Rickettsia parkeri strain Atlantic rainforest in ticks (Acari: Ixodidae) of wild birds in Arauca, Orinoquia region of Colombia

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ABSTRACT

Birds are important hosts for the development of the immature stages of several tick species that are vectors for disease-causing microorganisms in animals and humans. Colombia has the highest number of bird species worldwide; however, there is scarce data on the role of birds in the circulation of ticks and their associated pathogens, such as rickettsiae. The department of Arauca has a high diversity of resident and migratory (boreal and austral) birds and ticks associated with the transmission of Rickettsia. The objective of this research was to identify tick species parasitizing birds and to detect Rickettsia species in these ectoparasites. We conducted samplings in the municipalities of Arauca, Cravo Norte, and Tame between November of 2018 and August of 2019. Birds were captured using mist nets and examined for the presence of tick species. The collected ticks were morphologically and molecularly identified. Furthermore, we detected rickettsiae in ticks by amplifying fragments of the citrate synthase (gltA) and outer membrane protein (ompB) genes. We captured 606 birds belonging to 25 families and 115 species. Tick infestation rate was 3.3% (20/606) in the birds captured and eight new associations between wild birds and ticks are reported for the American continent. We identified four tick species: Amblyomma nodosum, Amblyomma longirostre, Amblyomma mixtum, and Amblyomma sp.. Moreover, we confirmed the presence of Rickettsia parkeri strain Atlantic rainforest in A. nodosum, a medically-relevant rickettsia due to cases of rickettsiosis in the American continent. This finding manifests the importance of wild birds as hosts and dispersal agents of ticks infected with pathogenic rickettsiae, as well as the need to monitor migratory birds in the Orinoquia and other regions of Colombia and America.

1. Introduction

Wild birds have an important role in the life cycle of several tick species since birds serve as hosts for the immature stages of larvae and nymphs and, in some cases of ornithophilic ticks (Ogrzewalska et al., 2009a; Flores et al., 2014; Ramos et al., 2015). Ticks can act as vectors or reservoirs of pathogenic bacteria, such as rickettsiae, which can be transmitted to animals and humans (Sonenshine et al., 2002; Parola

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Ticks have limited locomotion capacity; therefore, their dispersal largely depends on hosts (Randolph, 1998). In this sense, bird hosts can disperse ticks between habitats or even continents during migrations (Baneth, 2014; Mukherjee et al., 2014; Cohen et al., 2015; Budacheti et al., 2017). In America, there are records of several tick species of the genera Amblyomma, Ixodes, Haemaphysalis (family Ixodidae), Ornithodoros, and Argas (family Argasidae) that parasitize resident and migratory birds (Barros-Battestii et al., 2006; Ogrzewalska et al., 2008, 2015; Mukherjee et al., 2014; Cohen et al., 2015). Colombia has the highest bird diversity in the world, with 1632 resident species and 139 migratory boreal and austral species (Avendaño et al., 2017). In Colombia, there are 58 tick species (43 Ixodidae and 15 Argasidae) (Rivera-Paz et al., 2018a), including eight species associated with wild birds: Ixodes auritulus (González-Acuna et al., 2005), Amblyomma calcaratum, Amblyomma dissimile, Amblyomma longirostre, Amblyomma nodosum, Amblyomma ovale, Amblyomma varium, and Haemaphysalis leporispalustris (Osorno-Messa, 1946; Martínez-Sánchez et al., 2020). The record of Ixodes brunesceus in Calotochaetes coccineus should be revised (Osorno-Messa, 1940). In addition, three Ixodes species, which yielded low identity DNA sequences (<95%) to any tick species in GenBank are also known (Martínez-Sánchez et al., 2020).

