

Rickettsial infection in ticks infesting wild birds from two eco-regions of Argentina

Infecção por riquetsias em carrapatos de aves silvestres em duas ecorregiões da Argentina

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Abstract

Several tick-borne *Rickettsia* species are recognized human pathogens in Argentina. Here we evaluated rickettsial infection in ticks collected on passerine birds during 2011-2012 in two eco-regions of Argentina. The ticks were processed by molecular analysis through polymerase chain reaction (PCR) detection and DNA sequencing of fragments of two rickettsial genes, *gltA* and *ompA*. A total of 594 tick specimens (532 larvae and 62 nymphs), representing at least 4 species (*Amblyomma tigrinum*, *Ixodes pararicinus*, *Haemaphysalis juxtakochi*, *Haemaphysalis leporispalustris*), were evaluated. At least one *A. tigrinum* larva, collected on *Coryphospingus cucullatus* in Chaco Seco, was infected with *Rickettsia parkeri*, whereas at least 12 larvae and 1 nymph of *I. pararicinus*, collected from *Troglodytes aedon*, *Turdus amaurochalinus*, *Turdus rufiventris*, *C. cucullatus* and *Zonotrichia capensis*, were infected with an undescribed *Rickettsia* agent, genetically related to several rickettsial endosymbionts of ticks of the *Ixodes ricinus* complex. *R. parkeri* is a recognized human pathogen in several American countries including Argentina, where a recent study incriminated *A. tigrinum* as the potential vector of *R. parkeri* to humans. Birds could play an important role in dispersing *R. parkeri*-infected *A. tigrinum* ticks. Additionally, we report for the first time a rickettsial agent infecting *I. pararicinus* ticks.

Keywords: *Rickettsia parkeri*, endosymbiont, *Amblyomma tigrinum*, *Ixodes pararicinus*, passeriformes.

Resumo

Algumas espécies de *Rickettsia* transmitidas por carrapatos são reconhecidos como patógenos humanos na Argentina. Este presente trabalho avaliou a infecção por *Rickettsia* em carrapatos coletados em aves passeriformes, durante 2011-2012, em duas ecorregiões da Argentina. Os carrapatos foram processados pela reação em cadeia da polimerase (PCR) e sequenciamento de DNA de dois genes de *Rickettsia*: *gltA* e *ompA*. Ao todo, 594 amostras de carrapatos (532 larvas e 62 ninfas), representando pelo menos 4 espécies (*Amblyomma tigrinum*, *Ixodes pararicinus*, *Haemaphysalis juxtakochi*, *Haemaphysalis leporispalustris*), foram avaliadas. Pelo menos uma larva de *A. tigrinum*, coletada de *Coryphospingus cucullatus* no Chaco Seco, estava infectada com *Rickettsia parkeri*, enquanto pelo menos 12 larvas e 1 ninfa de *I. pararicinus*, coletadas de *Troglodytes aedon*, *Turdus amaurochalinus*, *Turdus rufiventris*, *C. cucullatus* e *Zonotrichia capensis* estavam infectadas com *Rickettsia* sp., geneticamente relacionada a vários endossimbiontes riquetsiais de carrapatos do complexo *Ixodes ricinus*. *R. parkeri* é reconhecidamente um patógeno humano em alguns países americanos, incluindo a Argentina, onde um estudo recente incriminou *A. tigrinum* como um provável vetor. Aves poderiam desempenhar um papel importante na dispersão de carrapatos *A. tigrinum* infectados por *R. parkeri*. Em adição, relata-se pela primeira vez a infecção por *Rickettsia* em *I. pararicinus*.

Palavras-chave: *Rickettsia parkeri*, endo-simbionte, *Amblyomma tigrinum*, *Ixodes pararicinus*, passeriformes.

