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Original article

Borrelia spp. in ticks and birds from a protected urban area in Buenos Aires city, Argentina

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ABSTRACT

This study was aimed to know epidemiological aspects of *Borrelia* spp. in a protected urban area of Buenos Aires city, Argentina, where thousands of people visit this area for recreational purposes. Ticks were collected from vegetation, birds and dogs. Three hundred and forty birds belonging to 43 species, 41 genera, 18 families and six orders were captured (90.3% corresponded to the order Passeriformes). One hundred and twenty ticks were collected from 47 birds (13.8%) belonging to 10 species (23.2%), all of them from to the order Passeriformes (Emberizidae, Furnariidae, Parulidae, Thraupidae, Troglodytidae, Turdidae). Ticks were identified as *Ixodes auritulus* (56 larvae, 44 nymphs and 8 females) and *Amblyomma aureolatum* (1 larva and 11 nymphs).

One thousand and ninety-one ticks collected from vegetation, 100 ticks collected from birds, and 89 ticks from dogs were tested for *Borrelia* infection by PCR trials targeting the flagellin (*fla*) and 16S rRNA genes. In addition, 101 blood and 168 tissue samples from birds were analyzed. Nine nymphs of *A. aureolatum* (2.1%) and four nymphs of *I. auritulus* (0.7%) collected from vegetation were positive. Five nymphs of *A. aureolatum* (45.4%), and five pools of larvae (minimum infection rate 13.5%), 18 nymphs (40.9%) and one female (14.3%) of *I. auritulus* collected on birds were also positive. The remaining samples were negative. The phylogenetic tree generated with *fla* sequences shows that seven of the eight different haplotypes of *Borrelia* detected in *I. auritulus* conform an independent lineage within the *Borrelia burgdorferi* sensu lato complex together with sequences of *Borrelia* sp. detected in *I. auritulus* from Canada and Uruguay. The *fla* sequences of *Borrelia* obtained from *A. aureolatum* and one sequence of a single specimen of *I. auritulus* conform a phylogenetic group with *Borrelia turcica*, *Borrelia* sp. isolated from a tortoise in Zambia, *Borrelia* spp. detected in *Amblyomma maculatum* from USA and *Amblyomma longirostre* from Brazil. The epidemiological risk that implies the infection with *Borrelia* genospecies associated with *I. auritulus* seems to be low because this tick is not aggressive to humans, but it helps to maintain borrelial spirochetes in the enzootic transmission cycles. The pathogenicity to humans of the *Borrelia* found in *A. aureolatum* is unknown; however, adults of this tick species are known to bite humans. This is the first report of the presence of *Borrelia* in *A. aureolatum*. Further investigations are necessary to know the risk of transmission of borreliosis by hard ticks in the study area.

1. Introduction

The genus *Borrelia* currently contains more than 30 genospecies, which are transmitted by arthropod vectors and exhibit different degrees of pathogenicity in mammalian and avian hosts (Adeolu and Gupta, 2014; Takano et al., 2011; Talagrand-Reboul et al., 2018). This genus was traditionally classified into two groups: Lyme borreliosis (LB) *Borrelia burgdorferi* sensu lato complex, transmitted principally by ticks of the genus *Ixodes*, and relapsing fever (RF) *Borrelia*, transmitted primarily by argasid ticks, a few species by ixodid ticks, and also by lice (Adeolu and Gupta, 2014; Takano et al., 2011; Talagrand-Reboul et al., 2018). The LB group consists of approximately 18 genospecies formally described, where the principal LB-causing agents include *Borrelia burgdorferi* sensu stricto (s.s.), *Borrelia afzelii* and *Borrelia garinii*, all of them transmitted by ticks belonging to the *Ixodes ricinus* complex (e.g. *Ixodes pacificus* and *Ixodes scapularis* in the United States, *Ixodes ricinus*

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in Europe, and *Ixodes persulcatus* in Europe and Asia) (Adeolu and Gupta, 2014; Takano et al., 2011; Talagrand-Reboul et al., 2018). In turn, according to the main host, LB can be divided into four subgroups: a) species adapted to mammals (e.g. *B. afzelii*); b) to birds (e.g. *B. garinii*, *Borrelia americana*); c) to reptilian hosts (e.g. *Borrelia lusitaniae*) or d) species with no specialized hosts (e.g., *B. burgdorferi* s.s.) (Mechai et al., 2016; Rudenko et al., 2014).

Within tick-borne RF (TBRF), there are four subgroups according to ecological aspects, a) Old-World TBRF borreliae (e.g., *Borrelia hispanica*), b) New-World TBRF borreliae (e.g. *Borrelia turicatae*), c) the worldwide avian TBRF borreliae (e.g., *Borrelia anserina*), (the three subgroups transmitted by soft ticks), and c) the worldwide hard tick-borne relapsing fever group (HTBRF) (e.g., *Borrelia miyamotoi*) (Cutler, 2010; Talagrand-Reboul et al., 2018). There also are studies which support a third major *Borrelia* group, the reptile-associated (REP) *Borrelia* spp. group, also transmitted by ixodid ticks (Lee et al., 2014; Loh et al., 2016; Takano et al., 2011, 2010).

