Braz. J. Vet. Parasitol., Jaboticabal
ISSN 0103-846X (Print) / ISSN 1984-2961 (Electronic)
Doi: http://dx.doi.org/10.1590/S1984-29612016045

Rickettsial infection in ticks infesting wild birds from two eco-regions of Argentina

Infecção por riquétsias em carrapatos de aves silvestres em duas ecorregiões da Argentina

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Received February 25, 2016
Accepted April 11, 2016

Abstract

Several tick-borne *Rickettsia* species are recognized human pathogens in Argentina. Here we evaluated rickettsial infection in ticks collected on passerine birds during 2011-2012 in two eco-regions of Argentina. The ticks were processed by molecular analysis through polymerase chain reaction (PCR) detection and DNA sequencing of fragments of two rickettsial genes, *gltA* and *ompA.* A total of 594 tick specimens (532 larvae and 62 nymphs), representing at least 4 species (*Amblyomma tigrinum, Ixodes pararicinus, Haemaphysalis juxtakochi, Haemaphysalis leporispalustris*), were evaluated. At least one *A. tigrinum* larva, collected on *Coryphospingus cucullatus* in Chaco Seco, was infected with *Rickettsia parkeri,* whereas at least 12 larvae and 1 nymph of *I. pararicinus,* collected from *Troglodytes aedon, Turdus amaurochalinus, Turdus rufiventris, C. cucullatus* and *Zonotrichia capensis,* were infected with an undescribed *Rickettsia* agent, genetically related to several rickettsial endosymbionts of ticks of the *Ixodes ricinus* complex. *R. parkeri* is a recognized human pathogen in several American countries including Argentina, where a recent study incriminated *A. tigrinum* as the potential vector of *R. parkeri* to humans. Birds could play an important role in dispersing *R. parkeri*-infected *A. tigrinum* ticks. Additionally, we report for the first time a rickettsial agent infecting *I. pararicinus* ticks.

Keywords: *Rickettsia parkeri*, endosymbiont, *Amblyomma tigrinum*, *Ixodes pararicinus*, passeriformes.

Resumo

Algumas espécies de *Rickettsia* transmitidas por carrapatos são reconhecidos como patógenos humanos na Argentina. Este presente trabalho avaliou a infecção por *Rickettsia* em carrapatos coletados em aves passeriformes, durante 2011-2012, em duas ecorregiões da Argentina. Os carrapatos foram processados pela reação em cadeia da polimerase (PCR) e sequenciamento de DNA de dois genes de *Rickettsia*: *gltA* e *ompA*. Ao todo, 594 amostras de carrapatos (532 larvas e 62 ninfas), representando pelo menos 4 espécies (*Amblyomma tigrinum, Ixodes pararicinus, Haemaphysalis juxtakochi, Haemaphysalis leporispalustris*), foram avaliadas. Pelo menos uma larva de *A. tigrinum*, coletada de *Coryphospingus cucullatus* no Chaco Seco, estava infectada com *Rickettsia parkeri*, enquanto pelo menos 12 larvas e 1 ninfa de *I. pararicinus*, coletadas de *Troglodytes aedon, Turdus amaurochalinus, Turdus rufiventris, C. cucullatus* e *Zonotrichia capensis* estavam infectadas com *Rickettsia* sp., geneticamente relacionada a vários endossimbiontes riquetsiais de carrapatos do complexo *Ixodes ricinus*. *R. parkeri* é reconhecidamente um patógeno humano em alguns países americanos, incluindo a Argentina, onde um estudo recente incriminou *A. tigrinum* como um provável vetor. Aves poderiam desempenhar um papel importante na dispersão de carrapatos *A. tigrinum* infectados por *R. parkeri*. Em adição, relata-se pela primeira vez a infecção por *Rickettsia* em *I. pararicinus*.

Palavras-chave: *Rickettsia parkeri*, endo-simbionte, *Amblyomma tigrinum*, *Ixodes pararicinus*, passeriformes.

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Bacteria of the genus *Rickettsia* are obligate intracellular organisms that infect invertebrate hosts worldwide. Some of them also infect and cause diseases (i.e., rickettsioses) in warm-blooded animals and humans, to whom they are transmitted by hematophagous vectors, mostly ticks (PAROLA et al., 2013). In Argentina, three species of the spotted fever group (SFG) rickettsiae, *Rickettsia rickettsii*, *Rickettsia parkeri* and *Rickettsia massiliae,* are recognized as agents of human diseases (PADDOCK et al., 2008; GARCÍA-GARCÍA et al., 2010; ROMER et al., 2011, 2014). A fourth human pathogen, *Rickettsia* sp. strain Atlantic rainforest has also been reported in Argentinean ticks (MONJE et al., 2015); however, no human cases of rickettsiosis attributed to this pathogen have been detected in Argentina. Additional rickettsial agents of unknown pathogenicity have been reported in Argentinean ixodid ticks, namely *Rickettsia bellii,* "*Candidatus* Rickettsia amblyommii", "*Candidatus* Rickettsia andeanae", *Rickettsia* sp. strain El Tunal, and *Rickettsia* sp. endosymbiont of *Amblyomma parvitarsum* (LABRUNA et al, 2007; PACHECO et al., 2007; SARACHO BOTTERO et al., 2015; TARRAGONA et al., 2015; OGRZEWALSKA et al., 2016).

