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Borrelia infection in *Ixodes pararicinus* ticks (Acari: Ixodidae) from northwestern Argentina

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ABSTRACT

The aim of this work was to describe for the first time the presence of *Borrelia burgdorferi* sensu lato infecting ticks in Argentina. Unfed specimens of *Ixodes pararicinus* collected from vegetation in Jujuy Province were tested for *Borrelia* infection by PCR targeting the gene flagellin (*fla*), the *rrfA-rrlB* intergenic spacer region (IGS) and the 16S rDNA (*rrs*) gene. One male and one female of *I. pararicinus* collected in Jujuy were found to be positive to *Borrelia* infection with the three molecular markers tested. Phylogenetically, the *Borrelia* found in *I. pararicinus* from Jujuy belongs to the *B. burgdorferi* s.l complex, and it was similar to one of the genospecies detected in *I. aragaoi* from Uruguay. Also, this genospecies is closely related to two genospecies known from USA, *Borrelia americana* and the *Borrelia* sp. genospecies 1. The epidemiological risk that implies the infection with *Borrelia* in *I. paracinus* ticks from Argentina appears to be low because the genospecies detected is not suspected of having clinical relevance and there are no records of *Ixodes* ticks biting humans in the southern cone of South America. Further studies are needed to assess accurately if there is risk of borreliosis transmitted by ticks in South America.

1. Introduction

Borrelia burgdorferi sensu lato is a species complex of spirochaetal bacteria which includes at least 20 genospecies, namely *Borrelia afzelii*, *Borrelia americana*, *Borrelia andersonii*, *Borrelia bavariensis*, *Borrelia bisettii*, *Borrelia burgdorferi* sensu stricto, *Borrelia californiensis*, *Borrelia carolinensis*, *Borrelia chilensis*, *Borrelia finlandensis*, *Borrelia garinii*, *Borrelia japonica*, *Borrelia kurtenbachii*, *Borrelia lusitaniae*, *Borrelia sinica*, *Borrelia spielmanii*, *Borrelia tanukii*, *Borrelia turdi*, *Borrelia valaisiana* and *Borrelia yangtze* (Casjens et al., 2011; Margos et al., 2011; Stanek and Reiter, 2011; Ivanova et al., 2013; Margos et al., 2014). *B. burgdorferi* s.s, *B. afzelii* and *B. garinii* are the major etiological agents of Lyme borreliosis, a tick-borne infectious disease of humans in the Holarctic region, although other species, such as *B. spielmanii*, *B. bavariensis*, *B. bisettii*, *B. lusitaniae* and *B. valaisiana*, have also been involved as causative agents of Lyme borreliosis in humans (Steere et al., 2005; Stanek and Reiter, 2011; Stanek et al., 2012). The remaining species of the *B. burgdorferi* s. l complex are of unknown pathogenicity.

Most of the *B. burgdorferi* s. l species are associated to hard ticks of the genus *Ixodes*, principally with species belonging to the *Ixodes ricinus* complex (Steere et al., 2005). This tick complex is formed by species mostly distributed in the Nearctic and Palearctic regions, as *I. ricinus, Ixodes scapularis, Ixodes jellisoni, Ixodes pacificus, Ixodes gibbosus, Ixodes hyatti, Ixodes kashmiricus, Ixodes kazakstani, Ixodes nipponensis, Ixodes muris, Ixodes minor* (this species is also present in the Neotropics), *Ixodes nuttallianus, Ixodes pavlovskyi, Ixodes persulcatus* and *Ixodes granulatus* (Keirans et al., 1999; Xu et al., 2003). Among these species, the principal vector of Lyme borreliosis are *I. scapularis* (Nearctic distribution), *I. ricinus* and *I. persulcatus* (Palearctic distribution) (Piesman and Gern, 2008; Stanek et al., 2012). The *I. ricinus* species complex is represented in South America by *Ixodes pararicinus*, *Ixodes affinis* (this species is also present in the Nearctic) and *Ixodes aragaoi* (Venzal et al., 2005; Onofrio et al., 2014), and possibly also by *Ixodes fuscipes*, as

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recently proposed Onofrio et al. (2014). To date, there are no records of these four species biting humans along their distribution in this continent (Guglielmone et al., 2014).

The only consistent reports of *B. burgdorferi* s.l in South America were made in Chile and Uruguay (Barbieri et al., 2013; Ivanova et al., 2013). *Borrelia chilensis* was described in Chile throughout the characterization of cultured spirochetes and borrelial DNA obtained from *Ixodes stilesi* ticks collected in Valdivia, Chile (Ivanova et al., 2013). In Uruguay, specimens of *I. aragaoi* (named as *I. pararicinus*, see Onofrio et al. (2014)) were found to be infected with two new genospecies of *B. burgdorferi* s.l, one phylogenetically related to *B. bisettii* and the other associated to *B. americana* (Barbieri et al., 2013). Cases of tick-borne borreliosis were not diagnosed so far in both the countries.

