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Characterization of *Anaplasma* spp. infection in dogs from Costa Rica

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ABSTRACT

A cross-sectional study combining serological and molecular techniques for detecting selected *Anaplasma* species was conducted between 2011 and 2012 in dogs and ticks sampled in all provinces of Costa Rica. Global *Anaplasma* spp. seroprevalence was 2.7% (11/408) by indirect immunofluorescence assay. The 16S rRNA PCR confirmed active *A. phagocytophilum* infection only in one dog (0.3%, 1/374); however, the same sample was negative to *groEL* PCR. Out of 122 *Rhipicephalus sanguineus* s.l. ticks analyzed, one (0.8%) was found positive to *A. phagocytophilum* 16S rRNA PCR but negative when tested by *groEL* PCR; this tick was collected from a seronegative and PCR negative dog. Both 16S rRNA sequences were 100% (510/510 bp) identical to *A. phagocytophilum* strains isolated in different countries from different hosts.

The presence of *A. platys* was established in four dogs (1%, 4/374) by both 16SrRNA and *groEL* PCR. Ticks collected from the same dogs tested negative by PCR. The 16S rRNA sequences were 100% identical to the corresponding sequences of *A. platys* strains isolated from dogs in Croatia and Brazil, however *groEL* sequences showed variable similarity levels (99–100%) with different strains of *A. platys* isolated in Chile, Japan and Thailand, pointing out the possible presence of different variants in Central America. Collectively data indicate low prevalence of *A. phagocytophilum* and *A. platys* in dogs from Costa Rica. Furthermore, infections seem to occur without clinical signs but with some hematological changes, and seem to resolve without treatment.

1. Introduction

Anaplasmosis is a tick-borne disease caused by bacteria belonging to the genus *Anaplasma* that infect different vertebrate species, including human. This genus includes obligate intracellular Gram-negative bacteria that mainly infect hematopoietic cells, and have a worldwide distribution (Dumler et al., 2001).

Human granulocytotropic anaplasmosis is caused by *Anaplasma phagocytophilum* (Dumler et al., 2001), which form morulae in the cytoplasm of neutrophilic granulocytes (Goddard, 2008), whereas *Anaplasma platys* is the causative agent of infectious canine cyclic thrombocytopenia (ICCT), and is found in platelets (Ramsey and Tennant, 2012). Ticks involved in the transmission of *A. phagocytophilum* are *Amblyomma* and *Ixodes* spp., while *Rhipicephalus* ticks are the vector of *A. platys* (Mullen and Durden, 2002). These ticks are present in Costa Rica (Álvarez et al., 2005).

The most common clinical signs in dogs infected with *A. phagocytophilum* are non-specific. A few patients show signs of coagulation disorders, such as petechiae, melena, or epistaxis (Rikihisa, 2011; Greene, 2012). Asymptomatic disease often coupled to marked thrombocytopenia (De Farias-Rotondano et al., 2012) is the most common outcome in dogs infected by *A. platys* (McGavin and Zachary, 2006; Ettinger and Feldman, 2010). Reinfection can occur with both agents, and antibody titers decrease observably eight months after bacteremia, approximately (Ettinger and Feldman, 2010; Woldehiwet, 2010).

Diagnosis relies on determining presence or exposure to the agent (Yabsley et al., 2008; De Farias-Rotondano et al., 2012). Serological tests, such as indirect immunofluorescence, are complicated by cross-reaction between different *Anaplasma* species (De la Fuente et al., 2006; Greene, 2012; Zobba et al., 2014). In contrast, polymerase chain reaction (PCR) allows establishing the presence of the pathogen (Parola, 2007; Forbes et al., 2009).

The first two cases of human granulocytotropic ehrlichiosis in Costa Rica were reported in 2007 in two hospitals of the Central Valley, based on clinical symptoms, detection of morulae in peripheral blood granulocytes, and recovery of patients after doxycycline treatment

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(Rojas-Solano and Villalobos-Vindas, 2007; Hernández-de Mezerville and Padilla-Cuadra, 2007). Studies that investigated a blood bank collected from dogs of the Central Valley of Costa Rica, with suspected ehrlichial and ICCT infection, established the presence of *A. platys* in 6.3% (19/300) samples, using a nested 16S rRNA PCR (Ábrego et al., 2009). Another study based on *Ehrlichia* and *Anaplasma* spp. (Rojas et al., 2014) 16S rRNA PCR, carried out in four regions of Costa Rica, revealed 10.0% (14/146) of dogs infected by *A. platys*. Recently, 16S rRNA/*groEL* amplification and sequence analyses provided evidence for *Anaplasma platys*, *Anaplasma phagocytophilum*, and *A. platys*-like infection in *Rhipicephalus sanguineus* s.l. ticks (Campos-Calderón et al., 2016).