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Bacteria of the genus *Rickettsia* are obligate intracellular organisms that infect invertebrate hosts worldwide. Some of them also infect and cause diseases (i.e., rickettsioses) in warm-blooded animals and humans, to whom they are transmitted by hematophagous vectors, mostly ticks (PAROLA et al., 2013). In Argentina, three species of the spotted fever group (SFG) rickettsiae, *Rickettsia rickettsii*, *Rickettsia parkeri* and *Rickettsia massiliae*, are recognized as agents of human diseases (PADDOCK et al., 2008; GARCÍA-GARCÍA et al., 2010; ROMER et al., 2011, 2014). A fourth human pathogen, *Rickettsia* sp. strain Atlantic rainforest has also been reported in Argentinean ticks (MONJE et al., 2015); however, no human cases of rickettsiosis attributed to this pathogen have been detected in Argentina. Additional rickettsial agents of unknown pathogenicity have been reported in Argentinean ixodid ticks, namely *Rickettsia bellii*, “*Candidatus Rickettsia amblyommii*”, “*Candidatus Rickettsia andeanae*”, *Rickettsia* sp. strain El Tunal, and *Rickettsia* sp. endosymbiont of *Amblyomma parvitarsum* (LABRUNA et al., 2007; PACHECO et al., 2007; SARACHO BOTTERO et al., 2015; TARRAGONA et al., 2015; OGRZEWALSKA et al., 2016).

Wild birds can be hosts of different stages of some species of ticks, commonly larvae and nymphs, and rarely adults (GUGLIELMONE et al., 2014; FLORES et al., 2014). Furthermore, wild birds are among the most mobile hosts, and therefore they may be regarded as hosts with relevant potential in the dispersion of ticks and tick-borne diseases, including rickettsial organisms (ELFVING et al., 2010; HORNOK et al., 2014; BERTHOVÁ et al., 2016). Among 127 species of Ixodidae established in the Neotropical Zoogeographic Region (GUGLIELMONE et al., 2014; NAVA et al., 2014a, b; KRAWCZAK et al., 2015), 39 are found in Argentina (GUGLIELMONE & NAVA, 2005, 2006; NAVA et al., 2009, 2014a, b). Most of these species belong to the genus *Amblyomma*, best represented in Argentina with 25 species. Ten species from three genera have been reported parasitizing wild birds in different Argentinean eco-regions (FLORES et al., 2014); however, bird ticks have never been searched for rickettsial infection in Argentina. Here, we have evaluated rickettsial infection in ticks collected on wild birds in two eco-regions of Argentina.

This study was conducted in two localities of the Chaco Seco eco-region (Chaco Seco1: 30°50'S, 62°54'W; and Chaco Seco 2: 30°22'S, 64°21'W) and one in the Yungas ecoregion (Parque Nacional El Rey: 24°43'S, 64°38'W) in Argentina as defined by Burkart et al. (1999). Bird collections were performed using mist nets, which remained active during morning and twilight hours, obtaining convenience samplings in 2011 and 2012, as previously reported (FLORES et al., 2014). Each individual bird was identified using Narosky & Yzurieta (2010), classified under Clements et al. (2015) criteria and examined for ticks using fine-tipped tweezers. After being processed, the birds were released, and the ticks obtained were stored in 70% ethanol until specific determination in the laboratory. The detailed procedures for the fieldwork, bird and tick taxonomic identification, and the results of the ticks infesting these birds have been published elsewhere (FLORES et al., 2014).

Ticks of the same developmental stage, collected from the same individual host, were processed in pools of 1 to 39 larvae (median: 4) or 1 to 4 nymphs (median: 1). Ticks from each

pool were crushed with a sterile pestle in a microtube, and then processed for DNA extraction by using the AxyPrep Body Fluid Viral DNA/RNA Miniprep Kit (Axygen Biosciences, USA) following the manufacturer's procedures. DNA samples were processed by PCR using primers CS-78 and CS-323 targeting a 398-bp fragment of the citrate synthase gene (*gltA*), which occurs in all *Rickettsia* species (LABRUNA et al., 2004). Samples positive by this first PCR assay were tested by a second PCR assay with primers Rr190.70p and Rr190.701, which amplifies a ≈630-bp fragment of the 190-kDa outer membrane protein gene (*ompA*) of the majority of the SFG *Rickettsia* species (ROUX et al., 1996). PCR products were DNA-sequenced and subjected to BLAST analyses (BLAST, 2015) to infer the closest similarities available in GenBank.