In Argentina, *Borrelia* sp. was detected in cattle and in the tick *Rhipicephalus* (*Boophilus*) *microplus* (Nájera, 1949; Hadani et al., 1985; Guglielmone et al., 1987), and *Borrelia anserina* was thought to be associated to *Argas persicus* (Boero, 1957). However, the knowledge on *B. burgdorferi* s.l in this country is inexistent. There are no records of *B. burgdorferi* s.l infecting ticks and although, a few suspected cases of human borreliosis were described (Stanchi and Balague, 1993; Battaglia et al., 2000), the clinical evidence of human borreliosis in Argentina is far from conclusive. In view of the above-mentioned information, herein we report, for the first time, the occurrence of a genospecies of *B. burgdorferi* s.l infecting ticks in Argentina.

2. Materials and methods

Tick collection was performed in Villa Monte (24°18′S, 64°31′W; 1900 m a.s.l), Santa Bárbara Department, Jujuy Province, northwestern Argentina, during September 2013. This locality belongs to the Yungas Phytogeographic Province as defined by Cabrera (1994) and it consists of environmental settings of cloud forest biomes where the 1500 mm of annual rainfall are concentrated in spring and summer. Questing ticks were collected from vegetation by using cloth flags and preserved in 96% ethanol. All ticks collected were determined as males and females of *I. pararicinus* according to the description of Keirans et al. (1985) and they are deposited in the Departamento de Parasitología Veterinaria, Facultad de Veterinaria, Universidad de la República, Montevideo, Uruguay (DPVURU 871).

For molecular analyses, 12 ticks (six females and six males) were processed for DNA extraction and screened by PCR for detection of *Borrelia* DNA following the methods detailed in Barbieri et al. (2013). Nested PCR was done targeting the flagellin gene (*fla*) of *Borrelia* spp. with the primers Fla LL (5′-ACA TAT TCA GAT GCA GAC AGA GGT- 3′) and FLA RL (5′-GCA ATC ATA GCC ATT GCA GAT TGT-3′) for the first reaction and with the primers Fla LS (5′-AAC AGC TGA AGA GCT TGG AAT G-3′) and FLA RS (5′-CTT TGA TCA CTT TC ATT CTA ATA GC-3′) (Barbour et al., 1996) for the nested reaction. Positive samples were further used to amplify a 225- to 255-bp fragment of the *rrfA-rrlB* intergenic spacer region (IGS) using primers IGSb (5′-GTT AAG CTC TTA TTC GCT GAT GGT A-3′) and IGSa (5′-CGA CCT TCT TCG CCT TAA AGC-3′) (Derdáková et al., 2003), and a 746-bp fragment of the 16S rDNA (*rrs*) gene with primers S5-F (5′-GAG GAA TAA GCT TTG TAG GA-3′) and S13-R (5′-GAC GTC ATC CTC ACC TTC CT-3′) (Le Fleche et al., 1997). DNA of *B. anserina* was employed as positive control.

The 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 5 (Tamura et al., 2011). A tree based on *Borrelia fla* partial sequences was generated with the Tamura 3-parameter model by using a discrete gamma-distribution (+G). Support for the topologies was tested by bootstrapping over 1000 replications. The number of variable nucleotide positions between sequences of *fla*, IGS and *rrs* were used to calculate pair-wise estimates of percent sequence divergence among sequences of *Borrelia* spp. found in different areas of the world. Gaps were excluded in the pairwise distance estimation.

3. Results

One male and one female of *I. pararicinus* collected in Villa Monte were found to be positive to *Borrelia* infection with the three molecular markers tested. The phylogenetic tree constructed with *fla* sequences is showed in Fig. 1. Two haplotypes of a *Borrelia* genospecies belonging to the *B. burgdorferi* s.l complex were identified from the *fla* sequences, one in the positive male (haplotype I) and the other in the positive female (haplotype II). Phylogenetically, both haplotypes were closely related with the haplotypes D and E of *Borrelia* sp. which were reported by Barbieri et al. (2013) infecting *I. aragaoi* ticks (named as *I. pararicinus*, see Onofrio et al. (2014)) from Uruguay (Fig. 1). Pairwise difference between haplotype I from Argentina (GenBank accession number: KJ994335) and haplotypes D (GenBank accession number: JX082314) and E (GenBank accession number: JX082315) from Uruguay was 0.3% and 0%, respectively, while the difference between haplotype II from Argentina (GenBank accession number: KJ994336) and haplotypes D and E from Uruguay was 1.9% and 1.6%, respectively. Pairwise difference between haplotypes I and II from Argentina was 1.6%.

