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Article

Molecular detection of rickettsial agents of hard ticks (Acari: Ixodidae) collected from wild birds of Panama

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Abstract

Ticks (Acari: Ixodidae) collected from wild birds in Panama were tested for the presence of tick-borne pathogens as *Rickettsia*, *Anaplasma, Ehrlichia*, *Bartonella, Borrelia*, *Hepatozoon* sp., and *Babesia*. Overall 124 ticks were found in 57 birds belonging to 28 species: *Amblyomma longirostre* (32 larvae, 1 nymph), *Amblyomma nodosum* (30 nymphs), *Amblyomma geayi* (15 larvae, 1 nymph), *Amblyomma varium* (5 larvae, 2 nymphs), *Amblyomma naponense* (2 larvae), *Amblyomma ovale* (2 larvae), and *Amblyomma calcaratum* (1 larva). DNA of *Rickettsia amblyommatis* was detected in 65% of *A. longirostre*, 69% of *A. geayi* and 14% of *A. varium.* Moreover, results from two larvae of *A. longirostre* showed DNA of unidentified *Rickettsia* sp. No DNA of *Borrelia, Bartonella,* Anaplasmataceae neither *Babesia* nor *Hepatozoon* was detected. These results expand knowledge about the host for immature *Amblyomma* ticks in Panama and show the first data of *Rickettsia* in ticks collected from birds in this country.

Keywords: birds, Ixodidae, *Rickettsia*, *Amblyomma*, Panama

Introduction

Birds develop a crucial role in ecosystems as seed dispersers, pollinators and as a source of nutrients for predators and variety of endo and ectoparasites (Whelan *et al.* 2008; Atkinson *et al.* 2009). Among the ectoparasites, hard ticks (Acari: Ixodidae) are frequently found on a variety of birds around the world (Guglielmone *et al.* 2014). Due to their high vagility, birds can cross different types of ecosystems and even continents. This means that they can spread infectious agents directly, or infected arthropod vectors in previously uncolonized locations (Hoogstraal 1961; Loss *et al.* 2016;

Kuo *et al.* 2017). Then, the search for potential infectious agents in ticks may offer clearer ideas about potential transmission risks.

In Panama, approximately 987 species of birds (Anonymous 2018) and 50 species of ticks have been reported (Bermúdez *et al.* 2018); but, with exception of some information about ticks parasitizing birds (Fairchild *et al.* 1966; Miller *et al.* 2016; Domínguez *et al.* 2019), not much is known about the relations between these both groups. Moreover, in this country, *Rickettsia rickettsii* is by far the main pathogenic agent transmitted by ticks, but there are also references of cases of relapsing fevers caused by *Borrelia* spp. and the circulation of other *Rickettsia* species with pathogenicity unknown to humans (Lopez *et al.* 2016; Bermúdez & Troyo 2018; Martínez-Caballero *et al.* 2018). This information comes from ticks harvested from mammals in natural and disturbed environments, and there is no information of ticks collected from wild birds in Panama.

Therefore, the scope of this paper is to screen the ticks for the presence of bacteria of the genera *Rickettsia*, *Ehrlichia* and *Anaplasma* (Rickettsiales: Rickettsiaceae, Anaplasmataceae), *Bartonella* (Rhizobiales: Bartonellaceae), *Borrelia* (Spirochaetales: Spirochaetaceae); and Apicomplexa protozoa of the genera *Babesia* (Babesidae) and *Hepatozoon* (Hepatozoidae), which have a potential impact on human and animal health and add new host- parasite associations.

Materials and methods

Samples origin. Ticks analyzed in this study belong to Ectoparasites Collection of the Zoological Collection "Dr. Eustorgio Méndez" of the Gorgas Memorial Institute of Health (ECZCDEM), and correspond to studies developed during 2007 to 2018 in different studies in: Summit Municipal Park (SMP) (9°03′58″ N, 79°38′44″ W), Gamboa (GA) (9°07´14.8" N, 79°41´27.89" W), Soberania National Park (SNP) (9°07´54.25" N, 79°45´25.75" W), Barro Colorado Natural Monument (BCNM) (9°09´07.57" N, 79°50´47.33" W), Achiote-San Lorenzo Natural Park (A-SLNP) (9°13´20.48" N, 80°01´08.70" W), Galeta island (GI) (9°24´03.00" N, 79°52´11" W), and Montijo Natural Reserve (MNR) (7°47´53.43" N, 80° 47´32.28" W). Because GI and MNR samples were donated to ECZCDEM, their databases were not included and it is not possible to compare the intensity of parasitism.