In South America, *Borrelia chilensis* and different haplotypes of new genospecies from the LB group were found in *Ixodes longiscutatus* from Brazil (Dall'Agnol et al., 2017), *Ixodes aragaoi* and *Ixodes auritulus* from Uruguay (Barbieri et al., 2013; Carvalho et al., 2019), *Ixodes stilesi* and *I. auritulus* from Chile (Ivanova et al., 2014; Muñoz-Leal et al., 2019), and *Ixodes pararicinus*, *Ixodes* sp. cf. *I. neuquenensis* and *Ixodes sigelos* from Argentina (Nava et al., 2014; Saracho Bottero et al., 2017). Regarding RF group, it was only found in *Ornithodoros* soft ticks in Bolivia and Brazil (Parola et al., 2011; Muñoz-Leal et al., 2018) and in *Amblyomma longirostre* from Brazil (Pacheco et al., 2019).

In Buenos Aires city, four species of ixodid ticks have been found: *Amblyomma aureolatum*, *Amblyomma triste*, *I. auritulus* and *Rhipicephalus sanguineus* sensu stricto[1] (Cicuttin et al., 2017). The first three species were found exclusively in the protected urban area Reserva Ecológica Costanera Sur (Cicuttin et al., 2017). Thousands of people visit this area for recreational purposes, and they are potentially exposed to tick bites. In view of this situation, this study was aimed to determine the presence of *Borrelia* spp. in four tick species known to be present in a protected urban area of Buenos Aires city.

2. Methods

2.1. Study area

This study was conducted in a protected urban area (Reserva Ecológica Costanera Sur; 34° 36′ S, 58° 21′ W) from Buenos Aires city with permissions of the authorities (numbers 30/09/2010, 01/2014, 20/2016, 32/2016 and 17/2018). This area borders with two crowded neighborhoods, Puerto Madero and Rodrigo Bueno, with contrasting socioeconomic characteristics, and the La Plata River. It is characterized by different environments of artificial origin, such as marshes, lagoons, pastures, thickets and forests, in addition to the beaches of the river. Birds represent the most diverse group of vertebrates. Regarding reptiles, the lizard *Salvator merianae* is a typical inhabitant of the Reserve. Mammals mainly include rodents from the families Muridae, Cricetidae and Caviidae, and opossums (family Didelphidae). Furthermore, stray dogs (*Canis lupus familiaris*), which circulate throughout the reserve and surrounding poor neighborhoods, constitutes an important component of the fauna in the area (Wais de Badgen, 2013).

2.2. Birds and tick collection

Birds were caught by using mist nets, which remained active during morning and twilight hours, in two-day convenience samplings per season (total 240 net-hours) between winter of 2016 and autumn of 2017. Each individual bird was determine using Narosky and Yzurieta (2010) and classified according to Clements et al. (2016), and examined for ticks using fine-tipped tweezers. All samples were stored in 70% ethanol. The ticks were identified following the taxonomic keys and specific descriptions presented in Nuttall (1916) and Nava et al. (2017) and by comparison with reference material deposited in the tick collection of Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria Rafaela, Argentina.

In addition, free-living ticks were monthly collected from vegetation in 2013 and 2014 by using cloth flags and carbon dioxide traps. Ticks attached to stray dogs were also collected. The detailed procedures for the fieldwork and tick taxonomic identification have been published elsewhere (Cicuttin et al., 2017). Two more samplings were carried out with cloth flags in September and October 2018.

2.3. Tissue samples of birds

Approximately 100 µl of blood was collected from the jugular vein from mainly large Passeriformes in good physical condition caught for tick collection. The blood samples were frozen at -20 °C. The birds were released into the same habitats in which they had just been caught. In addition, birds found dead in the area and derived for diagnosis of zoonoses at Instituto de Zoonosis Luis Pasteur (Buenos Aires) between 2011 and 2017, were also included in this study.

2.4. DNA extraction and PCR amplification

For DNA extraction, larvae were grouped in pools of 1–10 specimens according to species, date and host of collection, while nymphs and adults were extracted individually. Pools of organs (spleen and liver) of each individual bird which was found dead were exanimated. DNA extraction was performed using the High Pure PCR Template Preparation Kit (Roche, Mannheim, Germany) following the manufacturer's instructions. The detection of *Borrelia* DNA was carried out by a nested PCR targeting a fragment of the flagellin gene (*fla*) of *Borrelia* spp. Positive samples were further used to amplify a 654 bp fragment of the 16S rRNA gene by a nested PCR. The PCRs were performed according to the indications of the authors (Barbour et al., 1996; Richter et al., 2002). Primers used are detailed in Table 1. For all PCR reactions, nuclease free water was used as negative control, and DNA of the *B. burgdorferi* s. s. (kindly provided by the Laboratorio de Espiroquetas y Patógenos Especiales, Instituto de Salud Carlos III, Spain) served as positive control.

2.5. Sequence comparison and phylogenetic analysis

PCR-products were purified using the Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, USA) and sequenced with a 3500 Genetic Analyzer sequencer (Applied Biosystems, Foster City, USA). The obtained sequences were edited using BioEdit Sequence Alignment Editor (Hall, 1999) with manual edition whenever it was necessary and aligned with the program Clustal W (Thompson et al., 1994). Phylogenetic analysis was performed with the maximum-likelihood (ML) method. The best-fitting substitution model was determined with the Bayesian Information Criterion using the ML model test implemented in MEGA 6 (Tamura et al., 2013). A tree based on *Borrelia fla* partial sequences was generated with the general time reversible model by using gamma distribution with invariant sites (GTR + G + I). Gaps were excluded in the pairwise comparison, and support for the ML topology was tested by bootstrapping over 1000 replications. The 16S sequences were compared with those sequences of *Borrelia* spp. deposited in GenBank by using BLAST (www.ncbi.nlm.nih.gov/blast).