A total of 594 tick specimens (532 larvae and 62 nymphs), representing at least 4 species, were evaluated in 124 pools for rickettsial infection (Table 1). A complete list of hosts for these ticks has been published elsewhere (FLORES et al., 2014). Two tick species were found to be infected by *Rickettsia*. One *Amblyomma tigrinum* pool of 4 larvae was infected with *R. parkeri*, as its *gltA* and *ompA* DNA partial sequences (308 bp and 551 bp, respectively) were 100% identical to *R. parkeri* strain Maculatum20 (GenBank accession numbers U59732 and U43802, respectively).

Thirteen pools of *Ixodes parvicinus* (12 out of 32 larval pools; and 1 out of 8 nymphal pools) yielded PCR products for both the *gltA* and the *ompA* genes. DNA sequences were successfully obtained for 5 pools, which generated identical sequences for each rickettsial gene. The *gltA* partial sequence was 100% (350/350-bp) identical to *Rickettsia* sp. strain BelizeIaff1 of *Ixodes affinis* (KU001172; from Belize), 99.7% (349/350-bp) identical to *Rickettsia* sp. strain 12G1 of *Rhipicephalus (Boophilus) microplus* (KF831359; from Ecuador) and 99.4% (348/350-bp) identical to *Rickettsia monacensis* of *Ixodes ricinus* (LN794217; from Europe) and *Rickettsia* sp. strain Barva1 of *Ixodes minor* (KF702332; from Costa Rica). The *ompA* partial sequences generated from the *I. parvicinus* ticks were 100% (587/587-bp) identical to *Rickettsia* sp. strain Barva2 of *Ixodes minor* (KF702334; from Costa Rica), 99.8% (586/587-bp) identical to *Rickettsia* sp. strain BelizeIaff2 of *I. affinis* (KU001175; from Belize), and then 98.9% (572/578-bp) identical to *Rickettsia* sp. endosymbiont of *Ixodes scapularis* (EF689735; from the United States).

The GenBank nucleotide sequence accession numbers for the partial sequences generated in this study are KU744411-KU744412 for the *gltA* and *ompA* genes of *R. parkeri* from *A. tigrinum* and KU744413-KU744414 for the *gltA* and *ompA* genes of *Rickettsia* sp. from *I. parvicinus*.

This study provides molecular evidence of two rickettsial agents infecting ticks that were parasitizing birds in Argentina. *R. parkeri* is a recognized human pathogen in several countries of the Americas (PAROLA et al., 2013), including Argentina, where a recent study incriminated *A. tigrinum* as a probable vector of *R. parkeri* to humans (ROMER et al., 2014). The tick *A. tigrinum* is known to occur in a variety of eco-regions among almost all South American countries (ESTRADA-PEÑA et al., 2005). Recent findings of *R. parkeri* infecting *A. tigrinum* adult ticks in Bolivia (TOMASSONE et al., 2010), Uruguay (LADO et al., 2014), and Argentina (ROMER et al., 2014) suggest that this pathogen could

Table 1. Ticks collected from birds and tested by PCR for rickettsial infection.

Tick species	Locality	Tick stage	No. ticks	No. pools	No. pools infected by rickettsia	MIR ^a	Bird host species of the infected ticks ^b
<i>Amblyomma tigrinum</i>	Chaco Seco 1	Larva	93	16	0	0	
		Nymph	18	12	0	0	
	Chaco Seco 2	Larva	89	14	1	1.1	<i>Coryphospingus cucullatus</i>
		Nymph	20	14	0	0	
<i>Ixodes pararicinus</i>	Yungas	Larva	229	32	12 ^c	5.2	<i>Troglodytes aedon</i> , <i>Turdus amaurochalinus</i> , <i>Turdus rufiventris</i> , <i>Coryphospingus cucullatus</i> , <i>Zonotrichia capensis</i>
		Nymph	11	8	1	9	<i>Turdus rufiventris</i>
<i>Haemaphysalis juxtakochi</i>	Yungas	Larva	78	10	0	0	
		Nymph	12	5	0	0	
<i>Haemaphysalis leporispalustris</i>	Yungas	Larva	23	4	0	0	
		Nymph	1	1	0	0	
<i>Haemaphysalis</i> sp.	Yungas	Larva	10	4	0	0	
<i>Amblyomma</i> sp.	Yungas	Larva	10	4	0	0	

