SPOTTED FEVER GROUP RICKETTSIAE IN AMBLYOMMA TICKS LIKELY TO INFEST HUMANS IN RURAL AREAS FROM NORTHWESTERN ARGENTINA

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Abstract This work was performed to detect Rickettsia species of the spotted fever group in Amblyomma ticks likely to infest humans in rural areas from northwestern Argentina. Free-living ticks were collected and determined as Amblyomma tigrinum, Amblyomma neumanni and Amblyomma tonelliae. Rickettsia infection was determined by polymerase chain reactions which amplify fragments of the rickettsial genes gltA and ompA. A high frequency (35/44, 79.5%) of Candidatus “Rickettsia andeanae” was observed in A. tigrinum ticks, and Candidatus “Rickettsia amblyommii” was found in three out of 14 nymphs of A. neumanni. All 14 Amblyomma tonelliae ticks were negative for riketttsiae. The infection with spotted fever group rickettsiae in ticks aggressive for humans reveals the potential risk of exposure to tick-borne pathogens of people inhabiting rural areas of northwestern Argentina.

Key words: spotted fever rickettsiae, ticks, Amblyomma, Argentina

Resumen Rickettssias del grupo de las fiebres manchadas en garrapatas del género Amblyomma, capaces de infestar humanos, en áreas rurales del noroeste de Argentina. El objetivo de este trabajo fue detectar rickettssias del grupo de las fiebres manchadas en garrapatas del género Amblyomma capaces de infestar humanos, en áreas rurales del noroeste de Argentina. Se colectaron garrapatas en su fase de vida libre que fueron determinadas como Amblyomma tigrinum, Amblyomma neumanni y Amblyomma tonelliae. La infección con Rickettsia fue determinada utilizando la reacción en cadena de la polimerasa para amplificar los genes gltA y ompA. Candidatus “Rickettsia andeanae” fue detectada en A. tigrinum con alta frecuencia (35/44, 79.5%), mientras que Candidatus “Rickettsia amblyommii” fue encontrada en tres de las 14 ninfas de A. neumanni. Los 14 especímenes de A. tonelliae fueron negativos. La infección de garrapatas capaces de infestar humanos con rickettsias del grupo de las fiebres manchadas evidencia el riesgo potencial al que están expuestos los humanos que habitan o frecuentan áreas rurales del noroeste argentino.

Palabras clave: rickettsias del grupo de las fiebres manchadas, garrapatas, Amblyomma, Argentina

Amblyomma (Acari: Ixodidae) is the tick genus with the highest richness of species in South America1. This is important for public health since most of the records of ticks biting humans in this continent correspond to Amblyomma species1, 2. Intracellular bacteria of the genus Rickettsia belonging to the spotted fever group are among the most important tick-borne human pathogens in South America, and they are principally vectored by Amblyomma species5. The taxa Amblyomma cajennense sensu lato, Amblyomma aureolatum, Amblyomma triste, Amblyomma tigrinum and Amblyomma ovale were involved as vectors of human pathogenic rickettsiae3, 4. In this sense, Amblyomma ticks were determined as vectors of Rickettsia spp. in most of the human cases of rickettsioses diagnosed in Argentina3, 5.

A total of 25 tick species of the genus Amblyomma were recorded for Argentina, where13 of them were recorded biting humans5, 10. Amblyomma ticks acquire epidemiological relevance in northern Argentina because they are prevalent in areas intended for recreational use (e.g. tourism) and economic activities (e.g. livestock production) where human presence is usual. Therefore, the aim of this work is to analyze Rickettsia infection in Amblyomma ticks likely to infest humans in rural areas from northwestern Argentina.

Materials and Methods

Free-living ticks were collected in two localities representative of the Yungas Phytogeographic Province (YP) and Prepuna Phytogeographic Province (PPP)11 in northwestern Argentina: I) El Carmen (YP) (24°23’S, 65°15’W), Jujuy Province; II) El Mollar (PPP) (26°57’, 65°42’W), Tucumán Province. Questing
ticks were collected from vegetation by using cloth flags and preserved in 96% ethanol. Species was determined following Estrada-Peña et al. and Nava et al., and also compared with known laboratory-reared specimens deposited in the tick collection of Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria Rafaela (INTA Rafaela), Argentina.