[1] Cicuttin et al. (2017) mentioned the presence of *R. sanguineus* s.l. in Buenos Aires City, but according to the information given by Nava et al. (2018), it can be inferred that the taxon present in this area corresponds to *R. sanguineus* s.s.

Table 1
Primers for detection of *Borrelia* spp.

Target gene	Name	Sequence 5´-3´	Reference
Flagellin	Fla-LL	ACA TAT TCA GAT GCA GAC AGA GGT	(Barbour et al., 1996)
	Fla-RL	GCA ATC ATA GCC ATT GCA GAT TGT	
	Fla-LS	AAC AGC TGA AGA GCT TGG AAT G	
	Fla-RS	CTT TGA TCA CTT TC ATT CTA ATA GC	
16S Rrna	16S1A	CTA ACG CTG GCA GTG CGT CTT AAG C	(Richter et al., 2002)
	16S1B	AGC GTC AGT CTT GAC CCA GAA GTT C	
	16S2A	AGT CAA ACG GGA TGT AGC AAT AC	
	16S2B	GGT ATT CTT TCT GAT ATC AAC AG	

3. Results

3.1. Ticks collected on birds

A total of 340 birds belonging to 43 species, 41 genera, 18 families and six orders were captured (90.3% corresponded to the order Passeriformes). Ticks were collected from 47 birds (13.8%) from 10 species (23.2%), all of them belonging to the order Passeriformes (Emberizidae, Furnariidae, Parulidae, Thraupidae, Troglodytidae, Turdidae) (Tables 2 and 3). The prevalence of tick infestation was 10.8% (8/74) in autumn, 29.0% (20/69) in winter, 13.5% (12/89) in spring and 6.5% (7/108) in summer.

One hundred and twenty ticks (57 larvae, 55 nymphs and 8 females) were collected from birds. Ticks were identified as *I. auritulus* (56 larvae, 44 nymphs and 8 females) and *A. aureolatum* (1 larva and 11 nymphs) (Tables 2, 3 and 4). Among the six families of infested passerines, the Turdidae family, represented by two species, accumulated 71.7% (86/120) of the total ticks collected on birds in this study. Co-infestation with *I. auritulus* and *A. aureolatum* was observed in three specimens of *Turdus rufiventris* and in two specimens of *Turdus amaurochalinus*.

3.2. Molecular detection of Borrelia spp.

One thousand and ninety one ticks collected from the vegetation (determined as larvae, nymphs and adults of *A. aureolatum*, *A. triste* and *I. auritulus*) were analyzed by nested PCR of the *Borrelia fla* fragment. Nine nymphs of *A. aureolatum* (2.1%) and four nymphs of *I. auritulus* (0.7%) were positive (Table 5). The positive nymphs of *A. aureolatum* were collected in winter, and spring, while positive-*I. auritulus* were found in spring, summer and autumn. One hundred ticks collected of birds were analyzed by nested PCR of the *Borrelia fla* fragment (Table 6). Five nymphs of *A. aureolatum* (45.4%) and five pools of larvae (minimun infection rate 13.5%), 18 nymphs (40.9%) and one female (14.3%) of *I. auritulus* were positive. The positive ticks were collected from 16 birds (of a total of 47 infested birds; 34.0%): 10 *T. rufiventris* (of a total of 17 infested; 58.8%), five *T. amaurochalinus* (of a total of 12 infested; 41.7%) and one *Saltator aurantiirostris* (of a total of 2 infested; 50.0%) (Suppl Table 1). By last, eighty-nine ticks collected on dogs (*A. aureolatum*: 82 females and 4 males; *A. triste*: 1 female; *R. sanguineus* s.s.: 1 female and 1 male) were analyzed by nested PCR of the *Borrelia fla* fragment. All samples were negative.

3.3. Tissue samples of birds

Of the 340 birds captured, 101 blood samples were obtained (Suppl Table 2). Considering the 47 birds that had ticks, 30 were sampled (15 *T. rufiventris*, 11 *T. amaurochalinus*, 2 *S. aurantiirostris*, 1 *Stephanophorus diadematus* and 1 *Furnarius rufus*), of which 15 had *Borrelia*-positive ticks (9 *T. rufiventris*, 5 *T. amaurochalinus* and 1 *S. aurantiirostris*). Plus, 168 tissue samples from dead birds were studied (Suppl Table 3). All samples were negative by nested PCR of the *Borrelia fla* fragment.

3.4. Sequence comparison and phylogenetic analysis

Forty-one amplicons were sequenced from the 42 positive samples subjected to the nested PCR of *fla* fragment.

The ML phylogenetic tree generated with *fla* sequences shows that seven of the eight different haplotypes of *Borrelia* detected in *I. auritulus* (haplotypes G1000, G754, G1138, G911, G1128, G1107 and G1002; GenBank accession numbers MK984824, MK984826-MK984831) conform an independent lineage within the *B. burgdorferi* s.l. complex (Fig. 1). These haplotypes are closely related to sequences of *Borrelia* sp. detected in *I. auritulus* in Canada and Uruguay (Fig. 1). All these sequences conform a monophyletic group with more than 80% of bootstrap support (Fig. 1).

