

Molecular Detection of the Human Pathogenic *Rickettsia* sp. Strain Atlantic Rainforest in *Amblyomma dubitatum* Ticks from Argentina

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Abstract

To date, three tick-borne pathogenic *Rickettsia* species have been reported in different regions of Argentina, namely, *R. rickettsii*, *R. parkeri*, and *R. massiliae*. However, there are no reports available for the presence of tick-borne pathogens from the northeastern region of Argentina. This study evaluated the infection with *Rickettsia* species of *Amblyomma dubitatum* ticks collected from vegetation and feeding from capybaras (*Hydrochoerus hydrochaeris*) in northeastern Argentina. From a total of 374 *A. dubitatum* ticks collected and evaluated by PCR for the presence of rickettsial DNA, 19 were positive for the presence of *Rickettsia bellii* DNA, two were positive for *Rickettsia* sp. strain COOPERI, and one was positive for the pathogenic *Rickettsia* sp. strain Atlantic rainforest. To our knowledge, this study is the first report of the presence of the human pathogen *Rickettsia* sp. strain Atlantic rainforest and *Rickettsia* sp. strain COOPERI in Argentina. Moreover, our findings posit *A. dubitatum* as a potential vector for this pathogenic strain of *Rickettsia*.

Key Words: *Rickettsia* sp. strain Atlantic rainforest—*Rickettsia* sp. strain COOPERI—*Hydrochoerus hydrochaeris*—Argentina.

Introduction

IN SOUTH AMERICA, CLINICAL CASES of human rickettsioses caused by tick-borne rickettsiae were reported in Argentina, Brazil, Colombia, and Uruguay (for review, see Parola et al. 2013). In Argentina, six species of *Rickettsia* were found infecting ticks, namely, *R. rickettsii*, *R. parkeri*, *R. massiliae*, *R. bellii*, ‘*Candidatus R. amblyommii*’, and ‘*Candidatus R. andeanae*’ (Parola et al. 2013). Of these, *R. rickettsii*, *R. parkeri*, and *R. massiliae* are associated with human diseases (Parola et al. 2013). The respective vectors of these three pathogens in Argentina are the ticks *Amblyomma cajennense* sensu lato (s.l.), *Amblyomma triste*, and *Rhipicephalus sanguineus* s.l. (Paddock et al. 2008, Monje et al. 2014, Cicuttin et al. 2014). In the past few years, new strains of *Rickettsia*, some of them closely related to *R. parkeri*, were described in Brazil infecting *Amblyomma nodosum* (strain NOD), *Amblyomma ovale* (strain Atlantic rainforest), and *Amblyomma dubitatum* (strains COOPERI and Pampulha) ticks (Parola et al. 2013). Of these, *Rickettsia* sp. strain At-

lantic rainforest is the only one currently associated with human cases of rickettsiosis (Spolidorio et al. 2010).

Amblyomma dubitatum feeds predominantly on capybaras (*Hydrochoerus hydrochaeris*), but all stages of this tick species were recorded biting humans (Labruna et al. 2007). In northeastern Argentina, capybaras are present at high densities living close to farms and urban settlements, resulting in an extensive human–wildlife interface that may represent a potential risk to public health. Due to the possible role of *A. dubitatum* as an enzootic vector of potentially pathogenic rickettsiae, this study evaluated the infection with *Rickettsia* species of *A. dubitatum* ticks collected from vegetation and feeding from capybaras in northeastern Argentina.

Material and Methods

Ticks were collected at five localities from northeastern Argentina (Table 1). Questing larvae, nymphs, and adults were collected from the vegetation using flannel cloth flags in four areas of Corrientes province. Feeding ticks were

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TABLE 1. RICKETTSIAL INFECTION IN *AMBLYOMMA DUBITATUM* TICKS COLLECTED FROM CAPYBARAS AND VEGETATION IN NORTHEASTERN ARGENTINA

Site	Coordinates	Total ticks (infected ticks)					Source	Rickettsia prevalence (%)		
		Adults		Nymphs	Larvae pools	Rickettsia bellii		Rickettsia sp.		
		Males	Females					strain Atlantic rainforest	Rickettsia sp. strain COOPERI	
Mburucuyá ^a	28°01'S 58°01'W	6	6	117	5	Vegetation	0	0	0	
Mercedes ^b	29°09'S 58°04'W	0	0	3	14 (8)	Vegetation	47.1	0	0	
Provincial Road 40, km 60	28°50'S 57°34'W	5 (1)	2 (1)	43 (5)	0	Vegetation	14.0	0	0	
Santa Bárbara	28°36'S 58°24'W	0	1	14 (1)	2	Vegetation	5.9	0	0	
Romang	29°30'S 59°44'W	67 (3,0,1) ^c	32 (0,1,1) ^c	1	0	Capybara	3.0	1.0	2.0	

^aIncludes one nymph of *Haemaphysalis juxtakochi*.

^bIncludes one nymph and one pool of two larvae of *Amblyomma triste*.