Genomic DNA was obtained by proteolytic digestion with proteinase K, extraction with phenol-chloroform-isooamyl alcohol and precipitation in absolute ethanol as described by Mangold et al. Initial screening for rickettsiae was carried out with real-time polymerase chain reactions (rt-PCR) assay using primers CS-5 (5’ GAGGAAAAATATCCAAATGTTGAT -3) and CS-6 (5’ AGGGTCTTCGTGCAATTTCTT -3), which amplify a 147-bp fragment of the rickettsial citrate synthase (gltA) gene. The rt-PCR assay has successfully yielded fluorogenic signals for all Rickettsia species. For sequencing purposes, rt-PCR-positive specimens were subjected to conventional PCR amplifications targeting fragments of two rickettsial genes, the above-mentioned citrate synthase gene (gltA) and the 190-kDa outer membrane protein (ompA). In turn, two different primers combinations were used for sequencing this latter gene in different groups of specimens (Table 1). One negative control (water) and two positive controls (DNA of Rickettsia parkeri in the analysis of A. neumanni and A. tonelliae, and DNA of Rickettsia massiliae in the analysis of A. tigrinum) were included in each PCR run. Products of the conventional PCR amplifications were purified by WIZARD SV PCR purification columns (Promega, Madison, Wis.) according to the manufacturer’s protocol, and the purified amplicons were submitted to sequencing.

Sequences were aligned with each other and with the corresponding sequences of the Rickettsia species available in GenBank, using the BioEdit Sequence Alignment Editor with the CLUSTAL W program. Pairwise comparison among sequences was performed by using MEGA version 5.0. A phylogenetic analysis was carried out with ompA sequences employing the maximum-likelihood method by using the program Mega 5.0. Best fitting substitution model (GTR (G+I)) was determined with the Akaike Information Criterion using the maximum-likelihood model test implemented in MEGA 5.0. Support for the topologies was tested by bootstrapping over 1,000 replications and gaps were excluded from the comparisons.

Results

One male and 14 nymphs of Amblyomma neumanni and six males and eight females of Amblyomma tonelliae were collected in El Carmen. Forty four adults of A. tigrinum (16 males, 28 females) were collected in El Mollar. All ticks were tested for Rickettsia species. Thirty five (14 males, 21 females) out of 44 specimens (79.5%) of A. tigrinum were found to be gltA positive by rt-PCR. DNA sequences were obtained from 21 gltA-positive A. tigrinum ticks. These sequences were identical among each other (GenBank accession number: KT878724) and they had more than 99.8% similarity with the corresponding sequences of Candidatus “R. andeanae” detected in Amblyomma parvum from Argentina (GenBank accession number: EF451001) and Amblyomma maculatum from Peru (GenBank accession number: GU169050). Three gltA-positive samples obtained from A. tigrinum were used to amplify a circa 500-bp fragment of ompA gene with primers Rr190.70p and Rr190.701R (Table 1). The sequences were identical among each other (GenBank accession number: KT878725) and with those ompA sequences of Candidatus “R. andeanae” reported from A. parvum in Argentina and Brazil (GenBank accession numbers: EF451004, KF030932). Three nymphs of A. neumanni were PCR-positive for both gltA and ompA genes. In these specimens, ompA amplicons were obtained with primers Rr190.70p and Rr190.602n (Table 1). All three DNA positive samples were sequenced. The similarity of the three gltA sequences from El Carmen (GenBank accession number: KT878726) with the corresponding gltA sequence of Candidatus “R. amblyommii” previously reported in A. neumanni ticks from Argentina (GenBank accession number: DQ517290) was 99.7%. In the same way, the three ompA sequences obtained from the positive samples of A. neumanni (GenBank accession number: KT878727) was 99.5 % similar to the sequence of Candidatus “R. amblyommii” also detected in A. neumanni/ticks from Argentina (GenBank accession number: KT878727).

### TABLE 1. – Primer pairs used for amplification and sequencing of rickettsial gene fragments

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primers</th>
<th>Nucleotide sequence (5’ --- 3’)</th>
<th>Product size in basepairs</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>gltA</td>
<td>CS-239</td>
<td>GCTTTTCTCATCTATGGCTATTAT</td>
<td>549</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>CS-1069</td>
<td>CAGGGCTTCGTGCAATTCTTT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ompA</td>
<td>Rr190.70p</td>
<td>ATGGCGAATATTCTCCCCAAA</td>
<td>532</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Rr190.602n</td>
<td>AGTGCAGCATCCGTCCCCCT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ompA</td>
<td>Rr190.70p</td>
<td>ATGGCGAATATTCTCCCCAAA</td>
<td>circa 500</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Rr190.701R</td>
<td>GTTCCGTTAATGGGCAGCATCT</td>
<td></td>
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</tbody>
</table>
number: DQ517292). All 14 *A. tonelliae* ticks were negative for *Rickettsia* species. The phylogenetic position of the *Rickettsia* strains detected in this study according to ompA sequences is shown in Fig. 1.

