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Notas

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Detección molecular de *Rickettsia bellii* en garrapatas *Ixodes loricatus* (Acari: Ixodidae) asociadas a roedores de la provincia de Buenos Aires, Argentina

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Abstract: The aim of this study was the detection of *Rickettsia* in ticks of sigmodontine rodents from Northeastern Buenos Aires province, Argentina. A total of 222 rodents were captured collecting 10 ticks identified as *Ixodes loricatus* Neumann, which were analysed by the real-time PCR and conventional PCR techniques. DNA of *Rickettsia bellii* was detected in nymphs obtained from the rodents *Akodon azarae* Fischer, *Oxymycterus rufus* Fischer and *Deltamys kempi* Thomas. This is the first report of *R. bellii* infecting *I. loricatus* in Argentina and the first report of this bacterium associated with ticks of sigmodontine rodents.

Keywords: Cricetidae, Ixodida, PCR, Vectors.

Resumen: El objetivo de este estudio fue detectar *Rickettsia* en garrapatas de roedores sigmodontinos del Noreste de la provincia de Buenos Aires, Argentina. Se capturaron 222 roedores colectando 10 garrapatas identificadas como *Ixodes loricatus* Neumann, las cuales fueron analizadas por las técnicas de PCR real-time y PCR convencional. Se detectó ADN de *Rickettsia bellii* en ninfas de obtenidas de los roedores *Akodon azarae* Fischer, *Oxymycterus rufus* Fischer y *Deltamys kempi* Thomas. Este es el primer reporte de *R. bellii* infectando *I. loricatus* en Argentina y el primer reporte de esta bacteria asociada a garrapatas de roedores sigmodontinos.

Palabras clave: Cricetidae, Ixodida, PCR, Vectores.

Ticks (Acari: Ixodidae) are hematophagous arthropods, parasites of vertebrates with more than 920 valid species worldwide, 52 of them reported from Argentina (Guglielmone et al*.,* 2014, 2015; Nava et al., 2017; Saracho-Bottero et al., 2021). Ticks have relevance as parasites themselves and as vectors of zoonotic diseases (Jongejan & Uilenberg, 2004). These arthropods can acquire pathogenic agents by feeding blood of infected animals or by transstadial and transovarial transmission. The pathogenic agents include *Rickettsia*, a group of gram-negative bacteria with worldwide distribution which includes zoonotic species usually transmitted by arthropod vectors (Parola et al., 2013). In Argentina, clinical cases of rickettsial diseases have been reported in different provinces, including Buenos Aires (Romer et al., 2020).

Sigmodontine rodents (Cricetidae) are common hosts of immature stages of ticks (Beldoménico et al., 2005). These mammals are widely distributed in Argentina inhabiting almost all habitat types, and some species are reservoirs zoonotic agents, such as rickettsias (Meerburg et al., 2009; Patton et al., 2015). Moreover, some of the ectoparasites associated with sigmodontines, such as ticks, are involved in the enzootic cycle of these bacteria. Because of the proximity of the habitats of sigmodontine with humans, these rodents are important from an epidemiological perspective (Meerburg et al., 2009; Guglielmone et al., 2014).

Herein, we analyze the presence of *Rickettsia* in ticks parasitic of sigmodontine rodents in two areas of northeastern Buenos Aires province, Argentina, where cases of rickettsiosis were reported but the vector remains unknown.

Samplings were carried out between 2017 and 2018 throughout nine collection campaigns in two localities with different degrees of anthropization situated in La Pampa biogeographic province (Morrone, 2006). One of these (Arana) is mostly rural, located in the suburbs of La Plata (35°00'S, 57°54'W), with a surface of five hectares mostly composed of pasturelands (five sampling campaigns). The other location (La Balandra) is situated on the margin of Río de la Plata, in the city of Berisso (34°56'S, 57°42'W) and it is characterized by forested wetlands intercalated with coastal strips emerging from water (four sampling campaigns). The sampling effort was 80 traps *per* night placed in a distance of five meters one from each other for 24 hours. Rodents were captured alive by using Sherman-like traps baited with oat.