DNA sequences of *rrs* and IGS were generated from the *Borrelia* sp. detected in the male of *I. pararicinus*. The analyses of *rrs* and IGS sequences confirmed the results obtained with the *fla* gene. Regarding the *rrs* sequences, pairwise divergence between the haplotype from Argentina (GenBank accession number: KJ994333) and the haplotypes K (same source of DNA that *fla* haplotype D; GenBank accession number: JX082320) and L (same source of DNA that *fla* haplotype E; GenBank accession number: JX082321) from Uruguay (see Barbieri et al., 2013) was 0.7% and 0.6%, respectively. Among all IGS sequences of *Borrelia* spp. available, the sequence from Argentina (GenBank accession number: KJ994334) was more similar (1.1% of genetic divergence) to the haplotypes M (same source of DNA that *fla* haplotype D; GenBank accession number: JX082316) and G (same source of DNA that *fla* haplotype E; GenBank accession number: JX082317) from Uruguay (see Barbieri et al., 2013).

4. Discussion

The findings presented in this work which are based on the analyses of three different loci constitute the first record of *B. burgdorferi* s.l infection in ticks in Argentina. The *Borrelia* found in *I. pararicinus* from Villa Monte is similar to one of the genospecies detected in *I. aragaoi* from Uruguay by Barbieri et al. (2013). This genospecies belongs to the *B. burgdorferi* s.l complex and phylogenetically is most closely related to two genospecies known from USA, *B. americana* and the *Borrelia* sp. genospecies 1 described in Postic et al. (2007) (Fig. 1). Hence, the presence of different genospecies of the *B. burgdorferi* s.l complex infecting *Ixodes* tick is confirmed for the southern cone of South America in Uruguay, Chile and Argentina (Barbieri et al., 2013; Ivanova et al., 2013, this work).

The high similarity among the genospecies of *B. burgdorferi* s.l recorded in Argentina and Uruguay is not unexpected because they were detected in two South American species of the *I. ricinus* complex, *I. aragaoi* and *I. pararicinus* which are closely related from