The *fla* sequences of *Borrelia* obtained from *A. aureolatum* (samples G747, G1127, G672 and G674; GenBank accession number MK984823) were identical among each other and conform a phylogenetic group together with *Borrelia turcica* found in *Hyalomma aegyptium* from Turkey, *Borrelia* sp. isolated from the tortoise *Geochelone pardalis babcocki* in Zambia, and *Borrelia* spp. detected in *Amblyomma maculatum* from USA and *A. longirostre* from Brazil (Fig. 1). All these *Borrelia* spp. belong to the REP *Borrelia* group. One sequence of a single specimen of *I. auritulus* (haplotype G1034; GenBank accession number MK984825) was also found to be associated with this group (Fig. 1).

Of the 42 positive samples to the nested PCR of the *fla* fragment, thirty nine were also positive to the nested PCR of the 16 s rRNA fragment and were sequenced. The three negative samples were a nymph of *I. auritulus* collected in *T. rufiventris*, a nymph of *I. auritulus* collected in *T. amaurochalinus* and a nymph of *A. aureolatum* collected in *T. rufiventris*.

The 16S sequences obtained (six different haplotypes; GenBank accession numbers MK984832, MK984834-MK984838) from the positives of *I. auritulus* had 99.2–100% identity with each other, and less than 99.2% with the remaining sequences of *Borrelia* belonging to the LB group available in GenBank. There are not available 16S sequences belonging to the *Borrelia* spp. previously found in *I. auritulus* from Canada and Uruguay for comparison.

The 16S sequences obtained from specimens of *A. aureolatum* were identical among each other (GenBank accession number MK984833) and had 99.0% identity with *B. turcica* (AB473539) found in *H. aegyptium* from Jordania, *Borrelia* sp. (AB529377) found in *Amblyomma geoemydae* from Japan, *Borrelia* sp. (MH628249) found in *H. aegyptium* from Turkey, and *Borrelia* sp. (AB473532) from blood of the tortoise *G. pardalis babcocki* from Zambia.

4. Discussion

This is the first report of the presence of bacteria of the genus *Borrelia* in *A. aureolatum* and the third one for *I. auritulus* in South America. The group of *Borrelia* sp. associated with *A. aureolatum* and *I. auritulus* (haplotype G1034) constitutes the second record of the REP *Borrelia* spp. group in South America. The haplotypes of *Borrelia* sp. belonging to the LB *B. burgdorferi* s.l. complex that were detected in *I. auritulus* ticks (see Fig. 1), appear to belong to the same genospecies

Table 2
Birds infested with *Ixodes auritulus*.