A total of 222 rodents were captured, and ticks were collected from their furs in the field and preserved in ethanol 96%. At the laboratory ticks were identified under stereoscopic binocular microscope by using the taxonomic keys and morphological descriptions presented in Nava et al. (2017). Afterwards, for molecular studies, ticks were processed individually. Tick genomic DNA was extracted with phenol-chloroform method, as described in Mangold et al. (1998). Tick DNA samples were tested for the presence of rickettsial citrate synthase (*gltA*) gene (primers CS5: GAGAGAAAATTATATCCAAATGTTGAT and CS6: AGGGTCTTCGTGCATTTCTT) by real-time PCR as

described by Labruna et al. (2004) and Guedes et al. (2005). Positive samples were further analysed by a battery of conventional PCR methods targeting a 834 bp fragment of *gltA* gene (primers CS-239: CTCTTCTCATCCTATGGCTATTAT and CS-1069: CAGGGTCTTCGTGCATTTCTT) (Labruna et al., 2004), a 512 bp fragment of outer membrane protein A (*ompA*) gene (primers Rr190.70p: ATGGCGAATATTTCTCCAAAA and Rr190.602n: AGTGCAGCATTCGCTCCCCCT) (Regnery et al., 1991) and a 862 bp fragment of outer membrane protein B (*ompB*) (primers 120-M59: CCGCAGGGTTGGTAACTGC and 120-807: CCTTTTAGATTACCGCCTAA) (Roux & Raoult, 2000). In all PCR methods, DNA of *R. parkeri* sensu stricto from Brazil was used as positive control. Positive PCR fragments were purified using a commercial kit (Wizard® SV Gel and PCR Clean-Up System, Promega) and sequenced in ABI 3130XL Genetic Analyser (INTA Castelar, Buenos Aires, Argentina). Obtained partial sequences were edited using BioEdit software (Hall, 1999) with manual edition whenever it was necessary, aligned with the program Clustal W (Thompson et al., 1994) and compared with sequences deposited in GenBank™ by using BLAST tools (https://blast.ncbi.nlm.nih.gov/Blast.cgi) Phylogenetic analyses were performed with Maximum-likelihood (ML) method by using the program Mega 5 (Tamura et al., 2011). Best fitting substitution models were determined with the Akaike Information Criterion using the ML model test implemented in MEGA 5 (Substitution models were T92 (G +I). Support for the topologies was tested by bootstrapping over 1,000 replications and excluding gaps.

Rodents were identified by Ulyses Pardiñas (IDEAus, CONICET) and Carlos Galliari (CEPAVE) and will be deposited at the Colección de Mamíferos del Centro Nacional Patagónico, Puerto Madryn, Chubut, Argentina.

The 222 rodents were determined as *Oxymycterus rufus* Fischer (n = 70), *Akodon azarae* Fischer (n = 66), *Oligoryzomys flavescens* (Waterhouse) (n = 35), *Scapteromys aquaticus* Thomas (n = 33); *Oligoryzomys nigripes* Olfers (n = 15) and *Deltamys kempi* Thomas (n = 3) (Cricetidae, Sigmodontinae). A total of ten ticks (nine nymphs and one larva) were collected and determined as *Ixodes loricatus* Neumann. Ticks collected on each host species and localities are shown in table I.

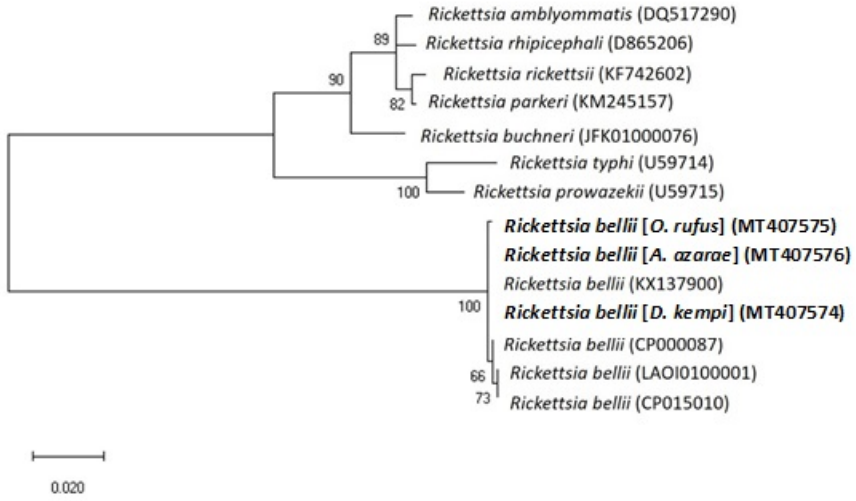

Fig. 1. Maximum-likelihood tree constructed from gltA partial sequences for different *Rickettsia* species.