In general terms, these areas correspond to lowlands tropical humid forests, with temperatures that vary between 20-34° C and high humidity (60–95% HR). The ecology of these sites has been described previously for SMP (Bermúdez *et al.* 2010), GA (Bermúdez-Mongue and Barrios 2011), SNP (Miambiente 2011), BCNM (Smithsonian 2019a), A-SLNP (Anam 2007), GI (Smithsonian 2019b).

Overall, birds were collected using mist nets (0600-1600 Hrs, depending of the weather), identified through specialized books on birds of Panama and Central America (Angher and Dean 2016), revised and released after to collect the ticks. All ticks were stored in plastic tubes containing 90% ethanol. All the investigations had permits granted by the Ministry of the Environment (SC/A-12-13, SE/A-100-2015, DAPVS-0743-2016 and STRI-IACUC 2012-1117-2015).

Tick identification. Identification of *Amblyomma* larvae was performed by molecular analysis as previously described (Ogrzewalska *et al.* 2012). For this purpose, representative specimens of each tick species were submitted to DNA extraction using the QIAamp® DNA Mini Kit and tested by a PCR assay targeting a portion of the tick mitochondrial 16S rRNA gene, as previously described (Mangold *et al.* 1998). Amplicons (~460 bp) were visualized on 1% agarose gels stained with Gel Red Nucleic Acid Gel Stain™ 10000× in DMSO. PCR products of the expected size were purified with ExoSAP-IT™ PCR Product Cleanup Reagent and sequenced in a 96-capillary 3730 xl DNA Analyzer® according to the protocols developed by Otto *et al.* (2008).

Partial sequences obtained were submitted to Blast analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and aligned with corresponding 16S rRNA sequences of different tick species available in the GenBank. Nymphs of *Amblyomma* species were identified following dichotomous keys prepared by Martins *et al.* (2010). However, these keys are for identification of Brazilian nymphs, therefore some nymphs were submitted as well to the same molecular identification procedure as larvae to confirm identification.

Molecular tests. Sixty-four larvae and 32 nymphs were individually tested by a set battery of PCR assays targeting bacteria of the genera *Rickettsia, Borrelia,* Anaplasmataceae family*, Bartonella,* and protozoa of the genera *Babesia* and *Hepatozoon.* PCR was performed using genus or family specific primers shown in Table 1. All reactions, with exception of PCR targeting genes of *Bartonella*, were performed with 25 µl per reaction, which contained 12.5 µl of DreamTaq™ Green PCR Master Mix, 8.0 µl of nuclease-free water, 1 µl of each primer at 10 µM (Invitrogen) and 2.5 µl of template DNA.

TABLE 1. List of primers used in the present study for PCR.

