *Amblyomma cajennense* sensu lato (s. l.) has been proposed as a vector for this species (O'Dwyer *et al.* 2001). Therefore, the presence of these hemoparasites in the Caribbean region is expected, considering that ticks of the fam-

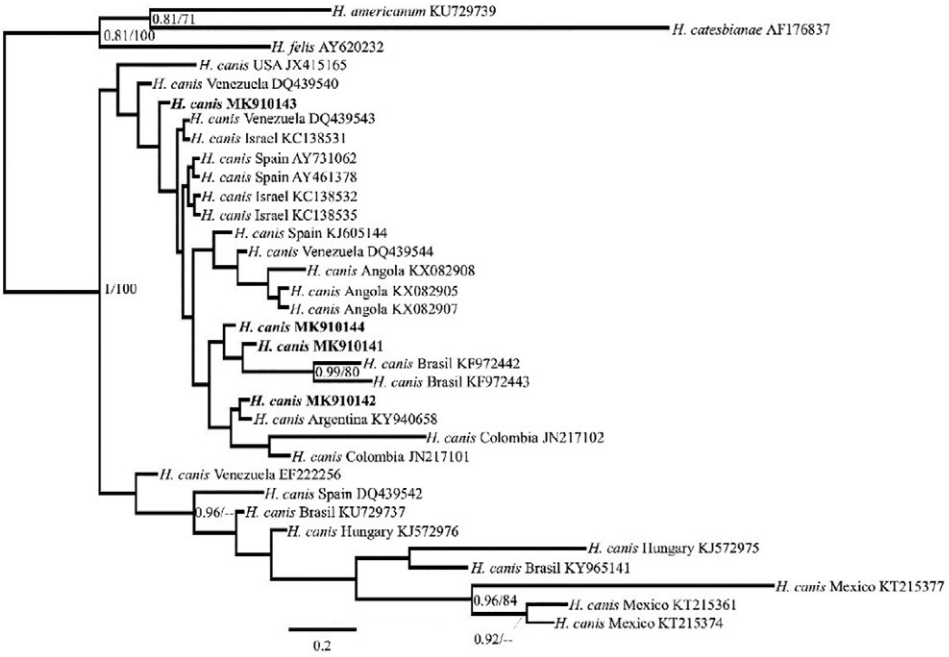

FIGURE 3. Tree topology of the Bayesian and maximum likelihood phylogenetic analyses including the sequences obtained in this study and the *Hepatozoon canis* 18S gene sequence downloaded from GenBank. The numbers correspond to the posterior probability/bootstrap values.

ily Ixodidae (*A. ovale*, *R. microplus* and *R. sanguineus*) have been found parasitizing dogs (Paternina *et al.* 2009; de Miranda *et al.* 2011), in addition to a report of *A. cajennense* s. l. on horses from the Tayrona National Natural Park (PNNT) (Santodomingo *et al.* 2019). However, *H. canis* has also been reported in areas free of *R. sanguineus* (Mitkova *et al.* 2017), which could be explained by the vertical transmission of *H. canis* reported by Murata *et al.* (1993).

With respect to cases of infection, a greater number of *H. canis* positive cases were found in males (8/12) than that in females (4/12), while for *Babesia,* the difference was greater only by one individual male (8/15 males and 7/15 females). Additionally, the cases of co-infection were greater for males (6/7) than for females (1/7). These results can be explained due to the preponderance of male dogs in the samples, or by an increased roaming behavior on these (Veneziano *et al.* 2018). However, in the present study, no significant correlations were found between positive samples and sex, which is in agreement with that reported by Aktas *et al.* (2015) and Ćoralić *et al.* (2018); denoting that these hemoparasites do not differentiate between sexes (O'Dwyer 2001; Mellanby *et al.* 2011).

The positive cases according to breed in our study were ranked as follows: the highest rate of infection by *Babesia* was found in mixed-breeds (20%), followed by

French Poodles, German Shepherds and Pit Bulls (all 13%) and other breeds (6,6%). For *H. canis*, the FI was highest in French Poodles (41.6%), followed by mixed-breeds and Schnauzers (both 25%), and Great Danes (8.3%). Rey-Valeiron *et al.* (2012) and Vezzani *et al.* (2017) documented a higher prevalence of *H. canis* in mixed-breed dogs in Venezuela and Argentina. Mestizo an French Poodles dogs probably presented a higher percentage of infection due the predominance of these races in our sampling, as these races are popular households in the region. Also, it is possible that these dogs are left in complete freedom by their owners, generating a greater encounter factor with the vector and increasing the probability of infection, unlike expensive breed dogs that generally are kept under strict supervision and care of their owners.