^a MIR: Minimum Infection Rate = No. PCR-positive pools / Total number of tested ticks × 100b. ^b a detailed list of bird hosts for all ticks of this table has been published elsewhere (FLORES et al., 2014). ^c 4 pools were from *Troglodytes aedon*, 1 from *Turdus amaurochalinus*, 5 from *Turdus rufiventris*, 1 from *Coryphospingus cucullatus* and 1 from *Zonotrichia capensis*.

have a wide distribution within the South American population of this tick species. Thus, it is likely that wild birds could play an important role in dispersing *R. parkeri*-infected *A. tigrinum* ticks, which could expand the area of human exposure to these ticks, consequently increasing the risk of human rickettsiosis. On the other hand, because all tick-infested birds of this study are considered to be non-migratory species (NAROSKY & YZURIETA, 2010), their specific role in dispersing infected ticks over long distances should be limited.

Additionally, we report for the first time a rickettsial agent infecting *I. pararicinus* ticks. Partial molecular characterization of this agent indicates that it is most closely related to SFG rickettsial agents associated with *I. affinis* (LOPES et al., 2016), *I. ricinus* (MAIOLI et al., 2012), *I. minor* (OGRZEWALSKA et al., 2015), and *I. scapularis* (PAROLA et al., 2013). These four *Ixodes* species, as well as *I. pararicinus*, belong to the *I. ricinus* species complex, based primarily on morphological and genetic relatedness (KEIRANS et al., 1985; BARBIERI et al., 2013). Interestingly, recent studies have indicated that tick members of the *I. ricinus* species complex are usually infected by species-specific closely related rickettsial organisms, usually considered endosymbionts (KURTTI et al., 2015). Moreover, the *I. ricinus* associated *R. monacensis* is considered by some authors as a human pathogen (PAROLA et al., 2013). Further studies on isolation and deeper molecular characterization are needed to elucidate the taxonomic status of the rickettsial endosymbiont of *I. pararicinus*.

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References

- Barbieri AM, Venzal JM, Marcili A, Almeida AP, González EM, Labruna MB. *Borrelia burgdorferi* sensu lato infecting ticks of the *Ixodes ricinus* complex in Uruguay: first report for the Southern Hemisphere. *Vector Borne Zoonotic Dis* 2013; 13(3): 147-153. <http://dx.doi.org/10.1089/vbz.2012.1102>. PMID:23402334.
- Basic Local Alignment Search Tool – BLAST [online]. Bethesda: National Center for Biotechnology Information; 2015 [cited 2016 Feb 25]. Available from: www.ncbi.nlm.nih.gov/blast
- Berthová L, Slobodník V, Slobodník R, Olekšák M, Sekeyová Z, Svitálková Z, et al. The natural infection of birds and ticks feeding on birds with *Rickettsia* spp. and *Coxiella burnetii* in Slovakia. *Exp Appl Acarol* 2016; 68(3): 299-314. <http://dx.doi.org/10.1007/s10493-015-9975-3>. PMID:26477038.
- Burkart RN, Bárbaro N, Sánchez R, Gómez D. *Eco-regiones de la Argentina* [online] Buenos Aires: Programa de Desarrollo Institucional Ambiental; 1999 [cited 2016 Feb 25]. Available from: http://www.sib.gov.ar/archivos/Eco-Regiones_de_la_Argentina.pdf