Organism	Gene	Primers (5′-3)				Product Size (bp)	References
		Forward		Reverse			
Anaplasmataceae	16S rRNA	EHR16SD	GGTACCYACAGA AGAAGTC	EHR16SR	TGCACTCATCGTTTACAG	345	Inokuma *et al.* (2000)
Rickettsia spp.	gltA	CSF1	CATCCTATGGCTA TTATGCTTGC	CSR1	TATACTCTCTATG(T/A)AC(A/ GT(A/G)AC C	885	Rozental *et al*. (2017)
Rickettsia spp.	gltA	CSF2	CTTACCGCTATTA GAATG ATT GC	CSR2	GAGCGA(T/ G)AGCTTCAAG(T/C)T CTA T	572	Rozental *et al*. (2017)
Rickettsia spp.	ompA	190.70	ATGGCGAATATTT CTCCAAAA	190.701	GTTCCGTTAATGGCAGCATC T	632	Roux *et al*. (1996)
Rickettsia spp	htrA	17k-5	GCTTTACAAAATT CTAAAAACCATAT A	17k-3	TGTCTATCAATTCACAACTT GCC	499	Labruna *et al.* (2004)
Rickettsia spp	ompB	120-M59	CCGCAGGGTTGGT AACTGC	120Ð807	CCTTTTAGATTACCGCCTAA	820	Roux and Raoult (2000)
Bartonella spp.	gltA	*gltA* F1	GCTATGTCTGCVT TCTATCAYGA	*gltA* R1	AGAACAGTAAACATTTCNG THGG	731	Rozental *et al*. (2017)
Bartonella spp.	gltA	*gltA* F2	ACDCTYGCYGCD ATGGCNATAA	*gltA* R1	AGAACAGTAAACATTTCN GTHGG	500	Rozental *et al*. (2017)
Babesia/ Hepatozoon spp.	18S rRNA	Bab18F1	GCGGTAATTCCAG CTCCAATAGCGTA TAT	Bab18R1	TCCGAATAATTCACCGGATC ACTCGAT	1150	Blanco *et al*. (2017)
Babesia/ Hepatozoon spp.	18S rRNA	Bab18F2	AGACGATCAGATA CCGTCGTAGTCCT A	Bab18R2	ATCACTCGATCGGTAGGAG CGACG	670	Blanco *et al*. (2017)
Borrelia spp.	Fla	BorFlaF1	TACATCAGCTATT AATGCTTCAAGAA	BorFlaR1	GCAATCATWGCCATTGCRG ATTG	729	Blanco *et al.* (2017)
Borrelia spp.	Fla	BorFlaF2	CTGATGATGCTGC TGGWATGG	BorFlaR2	TCATCTGTCATTRTWGCATC TT	410	Blanco *et al*. (2017)

To determine the presence of *Rickettsia* in the ticks all DNA samples were first analyzed with primers targeting the *gltA* gene. Samples yielding visible amplicons were also tested with primers *ompA* gene (Table 1). To fully characterize obtained unidentified *Rickettsia* species attempts to amplify other genes were made using primers targeting the 17-kDa rickettsial gene (*htrA*) 17k-3, and 17k-5 primers (Labruna *et al.* 2004) and the outer membrane B (*ompB*) gene using 120-M59 and 120- 807 primers (Roux & Raoult 2000).

For detection of *Bartonella* a semi nested screening protocol was performed by cloning the gltA gene, which contained 14.05 µl of nuclease-free water, 0.5 µl of dNTPs, 2.5 µl of buffer PCR 10X, 4.0 µl of $MgCl^2$ 50 mM, 0.2 µl AmpliTaq Gold Polymerase, 0.5 µl of each primers 10 nM forward and reverse (Table 1), with total of 22 µl of mix reaction and 3 µl genomic DNA, for second protocol

the same mix reaction was used but with other primers and 3µl of amplicons obtained in first step (Rozental *et al.* 2017).

In each PCR assay, negative controls (water) and an appropriate positive control sample (DNA of *R. rickettsii, Anaplasma platys, Bartonella henselae, Borrelia afzelii* or *Babesia vogeli*) were run together with the tick samples. The protocols for purification and sequencing of products were the same as described above.

Results

In total 237 birds belonging to the orders Columbiformes (one species), Strigiformes (one species), Accipitriformes (one species), Apodiformes (one species), Coraciiformes (one species), and Passeriformes (12 families and 23 species), were captured from seven localities in Panama, of these 57 (24.9%) were infested with ticks (Table 2). Altogether 124 ticks representing seven species were found: *Amblyomma longirostre* (32 larvae, 1 nymphs), *Amblyomma nodosum* (30 nymphs), *Amblyomma geayi* (15 larvae, 1 nymphs), *Amblyomma varium* (5 larvae, 2 nymphs), *Amblyomma naponense* (2 larvae), *Amblyomma ovale* (2 larvae), and *Amblyomma calcaratum* (1 larva). Of these, *A. longirostre* was collected from 13 species of birds, *A. nodosum* on from 11 species of birds, *A. geayi* on nine species of birds, *A. varium* on four species of birds, and *A. naponense*, *A. ovale* on two and *A. calcaratum* on one species of birds (Table 3). All of these ticks were identified by molecular methods by sequencing the fragment of 16S rDNA. GenBank similarities with sequences available and number accession numbers of obtained sequences are presents in the Table 4. In total 26 larvae and 11 nymphs were identified only to the genus *Amblyomma* sp.