In this paper, we investigate the presence of antibodies against *Anaplasma* spp. and DNA of *A. phagocytophilum* and *A. platys* in blood samples of seemingly healthy dogs and in ticks collected from the same animals nationwide, and we molecularly characterize the bacterial strains locally isolated.

2. Material and Methods

2.1. Ethical approval

This article does not contain any studies with human participants. The authors followed all applicable international and institutional guidelines for dogs' blood sampling.

2.2. Study design, sample size and analyzed population

A cross-sectional study was conducted to investigate the presence of *A. phagocytophilum* and *A. platys* in dog blood samples and in ticks collected from the same animals, using serological and molecular assays. The total sample size was estimated to be 369 individuals (5% prevalence, 95% confidence, 2.5% expected error, and a population higher than forty thousand dogs), calculated with Win Episcope 2.0 (Thrusfield et al., 2001). The canine population was analyzed in accordance with the provincial distribution of households. Interviews, clinical records and sampling was performed ultimately from 408 dogs. Population study, sampling methodology and period of study are described in Barrantes-González et al. (2016).

2.3. Interview, clinical examination and sampling

Each owner walking its dog and consenting to participate, was interviewed to obtain information about place of origin, age, breed, household variables (dog residence in the house), and suggestive clinical signs of anaplasmosis (petechiae, melena, epistaxis) and tick infestation in the past, among others. In addition, dog ticks were collected manually of all anatomical sites during 10 min, and a clinical examination was carried out, determining body condition, attitude, capillary refill time, temperature, color of mucous membranes, and suggestive clinical signs of anaplasmosis. Finally, blood samples were collected from each dog, stored at 4 °C until completion of blood analysis and serum separation, and frozen at −20 °C until performing the serological and molecular tests. Ticks were stored in 70% alcohol. in stray dogs living in recreational parks, consent from the municipal council was obtained. Only clinical exam and sampling was performed on these dogs.

2.4. Blood analysis

The microcentrifuge HETTICH® (5 min × 18,600*g*) and hematocrit reader DAMON/IEC was used to determine hematocrit values. Blood smears were stained with Giemsa as described by Cowell et al. (2008), to determine complete blood count (CBC). A total of 374 blood samples of dogs were analyzed.

2.5. Classification of ticks

Taxonomic classification of ticks was performed as described by Barros-Battesti et al. (2006), Nava et al. (2012, 2014). A total of 120 dogs out of 408 dogs were found infested with ticks, 112 dogs with *Rhipicephalus sanguineus* s.l., four with *Amblyomma ovale*, one with *Amblyomma mixtum*, one with *Ixodes boliviensis*, and two dogs with mixed infestation (*R. sanguineus* s.l. - *A. ovale*, and *R. sanguineus* s.l. - *A. mixtum*, respectively). Ticks from each dog were separated in microcentrifuge tubes containing 70% alcohol by species, sex and stage, and stored at room temperature until DNA extraction.

2.6. Indirect immunofluorescense assay (IFA)

The *Ehrlichia canis* and *Anaplasma phagocytophilum* MIF Canine IgG Antibody Kit from Fuller Laboratories® (California, USA) was used as recommended by the manufacturer. Each slide contained two separate antigen spots, with elementary bodies of each antigen (*E. canis* and *A. phagocytophilum*). In the present study, only the results of *A. phagocytophilum* IFA are shown, *E. canis* IFA results were reported previously (Barrantes-González et al., 2016). This assay reported a sensitivity and specificity of 100% for the *A. phagocytophilum* IFA (Naroo Ditech Inc., 2016). To determine the global seroprevalence of *A. phagocytophilum* one serial dilution (1:80) of 408 sera was analyzed. Serum samples that did not show fluorescence to *A. phagocytophilum* antigen in dilutions of 1:80 were considered negative, serum samples that showed fluorescence in dilutions of 1:80 were considered positive (Naroo Ditech Inc., 2016).

