Ticks and Tick-borne Diseases 6 (2015) 173–177

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Original article

Rickettsia infection in *Amblyomma tonelliae*, a tick species from the *Amblyomma cajennense* complex

Evelina L. Tarragona[a,b,*], Gabriel L. Cicuttin[c], Atilio J. Mangold[a], Mariano Mastropaolo[b], M. Nazarena De Salvo[c], Santiago Nava[a]

[a] Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria Rafaela and Consejo Nacional de Investigaciones Científicas y Técnicas, CC 22, CP 2300 Rafaela, Santa Fe, Argentina
[b] Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral, RP Kreder 2805, S3080HOF, Esperanza, Santa Fe, Argentina
[c] Instituto de Zoonosis Luis Pasteur-Ministerio de Salud-Gobierno de la Ciudad Autónoma de Buenos Aires, Av Díaz Vélez 4821 C1405DCD CABA, Argentina

ARTICLE INFO

Article history:
Received 14 August 2014
Received in revised form
20 November 2014
Accepted 29 November 2014
Available online 18 December 2014

Keywords:
Amblyomma tonelliae
Rickettsia
Argentina

ABSTRACT

The present study was performed to evaluate the *Rickettsia* infection in *Amblyomma tonelliae* ticks from Argentina. All ticks were subjected to DNA extraction and tested by a battery of PCRs to amplify fragments of four rickettsial genes, *23S-5S*, *gltA*, *ompA* and *htrA*. Two ticks were positive. The *Rickettsia* detected in one tick represents a new lineage which is named *Rickettsia* sp. strain El Tunal. This new strain belongs to the canadensis group because it is closely related to *Rickettsia monteiroi*, *Rickettsia canadensis* and *Candidatus* "Rickettsia tarasevichiae". They clustered together on a high supported clade with both *gltA* and *htrA* genes. The other positive tick was infected with *Candidatus* "Rickettsia amblyommii". The results presented in this study constitute the first records of *Rickettsia* infection in *A. tonelliae* ticks. However, the medical relevance of these findings should be considered cautiously because the pathogenicity of *Rickettsia* sp. strain El Tunal and *Candidatus* "R. amblyommii" remains undetermined.

Introduction

Bacteria of the genus *Rickettsia* are obligate intracellular parasites of order Rickettsiales in the alpha subdivision of the class Proteobacteria. They are transmitted by arthropod vectors and infect cells of the mammalian endothelial system. The genus *Rickettsia* was traditionally divided into the typhus group and spotted fever group, but currently they are classified into different groups: spotted fever group (SFG), typhus group (TG), *bellii* group (TRG), *canadensis* group and ancestral groups (Gillespie et al., 2007; Weinert et al., 2009). In Argentina, 49 species of ticks have been recorded to date, and *Amblyomma* is the genus with the highest specific richness (25 species) (Guglielmone and Nava, 2005, 2006; Nava et al., 2009, 2014a,b). With the exception of the records of *Rickettsia massiliae* in *Rhipicephalus sanguineus* sensu lato (Cicuttin et al., 2014), all rickettsiae vectored by ticks in this country were found to be associated to *Amblyomma* species (Labruna et al., 2007; Pacheco et al., 2007; Nava et al., 2008; Paddock et al., 2008; Tomassone et al., 2010; Cicuttin and Nava, 2013; Romer et al., 2014). Furthermore, most of the ticks recorded on humans in Argentina belong to the genus *Amblyomma* (Guglielmone et al., 1991, 2006; Nava et al., 2006).

The taxon *Amblyomma cajennense* was considered by a long time as a monospecific entity distributed in the Nearctic and Neotropical Regions from southern United States to northern Argentina (Estrada-Peña et al., 2004), but currently it has been demonstrated that *A. cajennense* is a complex formed by six species, namely *A. cajennense* sensu stricto, *Amblyomma interandinum*, *Amblyomma mixtum*, *Amblyomma patinoi*, *Amblyomma sculptum* and *Amblyomma tonelliae* (Beati et al., 2013; Nava et al., 2014a). In South America, ticks of *A. cajennense* complex are the principal vector of the human pathogen *Rickettsia rickettsii* (Guedes et al., 2005; Paddock et al., 2008; Labruna, 2009), and they were determined as the ticks with the most number of records on humans (Guglielmone et al., 2006). However, the taxonomic rearrangement of the *A. cajennense* complex aforementioned put in evidence the necessity of new studies about the role as vector of all its members, including the two species of this complex present in Argentina, *A. sculptum* and *A. tonelliae*. In light of this new perspective, the aim of this study is to evaluate the rickettsial infection in *A. tonelliae* ticks collected in northern Argentina.