Among 87 ticks (32 *A. longirostre*, 27 *A. nodosum*, 16 *A. geayi*, 7 *A. varium*, 2 *A. ovale*, 2 *A. naponense*, 1 *A. calcaratum*) tested, no DNA of *Borrelia, Bartonella,* Anaplasmataceae neither *Babesia* nor *Hepatozoon* was detected. However, in 65.6% (21/32) of *A. longirostre*, 68.7% (11/16) of *A. geayi* and in 14.2% (1/7) of *A. varium* was detected DNA of *Rickettsia amblyommatis* (Table 3). All 33 sequences of the fragment of *gltA* gene were identical to each other and 100% (548/548 bp) identical to *Rickettsia amblyommatis* strain AL (EU274654) and strain Aranha (AY360216). In total 20 sequences of the fragment *ompA* gene were obtained being 99.1%-99.8% identical to each other 98.3% (595/601 bp), 98.8% (591/598 bp) and 99.3% (594/598 bp) *R. amblyommatis* isolate An13 (CP015012) complete genome. The sequences were deposited in the GenBank under the following numbers: MH818420 (fragment of the *gltA* gene), MH818422 - MH818425 (fragments of the *ompA* gene).

In two larvae of *A. longirostre* collected from *Oncostoma olivaceum* (Tyrannidae) DNA of unidentified *Rickettsia* sp was detected (Table 2). The *gltA* partial sequence was 92.3% (513/552 bp) identical to *Rickettsia* sp. IrLB1/Warsaw (KT834984),'*Candidatus* Rickettsia mendelii' (KJ882311) and uncultured *Rickettsia* (JN849396) all detected in ticks *Ixodes ricinus* in Europe. Portions of the *ompA, ompB,* and *htrA* genes were not amplifiable in these samples. The *gltA* gene fragment sequence was deposited in the GenBank under the number MH818421. This *Rickettsia* is provisionally denominated *Rickettsia* sp. C325.

Discussion

Our study showed seven species of ticks parasitizing wild birds in seven localities in Panama. Along their distributions, birds appear to be the main hosts to immature *A. longirostre* and *A. nodosum*, and in lesser extension to *A. varium*, *A. geayi* or *A. calcaratum* (Guglielmone *et al.* 2014; Ogrzewalska

& Pinter 2016). Parasitism by adult *A. longirostre* adults is commonly associated with porcupine *Coendou* spp. (Erethizontidae); adult *A. varium* and *A. geayi* are found parasitizing sloths (Megalonychidae, Bradypodidae), and *A. nodosum* and *A. calcaratum* adult ticks are found on anteaters (Myrmecophagidae). These species were previously reported in birds from Panama (Fairchild *et al.* 1966; Miller *et al.* 2016; Domínguez *et al.* 2019).

TABLE 2. Number of birds infested with ticks captured in Panama during 2007 to 2018.