2.7. Polymerase chain reaction (PCR) and sequencing

Extraction of DNA from blood samples was performed with the Wizard Genomic kit from Promega®, Wisconsin, USA, whereas extraction of DNA from ticks was performed with the DNeasy Blood and Tissue kit from QIAGEN®, California, USA, as recommended by the manufacturers, additionally tick samples were homogenized and pretreated with proteinase K before DNA extraction. Ticks from each dog were analyzed in groups of the same species. In case of finding individuals of the same species but of different sex or stage, groups of ticks were analyzed according to the following priority: females > nymphs > males > larvae. A total of 374 blood samples and 122 groups of ticks were analyzed by PCR.

Nested PCRs were carried out to amplify fragments of the 16S rRNA gene from *A. phagocytophilum* (Massung et al., 1998) and from *A. platys* (Martin et al., 2005). In the first reactions, primers ge3a/ge10r and 8F/1448R were used for *A. phagocytophilum* and *A. platys*, respectively (Table 1). Each reaction consisted of Dream Taq™ PCR Master Mix 2X (Fermentas®), primers (1 μM), 2 μl of DNA (20 ng), nuclease free water (Fermentas®), in a final volume of 25 μl. The PCR amplification for *A. phagocytophilum* was performed with an initial denaturalization at 95 °C (2 min), followed by 40 cycles of 94 °C (30 s), 55 °C (30 s) and 72 °C (60 s), followed by a final extension at 72 °C (5 min). The amplification profile for *A. platys* PCR consisted in denaturalization at 95 °C (2 min), followed by 40 cycles of 94 °C (60 s), 45 °C (60 s) and 72 °C (120 s); followed by a final extension at 72 °C (5 min). In the nested reaction, Ge9f/Ge2 and EHR16SR/PLATYS primers were used for *A. phagocytophilum* and *A. platys*, respectively (Table 1). The same conditions as described above were used for *A. phagocytophium*, changing only quantity of DNA (1 μl) and number of cycles (30). The nested PCR profile for *A. platys* involved initial denaturalization at 94 °C (1 min), followed by 40 cycles of denaturalization 94 °C (30 s), annealing 55 °C (30 s) and extension at 72 °C (30 s); final extension consisted in 72 °C (5 min).

Positive samples in the 16S rRNA PCR of *A. phagocytophilum* or *A. platys* underwent a nested PCR for amplification of a fragment of *groEL* gene (Alberti et al., 2005). In the first reaction, primers EphplgroEL F/

Table 1
Primers used for amplifying *A. phagocytophilum* and *A. platys*.

Pathogen (Reference)	Gen	Primers	Sequences (5′-3′)	Fragment length (bp)
A. phagocytophilum (Massung et al., 1998)	16S rRNA	Ge3a	CACATGCAAGTCGAACGGATTATTC	932
		Ge10r	TTCCGTTAAGAAGGATCTAATCTCC	
		Ge9f	AACGGATTATTCTTTATAGCTTGCT	546
		Ge2	GGCAGTATTAAAAGCAGCTCCAGG	
A. platys (Martin et al., 2005)	16S rRNA	8F	AGTTTGATCATGGCTCAG	1400
		1448R	CCATGGCGTGACGGGCAGTGTG	
		EHR16SR	TAGCACTCATCGTTTACAGC	678
		PLATYS	GATTTTTGTCGTAGCTTGCTATG	
Anaplasma spp. (Alberti et al., 2005)	*groEL*	EphplgroEL-F	ATGGTATGCAGTTTGATCGC	624
		EphplgroEL-R	TCTACTCTGTCTTTGCGTTC	
A. platys (Zobba et al., 2014)	*groEL*	EphplgroEL-F	ATGGTATGCAGTTTGATCGC	515
		EplgroEL	CATAGTCTGAAGTGGAGGAC	
A. phagocytophilum (Alberti et al., 2005)	*groEL*	EphplgroE-F	ATGGTATGCAGTTTGATCGC	573
		EphgroEL	TTGAGTACAGCAACACCACCGGAA	

EphplgroEL R (Table 1) were used. Each reaction consisted of 25 μl Dream Taq™ PCR Master Mix 2X (Fermentas®), primers (1 μM), 5 μl of DNA, and nuclease free water (Fermentas®) in a final volume of 50 μl. The temperature profile included initial denaturalization at 94 °C (3 min), 30 cycles of denaturalization (30 s, 94 °C), annealing (30 s, 52 °C), extension (60 s, 72 °C), and a final extension at 72 °C for 10 min. Same conditions were used in the nested reactions, changing primers (Table 1), and annealing (30 s, 55 °C) and extension (30 s, 72 °C) temperatures and time.