- Clements JF, Schulenberg TS, Iliff MJ, Sullivan BL, Wood CL, Roberson D, et al. *The ebird/Clements checklist of birds of the world: v2015* [online]. Ithaca: Cornell Lab of Ornithology; 2015 [cited 2016 Feb 25]. Available from: <http://www.birds.cornell.edu/clementschecklist/download/>
- Elfving K, Olsen B, Bergström S, Waldenström J, Lundkvist A, Sjöstedt A, et al. Dissemination of spotted fever *Rickettsia* agents in Europe by migrating birds. *PLoS One* 2010; 5(1): e8572. <http://dx.doi.org/10.1371/journal.pone.0008572>. PMID:20052286.
- Estrada-Peña A, Venzal JM, Mangold AJ, Cafrune MM, Guglielmone AA. The *Amblyomma maculatum* Koch, 1844 (Acari: Ixodidae: Amblyomminae) tick group: diagnostic characters, description of the larva of *A. parvitarsum* Neumann, 1901, 16S rDNA sequences, distribution and hosts. *Syst Parasitol* 2005; 60(2): 99-112. <http://dx.doi.org/10.1007/s11230-004-1382-9>. PMID:15841347.
- Flores FS, Nava S, Batallán G, Tauro LB, Contigiani MS, Diaz LA, et al. Ticks (Acari: Ixodidae) on wild birds in north-central Argentina. *Ticks Tick Borne Dis* 2014; 5(6): 715-721. <http://dx.doi.org/10.1016/j.ttbdis.2014.05.004>. PMID:25108788.
- García-García JC, Portillo A, Núñez MJ, Santibáñez S, Castro B, Oteo JA. A patient from Argentina infected with *Rickettsia massiliae*. *Am J Trop Med Hyg* 2010; 82(4): 691-692. <http://dx.doi.org/10.4269/ajtmh.2010.09-0662>. PMID:20348520.
- Guglielmone AA, Nava S. Las garrapatas argentinas del género *Amblyomma* (Acari: Ixodidae): distribución y hospedadores. *Rev Inv Agropec* 2006; 35(3): 133-153.
- Guglielmone AA, Nava S. 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* 2005; 34(2): 123-141.
- Guglielmone AA, Robbins RG, Apanaskevich DA, Petney TN, Estrada-Peña A, Horak IG. *The hard ticks of the world (Acari: Ixodida: Ixodidae)*. Heidelberg: Springer; 2014
- Hornok S, Kováts D, Csörgő T, Meli ML, Gönczi E, Hadnagy Z, et al. Birds as potential reservoirs of tick-borne pathogens: first evidence of bacteraemia with *Rickettsia helvetica*. *Parasit Vectors* 2014; 7(1): 128. <http://dx.doi.org/10.1186/1756-3305-7-128>. PMID:24679245.
- Keirans JE, Clifford CM, Guglielmone AA, Mangold AJ. *Ixodes (Ixodes) parvicinus*, n. sp. (Acari: Ixodoidea: Ixodidae), a South American cattle tick long confused with *Ixodes ricinus*. *J Med Entomol* 1985; 22(4): 401-407. <http://dx.doi.org/10.1093/jmedent/22.4.401>. PMID:4045937.
- Krawczak FS, Martins TF, Oliveira CS, Binder LC, Costa FB, Nunes PH, et al. *Amblyomma yucumense* n. sp. (Acari: Ixodidae), a parasite of wild mammals in southern Brazil. *J Med Entomol* 2015; 52(1): 28-37. <http://dx.doi.org/10.1093/jmedent/tju007>. PMID:26336277.
- Kurtti TJ, Felsheim RF, Burkhardt NY, Oliver JD, Heu CC, Munderloh UG. *Rickettsia buchneri* sp. nov., a rickettsial endosymbiont of the blacklegged tick *Ixodes scapularis*. *Int J Syst Evol Microbiol* 2015; 65(3): 965-970. <http://dx.doi.org/10.1099/ijs.0.000047>. PMID:25563918.
- Labruna MB, Pacheco RC, Nava S, Brandão PE, Richtzenhain LJ, Guglielmone AA. Infection by *Rickettsia bellii* and *Candidatus "Rickettsia amblyommii"* in *Amblyomma neumanni* ticks from Argentina. *Microb Ecol* 2007; 54(1): 126-133. <http://dx.doi.org/10.1007/s00248-006-9180-3>. PMID:17264991.