* Corresponding author at: CC 22, CP 2300 Rafaela, Santa Fe, Argentina. Tel.: +54 03492440121; fax: +54 03492440114.
E-mail address: tarragona.evelina@inta.gob.ar (E.L. Tarragona).

http://dx.doi.org/10.1016/j.ttbdis.2014.11.010
1877-959X/

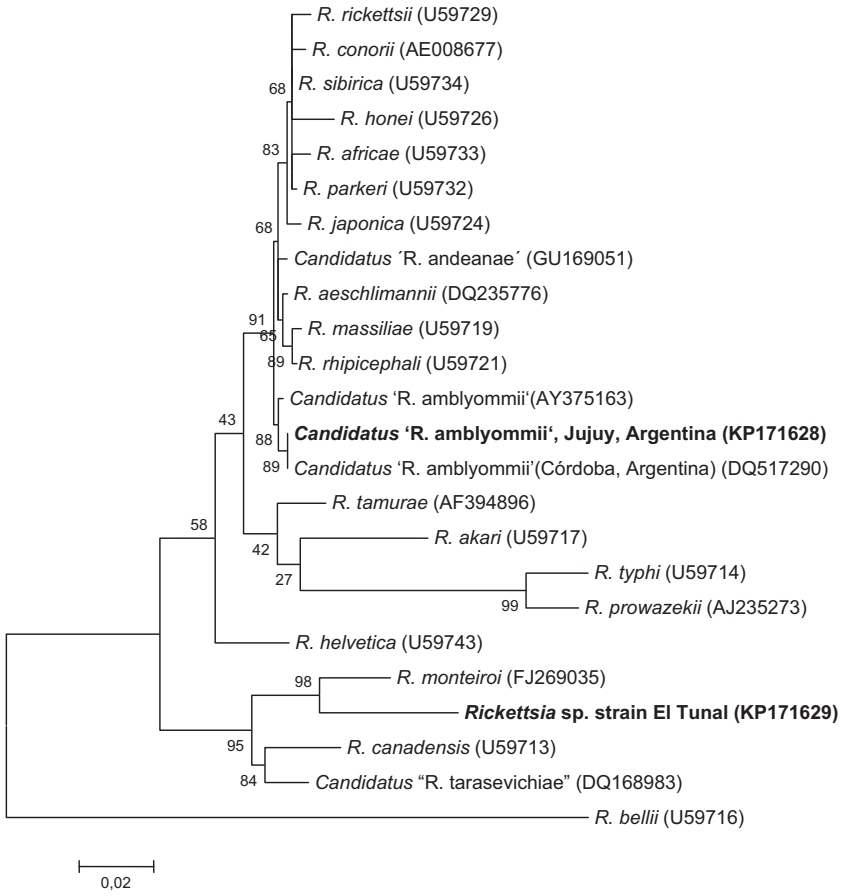

Fig. 1. Maximum-likelihood tree constructed from *gltA* partial sequences. Numbers represent bootstrap support generated from 1000 replications. GenBank accession numbers are in brackets.

Materials and methods

On January 2012, free-living adult ticks were collected from vegetation by dragging in El Tunal, Salta Province (ETS) (25° 14′S, 64° 25′W), and in a site (PSJ) (23° 49′S, 64° 13′W) located 20 km northeastern from Palma Sola, Jujuy Province. Both localities belong to the Chaco Phytogeographic Province according to the definition given by Cabrera (1994). All ticks were determined as *A. tonelliae* following the descriptions of Nava et al. (2014a).

Genomic DNA was extracted from each tick using the AxyPrep Multisource Genomic DNA MiniPrep Kit (Axygen Biosciences, USA). Detection of *Rickettsia* spp. was based on a PCR that amplifies a ca. 400-bp fragment of the 23S-5S intergenic spacer. PCR-positive samples were tested by a battery of PCRs to amplify fragments of three rickettsial genes, those for citrate synthase gene (*gltA*), 190-kDa outer membrane protein (*ompA*) and 17-kDa protein (*htrA)* (see details in Table 1). All amplicons were purified and sequenced. The sequences were edited using BioEdit Sequence Alignment Editor (Hall, 1999) with manual edition whenever it was necessary, aligned with the program Clustal W (Thompson et al., 1994) and compared with those sequences deposited in GenBank. Phylogenetic analyses were performed with maximum-likelihood (ML) methods by using the program Mega 5.0 (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 GTR (G+I) for *gltA* and *ompA*, and GTR+G for *htrA*. Support for the topologies was tested by bootstrapping over 1000 replications and gaps were excluded from the comparisons.

Results

A total of 68 adults of *A. tonelliae* were collected, 32 in ETS and 36 in PSJ. All ticks were tested individually by PCR. One tick of each locality was positive for 23S-5S intergenic spacer. Positive samples were used to amplify fragments of *gltA* and *ompA* genes as described in materials and methods. The positive sample from PSJ yielded expected PCR products for these two rickettsial genes, while the sample from ETS only yielded expected PCR products for *gltA* (the primers used to amplify *omp*A gen are specific for SFG Rickettsiae). In this case, a partial sequence of *htrA* was also obtained in order to confirm the results reached with *gltA*.