Ticks can transmit bacteria that cause rickettsial diseases (Sonen-shine and Clifford, 1973; Ogrzewalska et al., 2009b; Cohen et al., 2015). In particular, these diseases are attributed to infection by Gram-negative bacteria of the genus Rickettsia, which comprise four groups: i) the typhus group (TG) with two members, namely Rickettsia prowazekii and Rickettsia typhi; ii) the spotted fever group (SFG) that contains more than 20 species, including Rickettsia rickettsii and Rickettsia parkeri; iii) the transitional group (TRG), which comprises Rickettsia akari and Rickettsia felis; and iv) the ancestral group (AG) that includes Rickettsia belli and Rickettsia canadensis (Quintero et al., 2013). Specifically, TG and SFG generate the highest level of concern for public health (Londono et al., 2017); for instance, R. rickettsii is the most pathogenic species in this genus, with reported lethality rates between 20% and 95% for the American continent (Abarca and Oteo, 2014). Particularly, in Colombia, there are lethality rates between 26.6% and 95% (Quintero et al., 2013; Miranda et al., 2017). R. rickettsii is the causal agent of Rocky Mountain spotted fever (RMSF), Brazilian spotted fever, or Tobia fever in Colombia (Labruna et al., 2011; Oteo et al., 2014). Moreover, there are two strains of R. parkeri known to cause rickettsiosis, namely R. parkeri sensu stricto and R. parkeri strain Atlantic rainforest (Londono et al., 2019). Like in other spotted fever group (SFG) rickettsiae, R. parkeri is transovarially (from female to eggs) and transestadially (from one life history stage to the next) transmitted to ticks. Also, R. parkeri can be horizontal acquired while tick feeds on a rickettsemic host (Godard, 2003; Walker and Ismail, 2008).

Historically, R. rickettsii has caused outbreaks of febrile illness in several regions of Colombia, such as the central (department of Cundinamarca) and southwestern regions (departments of Cordoba and Antioquia); therefore, these two regions of considered endemic areas for rickettsiosis (Patiño et al., 1937; Patiño, 1941; Acosta et al., 2006; Hidalgo et al., 2007a, Hidalgo et al., 2007b, 2011; Velez et al., 2012). Additionally, the Orinoquia region has also been proposed as an endemic area for rickettsial disease associated with the SFG, due to the confirmed circulation of rickettsiae and their vector Amblyomma mixtum in this region (Miranda et al., 2011; Riveros-Pinilla et al., 2015; River-a-Paz et al., 2016; Gómez-Quintero et al., 2017; Rivera-Paz et al., 2018b). In the last decade, there were reports of the pathogenic strain Rickettsia parkeri Atlantic rainforest associated with Amblyomma ovale in the departments of Cordoba and Antioquia (northwestern region). R. parkeri strain Atlantic rainforest is phylogenetically closely related to R. parkeri, Rickettsia africae, and Rickettsia sibirica (Spolidorio et al., 2010; Londono et al., 2014; Nieri-Bastos et al., 2018). R. africae and R. sibirica are pathogenic species distributed in the Old World, and the symptoms they cause are similar to those caused by New World species such as R. parkeri sensu stricto, and R. parkeri strain Atlantic rainforest (Paddock et al., 2004; Parolai et al., 2005; Pacheco et al., 2012). Using genetic evidence, Nieri-Bastos et al. (2018), defined that Atlantic rainforest is R. parkeri strain found in the southern part of South America that is transmitted by ticks of the A. ovale complex (i.e., A. ovale and Amblyomma aureolatum). Other tick species of the Amblyomma maculatum complex (A. maculatum, Amblyomma triste, and Amblyomma tigrinum), A. nodosum, Amblyomma parvitarsum and Dermacentor parmauproctus have been involved in the transmission of R. parkeri in America (Paddock et al., 2004, 2017; Nieri-Bastos et al., 2018; Londono et al., 2019).

A. ovale was found parasitizing resident and migratory birds (e.g., Fornicivora grisae and Parkesia novoboracensis) in Colombia (Martínez-Sánchez et al., 2020). However, R. parkeri strain Atlantic rainforest has not been detected in ticks associated with birds, although it has been detected in ticks on wild and domestic mammals in the northwestern region of Colombia (Londono et al., 2014, 2017). The only reports of genus Rickettsia associated with ticks in the Orinoquia region, are the reports of R. rickettsii on ticks of domestic animals (Rivera-Paz et al., 2018b).

Given that migratory boreal and austral birds converge in the Colombian Orinoquia region (McNish, 2007; Ocampo-Penuela, 2010), these bird species could serve as hosts and dispersal agents of ticks infected with rickettsiae. The department of Arauca in the Orinoquia (Eastern Plains) is an ideal site to study the associations among birds, ticks, and rickettsiae. Given the above, this study aimed to identify tick species associated with wild birds and detect ticks infected with rickettsiae.