Order	Family	Species	SMP[a]	GA[a]	SNP[a]	BCNM[a]	A-SLNP[a]	GI[a]	MNR[a]	Total*
Columbiformes	Columbidae	*Leptotila cassini*	0	0/1	0/1	1/2	0	1/3	0	2/7
Strigiformes	Strigidae	*Megascops choliba*	1/1	0	0	0	0	0	0	1/1
Accipitriformes	Accipitridae	*Buteogallus* sp.	1/1	0	0	0	0	0	0	1/1
Apodiformes	Trochilidae	*Phaethornis longirostris*	0	0/2	1/3	0	0	0	0	1/5
Coraciiformes	Momotidae	*Baryphthengus martii*	0	0	0	2/4	0	0	0	2/4
Passeriformes	Thamnophilidae	*Myrmotherula schisticolor*	0	1/1	0	0	0	0	0	1/1
		Myrmeciza exsul	0	0	0	1/3	0	0/2	0	1/5
		Gymnopithys leucaspis	0	0	2/4	1/4	1/7	0	0	4/15
	Formicariidae	*Hylophylax naevaoides*	0	0	2/4	1/9	0	0/4	0	3/17
	Dendrocolaptidae	*Glyphorynchus spirurus*	0	0	2/4	1/5	0	0	0	3/9
		Xiphorhynchus susurrans	0	0	1/6	0	0	1/8	0	2/14
	Pipridae	*Manacus vitellinus*	0	1/4	1/3	0	0	0/4	0	1/11
		Ceratopipra mentalis	0	2/6	2/12	2/13	0	0/4	0	6/35
	Rhynchocyclidae	*Mionectes oleagineus*	0	0	1/10	2/4	0	1/4	0	4/18
	Tyrannidae	*Oncostoma olivaceum*	0	1/4	1/2	0	0	0	0	2/6
	Troglodytidae	*Cyphorhinus phaeocephalus*	0	1/1	1/7	0	0	1/9	0	3/17
	Turdidae	*Turdus grayi*	0	3/5	0	0	0	0	0	3/5
		Catharus ustulatus	0	0/1	1/2	1/9	0/5	1/6	0	3/23
	Parulidae	*Basileuterus rufifrons*	0	0	0	0	0	0	1/1	1/1
	Thraupidae	*Eucometis penicillata*	0	3/5	2/5	0	0	0/2	0	6/12
	Cardinalidae	*Cyanocompsa cyanoides*	0	0	1/3	0	0	1/7	0	2/10
		Habia fuscicauda	0	2/3	0/2	0	0	0	0	2/5
		Saltator striatipectus	0	1/1	0	0	0	0	0	1/1
	Fringillidae	*Euphonia laniirostris*	0	2/2	0	0	0	0/12	0	2/14
		not identified 1	1/1	0	0	0	0	0	0	1/1
		not identified 2	1/1	0	0	0	0	0	0	1/1
		not identified 3	1/1	0	0	0	0	0	0	1/1
		not identfied 4	1/1	0	0	0	0	0	0	1/1

*No. infested/ No. Captured

[a] Summit Municipal Park (SMP), Gamboa (Ga), Soberania National Park (SNP), Barro Colorado Natural Monument (BCNM), Achiote-San Lorenzo Natural Park (A-SLNP), Galeta island (GI), and Montijo Natural Reserve (MNR).

TABLE 3. Rickettsial infection in ticks collected from wild birds.