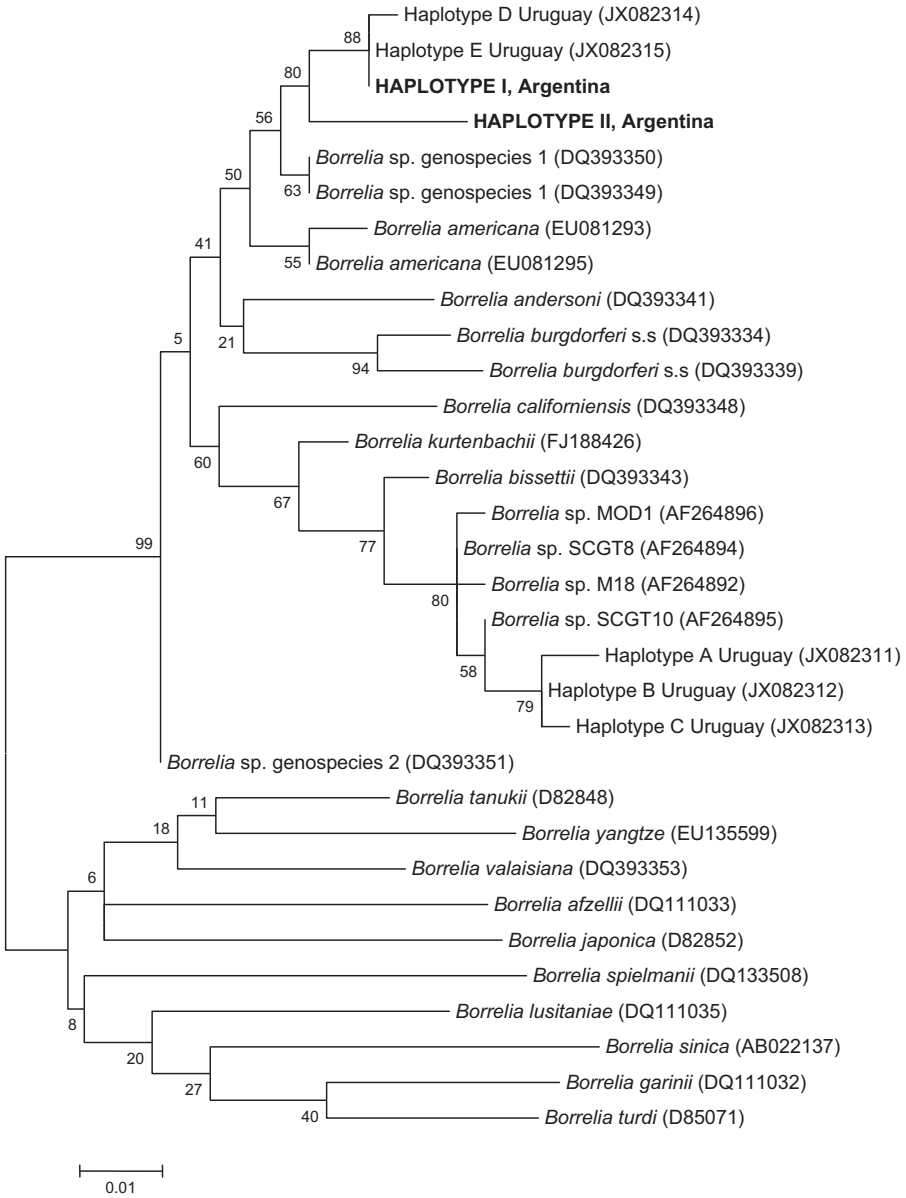

Fig. 1. Maximum-likelihood tree constructed from *Borrelia fla* partial sequences. Numbers represent bootstrap support generated from 1000 replications. GenBank accession numbers are in brackets.

a phylogenetic and morphological perspective (Venzal et al., 2005; Onofrio et al., 2014). *Ixodes aragaoi* was considered to be exclusively distributed in Brazil but a recent taxonomic work (Onofrio et al., 2014) determined that ticks previously considered as *I. parariccinus* in Uruguay correspond, in fact, to *I. aragaoi.* Consequently, the distribution of *I. parariccinus* is restricted to Argentina and Colombia while *I. aragaoi* is present in southern Brazil and Uruguay (Guglielmone et al., 2003; Onofrio et al., 2014). Far different is the case of *B. chilensis*, a genospecies isolated from *I. stilesi* in Chile (Ivanova et al., 2013). This tick species does not belong to the *I. ricinus* complex and it is very different to *I. aragaoi* and *I. parariccinus* regarding distribution (exclusively in Chile), morphology and phylogenetic position (Guglielmone et al., 2006b). Therefore, it can be affirmed that in the southern cone of South America the genospecies of the *B. burgdorferi* s.l complex are not exclusively associated to ticks of the *I. ricinus* complex. Future studies on *Borrelia* infection in South American *Ixodes* ticks should include different species regardless of their belonging to the *I. ricinus* complex.

Considering the currently available information, the epidemiological risk that implies the infection with *Borrelia* in *I. paracinus* ticks from Argentina appears to be low. Although the *Borrelia* genospecies detected in *I. parariccinus* belong to the *B. burgdorferi* s.l complex, it is phylogenetically related to other *Borrelia* genospecies (*B. americana* and *Borrelia* sp. genospecies 1) which are not suspected of having clinical relevance (Stanek and Reiter, 2011). Additionally and unlike what occur in the Holarctic region where tick bites in humans by *Ixodes* spp. are very common, the medical relevance of the genus *Ixodes* in South America is extremely low (Guglielmone et al., 2006a). In fact, there are no records of *Ixodes*

ticks biting humans in the southern cone of South America (Guglielmone et al., 2014). Eco-epidemiological information available for Argentina supports that mentioned in the preceding lines. The populations of *I. paracinus* in Argentina are mostly concentrated in areas belonging to the Yungas Phytogeographic Province sensu Cabrera (1994) in Salta, Jujuy and Tucumán Provinces (Guglielmone and Nava, 2005). Adults of *I. parariccinus* have a host range that includes cattle, horses, *Mazama gouazoubira* and *Tayassu tajacu* and immature stages are associated to small rodents and passerine birds (Guglielmone and Nava, 2005; Nava and Guglielmone, 2013), but cases of human infestation by this tick have not been recorded. The evidence of human borreliosis in Argentina (Stanchi and Balague, 1993; Battaglia et al., 2000) is based on poorly obtained data. These diagnostics did not reach with molecular detection or culture of spirochetes but they were based on non-specific serology with no indication of tick bites in the patients. Thus, the suspected cases of human borreliosis diagnosed in Argentina by Stanchi and Balague (1993) and Battaglia et al. (2000) should be considered warily until new and solid evidence becomes available. In view of the above-mentioned considerations, it is evident that studies focussing on isolation and molecular detection of *Borrelia* from ticks and on epidemiological issues of the tick vector (ecology, vectorial capacity to transmit *Borrelia*, determination of species prone to bite humans, geographic distribution) are needed to assess accurately if there is risk of borreliosis transmitted by ticks in the southern cone of South America.

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