Rickettsia sp. detected in *A. tonelliae* from ETS was closely related to *Rickettsia* species belonging to the canadensis group, namely *Rickettsia monteiroi*, *Rickettsia canadensis* and *Candidatus* "Rickettsia tarasevichiae". They clustered together on a high supported clade with both *gltA* and *htrA* genes (Figs. 1 and 2). However, *gltA* sequence of *Rickettsia* sp. detected in *A. tonelliae* from ETS (Genbank accession number: KP171629) differed by more than 4.8% with the *gltA* sequences of *R. monteiroi* (FJ269035), *R. canadensis* (U59713) and *Candidatus* "R. tarasevichiae" (DQ168983), and the genetic divergence between *htrA* sequences from El Tunal (Genbank accession number: KP171630) and *htrA* sequences of *R. monteiroi* (FJ269036), *R. canadensis* (AF445381) and *Candidatus* "R. tarasevichiae" (JX996052) was always higher than 11.5%.

The *gltA* partial sequence from PSJ (Genbank accession number: KP171628) was identical to the corresponding sequence of *Candidatus* "R. amblyommii" strain An13 (DQ517290) detected in *Amblyomma neumanni* from Argentina, and they formed a

Table 1
Primer pairs used for amplification of rickettsial genes.

Gene	Primers	Nucleotide sequence (5′–3′)	Product size	Reference or source
23s-5s	RCK/23-5- RCK/23-5-	GATAGGTCRGRTGTGGAAGCAC TCGGGAYGGGATCGTGTGTTTC	388	Jado et al. (2006)
gltA	RpCS.415 RpCS.1220	GCTATTATGCTTGCGGCTGT TGCATTTCTTTCCATTGTGC	806	De Sousa et al. (2006)
htrA	17k-5 17k-3	GCTTTACAAAATTCTAAAAACCATATA TGTCTATCAATTCACAACTTGCC	549	Labruna et al. (2004a)
ompA	Rr190.70p Rr190.602n	ATGGCGAATATTTCTCCAAAA AGTGCAGCATTCGCTCCCCCT	532	Regnery et al. (1991)

highly supported clade with *Candidatus* "R. amblyommii" strain AcaIII from Brazil (AY375163) (Fig. 1). Phylogenetic analysis of *ompA* sequences confirmed the results obtained with *gltA*, because the sequence (Genbank accession number: KP171631) from PSJ was closely related to those of *Candidatus* "R. amblyommii" from Argentina (DQ517292) and USA (AY062007) (Fig. 3).

Discussion

Sequences analyses of three different loci have showed that the bacteria detected in *A. tonelliae* ticks from ETS represent a new lineage within the genus *Rickettsia*, which is named *Rickettsia* sp. strain El Tunal. Phylogenetically, this rickettsia is closely related to *R. monteiroi*, and they form a clade together with *R. canadensis* and *Candidatus* "R. tarasevichiae" (Figs. 1 and 2). This fact indicates that *Rickettsia* sp. strain El Tunal belongs to the canadensis group. The genetic divergence between *Rickettsia* sp. strain El Tunal and the three species of the canadensis group for both *gltA* and *htrA* sequences (see results) are considered to be higher than those expected values for intraspecific polymorphism (Fournier and Raoult, 2009; Pacheco et al., 2011). However, data from additional loci and isolation in a pure culture are needed to perform a complete characterization of this new strain of *Rickettsia* detected in *A. tonelliae* ticks from Argentina. *Rickettsia monteiroi*, *R. canadensis*, *Candidatus* "R. tarasevichiae" and *Rickettsia* sp. strain El Tunal constitute a well-supported phylogenetic group sufficiently distant from the remaining *Rickettsia* species, although they were found in different localities distant from each other and associated to tick species without a phylogenetic relationship. *Rickettsia monteiroi* was isolated from *Amblyomma incisum* ticks in Brazil (Pacheco et al., 2011), *R. canadensis* was detected in *Haemaphysalis leporispalustris* ticks from North and Central America (McKiel et al., 1967; Parola et al., 2013), and *Candidatus* "R. tarasevichiae" was found associated to *Ixodes persulcatus* and *Haemaphysalis japonica* in Russia and Japan (Eremeeva et al., 2006; Mediannikov et al., 2006; Inokuma et al., 2007). Even though some of their potential vectors may be aggressive to humans, as in the cases of *A. incisum* and *A. tonelliae* (Guglielmone et al., 2006; Nava et al., 2014a), the pathogenicity of *R. monteiroi*, *R. canadensis* and *Rickettsia* sp. strain El Tunal remains uncertain to date.