On the other hand, for the present study, the FI of individuals positive for *Babesia* was 11.48% (76 samples) in dogs < 2 years of age, 15.51% (58 samples) in dogs 2-5 years of age, and 12% (25 samples) in dogs > 5 years of age. While for *H. canis* was 10.76% (65 samples) in dogs < 2 years of age, 11.53% (52 samples) in dogs 2-5 years of age, and 38% (18 samples) in dogs > 5 years of age. These outcomes could suggest a greater susceptibility in adult dogs (> 2-5 years) to Babesia and in older adult dogs (> 5 years) to *H. canis*. The above, could be explained by the immunologic status of the host (which changes by age) or an increased exposure to the tick vectors in adult and older dogs, having more opportunities and more time to become infected (Farkas *et al.* 2014; Aktas *et al.* 2015; Tsegay *et al.* 2016). However, our study did not find statistically significant correlations between age and positive cases for *Babesia* or *H. canis*. Moreover, there is evidence of a higher susceptibility to *Babesia* and *H. canis* in puppies and young dogs, due to an immature immune system (Baneth and Weigler 1997; Ivanov and Tsachev 2008).

The phylogenetic analyses grouped our *Babesia* sp. sequences with the sequences of *B. canis vogeli* (Fig. 2), which is the only subspecies reported in domestic dogs to date in Colombia (Vargas-Hernández *et al.* 2012a; Galván *et al.* 2018) and some Latin American countries (Jarquín-Díaz *et al.* 2016; Da Silva *et al.* 2016). This subspecies has been widely reported in canines worldwide (Adao *et al.* 2017; Ribeiro *et al.* 2017), mainly in tropical and subtropical areas (Southern United States, Southern Cone of America, Southern Africa, Souther Europe, Middle East, Central America and the Caribbean) (Shaw *et al.* 2001).

This phenomenon could be explained by the cosmopolitan distribution of its main vector (*R. sanguineus*) (Dantas-Torres 2010). Therefore, the other samples positive for *Babesia* sp. most likely correspond to this subspecies, given that the other two *Babesia* sp. reported in dogs (*B. canis canis* and *B. canis rossi*) are transmitted by *Dermacentor reticulatus* in Europe and *Haemaphysalis leachi* in southern Africa, respectively (Carret *et al.* 1999; Földvári *et al.* 2005), and none of these vectors have been reported in Colombia (Rivera-Páez *et al.* 2018).

On the other hand, our *H. canis* sequences grouped into one clade with other *H. canis* sequences (Figure 3). However, the statistical support was not strong enough to make inferences about the phylogenetic relationships among the different sequences obtained and included.

H. canis has been described as the causative agent of canine hepatozoonosis in Colombia (Vargas-Hernandez *et al.* 2012b). However, in Brazil, Criado-Fornelio *et al.* (2006) and André *et al.* (2010) reported a species similar to *Hepatozoon americanum*

in wild canids. While *H. americanum* had only been reported in northern America (Baneth 2011), Gomes *et al.* (2016) provided the first molecular evidence of this species infecting domestic dogs in Brazil. Therefore, perhaps other species of *Hepatozoon* sp. are parasitizing dogs in South America (de Miranda *et al.* 2011).

The FI of *Babesia* sp. in the present study was 8.82%, while the FI of *H. canis* was estimated at 7.10%. Although *H. canis* was not frequent in the Ciénaga samples (p-values = 0.02), *Babesia* sp. frequency was significantly higher (FI = 23.52%) than that in the Santa Marta locality (FI = 5.14%) in terms of FI (p-values = 0.042). Our results differ to those of Vargas-Hernandez *et al.* (2012a, 2012b), who reported higher prevalence values for *H. canis* (31.8%; 29/91 canines) than for *B. canis vogeli* (5.5%; 5/91 dogs) and differ to those Galván *et al*. (2018), who reported higher FI of *B. canis vogeli* (26%; 11/42 dogs). The high frequency of *Babesia* infection in this latest work compared to ours was expected, as their study included dogs that presented clinical signs related to tick-borne diseases. The FI of *Babesia* sp., which was slightly higher (8.87%) than that in the study by Vargas-Hernández *et al.* (2012a) (5.5%), could suggest a high presence of this species for the Colombian Caribbean region, which would be supported by the findings of Galván *et al*. (2018).

The low prevalence of *H. canis* in our study could be explained in terms of the sampling, the characteristics of the study population, social factors (raising and caring for pets), the immune status of the dogs, climatic conditions and geographic location, all of which are factors that influence the abundance and distribution of the vector ticks (de Miranda *et al.* 2014). Moreover, *H. canis* can remain with intermittent or low levels of parasitemia, making it difficult for detection, therefore, it would be recommended to implement nested-PCR or real-time PCR to increase chances of detection, as it is rare to find dogs with high levels of *Hepatozoon* infection (Gomes *et al*. 2016).

The findings of coinfections in the present study denote a problem that veterinarians may face when determining positive cases, not only because they complicate the diagnosis but also because coinfections make an individual more susceptible to infections by other hemoparasites (such as *Ehrlichia*, *Rickettsia*, *Anaplasma*, among others) (Rojas *et al*. 2014; Happi *et al*. 2018). The veterinary community is encouraged to consider the information presented here in their differential diagnoses associated with companion vector-borne diseases (CVBD).

CONCLUSIONS

The first pilot study for the detection and prevalence of *Babesia* and *Hepatozoon canis* species was conducted in dogs from two locations in the department of Magdalena (Colombia). The presence of *B. canis vogeli* and *H. canis* was corroborated by sequencing PCR products and making phylogenetic inferences from the blood samples of dogs examined from both locations. Our results are relevant for the future studies and monitoring of these pathogens in the Colombian Caribbean region and at the national territory.

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