^cNumber of ticks infected with *R. bellii*, *Rickettsia* sp. strain Atlantic rainforest, and *Rickettsia* sp. strain COOPERI, respectively.

obtained from wild capybaras (around ten) belonging to populations close to Romang city, Santa Fe province (Table 1). Of these latter, only males, nonengorged females, and nonengorged nymphs were analyzed. Ticks were determined by S.N. and A.A.G. by using standard taxonomic keys and morphological descriptions. All adults and nymphs were analyzed individually, and larvae were divided in pools according to collection site. Ticks were processed for DNA extraction by a boiling technique (Monje et al. 2014) and tested for rickettsial infection by conventional PCR targeting gene *gltA* using primers CS-78/CS-323 (Labruna et al. 2004). Positive samples were further tested with primers targeting genes *gltA* (larger fragment) and *ompA* (Labruna et al. 2004). Reactions were performed in triplicate, and PCR products were purified and sequenced. Resultant sequences were compared to GenBank data by BLAST analysis.

Results

Most of the ticks were identified as *A. dubitatum* (119 adults [78 males, 41 females], 176 nymphs, and 79 larvae). One nymph and two larvae were identified as *A. triste*, and one nymph was identified as *Haemaphysalis juxtakochi* (all questing). Eight adult ticks, six nymphs, and eight larvae pools tested positive for *gltA* PCR using primers CS-78/CS-323 (Table 1). The few *A. triste* and *H. juxtakochi* samples were PCR negative. Only three adult ticks collected on *H. hydrochaeris* from Romang (Ad106 in May, 2012; Ad144 and Ad170 in November, 2012) were positive for both the larger fragment of *gltA* and *ompA* genes. Moreover, only *gltA* PCR products were obtained in the rest of the positive samples ($n=19$) that were 100% identical to *R. bellii* (acc. no. CP000087). Sequences from sample Ad106 were identified as *Rickettsia* sp. strain Atlantic rainforest, because its *gltA* (787 bp) and *ompA* (592 bp) gene fragments were 100% equal to corresponding sequences (JQ906783 and JQ906784, respectively). Sequences from Ad144 and Ad170 were 100% identical to each other for the rickettsial gen *ompA* and were identified as *Rickettsia* sp. strain COOPERI. BLAST analysis showed that the *ompA* sequence from ticks Ad144 and Ad170 (592 bp) was 99.8% identical to strain COOPERI (AY362706). The *gltA* sequence (758 bp) from tick Ad170 was 99.9% identical to strain COOPERI (AY362704) and 99.7% identical to *R. parkeri* (CP003341). Surprisingly, sample

Ad144 was co-infected with *R. bellii* because its *gltA* fragment was 100% identical to *R. bellii* (CP000087). Novel sequences were deposited in GenBank (KM116015-KM116018).

Discussion

In the present study, we report the presence of the human pathogen *Rickettsia* sp. strain Atlantic rainforest (Spolidorio et al. 2010) for the first time in Argentina. Moreover, our findings posit *A. dubitatum* as a potential vector for strain Atlantic rainforest, which was previously reported infecting *A. ovale* ticks in southeastern Brazil (Szabo et al. 2013).

Of five *A. dubitatum* populations examined, *R. bellii* was found in four, with prevalences that ranged from 3.0% to 47.1%. *Rickettsia* sp. strain Atlantic rainforest and *Rickettsia* sp. strain COOPERI were found only in Romang, where the prevalences were 1.0% and 2.0%, respectively (Table 1). No evidence of rickettsial DNA was observed in the samples from site "Mburucuyá," although over 100 ticks were analyzed. Previous studies failed to detect *Rickettsia* sp. strain Atlantic rainforest in various populations of *A. dubitatum* in Brazil, even when thousands of adult ticks were tested (Labruna et al. 2004, Pacheco et al. 2009, Almeida et al. 2011). In those studies, *A. dubitatum* ticks were infected chiefly by *R. bellii*, *Rickettsia* sp. strain COOPERI, and *Rickettsia* sp. strain Pampulha (Labruna et al. 2004, Pacheco et al. 2009, Almeida et al. 2011). In addition, *R. parkeri* was recently detected in *A. dubitatum* ticks from Uruguay (Lado et al. 2014). Altogether, these results suggest that different populations of *A. dubitatum* throughout Argentina, Brazil, and Uruguay are capable of maintaining a variety of rickettsiae, including the human pathogens *Rickettsia* sp. strain Atlantic rainforest and *R. parkeri*. However, because the evidence of the presence of pathogenic *Rickettsia* was obtained from *A. dubitatum* ticks feeding on hosts, these results should be complemented with data from free-living ticks to confirm the vector capacity of *A. dubitatum* ticks.

Finally, further studies are warranted to elucidate the determinants of the distinctive geographical distribution of *Rickettsia* spp. observed in the region and to determine the potential pathogenicity and clinical significance of *Rickettsia* sp. strain COOPERI. Moreover, the fact that *Rickettsia* sp. strain Atlantic rainforest is pathogenic (Spolidorio et al. 2010) and *A. dubitatum* can parasitize humans in all its

feeding stages (Labruna et al. 2007) indicates that human cases of spotted fever caused by this pathogen may occur in northeastern Argentina.

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Author Disclosure Statement

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