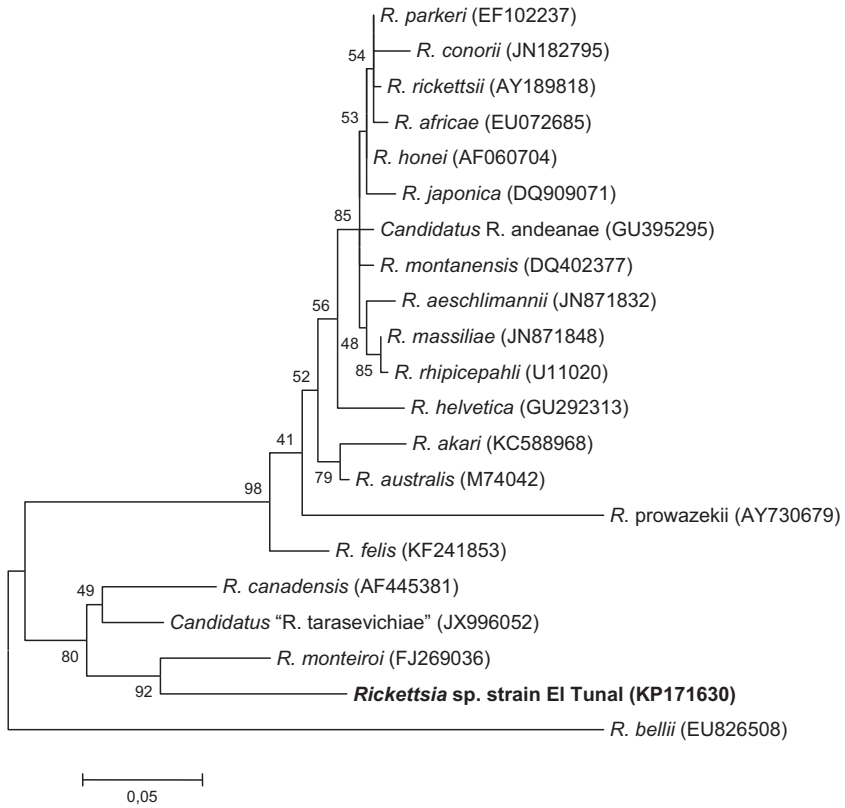

Fig. 2. Maximum-likelihood tree constructed from *htrA* partial sequences. Numbers represent bootstrap support generated from 1000 replications. GenBank accession numbers are in brackets.