Partial sequences generated in this study are written in bold letters. Numbers represent bootstrap support generated from 1,000 replications. GenBank™ accession numbers are given in brackets.

Out of these ten specimens of *I. loricatus* screened in real-time PCR, three nymphs showed amplification for the *gltA* gene. These samples were also positive in the conventional PCR showing amplicons of the expected sizes for the *gltA* gene. No amplification was observed in the *ompA* and *ompB* PCR tests of these three ticks.

The three positive *gltA* samples were obtained from three *I. loricatus* nymphs collected each one on *D. kempi, O. rufus* and *A. azarae,* respectively. Sequences of *gltA* were deposited in the GenBank™ (Accession numbers: MT407574; MT407575 & MT407576 respectively). These sequences matched with more than 99% of similarity with *gltA* sequences available in GenBank™ of *R. bellii*. The phylogenetic tree constructed with the sequences obtained in this work (Fig. 1) clearly shows that they are grouped with *R. bellii* (Accession numbers KX137900, CP000087, LAOI100001, CP015010) and separated from other rickettsial groups.

The present study evaluates the rickettsial infection in *I. loricatus* ticks collected on sigmodontine rodents in the northeastern region of Buenos Aires province. *Ixodes loricatus* is distributed in areas of Argentina, Brazil, Paraguay and Uruguay belonging to the Pampa, Chaco and Parana forest biogeographic provinces *sensu* Morrone (2006), with adults usually found in marsupials (Didelphidae) and their immature stages in sigmodontine rodents (Cricetidae) and also in marsupials (Didelphidae) (Nava et al., 2017). In this work, *I. loricatus* was the only tick species collected, which is in accordance with the literature that indicates that this specie is the most prevalent in sigmodontine rodents from Buenos Aires (Beldoménico et al., 2005). Even though a small number of ticks was collected and tested (n = 10), 30% of them were positive for *R. bellii* suggesting a high prevalence of this bacterium in *I. loricatus* from the study area.

According to previous reports, *R. bellii* was only isolated in ticks and it is widely distributed in America as resumed by Krawczak et al. (2018). In Argentina, *R. bellii* was detected in free living ticks of *Amblyomma sculptum* Berlese, *A. ovale* Koch, *A. neumanni* Ribaga, *A. tigrinum* Koch, *Haemaphysalis juxtakochi* Cooley and *A. dubitatum* Neumann collected on *Hydrochoerus hydrochaeris* L. (Caviidae) (Nava et al., 2017; Sebastian et al., 2017).

This is the first report of *R. bellii* infecting *I. loricatus* in Argentina and the first isolation of this bacterium in ticks associated with sigmodontine rodents. Nevertheless, additional research is required to determine the role of these mammals and their ectoparasites in the enzootic cycle of *R. bellii*. Until the epidemiological relevance of this species is fully clarified, the importance of its isolation should not be underestimated.

Hosts (n)	Prevalence	Larvae	Nymphs	Locality (n)
Oxymycterus rufus (70)	6% (4/70)	-	4	Arana (3); La Balandra (1)
Oligoryzomys nigripes (15)	13% (2/15)	-	2	La Balandra (2)
Akodon azarae (66)	3% (2/66)	1	1	Arana (2)
Oligoryzomys flavescens (35)	3% (1/35)	-	1	La Balandra (1)
Deltamys kempi (3)	33% (1/3)	-	1	La Balandra (1)

Table I. *Ixodes loricatus* ticks collected with their prevalence values (%) followed by number of parasitized hosts / number of hosts examined between parentheses in every host species, and number of ticks collected *per* locality

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