Order, family, species	n	Birds infested with *Ixodes auritulus* / Birds captured (% Prevalence)			
		Autumn	Winter	Spring	Summer
Columbiformes	**16**	**0 / 1**	**0 / 2**	**0 / 3**	**0 / 10**
Columbidae	**16**	**0 / 1**	**0 / 2**	**0 / 3**	**0 / 10**
Leptotila verreauxi	4	–	0 / 2	–	0 / 2
Zenaida auriculata	12	0 / 1	–	0 / 3	0 / 8
Coraciiformes	**6**	**0 / 1**	**0 / 1**	–	**0 / 4**
Picidae	**6**	**0 / 1**	**0 / 1**	–	**0 / 4**
Colaptes melanochloros	1	–	–	–	0 / 1
Picoides mixtus	5	0 / 1	0 / 1	–	0 / 3
Cuculiformes	**1**	**0 / 1**	–	–	–
Cuculidae	**1**	**0 / 1**	–	–	–
Coccyzus melacoryphus	1	0 / 1	–	–	–
Passeriformes	**307**	**8 / 71 (11.3)**	**17 / 66 (25.7)**	**10 / 80 (12.5)**	**7 / 90 (7.8)**
Emberizidae	**78**	**1 / 17 (5.9)**	**7 / 19 (36.8)**	**0 / 19**	**0 / 23**
Cyanocompsa brissonii	3	–	–	0 / 2	0 / 1
Cyanoloxia glaucocaerulea	1	–	–	0 / 1	–
Poospiza melanoleuca	5	–	0 / 2	–	0 / 3
Poospiza nigrorufa	20	0 / 5	2 / 4 (50)	0 / 6	0 / 5
Saltator aurantiirostris	11	0 / 3	2 / 5 (40)	–	0 / 3
Sicalis flaveola	4	–	0 / 1	0 / 2	0 / 1
Sporophila caerulescens	3	0 / 1	–	–	0 / 2
Zonotrichia capensis	31	1 / 8 (12.5)	3 / 7 (42.8)	0 / 8	0 / 8
Fringillidae	**10**	**0 / 2**	–	**0 / 3**	**0 / 5**
Carduelis magellanica	10	0 / 2	–	0 / 3	0 / 5
Furnariidae	**28**	**2 / 9 (22.2)**	**0 / 6**	**0 / 2**	**1 / 11 (9.1)**
Furnarius rufus	14	0 / 4	0 / 4	0 / 1	1 / 5 (20)
Phacellodomus striaticollis	3	2 / 2 (100)	–	–	0 / 1
Synallaxis frontalis	11	0 / 3	0 / 2	0 / 1	0 / 5
Hirundinidae	**2**	–	–	–	**0/ 2**
Progne tapera	2	–	–	–	0 / 2
Icteridae	**12**	**0 / 3**	**0 / 3**	**0 / 5**	**0 / 1**
Agelaioides badius	6	0 / 1	0 / 3	0 / 2	–
Cacicus solitarius	2	0 / 1	–	–	0 / 1
Icterus cayanensis	1	0 / 1	–	–	–
Molothrus bonariensis	3	–	–	0 / 3	–
Mimidae	**3**	–	–	**0/ 2**	**0 / 1**
Mimus saturninus	3	–	–	0 / 2	0 / 1
Parulidae	**30**	**0 / 9**	**0 / 7**	**2 / 11 (18.2)**	**0 / 3**
Basileuterus culicivorus	16	0 / 9	0 / 5	–	0 / 2
Geothlypis aequinoctialis	12	–	–	2 / 11 (18.2)	0 / 1
Parula pitiayumi	2	–	0 / 2	–	–
Polioptilidae	**10**	**0 / 2**	**0 / 2**	**0 / 1**	**0 / 5**
Polioptila dumicola	10	0 / 2	0 / 2	0 / 1	0 / 5
Thraupidae	**6**	**0 / 2**	**1 / 3 (33.3)**	**0 / 1**	–
Stephanophorus diadematus	1	–	1 / 1 (100)	–	–
Tachyphonus rufus	2	–	0 / 2	–	–
Thraupis bonariensis	3	0 / 2	–	0 / 1	–
Troglodytidae	**23**	**1 / 6 (16.7)**	**3 / 5 (60)**	**0 / 7**	**0 / 5**
Troglodytes aedon	23	1 / 6 (16.7)	3 / 5 (60)	0 / 7	0 / 5
Turdidae	**62**	**4 / 15 (26.7)**	**6 / 20 (30)**	**8 / 18 (44.4)**	**6 / 9 (66.7)**
Turdus amaurochalinus	27	1 / 5 (20)	1 / 4 (25)	3 / 12 (25)	4 / 6 (66.7)
Turdus rufiventris	35	3 / 10 (30)	5 / 16 (31.2)	5 / 6 (83.3)	2 / 3 (66.7)
Tyrannidae	**39**	**0 / 6**	**0 / 1**	**0 / 10**	**0 / 22**
Elaenia parvirostris	13	0 / 1	–	0 / 2	0 / 10
Myiodynastes maculatus	2	–	–	–	0 / 2
Myiophobus fasciatus	10	0 / 1	–	0 / 5	0 / 4
Pachyramphus polychopterus	3	–	–	–	0 / 3
Pitangus sulphuratus	5	0 / 2	0 / 1	0 / 1	0 / 1
Serpophaga subcristata	4	0 / 2	–	0 / 2	–
Tirannus melancholicus	2	–	–	–	0 / 2
Vireonidae	**4**	–	–	**0/ 1**	**0 / 3**
Vireo olivaceus	4	–	–	0 / 1	0 / 3
Strigiformes	**1**	–	–	–	**0/ 1**
Strigidae	**1**	–	–	–	**0/ 1**
Glaucidium brasilianum	1	–	–	–	0 / 1
Trochiliformes	**9**	–	–	**0/ 6**	**0 / 3**
Trochilidae	**9**	–	–	**0/ 6**	**0 / 3**
Chlorostilbon aureoventris	9	–	–	0 / 6	0 / 3
Total	**340**	**8 / 74 (10.8)**	**17 / 69 (24.6)**	**10 / 89 (11.2)**	**7 / 108 (6.5)**
Birds per season (%)		**21.7**	**20.3**	**26.2**	**31.8**

that those *Borrelia* genospecies detected in *I. auritulus* from Canada (Isolate Cn186) and Uruguay (haplotype UY3). Muñoz-Leal et al. (2019) also found a *Borrelia* genospecies belonging to the LB *B. burgdorferi* s.l. complex (*Borrelia* sp. Navarino) in Chile, but it is not related to the other *Borrelia* genospecies from the Southern Cone of South America in Uruguay and Argentina (Carvalho et al., 2019; this work).

Birds are important dispersers of ticks, and some of them are possible vectors of *B. burgdorferi* s. l. (Newman et al., 2015; Rudenko et al., 2014; Scott et al., 2015). Wild birds, principally those foraging primarily on the ground and in the shrub layer, are frequently infested by ticks (Carvalho et al., 2019; Nava et al., 2006). In our study, as expected, ticks were found on birds foraging in low forest stratum.

Ixodes auritulus is a tick species associated with birds and probably represents a species complex that parasitize a great variety of birds in the Afrotropical, Australasian, Nearctic and Neotropical Zoogeographic Regions (Guglielmone et al., 2014; Nava et al., 2017). There are no records of *I. auritulus* on domestic animals and humans (Guglielmone et al., 2014; Nava et al., 2017). In Argentina, *I. auritulus* has been found in the Paraná Delta from the Buenos Aires province, Buenos Aires city and provinces from the Patagonia region parasitizing mainly Passeriformes (Cicuttin, 2016; Flores et al., 2014; Guglielmone and Nava, 2005). We captured 42 passerine birds (57.1% from family Turdidae) infested with *I. auritulus*. *Troglodytes aedon* and *Zonotrichia capensis* were the birds with second rating of infestation. The present study adds *S. aurantiirostris*, *Phacellodomus striaticollis*, *Furnarius rufus* and *Geothlypis aequinoctialis* as hosts for *I. auritulus*.