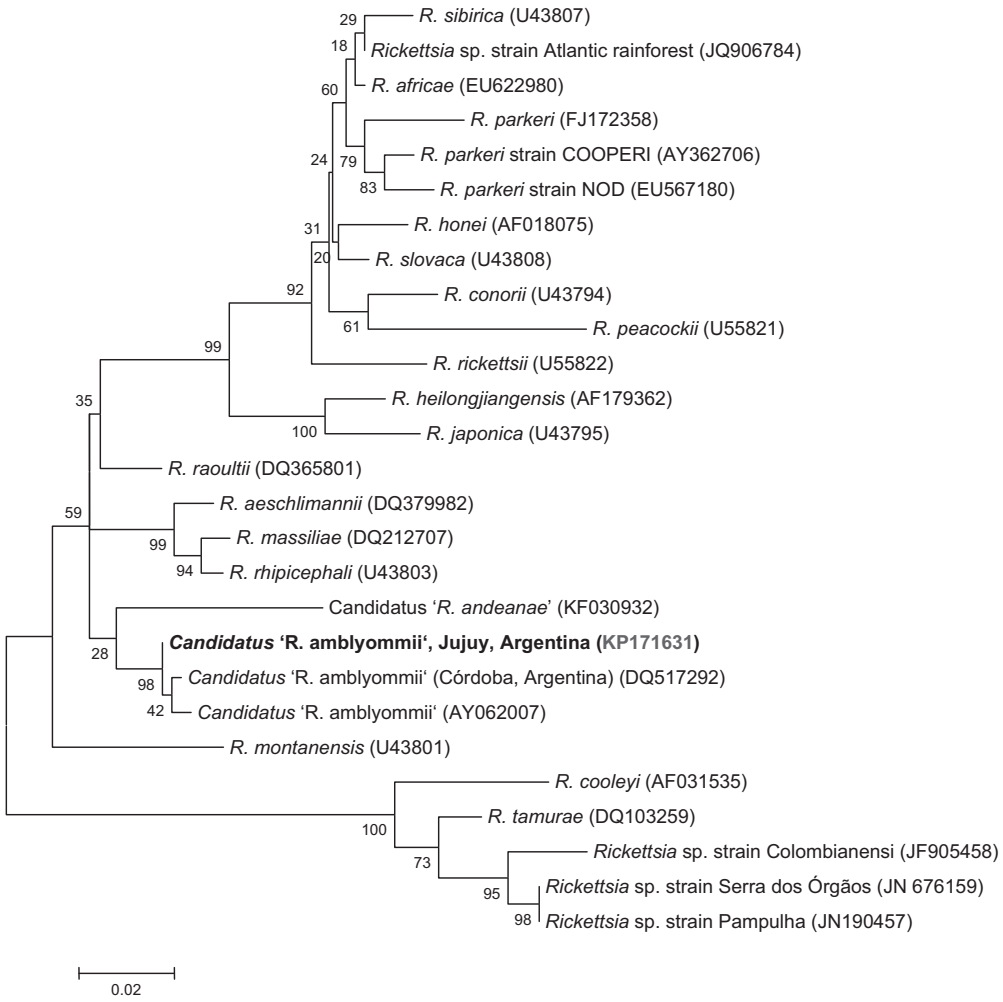

Fig. 3. Maximum-likelihood tree constructed from *ompA* partial sequences. Numbers represent bootstrap support generated from 1000 replications. GenBank accession numbers are in brackets.

Candidatus "R. tarasevichiae" constitutes an exception, because it has been involved as a human pathogen in China (Jia et al., 2013).

The finding of *Candidatus* "R. amblyommii" in *A. tonelliae* constitutes the first record of this association. *Candidatus* "R. amblyommii" has been previously detected in Argentina infecting *A. neumanni* ticks in Córdoba Province (Labruna et al., 2007). This rickettsia is widely distributed in America where it was reported in Argentina, Brazil, Costa Rica, French Guiana, Panama and USA associated to several tick species of the genera *Amblyomma*, *Dermacentor* and *Rhipicephalus* (Labruna et al., 2011; Parola et al., 2013; Saraiva et al., 2013), also including species of the *A. cajennense* complex. In fact, *Candidatus* "R. amblyommii" was detected in *A. mixtum*[1], *A. sculptum*[1], *A. cajennense* s.s[1] and now, in *A. tonelliae* (Labruna et al., 2004b; Bermúdez et al., 2011; Hun et al., 2011; Alves et al., 2014; this work). The ubiquity of *Candidatus* "R. amblyommii" implicates a health risk because it is found in different potential vectors along a wide geographic range which increase the probability of its transmission to humans. In this sense, Apperson et al. (2008) have suggested that some cases of rickettsiosis in USA may have been caused by *Candidatus* "R. amblyommii", but this hypothesis was not confirmed yet, and the *Candidatus* "R. amblyommii" pathogenicity remains undetermined (Parola et al., 2013).

Rickettsia rickettsii is by far the most pathogenic *Rickettsia* species in South America, and ticks of the *A. cajennense* complex are their principal vectors (Labruna, 2009). Infection with *R. rickettsii* was not detected among the *A. tonelliae* ticks tested during this study. Prevalence of *R. rickettsii* in natural populations of *A. cajennense* s.l has usually been shown to be relatively low (around 1%) or inexistent (Guedes et al., 2005; Sangioni et al., 2005; Labruna, 2009). Therefore, the negative results presented in this work are not unexpected, because only 68 ticks were evaluated for *Rickettsia* infection. Labruna et al. (2014) have confirmed that a Central/South American clade of *R. rickettsii* is found in *A. patinoi* of Colombia and *A. sculptum* of Brazil but it is unknown if this vector-pathogen association occurs in the remaining four species of *A. cajennense* complex. In Argentina, fatal cases of spotted fever caused by *R. rickettsii* were reported in northwestern areas where specimens of *A. cajennense* s.l were found to be infected with this *Rickettsia* species (Paddock et al., 2008). In such areas, two species of the *A. cajennense* complex (*A. tonelliae* and *A. sculptum*) occur in sympatry, but after the reassessment of the taxonomic status of *A. cajennense*, is not possible to determine if the ticks analyzed by Paddock et al. (2008) corresponded to *A. tonelliae* or *A. sculptum*. In this point further investigations are needed to determine if *A. tonelliae* could be a potential vector of *R. rickettsii* along its distribution in Argentina.

[1] All these species originally named as *A. cajennense* (see Nava et al., 2014a).

Acknowledgements

We acknowledge the financial support of INTA, Asociación Cooperadora INTA Rafaela, CONICET, Fundación Bunge & Born (FBBEI14/12) and Agencia Nacional de Promoción Científica y Tecnológica (PICT 1298). We are very grateful to Marcelo B. Labruna for his comments on the molecular diagnosis.

References

Alves, A.S., Melo, A.L.T., Amorin, M.V., Borges, A.M.C.M., Silva, L.G.E., Martins, T.F., Labruna, M.B., Aguiar, D.M., Pacheco, R.M., 2014. Seroprevalence of *Rickettsia* spp. in equids and molecular detection of '*Candidatus* Rickettsia amblyommii' in *Amblyomma cajennense* sensu lato ticks from the Pantanal Region of Mato Grosso. Braz. J. Med. Entomol. 41, 1252–1257.