DNA from HL-60 infected cells with *A. phagocytophilum*, strain Trestom was used as positive control for *A. phagocytophilum* (kindly donated by the Center of Disease Control, Atlanta, USA), a PCR positive blood sample from a dog confirmed by sequencing was used as positive control for *A. platys* (Ábrego et al., 2009), 16S rDNA amplification (Black and Piesman, 1994) served as an internal sample control to rule out PCR/extraction inhibition, and nuclease free water (Fermentas®) was used as negative control. The products obtained in the PCR were visualized in 2% agarose gel electrophoresis, stained with GelRed DNA Stain (Biotium®). Thermo Scientific GeneRuler 100 bp Plus DNA Ladder (Sm0321) molecular weight marker was included.

In order to confirm all positive PCR results, amplified products were purified using the QIAquick® (QIAGEN) kit, according to the manufacturer's instructions. Positive samples were sent to Macrogen (Seoul, Korea) for sequencing. Partial sequences were aligned with BioEdit Sequence Aligment Editor® (Hall, 1999) and compared using the BLASTn algorithm with the database of NCBI (National Center for Biotechnology Information).

2.8. Statistical analysis

Data obtained from the interview, clinical examination and results of diagnostic tests (serology and PCR) were entered in a digital database. Seroprevalences for *A. phagocytophilum* and PCR prevalences for *A. phagocytophilum* and *A. platys* were established. Finally, a descriptive analysis was performed for all seropositive and PCR positive samples.

3. Results

From 408 sera tested, 11 (2.7%) reacted positive in IFA. Seropositive dogs were detected in the provinces of Heredia (2/31, 6.5%), Guanacaste (2/42, 4.8%), Puntarenas (1/30, 3.3%), San José (5/179, 2.8%), and Limón (1/53, 1.9%). No seropositive dogs were detected in Alajuela (0/41) and Cartago (0/32) provinces. Most seropositive dogs were mixed-breed (8/11, 72.7%), females (7/11, 63.6%), and between one and three years old (8/11, 72.7%). Three dogs (27.3%) did not have an owner and were residents of recreational parks, three dogs (27.3%) were held outside and five dogs (45.4%) mainly inside the owner's home. Six dogs (54.5%) showed regular body condition. Seven out of eight dog owners (87.5%) reported past infestation of their animals with ticks, only on two seropositive dogs (18.2%) were found infested with *R. sanguineus* s.l. ticks. None showed clinical signs, nine dogs (81.8%) showed altered hematological values (Table 2), specifically, eosinophilia was determined in six (54.5%) cases. Of these six dogs, only two dogs (6 and 11) showed gastrointestinal parasites in feces. None of the owners suspected anaplasmosis in their animals, and none of the seropositive dogs were treated with doxycycline. None of the seropositive dogs or their ticks were positive by the 16S rRNA PCR for *A. phagocytophilum* or *A. platys*.

Of 374 blood samples tested by PCR for *A. phagocytophilum*, only one (0.3%) dog (C160), which was sampled in the Guanacaste province, amplified a segment of 16S rRNA but not *groEL* (Table 3). It was a female, mixed-breed dog, approximately 10 years old and with no known owner, resident of the Central Park of Cañas. The animal showed good physical condition, pink membranes, and did not show any clinical signs. No ticks were found on the dog. Hematological values were not determined and IFA was negative.