The prevalence of *B. burgdorferi s.l.* in *I. auritulus* ticks collected on birds in previous studies from Canada varied from 6.2 to 33.3% (Morshed et al., 2005; Scott et al., 2010, 2015, 2012), whereas in Uruguay, Carvalho et al. (2019)) found 12.2% positive of *I. auritulus* collected on birds (nymphs and females) and 5.3% from vegetation (nymphs and females). In our study, we found 27.3% positive for ticks collected on birds (with samples positives in all stages) and 0.7% (28.6% in nymphs and no detection in larvae) for questing ticks. Considering that the transovarial transmission of the LB spirochete appears to be rare (Rollend et al., 2013), it is possible to suggest that the spirochete was acquired during blood meals on birds, maintain infection through the molt.

The finding of *Borrelia* in *I. auritulus* during this study adds a putative new species to the list of *B. burgdorferi* s.l. genospecies reported for Argentina. Different haplotypes of *B. burgdorferi* s.l. were detected in *I. pararicinus* ticks from northern area of this country (Flores et al., 2018; Nava et al., 2014; Saracho Bottero et al., 2017) and one haplotype was detected in *Ixodes* sp. cf. *I. neuquenensis* and *I. sigelos* ticks from

Table 3
Birds infested with *Amblyomma aureolatum*.

Order, family, species	n	Birds infested with *Amblyomma aureolatum* / Birds captured (% Prevalence)			
		Autumn	Winter	Spring	Summer
Passeriformes	**307**	**0 / 71**	**6 / 66 (9.1)**	**3 / 80 (3.7)**	**0 / 90**
Turdidae	**62**	**0 / 15**	**6 / 20 (30)**	**3 / 18 (16.7)**	**0 / 9**
Turdus amaurochalinus	27	0 / 5	2 / 4 (50)	2 / 12 (16.7)	0 / 6
Turdus rufiventris	35	0 / 10	4 / 16 (25)	1 / 6 (16.7)	0 / 3
Total	**340**	**0 / 74**	**6 / 69 (8.7)**	**3 / 89 (3.4)**	**0 / 108**

Table 4
Ixodid ticks collected on birds.

Birds (family, species)	I/C	Species of ticks	Autumn			Winter			Spring			Summer			All seasons				Mean intensity	Mean abundance
			L	N	F	L	N	F	L	N	F	L	N	F	L	N	F	Total		
Emberizidae																				
Zonotrichia capensis	4/31	*Ixodes auritulus*	0	1	0	5	0	0	0	0	0	0	0	0	5	1	0	6	1.50	0.19
Poospiza nigrorufa	2/20	*I. auritulus*	0	0	0	1	1	0	0	0	0	0	0	0	1	1	0	2	1.00	0.10
Saltator aurantiirostris	2/11	*I. auritulus*[a]	0	0	0	2	2	0	0	0	0	0	0	0	2	2	0	4	2.00	0.36
Furnariidae																				
Phacellodomus striaticollis	2/3	*I. auritulus*[a]	0	2	0	0	0	0	0	0	0	0	0	0	0	2	0	2	1.00	0.67
Furnarius rufus	1/14	*I. auritulus*[a]	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1.00	0.07
Parulidae																				
Geothlypis aequinoctialis	2/12	*I. auritulus*[a]	0	0	0	0	0	0	3	0	0	0	0	0	3	0	0	3	1.50	0.25
Thraupidae																				
Stephanophorus diadematus	1/1	*I. auritulus*	0	0	0	4	0	0	0	0	0	0	0	0	4	0	0	4	4.00	4.00
Troglodytidae																				
Troglodytes aedon	4/23	*I. auritulus*	0	1	0	9	1	1	0	0	0	0	0	0	9	2	1	12	3.00	0.52
Turdidae																				
Turdus rufiventris[b]	5/35	*Amblyomma aureolatum*	0	0	0	0	7	0	0	1	0	0	0	0	0	8	0	8	1.60	0.23
	15/35	*I. auritulus*	5	4	0	9	6	0	8	18	1	0	0	2	22	28	3	53	3.53	1.51
Turdus amaurochalinus[c]	4/27	*A. aureolatum*	0	0	0	1	1	0	0	2	0	0	0	0	1	3	0	4	1.00	0.15
	9/27	*I. auritulus*	0	1	0	0	0	1	9	4	0	1	3	2	10	8	3	21	2.33	0.78
Total	**47/340**		**5**	**9**	**0**	**31**	**18**	**2**	**20**	**25**	**1**	**1**	**3**	**5**	**57**	**55**	**8**	**120**	**2.55**	**0.35**
Ticks per season (%)			**14 (11.7)**			**51 (42.5)**			**46 (38.3)**			**9 (7.5)**								

I/C, infested/captured; L, larvae; N, nymphs; F, females; [a]New host species-parasite species association; [b]Three had co-infestation; [c]One had co-infestation.

Table 5
Results for nested PCR *fla* fragment of *Borrelia* for ticks collected on vegetation.

Tick		n	Pools	Positive n (%)	Sequences obtained (%)
Amblyomma aureolatum	Larvae	368	46	0	–
	Nymphs	52	–	9 (17.3)	9 (100)
	Total	420	–	9 (2.1)	9 (100)
Ixodes auritulus	Larvae	591	69	0	–
	Nymphs	14	–	4 (28.6)	4 (100)
	Females	1	–	0	–
	Total	606	–	4 (0.7)	4 (100)
Amblyomma triste	Males	21	–	0	–
	Females	44	–	0	–
	Total	65	–	0	–
Total	–	1091	–	13 (1.2)	13 (100)

Table 6
Results for nested PCR *fla* fragment of *Borrelia* for ticks collected on birds.