Apperson, C.S., Engber, B., Nicholson, W.L., Mead, D.G., Engel, J., Yabsley, M.J., Dail, K., Johnson, J., Watson, D.W., 2008. Tick-borne diseases in North Carolina: is "*Rickettsia amblyommii*" a possible cause of rickettsiosis reported as Rocky Mountain spotted fever. Vector Borne Zoonotic Dis. 8, 1–9.

Beati, L., Nava, S., Burkman, E.J., Barros-Battesti, D., Labruna, M.B., Guglielmone, A.A., Cáceres, A.G., Guzman Cornejo, C., Léon, R., Durden, L.A., Faccini, J.L.H., 2013. *Amblyomma cajennense* (Fabricius, 1787) (Acari: Ixodidae), the Cayenne tick: phylogeography and evidence for allopatric speciation. BMC Evol. Biol. 13, 267.

Bermúdez, S.E., Zaldívar, Y.L., Spolidorio, M.G., Moraes-Filho, J., Miranda, R.J., Caballero, C.M., Mendoza, Y., Labruna, M.B., 2011. Rickettsial infection in domestic mammals and their ectoparasites in El Valle de Antón, Coclé, Panamá. Vet. Parasitol. 177, 134–138.

Cabrera, A.L., 1994. In: Tomo, II. (Ed.), Enciclopedia Argentina de Agricultura y Jardinería. Fascículo 1. Regiones fitogeográficas Argentinas. ACME, Buenos Aires, Argentina, p. 85.

Cicuttin, G., Nava, S., 2013. Molecular identification of *Rickettsia parkeri* infecting *Amblyomma triste* ticks in na área of Argentina where cases of rickettsiosis were diagnosed. Mem. Inst. Oswaldo Cruz. 108, 123–125.

Cicuttin, G.L., Brambati, D.F., Rodríguez Eugui, J.I., Lebrero, C.G., De Salvo, M.N., Beltrán, F.J., Gury Dohmen, F.E., Jado, I., Anda, P., 2014. Molecular characterization of *Rickettsia massiliae* and *Anaplasma platys* infecting *Rhipicephalus sanguineus* ticks and domestic dogs, Buenos Aires (Argentina). Ticks Tick Borne Dis. 5, 484–488.

De Sousa, R., Barata, C., Vitorino, L., Santos-Silva, M., Carrapato, C., Togal, J., Walker, D., Bacellar, F., 2006. *Rickettsia sibirica* isolation from a patient and detection in ticks. Port. Emerg. Inf. Dis. 12, 1103–1108.

Eremeeva, M.E., Oliveira, A., Robinson, J.B., Ribakova, N., Tokarevich, N.K., Dasch, G.A., 2006. Prevalence of bacterial agents in *Ixodes persulcatus* ticks from the Vologda Province of Russia. Ann. N. Y. Acad. Sci. 1078, 291–298.

Estrada-Peña, A., Guglielmone, A.A., Mangold, A.J., 2004. The distribution and ecological "preferences" of the tick *Amblyomma cajennense* (Acari: Ixodidae), an ectoparasite of humans and other mammals in the Americas. Ann. Trop. Med. Parasitol. 98, 283–292.

Fournier, P.E., Raoult, D., 2009. Current knowledge on phylogeny and taxonomy of *Rickettsia* spp. Ann. N. Y. Acad. Sci. 1166, 1–11.

Gillespie, J.J., Beier, M.S., Rahman, M.S., Ammerman, N.C., Shallom, J.M., Purkayastha, A., Sobral, B.S., Azad, A.F., 2007. Plasmids and rickettsial evolution: insight from *Rickettsia felis*. PLoS ONE 2, 266.

Guedes, E., Leite, R.C., Prata, M.C.A., Pacheco, R.C., Walker, D.H., Labruna, M.B., 2005. Detection of *Rickettsia rickettsii* in the tick *Amblyomma cajennense* in a new Brazilian spotted fever-endemic area in the state of Minas Gerais. Mem. Inst. Oswaldo Cruz. 100, 841–845.

Guglielmone, A.A., Nava, S., 2005. Las garrapatas de la familia Argasidae y de los géneros *Dermacentor, Haemaphysalis, Ixodes y Rhipicephalus* (Ixodidae) de la Argentina: distribución y hospedadores. Rev. Inv. Agropec. 34, 123–141.

Guglielmone, A.A., Nava, S., 2006. Las garrapatas argentinas del género *Amblyomma* (Acari: Ixodidae): distribución y hospedadores. Rev. Inv. Agropec. 35, 135–155.