Birds Species	Ticks Species	L	N	Tested ticks[a]	*Rickettsia* species	Sites[a]
Leptotila cassini	*Amblyomma geayi*	1	0	1/1	*R. amblyommatis*	BCNM
Megascops choliba	*Amblyomma geayi*	1	0	1/0		
	Amblyomma sp.	1	0	0/-		
Buteogallus sp.	*Amblyomma nodosum*	0	1	0/-		
Phaethornis longirostris	*Amblyomma* sp.	1	0	0/-		
Baryphthengus martii	*Amblyomma geayi*	3	0	3/3	*R. amblyommatis*	BCNM
	Amblyomma sp.	2	0	0/-		
Myrmotherula schisticolor	*Amblyomma nodosum*	0	5	5/0		
	Amblyomma sp.	0	5	0/-		
Myrmeciza exsul	*Amblyomma ovale*	1	0	1/0		
Gymnopithys leucaspis	*Amblyomma longirostre*	1	0	1/1	*R. amblyommatis*	A-SLNP
	Amblyomma geayi	2	0	2/0		
	Amblyomma varium	3	0	3/0		
Hylophylax naevaoides	*Amblyomma* sp.	0	1	1/0		
Glyphorynchus spirurus	*Amblyomma longirostre*	2	0	2/2	*R. amblyommatis*	SNP
	Amblyomma sp.	1	0	0/-		
Xiphorhynchus susurrans	*Amblyomma nodosum*	0	1	0/-		
	Amblyomma sp.	0	1	0/-		
Manacus vitellinus	Amblyomma geayi	2	0	2/2	*R. amblyommatis*	GI
	Amblyomma nodosum	0	1	1/0		
	Amblyomma varium	1	0	1/1	*R. amblyommatis*	GI
	Amblyomma sp.	1	0	0/-		
Ceratopipra mentalis	*Amblyomma longirostre*	2	0	2/0		
	Amblyomma geayi	2	1	3/3	*R. amblyommatis*	PNS
	Amblyomma nodosum	0	2	2/0		
	Amblyomma sp.	3		2/0		
Mionectes oleagineus	*Amblyomma longirostre*	5	0	5/4	*R. amblyommatis*	BCNM
	Amblyomma sp.	2	0	0/-		
Oncostoma olivaceum	*Amblyomma longirostre*	2	0	2/2	*Rickettsia* st. C325	GA
	Amblyomma sp.	0	1	0/-		
Cyphorhinus phaeocephalus	*Amblyomma longirostre*	1	0	1/1	*R. amblyommatis*	GA
	Amblyomma naponense	2	0	2/0		
	Amblyomma ovale	1	0	1/0		
	Amblyomma varium	1	1	2/0		
	Amblyomma sp.	9	1	2/0		
Turdus grayi	*Amblyomma nodosum*	0	2	2/0		
	Amblyomma sp.	1	0	0/-		
Catharus ustulatus	*Amblyomma longirostre*	2	0	2/2	*R. amblyommatis*	BCNM
	Amblyomma calcaratum	1	0	1/0		
	Amblyomma sp.	1	0	0/-		
Basileuterus rufifrons	*Amblyomma longirostre*	1	0	1/1	*R. amblyommatis*	MNR

……continued on the next page

TABLE 3. (Continued)

Birds Species	Ticks Species	L	N	Tested ticks[a]	*Rickettsia* species	Sites[a]
Eucometis penicillata	*Amblyomma longirostre*	11	0	11/8	*R. amblyommatis*	GA, PNS
	Amblyomma geayi	1	0	1/0		
	Amblyomma nodosum	0	3	3/0		
Cyanocompsa cyanoides	*Amblyomma longirostre*	1	0	1/1	*R. amblyommatis*	PNS
	Amblyomma geayi	2	0	2/1	*R. amblyommatis*	GI
	Amblyomma sp.	3	0	1/0		
Habia fuscicauda	*Amblyomma nodosum*	0	1	1/0		
	Amblyomma varium	0	1	1/0		
Saltator striatipectus	*Amblyomma nodosum*	0	7	7/0		
Euphonia laniirostris	*Amblyomma longirostre*	3	0	3/1	*R. amblyommatis*	GA
	Amblyomma geayi	1	0	1/1	*R. amblyommatis*	GA
	Amblyomma longirostre	1	0	1/0		
not identified 1	*Amblyomma longirostre*	0	1	0/-		
not identified 2	*Amblyomma nodosum*	0	7	6/0		
not identified 3	*Amblyomma* sp.	1	1	1/0		
not identified 4	*Amblyomma* sp.	0	1	0/-		

In regards to the finding of *Rickettsia* in these ticks, this study presents the first data from Panama. Although Miller *et al*. (2016) indicated that they did not find evidence that Panamanian wild birds were involved in the ecology of the Spotted Fever Group *Rickettsia*, these authors did not perform molecular analyzes on their ticks, nor did they develop experimental studies that allowed them to affirm or deny this fact. In Central America, studies on tick parasitizing birds developed in Honduras (Novakova *et al.* 2015) and Costa Rica (Ogrzewalska *et al.* 2015), revealed the presence of *R. amblyommatis* in *A. longirostre*, *Rickettsia bellii* in *Amblyomma sabanerae*, and *Rickettsia* sp. in *Ixodes minor*. Together, these data report at least five species of rickettsiae in ticks collected of birds from Central America, and until now, none of these reports have detected pathogenic species such as *R. rickettsii* or *Rickettsia parkeri*.