Wild birds can be hosts of different stages of some species of ticks, commonly larvae and nymphs, and rarely adults (GUGLIELMONE et al., 2014; FLORES et al., 2014). Furthermore, wild birds are among the most mobile hosts, and therefore they may be regarded as hosts with relevant potential in the dispersion of ticks and tick-borne diseases, including rickettsial organisms (ELFVING et al., 2010; HORNOK et al., 2014; BERTHOVÁ et al., 2016). Among 127 species of Ixodidae established in the Neotropical Zoogeographic Region (GUGLIELMONE et al., 2014; NAVA et al., 2014a, b; KRAWCZAK et al., 2015), 39 are found in Argentina (GUGLIELMONE & NAVA, 2005, 2006; NAVA et al., 2009, 2014a, b). Most of these species belong to the genus *Amblyomma*, best represented in Argentina with 25 species. Ten species from three genera have been reported parasitizing wild birds in different Argentinean eco-regions (FLORES et al., 2014); however, bird ticks have never been searched for rickettsial infection in Argentina. Here, we have evaluated rickettsial infection in ticks collected on wild birds in two eco-regions of Argentina.

This study was conducted in two localities of the Chaco Seco eco-region (Chaco Seco1: 30°50'S, 62°54'W; and Chaco Seco 2: 30°22'S, 64°21'W) and one in the Yungas ecoregion (Parque Nacional El Rey: 24°43'S, 64°38'W) in Argentina as defined by Burkart et al. (1999). Bird collections were performed using mist nets, which remained active during morning and twilight hours, obtaining convenience samplings in 2011 and 2012, as previously reported (FLORES et al., 2014). Each individual bird was identified using Narosky & Yzurieta (2010), classified under Clements et al. (2015) criteria and examined for ticks using fine-tipped tweezers. After being processed, the birds were released, and the ticks obtained were stored in 70% ethanol until specific determination in the laboratory. The detailed procedures for the fieldwork, bird and tick taxonomic identification, and the results of the ticks infesting these birds have been published elsewhere (FLORES et al., 2014).

Ticks of the same developmental stage, collected from the same individual host, were processed in pools of 1 to 39 larvae (median: 4) or 1 to 4 nymphs (median: 1). Ticks from each pool were crushed with a sterile pestle in a microtube, and then processed for DNA extraction by using the AxyPrep Body Fluid Viral DNA/RNA Miniprep Kit (Axygen Biosciences, USA) following the manufacturer's procedures. DNA samples were processed by PCR using primers CS-78 and CS-323 targeting a 398-bp fragment of the citrate synthase gene (*gltA*), which occurs in all *Rickettsia* species (LABRUNA et al., 2004). Samples positive by this first PCR assay were tested by a second PCR assay with primers Rr190.70p and Rr190.701, which amplifies a ≈630-bp fragment of the 190-kDa outer membrane protein gene (*ompA*) of the majority of the SFG *Rickettsia* species (ROUX et al., 1996). PCR products were DNA-sequenced and subjected to BLAST analyses (BLAST, 2015) to infer the closest similarities available in GenBank.

A total of 594 tick specimens (532 larvae and 62 nymphs), representing at least 4 species, were evaluated in 124 pools for rickettsial infection (Table 1). A complete list of hosts for these ticks has been published elsewhere (FLORES et al., 2014). Two tick species were found to be infected by *Rickettsia.* One *Amblyomma tigrinum* pool of 4 larvae was infected with *R. parkeri,* as its *gltA* and *ompA* DNA partial sequences (308 bp and 551 bp, respectively) were 100% identical to *R. parkeri* strain Maculatum20 (GenBank accession numbers U59732 and U43802, respectively).

Thirteen pools of *Ixodes pararicinus* (12 out of 32 larval pools; and 1 out of 8 nymphal pools) yielded PCR products for both the *gltA* and the *ompA* genes. DNA sequences were successfully obtained for 5 pools, which generated identical sequences for each rickettsial gene. The *gltA* partial sequence was 100% (350/350-bp) identical to *Rickettsia* sp. strain BelizeIaff1 of *Ixodes affinis* (KU001172; from Belize), 99.7% (349/350-bp) identical to *Rickettsia* sp. strain 12G1 of *Rhipicephalus (Boophilus) microplus* (KF831359; from Ecuador) and 99.4% (348/350-bp) identical to *Rickettsia monacensis* of *Ixodes ricinus* (LN794217; from Europe) and *Rickettsia* sp. strain Barva1 of *Ixodes minor* (KF702332; from Costa Rica). The *ompA* partial sequences generated from the *I. pararicinus* ticks were 100% (587/587-bp) identical to *Rickettsia* sp. strain Barva2 of *Ixodes minor* (KF702334; from Costa Rica), 99.8% (586/587-bp) identical to *Rickettsia* sp. strain BelizeIaff2 of *I. affinis* (KU001175; from Belize), and then 98.9% (572/578-bp) identical to *Rickettsia* sp. endosymbiont of *Ixodes scapularis* (EF689735; from the United States).