From 122 groups of ticks analyzed, only one *R. sanguineus* s.l. (0.8%) tested positive in PCR 16S rRNA for *A. phagocytophilum*, but negative in *groEL* PCR (Table 3). This tick was collected from a male Pekinese dog (C25), five years old, walking with his owners in a recreational park in the San José province. The dog showed a good body condition, pink mucous membranes, and no clinical signs were recorded. Although the hematological values were within the reference values, mean corpuscular hemoglobin concentration was slightly diminished and number of banded neutrophils slightly increased. According to its owners, the dog lived inside the house, sleeping in the bed with the owners, and had had ticks several times during its life; it was never treated with doxycycline.

Of a total of 374 blood samples tested in PCR for *A. platys*, four dogs (1%) were found positive in 16S rRNA and *groEL* PCR, two dogs (C140 and C126) were from the Guanacaste province, and two (C346 and C329) from the Puntarenas province. All dogs were under three years old, three dogs were in good body condition, three individuals were infested with ticks, two had pale or very pale mucous membranes and reported history of having ticks in the past, owners also reported, that the dogs never had received treatment with doxycycline. However, all showed at least three abnormal hematological values, especially low hemoglobin levels (4), low hematocrit (3), low platelet counts (3), and high eosinophil counts (3). The four PCR positives dogs were seronegative.

Finally, all 122 groups of ticks analyzed by PCR 16S rRNA for *A. platys* showed negative results.

After sequencing, both *A. phagocytophilum* 16S rRNA sequences from the tick C25 and the dog C160 were identical and showed 100% of homology (510/510 bp) with sequences of *A. phagocytophilum* isolated from *R. sanguineus* s.l. tick in Costa Rica [KU534874] and from dog in the United States [CP006618.1] (Table 3). One additional blood sample (C140) showed positive results on the 16S rRNA PCR for *A.*

Table 2
Hematological values of dogs seropositive to *A. phagocytophilum*.

Hematological values (Reference values)	Hct (36–47)	Hgb (11–16)	MCHC (32–36)	WBC (6000–12,000)	N. Ban (0−300)	N. Seg (3000–9000)	Eos (100–750)	Bas (0−10)	Linf (1000–4800)	Mon (60–840)	PTL (200000–500,000)
Dog 1	**51**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Dog 2	46	16	34	9900	99	6435	594	0	1980	792	ND
Dog 3	41	14	34	9050	0	4978	**1539**	0	2444	91	270,846
Dog 4	**34**	11	32	**20,400**	0	8364	612	0	**11,016**	408	274,516
Dog 5	45	15	33	**5350**	0	3264	0	0	2033	**54**	**198,180**
Dog 6	**35**	11	**31**	**23,600**	**472**	**11,800**	**5900**	0	**4956**	472	ND
Dog 7	**35**	15	**42**	9050	**362**	3982	**1810**	**91**	2353	453	ND
Dog 8	45	15	33	**20,100**	0	8241	**5628**	**402**	**5427**	402	**165,150**
Dog 9	46	15	33	**17,300**	**346**	**10,726**	**865**	0	**5017**	346	337,640
Dog 10	**49**	**17**	34	6150	0	3629	308	0	2214	0	395,626
Dog 11	**30**	**9**	**30**	9800	**490**	6174	**1078**	0	1862	196	**88,080**

Hct: Hematocrit, **Hgb:** Hemoglobin, **MCHC:** Mean Corpuscular Hemoglobin Concentration, **WBC:** White Blood Cells, **N. Ban:** Banded Neutophils, **N. Seg:** Segmented Neutophils, **Eos:** Eosinophils, **Bas:** Basophils, **Linf:** Lymphocytes, **Mon:** Monocytes, **PTL:** Platelet, **ND**: not determined.
Hematological values out of reference range are shown in bold.

phagocytophilum and sequencing determined 99% identity (322/324 bp) with different *Anaplasma* species: *A. phagocytophilum* isolated from dromedaries in Tunisia [KC455366.1], *A. platys* from a dog from Malaysia [KU500911.1], *A. odocoilei* from a deer from USA [KT870139.1], and *Candidatus Anaplasma camelli* detected in dromedaries from Saudi Arabia [KF843827.1] (Table 3).