Guglielmone, A.A., Mangold, A.J., Viñabal, A.E., 1991. Ticks (Ixodidae) parasitizing humans in four provinces of northwestern Argentina. Ann. Trop. Med. Parasitol. 85, 539–542.

Guglielmone, A.A., Beati, L., Barros-Battesti, D.M., Labruna, M.B., Nava, S., Venzal, J.M., Mangold, A.J., Szabó, M.J.P., Martins, J.R., González Acuña, D., Estrada-Peña, A., 2006. Ticks (Ixodidae) on humans in South America. Exp. Appl. Acarol. 40, 83–100.

Hall, T.A., 1999. BioEdit: a user friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95–98.

Hun, L., Troyo, A., Taylor, L., Babrbieri, A.M., Labruna, M.B., 2011. First report of the isolation and molecular characterization of *Rickettsia amblyomii* and *Rickettsia felis* in Central America. Vector Borne Zoonotic Dis. 11, 1395–1397.

Inokuma, H., Ohashi, M., Jilintai, Tanabe, S., Myahara, K., 2007. Prevalence of tick-borne *Rickettsia* and *Ehrlichia* in *Ixodes persulcatus* and *Ixodes ovatus* in Tokachi district, eastern Hokkaido, Japan. J. Vet. Med. Sci. 69, 661–664.

Jado, I., Escudero, R., Gil, H., Jiménez-Alonso, M.I., Sousa, R., García-Pérez, A.L., Rodríguez-Vargas, M., Lobo, B., Anda, P., 2006. Molecular method for identification of *Rickettsia* species in clinical and environmental samples. J. Clin. Microbiol. 44, 4572–4576.

Jia, N., Zheng, Y.C., Jiang, J., Ma, F., cao, L.W.C., 2013. Human infection with *Candidatus* Rickettsia tarasevichiae. N. Engl. J. Med. 369, 1178–1180.

Labruna, M.B., 2009. Ecology of Rickettsia in South America. Ann. N. Y. Acad. Sci. 1166, 156–166.

Labruna, M.B., Mcbride, J.W., Bouyer, D.H., Camargo, L.M.A., Camargo, E.P., Walker, D.H., 2004a. Molecular evidence for a spotted fever group *Rickettsia* species in the tick *Amblyomma longirostre* in Brazil. J. Med. Entomol. 41, 533–535.

Labruna, M.B., Whitworth, T., Bouyer, D.H., McBride, J., Camargo, L.M.A., Camargo, E.P., Popov, V., Walker, D.H., 2004b. *Rickettsia belli* and *Rickettsia amblyommii* in *Amblyomma* ticks from the state of Rondonia, Western Amazon. Braz. J. Med. Entomol. 41, 1073–1081.

Labruna, M.B., Pacheco, R.C., Nava, S., Brandao, P.E., Richtzenhain, L.J., Guglielmone, A.A., 2007. Infection by *Rickettsia bellii* and "*Rickettsia amblyommii*" in *Amblyomma neumanni* ticks from Argentina. Microb. Ecol. 54, 126–133.

Labruna, M.B., Mattar, S., Nava, S., Bermudez, S., Venzal, J.M., Dolz, G., Abarca, K., Romero, L., de Sousa, R., Oteo, J., Zavala-Castro, J., 2011. Rickettsioses in Latin America, Caribbean, Spain and Portugal. Rev. MVZ Córdoba 16, 2435–2457.

Labruna, M.B., Santos, F.C.P., Ogrzewalska, A., Nascimento, E.M.M., Colombo, S., Marcili, A., Angeramid, R.N., 2014. Genetic identification of rickettsial isolates from fatal cases of Brazilian spotted fever and comparison with *Rickettsia rickettsii* isolates from the American continents. J. Clin. Microbiol. 52 (10), 3788–3791.

McKiel, J.A., Bell, E.J., Lackman, D.B., 1967. *Rickettsia canada*: a new member of the typhus group rickettsiae isolated from *Haemaphysalis leporispalustris* Ticks in Canada. Can. J. Microbiol. 13, 503–510.

Mediannikov, O., Sidelniov, Y., Ivanov, L., Fournier, P.E., Tarasevich, I., Raoult, D., 2006. Far eastern tick-borne rickettsiosis. Identification of two new cases and tick vector. Ann. N. Y. Acad. Sci. 1078, 80–88.

Nava, S., Caparrós, J.A., Mangold, A.J., Guglielmone, A.A., 2006. Ticks (Acari: Ixodida: Argasidae, Ixodidae) infesting humans in northwestern Córdoba Province, Argentina. Medicina (Buenos Aires) 66, 225–228.

Nava, S., Elshenawy, Y., Eremeeva, M.E., Sumner, J.W., Mastropaolo, M., Paddock, C.D., 2008. *Rickettsia parkeri* in Argentina. Emerg. Infect. Dis. 14, 1894–1897.