2. Materials and methods

2.1. Study area

This study was conducted in eight localities in the municipalities of Arauca, Cravo Norte, and Tame, located in the department of Arauca in the Colombian Orinoquia (Table 1, Fig. 1). The region shows a typical savanna climate with a well-defined wet season between June and July and a very dry season between December and April. This precipitation pattern leads to occasional periods of flooding and drought in the Orinoquia savanna during the corresponding seasons. This region also shows high temperatures year-round with a mean of 27 °C (McNish, 2007).

The sampled localities are divided into two sub-regions: the flooded savanna (Sabana Inundable) and the Llanos foothills (Piedemonte Llanero) (Rodríguez-Durán, 2019). The flooded savanna comprises estuaries, gallery forests, and isolated forests locally known as “matas de monte” (McNish, 2007). The Llanos foothills are a sub-region between 250 m and 500 m of elevation, adjacent to the Eastern mountain range of Colombia. Both sub-regions are destined for agropecuaria activities, such as rice, oil palm, corn, and plantain crops and extensive livestock farming (Viloria de la Hoz, 2009). We conducted samplings in the eight localities to identify bird species parasitized by ticks and to detect possible infections of these ticks with rickettsiae. Localities 1–5 were sampled between November and December of 2018, locality 6 was sampled in March, and localities 7 and 8 were sampled between July and August of 2019 (Table 1, Fig. 1). The sampling dates coincided with the arrival of migratory birds from the northern (migratory boreal) and southern (austral migratory) hemispheres.

2.2. Bird captures and tick collection

Birds were captured using eight mist nets at each locality (12 m long x 2 m wide, 36 mm mesh). The mist nets were randomly installed and opened between 6:00 and 17:00 h. The birds were taxonomically classified according to Remsen et al. (2020). The residency status of the birds (i.e., resident, migratory boreal or austral) was defined based on Avendaño et al. (2017). Each bird was completely examined for ticks for...
The ticks were collected with entomological tweezers and conserved in Eppendorf tubes with 96% ethanol. The birds were marked with a small cut in the first rectrix of the tail to avoid re-counting the individuals and then, released at the capture site.

### 2.3. Identification of ticks and rickettsiae

The ticks collected were taxonomically identified based on their external morphology according to the literature and taxonomical keys (Kohls, 1956; Jones et al., 1972; Barros-Battesti et al., 2006; Mehlhorn, 2008; Martins et al., 2010; Nava et al., 2014, 2017). The prevalence of tick infestation in birds was calculated as (Number of infested individuals/Number of examined individuals) x 100.

The nymphs and larvae were molecularly confirmed through PCR amplification of two mitochondrial gene fragments. First, DNA extraction was performed using the DNeasy Blood and Tissue (Qiagen) and Wizard® Genomic DNA Purification (Promega) kits, according to the manufacturer’s instructions. Next, we amplified a 460 bp fragment of the 16S rDNA gene with primers 16S F 5′-CCGGTCTGAACTCA-GATCAAGT-3′ and 16S R 5′-CTGCTCAATGATTTTTAATTGCTGTGG-3′ (Norris et al., 1996; Mangold et al., 1998), as well as a 700 bp fragment of the cytochrome oxidase subunit I (COI) using primers LCO1490 F 5′-GGTCACAAGAGTAAAATATTGTTGG-3′ and HCO2198 R 5′-TAAACTTCAGGTGACCAAAAAATCA-3′ (Folmer et al., 1994). The voucher specimens of the ticks were deposited in the ectoparasite collection of the Museo de Historia Natural de la Universidad de Caldas.

| Municipality | Locality | Locality number | Geographical coordinates | Altitude (m above sea level) | Habitat type |
|--------------|----------|-----------------|--------------------------|-----------------------------|--------------|
| Arauca       | Las Plumas 1 | 06°36′40″ N 70°31′51″ W | 120 | Floodplain forest, Gallery forest |
| Arauca       | Las Plumas 2 | 06°37′01″ N 70°31′30″ W | 123 | Floodplain forest, Medano |
| Arauca       | Las Plumas 3 | 06°36′15″ N 70°29′52″ W | 123 | Floodplain forest |
| Arauca       | Las Plumas 4 | 06°36′15″ N 70°29′52″ W | 112 | Floodplain forest |
| Cravo Norte  | El Deleite 5 | 06°32′15″ N 70°31′14″ W | 111 | Floodplain forest |
| Tame         | Sacta Ises 6 | 06°24′52″ N 71°52′04″ W | 203 | Agricultural area |
| Arauca       | Km 9, via Arauca-Arasquita 7 | 07°00′53″ N 70°44′36″ W | 120 | Wooded areas; High stubble |
| Arauca       | El Socorro 8 | 06°46′39″ N 70°42′25″ W | 134 | Floodplain forest, Gallery forest |