Sequences of two *A. platys* 16S rRNA PCR positives samples (C140 and C346 [KY389142]) were identical between them and showed 100% (695/695 bp) sequence similarity with *A. platys* detected in dogs from Croatia [KY114935.1] and Cuba [KP903296.1]. The other two samples (C126 and C329 [KY389143]) were also identical between them, and showed 100% (695/695 bp) identity with *A. platys* isolated in dogs from Cuba [KP903294.1] and ticks from the Democratic Republic of Congo [AF478131.1] (Table 3). The difference in the percentage of identity between these two groups resulted from one nucleotide substitution (adenine for guanine). Sequences of samples of the first group (C140 and C346), showed also a 100% nucleotide identity with the partial sequence of *A. platys* detected previously in a dog in Costa Rica (Ábrego et al., 2009).

Finally, sequencing of the *groEL A. platys* segment showed variable percentages of identity with different sequences. Sample C126 [KY389144] showed 100% (438/438 bp) homology with *A. platys* detected in dogs from Thailand and Chile [KU765205.1, EF201806.1]. On the other hand, samples C140, C329 [KY389145] and C346 [KY389146] showed 99% of homology with the same sequences from Thailand and Chile but there were three nucleotide differences among them.

Ticks collected from PCR-positive dogs were negative for PCR for both agents. The *R. sanguineus* s.l. tick that was found positive by *A. phagocytophilum* PCR 16S rARN was found on a seronegative and PCR negative dog.

4. Discussion and conclusions

This work represents the first cross-sectional study in dogs from Costa Rica combining different diagnostic techniques to determine the seroprevalence of and infection with *Anaplasma* spp. with analysis of attached ticks. The seroprevalence and prevalence established in this study show the presence and wide distribution of *Anaplasma* spp. in dogs and their ticks in the country.

A low national seroprevalence (2.7%, 11/408) and prevalences of *Anaplasma* spp. ranging between 0% and 6.5% in the different provinces were determined for the first time. The overall seroprevalence was similar to the *A. phagocytophilum* seroprevalences reported in North America and in the Caribbean (Qurollo et al., 2014). Since cross-reactions of *A. phagocytophilum* and *A. platys* occur (De la Fuente et al., 2006; Greene, 2012; Taylor et al., 2007), some positive results could be due to the

Table 3
Sequences of 16S rARN and *groEL* genes of *A. phagocytophilum* and *A. platys* determined in blood samples from dogs and *R. sanguineus* s.l. tick in Costa Rica.

Sample (Province)	16S rARN *A. phagocytophilum*	16S rARN *A. platys*	*groEL A. phagocytophilum*	*groEL A. platys*/*A. platys* like
R. sanguineus s. l. C25 (San José)	100% *A. phagocytophilum* (KU534874, CP006618.1)	Negative	Negative	Negative
Dog C160 (Guanacaste)	100% *A. phagocytophilum* (KU534874, CP006618.1)	Negative	Negative	Negative
Dog C140 (Guanacaste)	99.0% *A. phagocytophilum* (KC455366.1) 99.0% *A. platys* (KU500911.1) 99.0% *A. odocoilei* (KT870139.1) 99.0% *Candidatus A. camelli* (KT870139.1)	100% *A. platys* (KY114935.1, KP903296.1)	Negative	99.0% *A. platys* (KU765205.1, EF201806.1)
Dog C346 (Puntarenas)	Negative	100% *A. platys* (KY114935.1, KP903296.1)	Negative	99.0% *A. platys* (KU765205.1, EF201806.1)
Dog C126 (Guanacaste)	Negative	100% *A. platys* (KP903294.1, AF478131.1)	Negative	100% *A. platys* (KU765205.1, EF201806.1)
Dog C329 (Puntarenas)	Negative	100% *A. platys* (KP903294.1, AF478131.1)	Negative	99.0% *A. platys* (KU765205.1, EF201806.1)

detection of antibodies against *A. platys*. The latter has been reported in higher percentage in dogs of Costa Rica respect to *A. phagocytophilum* (Ábrego et al., 2009; Dolz et al., 2013; Rojas et al., 2014). However, a wide distribution of *Anaplasma* spp. infections was established, since seropositive animals were determined in five out of seven provinces of Costa Rica. All seropositive dogs were PCR negative, and owners did not remember having observed clinical signs of anaplasmosis in their pets, also, their dogs were never treated with doxycycline, concluding, that the disease may have occurred subclinically, and self-resolved. These findings are consistent with previous reports, which describe few nonspecific clinical signs in infected dogs (Greene, 2012; Lillini et al., 2006; Stuen, 2007). However, seropositive dogs showed anemia, leukocytosis, lymphocytosis, eosinophilia and thrombocytopenia, consistently with hematological findings reported in the literature (Lillini et al., 2006; Rikihisa, 2011; Greene, 2012), indicating that dogs were recovering from or experiencing infection. However, co-infections with other tick-borne agents, such as *Ehrlichia* spp., *Rickettsia* spp., *Babesia* spp. and hemotropic Mycoplasmas were not considered.