### Discussion

The finding of *Candidatus* “*R. andeanae*” infecting *A. tigrinum* represents the first record of this association for Argentina. This result is not unexpected because *Candidatus* “*R. andeanae*” was already reported in *A. tigrinum* ticks from Chile\(^22\), and also in other American countries (including Argentina) associated to different species of *Amblyomma* as *A. triste*, *A. parvum*, *A.pseudoconcolor* and *A. maculatum\(^{23-28}\). However, the high prevalence detected in this work is noteworthy. Paddock et al.\(^{28}\) described a high prevalence of *Candidatus* “*R. andeanae*” in *A. maculatum* from USA (47% in ticks from Kansas, 73% in ticks from Oklahoma), and they suggest that those high levels of *Candidatus* “*R. andeanae*” infection may be responsible for the exclusion of the human pathogenic *Rickettsia parkeri* from their shared tick host. In this sense, Romer et al.\(^{5}\) found *A. tigrinum* ticks infected with *R. parkeri* in a tick population from Córdoba Province Argentina, where no ticks were found to be positive to *Candidatus* “*R. andeanae*”. Contrarily, the *A. trigrinum* ticks analyzed during this study were positive to *Candidatus* “*R. andeanae*” but not to *R. parkeri*. Altogether, these results support the idea of a rickettsial interference between *Candidatus* “*R. andeanae*” infecting *A. tigrinum*. For the phylogenetic relationships of the *Rickettsia* strains detected in this study, see Fig. 1.

![Fig. 1: Maximum-likelihood tree constructed from ompA partial sequences. Numbers represent bootstrap support generated from 1000 replications. GenBank accession numbers are in brackets.](image_url)
and R. parkeri. However, this phenomenon does not appear to be everywhere present, as coinfection of A. maculatum ticks with R. parkeri and Candidatus “R. andeanae” has been described. The prevalence of Candidatus “R. andeanae” reported by Ferrari et al. was ~ 3%, a value much lower than both values reported by Paddock et al. and those found in this work. Probably, the exclusion of R. parkeri by Candidatus “R. andeanae” may occur only when a tick presents high levels of infection with Candidatus “R. andeanae”.

A better knowledge of the pathogens carried by A. neumannii is relevant in Argentina because this is one of the tick species with the highest numbers of human infections reported in the country. Candidatus “R. amblyommii” was previously reported in A. neumannii ticks from Córdoba and Salta Provinces (M. Mastropaolo and S. Nava, unpublished observations). With the exception of a sole report of the non-pathogenic Rickettsia bellii in Córdoba Province, no other tick-borne bacteria has been detected to date in A. neumannii.

The results of this work together with data obtained in previous studies indicate that A. neumannii infection with Candidatus “R. amblyommii” is probably an ubiquitous phenomenon along the distribution of this tick species. All parasitic stages of A. neumannii and adult forms of A. tigrinum were recorded biting humans in Argentina. We find here these two tick species being infected with spotted fever group rickettsiae in rural areas of northwestern Argentina. Both Candidatus “R. andeanae” and Candidatus “R. amblyommii” are currently considered of unknown pathogenicity. Phylogenetically, however, they belong to the spotted fever group, which includes the tick-borne pathogenic Rickettsia species. In fact, Apperson et al. have suggested that Candidatus “R. amblyommii” might have been involved as the pathogenic agent in some human cases of rickettsiosis in USA. We conclude that the infection with spotted fever group rickettsiae in ticks known to be aggressive to humans alerts on a potential risk of exposure to tick-borne pathogens for livestock producers and people participating in outdoor recreational activities in the area represented by the study sites.

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Conflict of interest: None to declare

References


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Sanatorio Santa Mónica (*). Qué bien han hecho en ponerle ese nombre de mansedumbre al infierno rojo, en el que a todos los semblantes los ha barnizado de amarillo la muerte, y donde entre los cuatro pabellones, dos de hombres, dos de mujeres, sumamos cerca de mil tuberculosos [...] Y el círculo de montañas allá, que superan otras crestas de montes más distantes [...] Y el río que cuando hay sol, destella chapas de luz entre lo verde. [...] El médico también está tuberculoso. “Un vértice del izquierdo, nada más”. El practicante también, “casi nada, el derecho reblandecido”; y así, todos los que nos movemos como espectros en este infierno que lleva un santo nombre, [...] Tomamos mate de la misma bombilla, porque ya no tememos al contagio y bacilo más o menos por “campo” importa poco. Las conversaciones languidecen a poco de iniciadas y, generalmente, guardamos silencio.

Roberto Arlt (1900-1942)