*Locality numbers indicated in Fig. 1.*
For the detection and molecular identification of *Rickettsia* species, we evaluated 30 ticks (14 larvae, 16 nymphs) and amplified a ~401 bp fragment of the citrate synthase (gltA) gene using primers CS-78 and CS-323 (Labruna et al., 2004). The gltA gene is present in all species of the genus *Rickettsia*. We performed a second PCR amplification on the samples that were positive for gltA using primers rompB-OF and rompB-OR, proposed by Choi et al. (2005), which amplify a fragment of 511 bp of the outer membrane protein (ompB) that is present in *Rickettsia* species of the spotted fever group (SFG) (Choi et al., 2005). In each set of reactions, negative (ultrapure water) and positive controls for *Rickettsia* vari DNA (kindly supplied by Dr. Marcelo Bahia Labruna) were included. The PCR products were visualized through 1.0% agarose gel electrophoresis run with TBE 1X (pH 8.0) buffer at 110 V/50 mA and stained with SYBR® Safe dye. The products were visualized on a Gel Doc-It2 310 (UVF) photodocumenter and purified using Wizard® SV Gel and PCR Clean-Up System (Promega), according to the manufacturer’s instructions. The purified amplicons were Sanger sequenced at Macrogen (MHN-UCa).

Species confirmation was performed using a Maximum Likelihood (ML) similarity analysis using the Kimura 3-parameter disdatabase in Geneious Prime®. The 15 different sequences obtained in this study are [MT471971-MT471980] for the 16S rRNA mitochondrial gene; [MT439632] for the COI mitochondrial gene; [MT471971-MT471980] for the COI mitochondrial gene; [MT439632] for the COI mitochondrial gene; [MT501330-MT501331] for the citrate synthase gltA gene; [MT501328-MT501329] for the outer membrane protein ompB gene.

### 4. Discussion

We found eight new associations between birds and ticks, including

### Table 2

| Localitya | Host Bird | Tick species (number of specimens/stage) | No. infested/No. tested (%) | Closest identity (gene: accession number) |
|-----------|-----------|----------------------------------------|-----------------------------|-----------------------------------------|
| 6         | Cracidae  | Oturals ruficauda                       | 1/1 (100)                   | A. mixtum [Col: MF363073.1] 100%       |
| 7,8       | Thamnophilidae | Formicivora grisea                       | 2/5 (40)                    | A. nodosum [16S: MH818417.1] 99.2%     |
| 7         | Furnariidae | Dendreps picus                          | 1/4 (25)                    | N.D.                                    |
| 7         | Tyrannidae | Camptostoma obsolatum                   | 1/20 (5)                    | N.D.                                    |
| 6         | Tyriridae  | Pachyrhamphus                           | 1/7 (14.3)                  | A. longirostre [16S: MH818419.1] 100%  |
| 7         | Trogloctidae | Trogloctes aedon                        | 2/10 (20)                   | A. nodosum [16S: FJ442402.1] 99.6%     |
| 4         | Turdidae   | Turdus philomelos                       | 1/4 (25)                    | N.D.                                    |
| 6         | Turdus aedon | Camphorhynx clypeus                    | 1/6 (12.5)                  | A. nodosum [16S: MH818417.1] 99.7%     |
| 6         | Turdus ignobilis | Amblyomma sp. (2/larvae)               | 1/7 (14.3)                  | A. nodosum [16S: MH818417.1] 99.7%     |
| 6         | Fringillidae | Eubophus lanio                         | 1/5 (20)                    | N.D.                                    |
| 7,8       | Icteridae  | Icterus chrysaet                         | 2/13 (15.4)                 | A. nodosum [16S: FJ442402.1] 100%      |
| 7         | Thraupidae | Ramphoculus carbo                       | 2/5 (40)                    | A. nodosum [16S: MH818417.1] 99.5%     |
| 7,8       | Thraupidae | Sporophila angolensis                   | 1/19 (5.3)                  | A. nodosum [16S: FJ442402.1] 99.6%     |
| 7,8       | Thraupidae | Sporophila intermedia                   | 2/9 (22.2)                  | A. nodosum [16S: FJ442402.1] 99.6%     |

a Locality numbers indicated in Table 1 and Fig. 1.

b Identification by external morphology.

c N.D: Not Done (poor DNA quality).