Although DNA presence of *A. phagocytophilum* in dogs was reported previously in two dogs, one white tailed deer (Dolz et al., 2013) and two *Rhipicephalus sanguineus* s.l. ticks (Campos-Calderón et al., 2016), a nationwide representative investigation of *A. phagocytophilum* was not carried out before.

In this study *A. phagocytophilum* active infection was recorded only in one stray dog (0.3%) living as a resident in a recreational park, without a known owner. Not much information could be collected from this dog; however, clinical examination showed no clinical signs, reinforcing our conclusion, that *A. phagocytophilum* infection seems to occur silently in dogs from Costa Rica, consistently with others' observations (Greene, 2012; Lillini et al., 2006; Stuen, 2007). However, this animal may represent a source of infection and dissemination of the bacteria for other dogs and people, especially since it was living in the recreational park without veterinary care or ectoparasite control.

From all groups of ticks analyzed, only one (0.8%) was positive to *A. phagocytophilum*. This *R. sanguineus* s.l tick was collected from a dog regularly visiting a recreational park in San José province, and is consistent with previous reports in which the presence of *A. phagocytophilum* in *R. sanguineus* s.l. ticks collected from dogs from Costa Rica was reported (Campos-Calderón et al., 2016). It also supports, what was proposed by Stuen (2007), that *R. sanguineus* s.l. can act as a vector for *A. phagocytophilum*. The Pekinese dog, from which the positive tick was collected, remained, according to his owners, indoors, and spent much time on the beds. This case shows that *A. phagocytophilum* transmission could occur through dogs that live with humans in their homes. However, the dog showed no antibodies and was PCR negative. The reason could be, that ticks had infested the dog recently (within 48 h), and that the bacteria was not yet transferred to the dog (Carrade et al., 2009; Ettinger and Feldman, 2010; Taylor et al., 2007), or otherwise, that *R. sanguineus* s.l. is not a competent vector for *A. phagocytophilum*.

The two positive samples (blood and tick) could only be amplified with the *A. phagocytophilum* 16S rRNA PCR. This finding is consistent with previous results (Campos-Calderón et al., 2016). The presence of *A. phagocytophilum* genetic variants or of novel *Anaplasma* species in Costa Rica remains cloudy and needs to be deeply investigated (Dugat et al., 2015; Nicholson et al., 2010).

Findings of *A. platys* DNA agreed with previous studies of Ábrego et al. (2009), in which 6.3% *A. platys* infection rate was observed in sick dogs, and of Rojas et al. (2014), who found *A. platys* infection rates varying from 0% to 19.4% in dogs from 4 different provinces. In this study, 4 dogs (1%) *A. platys* active infection was recorded, indicating a low national *A. platys* prevalence, like that reported by Qurollo et al. (2014) in North America and the Caribbean. Positive dogs were found in Guanacaste (2) and Puntarenas (2) province, which coincides with reports of Ábrego et al. (2009), who found positive dogs in Guanacaste, and Rojas et al. (2014), who reported positive dogs in Guanacaste and Puntarenas. From all groups of ticks analyzed, none was positive for *A. platys*.

The four dogs positive to *A. platys* by 16S rRNA PCR were also positive by *groEL* PCR, showing variable percentages of identity. This is in accordance with our previous results (Campos-Calderón et al., 2016), and supports the presence of different *A. platys* genetic variants in our country.

Finding *A. phagocytophilum* in blood and ticks collected from dogs shows that there is a risk for dogs and people to get infected with these agents, when living in contact with infected ticks. Although anaplasmosis was reported only in the provinces of Puntarenas and Guanacaste, serologic results indicated a wider distribution of anaplasmosis in the country. Infected dogs seemed to devevelop no clinical signs and sub clinical disease remained unnoticed by the owners.

Conflict of interest

The authors stated no conflict of interest.

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