Tick		n	Pools	Positive n (%)	Sequences obtained (%)
Ixodes auritulus	Larvae	37	22	5 pools[a] (13.5[b])	5 (100)
	Nymphs	44	–	18 (40.9)	18 (100)
	Females	7	–	1 (14.3)	1 (100)
	Total	88	–	24 (27.3[b])	24 (100)
Amblyomma aureolatum	Larvae	1	1	0 (0)	–
	Nymphs	11	–	5 (45.4)	4 (80)
	Total	12	–	5 (41.7)	5 (100)
Total		100	–	29 (29.0[b])	28 (96.5)

[a] Corresponding to 7 larvae.
[b] MIR = minimum infection rate.

the Patagonian region (Sebastian et al., 2016). Interestingly, several of the *Borrelia*-positive ticks were collected on birds form the family Turdidae, indicating the possible role of this bird family as a reservoir of the LB group.

Amblyomma aureolatum is distributed in Argentina, Brazil, French Guiana, Paraguay, Surinam, and Uruguay (Nava et al., 2017). The principal hosts for immature and adult stages are passerine birds and carnivorous, respectively, but the range of hosts is wide, especially for adult ticks, and it includes humans (Nava et al., 2017). *Amblyomma aureolatum* is vector of the human pathogens *Rickettsia rickettsii* and *Rickettsia parkeri* strain Atlantic rainforest (Nava et al., 2017). In Argentina there are records of this tick in the provinces of Misiones, Chaco, Entre Ríos, Santa Fe, Buenos Aires and Buenos Aires city (Buenos Aires city does not belong to the province of the same name) (Colombo et al., 2016). We captured nine passerine birds from the family Turdidae infested with *A. aureolatum*. Previous studies indicate that the family Turdidae is a main host of *A. aureolatum* (Guglielmone

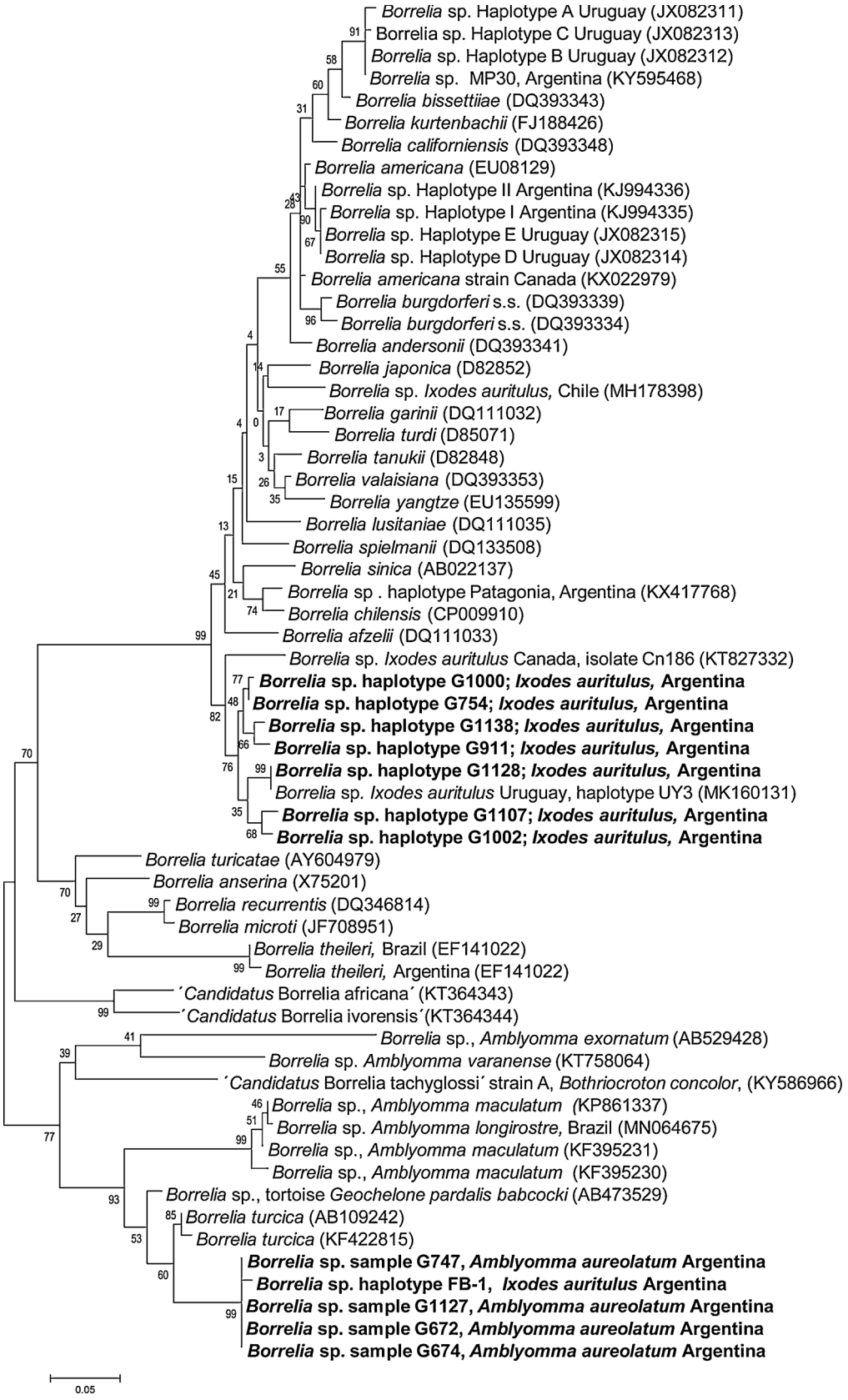

Fig. 1. Maximum-likelihood tree constructed from *Borrelia fla* partial sequences. Numbers represent bootstrap support generated from 1000 replications. GenBank accession numbers are in brackets.

et al., 2003).