D Larvae were morphologically assigned to the same morphotype and a selected number of individuals were randomly selected for molecular identification.
Tick species infected with Rickettsia in the department of Arauca and results of BLAST searches for the DNA sequences of the rickettsiae detected in this study.

| Host Bird | Tick species | No. infected/No. tested (%) | Closest identity (%) in GenBank (accession number) according to the Rickettsia gene |
|-----------|--------------|------------------------------|---------------------------------------------------------------------------------|
| F. grisea | A. nodosum   | 1/30 (3.33)                  | R. parkeri strain Atlantic rainforest [MN027564] 100% (CP0940325)                |
| S. angolensis | A. nodosum | 1/30 (3.33)                  | R. parkeri strain Atlantic rainforest [MN027564] 100% (CP0940325)                |

Tick species infected with Rickettsia.

Five between bird species T. aedon, I. chrysater, S. angolensis, S. intermedia, S. coerulescens and the tick species A. nodosum, as well as two associations between O. ruficauda and C. obsoletum and ticks A. mixtum and A. longirostre, respectively. Moreover, we provide the first report of infestation of C. griseus with ticks of the genus Amblyomma. On the other hand, seven interactions found here were previously reported in the literature (Tolesano-Pascoli et al., 2010; Ogrzewalska et al., 2011b; Luz et al., 2012; Pascoal et al., 2012; Torga et al., 2013; Ramos et al., 2015; Lugarini et al., 2015; Lima et al., 2018; Martínez-Sánchez, 2020). These findings support the assumption of the important role of wild birds in the life cycle of several Neotropical tick species by serving as hosts for immature stages (Ogrzewalska et al., 2009a, 2009b; Budacheti et al., 2017; Nava et al., 2014; Lugarini et al., 2015). Additionally, the tick species A. nodosum and A. longirostre had not been recorded in the department of Arauca; therefore, our results expand the known distribution of these species in Colombia (Osorno-Mesa, 1940; Luque, 1948; Wells et al., 1981; Benavides-Montañal et al., 2018; Rivera-Páez et al., 2018a; Acevedo-Gutiérrez et al., 2020).

We found that 93% of the infested bird species were Passeriformes, which agrees with previous research conducted in Brazil (Labruna et al., 2007; Ogrzewalska et al., 2009a; Lugarini et al., 2015; Luz et al., 2017). Furthermore, these studies state that Passeriformes are important hosts for ticks, such as A. longirostre and A. nodosum, due to the high frequency in which immature stages of both tick species parasitize Passeriformes.

Therefore, Passeriformes are considered their primary hosts (Nava et al., 2017).

In this study the birds parasitized by A. longirostre (C. obsoletum and Pachyurus polyxanthias) seek food in canopy and subcanopy (Hilty and Brown, 1986; Restall et al., 2007; Del Hoyo et al., 1992–2011), therefore, it has been hypothesized that this tick completes its life cycle in tree canopies (Labruna et al., 2007; Nava et al., 2017; Suzin et al., 2020). Other bird species parasitized by A. nodosum (Table 2) inhabit in lower strata, such as in the understory and ground (Hilty and Brown, 1986; Restall et al., 2007; Del Hoyo et al., 1992–2011), which seems to be related to tick infestation as mentioned by Labruna et al. (2007).

Other bird species belonging to the orders Gruiformes, Ciconiiformes, and Galliformes were found as hosts for immature stages of ticks of the Amblyomma cajennense complex (Labruna et al., 2007; Ogrzewalska et al., 2009a; Acevedo-Gutiérrez et al., 2020). Similarly, we found O. ruficauda (Galliforme) infected with the species A. mixtum, which belongs to the Amblyomma cajennense complex. The association between O. ruficauda and A. mixtum is epidemiologically relevant since this tick species is a vector of R. rickettsii (Rivera-Páez et al., 2016, 2018a; Bermúdez and Troyo, 2018), one of the most pathogenic rickettsiae on the Americas (Parola et al., 2013; Labruna et al., 2014). The distribution of O. ruficauda in northeastern and eastern Colombia (Hilty and Brown, 1986; Ayerbe-Quíñones, 2018), might play an important role as hosts and dispersers of ticks infected with rickettsia in these regions where the presence of R. rickettsii and its vector ticks has been reported (Rivera-Páez et al., 2016, 2018b).