During our study, *R. amblyommatis* or a closely related species was detected in *A. longoristre* collected from seven species of birds: *A. geayi* from four species of birds, and *A. varium* from one species of bird. *Rickettsia amblyommatis* has a wide distribution and has been found infecting at least 10 species of ticks in seven countries of America (Labruna *et al.* 2011; Karpathy *et al.* 2016), and it is considered the most common *Rickettsia* in Panama (Bermúdez *et al.* 2016; Bermudez & Troyo 2018).

Compared to pathogenic rickettsiae as *R. rickettsii* or *R. parkeri*, *R. amblyommatis* is reported in more species of ticks and also the rate of infection in ticks is higher. Moreover, according Macaluso *et al.* (2002), high rates of *R. amblyommatis* infection in ticks could inhibit the transovarial transmission of other *Rickettsia*. This fact could explain that this species is more frequent than other *Rickettsia* in ticks. On the other hand, its pathogenicity in vertebrates remains unknown, though it has associated with a rash in humans (Billiter *et al.* 2007), and there are serological evidence of exposure of humans and animals (Labruna *et al.* 2007; Bermúdez *et al.* 2012; Costa *et al.* 2017).

In our study, from two *A. longirostre* larvae we amplified a fragment of *gltA* gene without no closeness with another *Rickettsia* reported in America, but with *Rickettsia* IrlB1/Warsaw and '*Candidatus* Rickettsia mendelii', found in two nymphs *Ixodes ricinus* feeding on wild birds. Portions of the *omp*A, *omp*B, *sca*4, and *htr*A genes were not amplifiable in these samples

(Hajduskova *et al*. 2016), thus we were not able to define the species nor the strain. Further studies will be needed to determine its taxonomic status and related ecology. Unfortunately, due to exhaustion of the DNA samples, it was not possible to continue testing them for the presence of other rickettsial genes.

Although various other bacteria as such *Anaplasma, Ehrlichia* or *Borrelia* (Schulze *et al.* 2005; Mixon *et al.* 2006; Hudman & Sargentini 2016; Pacheco *et al*. 2019; Cicuttin *et al*. 2019), and protozoa like *Hepatozoon* sp. and *Babesia* sp. (Tomassone *et al.* 2005; Ogrzewalska *et al.* 2018), have been previously found in *Amblyomma* ticks, in our study no other microorganisms were detected in the ticks. The reason may be related with the low prevalence of these pathogens in ticks or for a not homogeneous distribution of these organisms at tick populations.

The *Borrelia* genus is divided into three phylogenetic clades: relapsing fever group (RFG), the Lyme diseases group (LDG), and the most recently described *Borrelia* group associated with reptiles and monotrema (Margos *et al*. 2018). Of these, RFG is mainly associated with Argasidae ticks and lice; while LDG is mainly associated with ticks of the genus *Ixodes*, with some exceptions in *Amblyomma*. In the Neotropical region, LDG infection was recently reported in *Ixodes auritulus* and *Amblyomma aureolatum* collected of birds and vegetation in Argentina (Cicuttin *et al*. 2019), and *A. longirostre* collected from birds of Brazil (Pacheco *et al*. 2019). Both findings indicate the importance of developing more studies that help to understand the diversity of this group in species of ticks of this region.

In the case of *Bartonella* it remains controversial whether ticks are involved in transmission of pathogenic *Bartonella* spp. to vertebrates under natural conditions (Angelakis *et al.* 2010; Telford & Wormer 2010). However, in Europe, questing *I. ricinus* ticks were found infected with *B. henselae* and *B. grahamii* what highlights the need for public awareness and draws attention to the possibility of an infection with zoonotic *Bartonella* spp. after a tick bite (Janecek *et al.* 2012).

Conclusions

Detection of Rickettsial agents found in ticks from wild birds in Panama, suggest similar results with other countries. In addition, during this study there was no evidence of pathogenic species. Moreover, these results highlight two aspects of the relationship tick-bird and tick-rickettsiae in natural environment of Panama. On the one hand, emphasize previous reports of the importance of birds as hosts to immature of *Amblyomma* spp. and also expand what is known about the relationship of these ticks with rickettsiae in Panama.

Finally, due to the findings of other groups of pathogens in Neotropical ticks, it is important to maintain new research that broadens the species and the number of individuals analyzed.

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