There are no previous studies on *Borrelia* spp. in this tick species. In the New World, the knowledge on the presence of *Borrelia* in *Amblyomma* ticks is restricted to a few findings in USA and Brazil. In USA, a novel *Borrelia* genospecies from the REP group was found in *A. maculatum,* a tick that does not feed on reptiles (Lee et al., 2014; Mitchell et al., 2016) and *Borrelia lonestari* (RF group) was found in *Amblyomma americanum* (Hudman and Sargentini, 2018; Mitchell et al., 2016). In Brazil, the presence of *Borrelia* sp. was reported in *A. longirostre* by Pacheco et al. (2019), but these authors did not include the sequences of the genospecies previously found in *A. maculatum* in their phylogenetic analysis, and they overlooked the close relationship between the *Borrelia* found in *A. maculatum* and that found in *A. longirostre*, such as is observed in the phylogenetic analysis presented in this work (see Fig. 1). The *Borrelia* found in our study in *A. aureolatum* and *I. auritulus* conform a monophyletic group with *B. turcica*, with *Borrelia* sp. detected in the tortoise *G. pardalis babcocki* in Zambia, and with the genospecies found in *A. maculatum* from USA and *A. longirostre* from Brazil (Fig. 1).

The prevalence of *Borrelia* sp. in *A. aureolatum* observed in this work varied from 2.1% (17.3% in nymphs and no detection in larvae) in questing ticks to 41.7% in ticks collected on birds. Transovarial transmission is an unusual event in RF group, but there are no specific studies for the HTBRF subgroup (Talagrand-Reboul et al., 2018). Considering the highest prevalence in ticks associated with birds with respect to questing ticks (and no detection in questing larvae ticks), our study suggests that the spirochete was acquired during blood meals of larvae on birds and transmitted transstadially to nymphs. However, this does not confirm the vectorial capacity of these species and the findings must always be carefully interpreted, especially considering that partially or completely engorged ticks collected on hosts will contain microorganisms of which they may or may not be vectors and that they may have been ingested with blood meals (Estrada-Peña et al., 2013). The positive-*I. auritulus* associated with REP *Borrelia* group may be due to blood meal, but also associated with the co-feeding with *A. aureolatum*. In this sense, it is known that spirochetes may pass directly from infected to noninfected ticks while the ticks are feeding simultaneously in close proximity (Richter et al., 2002).

The importance of birds as reservoir hosts of LB around the world is clearly recognized (Mechai et al., 2016; Newman et al., 2015; Rudenko et al., 2014) and many studies highlighted the potential role of birds belonging to the genus *Turdus* (Norte et al., 2013; Saracho Bottero et al., 2017; Scott et al., 2012). Vertebrate host specificity is variable between RF *Borrelia* spp., because different species can infect small mammals and human, as well as birds, domestic or wild mammals without clear lineage specificity (Cutler, 2010; Talagrand-Reboul et al., 2018). However, two host associations are reported: *B. anserina* in birds and *Borrelia duttonii/Borrelia recurrentis* associated with humans (Cutler, 2010; Talagrand-Reboul et al., 2018). Remarkably, the hosts of the HTBRF subgroup are not clearly identified (Cutler, 2010; Talagrand-Reboul et al., 2018). By last, REP *Borrelia* spp. were isolated or detected from reptiles (Takano et al., 2010, 2011). In our study, three bird species were found harboring *Borrelia* LB-positive ticks, namely *T. rufiventris*, *T. amaurochalinus* and *S. aurantiirostris*, and two bird species harboring *Borrelia* REP-positive ticks, namely *T. rufiventris* and *T. amaurochalinus*. In northwestern Argentina, Saracho Bottero et al. (2017) and Flores et al. (2018) have obtained positive samples for *B. burgdorferi* s. l. from *I. pararicinus* collected on *T. rufiventris*. On the other hand, all the samples of birds studied in our work were negative. It should be considered that the tissue tropism of *Borrelia* in birds is not clearly known and, due to its nucleated erythrocytes, bird blood contains large amounts of DNA that can make it difficult the detection of pathogens (Newman et al., 2015).

Regarding the importance in public health, the epidemiological risk that implies the infection with *Borrelia* genospecies associated with *I. auritulus* seems to be low because this tick is not aggressive to humans, but it helps to maintain borrelial spirochetes in the enzootic transmission cycles. On the other hand, the species of *Borrelia* found in *A. aureolatum*, as well as the phylogenetic group of related spirochetes, are of pathogenicity unknown to humans. However, the existence of a novel *Borrelia* sp. in *A. aureolatum* is significant from public health perspective because adults of this tick species are known to bite humans (Guglielmone and Robbins, 2018). Further investigations are necessary to better understand the biology, ecology, pathogenicity (if any), and infectivity of this organism to humans, domestic animals, and wildlife.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ttbdis.2019.101282.

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