Amblyomma nodosum represented 98.5% (458/465) of the ticks found in this study, which supports the hypothesis that this species is highly associated with Passeriformes in the Neotropics (Labruna et al., 2007; Ogrzewalska et al., 2009b; Lugarini et al., 2015; Lima et al., 2018). Particularly, two individuals of the species T. aedon showed high parasite loads of A. nodosum. In this regard, several life history traits of this bird species could favor its infestation with ticks; for example, the foraging behavior in the low strata of the vegetation and the elaboration of the nests in cavities (Kroodsma and Brewer, 2005). These sites likely provide favorable microclimatic conditions for tick establishment and survival (Pfäffle et al., 2013). Additionally, the two T. aedon individuals infested with ticks were found during the incubation period so the larvae could have been acquired in the nest (Johnson and Albrecht, 2020; Pacjeck et al., 1998). In the region, the high percentage of birds infested with immature stages of A. nodosum (75%) agrees with observations from other locations in America (Labruna et al., 2007; Ogrzewalska...
et al., 2009b; Lugarini et al., 2015; Lima et al., 2018). Conversely, adult ticks have been observed on mammals; for instance, in the Orinoquia, adult ticks are associated with mammal species of the order Pilosa, which are commonly found in the region (Ayua-Cuer, et al., 2019). These mammals are known hosts for A. nodosum (Luque, 1948; Witter et al., 2016; Moerbeck et al., 2018).

The detection of R. parkeri strain Atlantic rainforest in A. nodosum, which, in turn, was found infesting F. grisea and S. angolensis birds in the department of Arauca, supports the hypothesis that the Colombian Orinoquia should be considered an endemic area for rickettsial disease associated with the SFG. *Rickettsia parkeri* (strains NOD and COOPERI) were detected in A. nodosum collected from birds (Ogrzewalska et al., 2009b, 2011a; Lugarini et al., 2015); however, our detection of *R. parkeri* strain Atlantic rainforest in *A. nodosum* is the first association reported in the American continent for these species. *R. parkeri* strain Atlantic rainforest appears to be associated with species of the *A. ovale* complex and the NOD strain to *A. nodosum* in South America (Nier-i-Bastos et al., 2018), which suggests a Neotropical origin of these strains.

Despite the distribution of *A. nodosum* includes the Neotropical region (Nava et al., 2014, 2017), there are no human reports of infestation by this ticks (Guglielmone et al., 2014; Nava et al., 2017; Moerbeck et al., 2018). In Colombia, *A. nodosum* has only been reported in the central and western areas of the country in the departments of Antioquia, Meta, Tolima, and Valle del Cauca (Osorno-Mesa, 1940; Luque, 1948; López and Parra, 1985; Benavides-Montaño et al., 2018) infesting anteaters (*Tamandua tetradactyla*). Osorno-Mesa (1940), and Martínez-Sánchez et al. (2020), reported records of infected wild birds from central Colombia. Our results extend the distribution of infested birds to the Eastern planes of the country. *R. parkeri* strain Atlantic rainforest has been recorded in Colombia in *A. ovale* ticks collected from domestic (dogs) and wild mammals (*Proechimys semispinosus*) (Londoño et al., 2014, 2017). However, no cases of rickettsial infections by this strain of *R. parkeri* have yet been reported in Colombia. In contrast, in other South American countries such as Brazil, clinical cases of *R. parkeri* strain Atlantic rainforest infections mainly associated with the transmission mediated by ticks of the *A. ovale* complex (*A. ovale* and *A. aureolatum*) have been reported in the last decade (Spolidorio et al., 2010; Nier-i-Bastos et al., 2016; Da Paixão-Sevá et al., 2019). Detection of *R. parkeri* strain Atlantic rainforest in ticks that parasitize wild birds, suggests the role that these vertebrates may have for the lodging and dispersal of ticks infected with rickettsia. In this context, it is necessary to carry out studies to expand the knowledge of the associations between birds, ticks, and rickettsiae.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2020.09.001.
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