The GenBank nucleotide sequence accession numbers for the partial sequences generated in this study are KU744411-KU744412 for the *gltA* and *ompA* genes of *R. parkeri* from *A. tigrinum* and KU744413-KU744414 for the *gltA* and *ompA* genes of *Rickettsia* sp. from *I. pararicinus.*

This study provides molecular evidence of two rickettsial agents infecting ticks that were parasitizing birds in Argentina. *R. parkeri* is a recognized human pathogen in several countries of the Americas (PAROLA et al., 2013), including Argentina, where a recent study incriminated *A. tigrinum* as a probable vector of *R. parkeri* to humans (ROMER et al., 2014). The tick *A. tigrinum* is known to occur in a variety of eco-regions among almost all South American countries (ESTRADA-PEÑA et al., 2005). Recent findings of *R. parkeri* infecting *A. tigrinum* adult ticks in Bolivia (TOMASSONE et al., 2010), Uruguay (LADO et al., 2014), and Argentina (ROMER et al., 2014) suggest that this pathogen could

Table 1. Ticks collected from birds and tested by PCR for rickettsial infection.

Tick species	Locality	Tick stage	No. ticks	No. pools	No. pools infected by rickettsia	MIR[a]	Bird host species of the infected ticks[b]
Amblyomma tigrinum	Chaco Seco 1	Larva	93	16	0	0	
		Nymph	18	12	0	0	
	Chaco Seco 2	Larva	89	14	1	1.1	*Coryphospingus cucullatus*
		Nymph	20	14	0	0	
Ixodes pararicinus	Yungas	Larva	229	32	12[c]	5.2	*Troglodytes aedon, Turdus amaurochalinus, Turdus rufiventris, Coryphospingus cucullatus, Zonotrichia capensis*
		Nymph	11	8	1	9	*Turdus rufiventris*
Haemaphysalis juxtakochi	Yungas	Larva	78	10	0	0	
		Nymph	12	5	0	0	
Haemaphysalis leporispalustris	Yungas	Larva	23	4	0	0	
		Nymph	1	1	0	0	
Haemaphysalis sp.	Yungas	Larva	10	4	0	0	
Amblyomma sp.	Yungas	Larva	10	4	0	0	

[a] MIR: Minimun Infection Rate = No. PCR-positive pools / Total number of tested ticks × 100b. [b] a detailed list of bird hosts for all ticks of this table has been published elsewhere (FLORES et al., 2014). [c] 4 pools were from *Troglodytes aedon,* 1 from *Turdus amaurochalinus*, 5 from *Turdus rufiventris*, 1 from *Coryphospingus cucullatus* and 1 from *Zonotrichia capensis*.

have a wide distribution within the South American population of this tick species. Thus, it is likely that wild birds could play an important role in dispersing *R. parkeri*-infected *A. tigrinum* ticks, which could expand the area of human exposure to these ticks, consequently increasing the risk of human rickettsiosis. On the other hand, because all tick-infested birds of this study are considered to be non-migratory species (NAROSKY & YZURIETA, 2010), their specific role in dispersing infected ticks over long distances should be limited.

Additionally, we report for the first time a rickettsial agent infecting *I. pararicinus* ticks. Partial molecular characterization of this agent indicates that it is most closely related to SFG rickettsial agents associated with *I. affinis* (LOPES et al., 2016), *I. ricinus* (MAIOLI et al., 2012), *I. minor* (OGRZEWALSKA et al., 2015), and *I. scapularis* (PAROLA et al., 2013). These four *Ixodes* species, as well as *I. pararicinus,* belong to the *I. ricinus* species complex, based primarily on morphological and genetic relatedness (KEIRANS et al., 1985; BARBIERI et al., 2013). Interestingly, recent studies have indicated that tick members of the *I. ricinus* species complex are usually infected by species-specific closely related rickettsial organisms, usually considered endosymbionts (KURTTI et al., 2015). Moreover, the *I. ricinus* associated *R. monacensis* is considered by some authors as a human pathogen (PAROLA et al., 2013). Further studies on isolation and deeper molecular characterization are needed to elucidate the taxonomic status of the rickettsial endosymbiont of *I. pararicinus.*

Acknowledgements

This work received financial support from Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP/CONICET Project no. 2013/50605-6) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET/FAPESP Project no. 5112/13).

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