Nava, S., Mangold, A.J., Mastropaolo, M., Venzal, J.M., Oscherov, E.B., Guglielmone, A.A., 2009. *Amblyomma boeroi* n.sp. (Acari: Ixodidae), a parasite of the Chacoan peccary *Catagonus wagneri* (Rusconi) (Artiodactyla: Tayassuidae) in Argentina. Syst. Parasitol. 73, 161–174.

Nava, S., Beati, L., Labruna, M.B., Cáceres, A.G., Mangold, A.J., Guglielmone, A.A., 2014a. Reassessment of the taxonomic status of *Amblyomma cajennense* (Fabricius, 1787) with the description of three new species. *Amblyomma tonelliae* n. sp., *Amblyomma interandinum* n. sp. and *Amblyomma patinoi* n. sp., and reinstatement of *Amblyomma mixtum* Koch, 1844 and *Amblyomma sculptum* Berlese, 1888 (Ixodida: Ixodidae). Ticks Tick Borne Dis. 5, 252–276.

Nava, S., Mastropaolo, M., Mangold, A.J., Martins, T.F., Venzal, J.M., Guglielmone, A.A., 2014b. Amblyomma hadanii n. sp. (Acari: Ixodidae), a tick from northwestern Argentina previously confused with *Amblyomma coelebs* Neumann, 1899. Syst. Parasitol. 88, 261–272.

Pacheco, R.C., Moraes-Filho, J., Nava, S., Brandao, P.E., Richtzenhain, L.J., Labruna, M.B., 2007. Detection of a novel spotted fever group rickettsia in *Amblyomma parvum* ticks (Acari: Ixodidae) from Argentina. Exp. Appl. Acarol. 43, 63–71.

Pacheco, R., Moraes-Filho, J., Marcili, A., Richtzenhain, L.J., Szabó, M.P.J., Catroxo, M.H.B., Bouyer, D.H., Labruna, M.B., 2011. *Rickettsia monteiroi* sp. nov., infecting the tick Amblyomma incisum in Brazil. Appl. Environ. Microbiol. 77, 5207–5211.

Paddock, C.D., Fernández, S., Echenique, G.A., Summer, J.W., Reeves, W.K., Zaki, S.R., Remondegui, C.E., 2008. Rocky Mountain spotted fever in Argentina. Am. J. Trop. Med. Hyg. 78, 687–692.

Parola, P., Paddock, C.D., Scolovschi, C., Labruna, M.B., Mediannokov, O., Kernif, T., Abdad, M.Y., Stenos, J., Bitam, I., Fournier, P.E., Raoult, D., 2013. Update on tick-borne rickettsioses around the world: a geographic approach. Clin. Microbiol. Rev. 26, 657–702.

Regnery, R.L., Spruill, C.L., Plikaytis, B.D., 1991. Genotypic identification of rickettsiae and estimation of intraspecies sequence divergence for portions of two rickettsial genes. J. Bacteriol. 173, 1576–1589.

Romer, Y., Nava, S., Govedic, F., Cicuttin, G., Denison, A.M., Singleton, J., Kelly, A.J., Kato, C.I., Paddock, C.D., 2014. *Rickettsia parkeri* rickettsiosis in different ecological regions of Argentina and its association with *Amblyomma tigrinum* as a potential vector. Am. J. Trop. Med. Hyg. 91 (6), 1156–1160.

Sangioni, L.A., Horta, M.C., Vianna, M.C.B., Gennari, S.M., Soares, R.M., Galvao, M.A.M., Schumaker, T.T.S., Ferreira, F., Vidotto, O., Labruna, M.B., 2005. Rickettsial infection in animals and Brazilian spotted fever endemicity. Emerg. Inf. Dis. 11, 265–270.

Saraiva, D.G., Nieri-Bastos, F., Horta, M.C., Soraes, H.S., Nicola, P.A., Pereira, L.C.M., Labruna, M.B., 2013. *Rickettsia amblyommii* infecting *Amblyomma auricularium* ticks in Pernambuco, northeastern Brazil: isolation, transovarial transmission, and transstadial perpetuation. Vector Borne Zoonotic Dis. 13, 615–618.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28, 2731–2739.

Thompson, J.D., Higgins, D., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acid Res. 22, 4673–4680.

Tomassone, L., Nuñez, P., Ceballos, L.A., Gurtler, R.E., Kitron, U., Farber, M., 2010. Detection of "*Candidatus* Rickettsia sp. strain Argentina" and *Rickettsia belli* in *Amblyomma* ticks (Acari: Ixodidae) from Northern Argentina. Exp. Appl. Acarol. 52, 93–100.

Weinert, L.A., Werren, J.H., Aebi, A., Stone, G.N., Jiggins, F.M., 2009. Evolution and diversity of *Rickettsia* bacteria. BMC Evol. Biol. 7, 6.