- Labruna MB, Whitworth T, Horta MC, Bouyer DH, McBride JW, Pinter A, et al. *Rickettsia* species infecting *Amblyomma cooperi* ticks from an area in the state of São Paulo, Brazil, where Brazilian spotted fever is endemic. *J Clin Microbiol* 2004; 42(1): 90-98. <http://dx.doi.org/10.1128/JCM.42.1.90-98.2004>. PMID:14715737.
- Lado P, Castro O, Labruna MB, Venzal JM. First molecular detection of *Rickettsia parkeri* in *Amblyomma tigrinum* and *Amblyomma dubitatum* ticks from Uruguay. *Ticks Tick Borne Dis* 2014; 5(6): 660-662. <http://dx.doi.org/10.1016/j.ttbdis.2014.04.021>. PMID:25108780.
- Lopes MG, May JM Jr, Foster RJ, Harmsen BJ, Sanchez E, Martins TF, et al. Ticks and rickettsiae from wildlife in Belize, Central America. *Parasit Vectors* 2016; 9(1): 62. <http://dx.doi.org/10.1186/s13071-016-1348-1>. PMID:26831147.
- Maioli G, Pistone D, Bonilauri P, Pajoro M, Barbieri I, Patrizia M, et al. Etiological agents of rickettsiosis and anaplasmosis in ticks collected in Emilia-Romagna region (Italy) during 2008 and 2009. *Exp Appl Acarol* 2012; 57(2): 199-208. <http://dx.doi.org/10.1007/s10493-012-9535-z>. PMID:22402940.
- Monje LD, Nava S, Eberhardt AT, Correa AI, Guglielmone AA, Beldomenico PM. Molecular detection of the human pathogenic *Rickettsia* sp. strain Atlantic rainforest in *Amblyomma dubitatum* ticks from Argentina. *Vector Borne Zoonotic Dis* 2015; 15(2): 167-169. <http://dx.doi.org/10.1089/vbz.2014.1741>. PMID:25700048.
- Narosky T, Yzurieta D. *Aves de Argentina y Uruguay*. Buenos Aires: Vázquez Mazzini; 2010.
- Nava S, Beati L, Labruna MB, Cáceres AG, Mangold AJ, Guglielmone AA. 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* 2014a; 5(3): 252-276. <http://dx.doi.org/10.1016/j.ttbdis.2013.11.004>. PMID:24556273.
- Nava S, Mangold AJ, Mastropaolo M, Venzal JM, Oscherov EB, Guglielmone AA. *Amblyomma boeroi* n. sp. (Acari: Ixodidae), a parasite of the chacoan peccary *Catagonus wagneri* (Rusconi) (Artiodactyla: Tayassuidae) in Argentina. *Syst Parasitol* 2009; 73(3): 161-174. <http://dx.doi.org/10.1007/s11230-009-9191-9>. PMID:19472076.
- Nava S, Mastropaolo M, Mangold AJ, Martins TF, Venzal JM, Guglielmone AA. *Amblyomma hadanii* n. sp. (Acari: Ixodidae), a tick from northwestern Argentina previously confused with *Amblyomma coelebs* Neumann, 1899. *Syst Parasitol* 2014b; 88(3): 261-272. <http://dx.doi.org/10.1007/s11230-014-9500-9>. PMID:24935128.
- Ogrzewalska M, Literák I, Capek M, Sychra O, Calderón VÁ, Rodríguez BC, et al. Bacteria of the genus *Rickettsia* in ticks (Acari: Ixodidae) collected from birds in Costa Rica. *Ticks Tick Borne Dis* 2015; 6(4): 478-482. <http://dx.doi.org/10.1016/j.ttbdis.2015.03.016>. PMID:25869035.
- Ogrzewalska M, Nieri-Bastos FA, Marcili A, Nava S, González-Acuña D, Muñoz-Leal S, et al. A novel spotted fever group *Rickettsia* infecting *Amblyomma parvitarsum* (Acari: Ixodidae) in highlands of Argentina and Chile. *Ticks Tick Borne Dis* 2016; 7(3): 439-442. <http://dx.doi.org/10.1016/j.ttbdis.2016.01.003>. PMID:26826974.
- Pacheco RC, Moraes-Filho J, Nava S, Brandão PE, Richtzenhain LJ, Labruna MB. Detection of a novel spotted fever group rickettsia in *Amblyomma parvum* ticks (Acari: Ixodidae) from Argentina. *Exp Appl Acarol* 2007; 43(1): 63-71. <http://dx.doi.org/10.1007/s10493-007-9099-5>. PMID:17768597.
- Paddock CD, Fernández S, Echenique GA, Sumner JW, Reeves WK, Zaki SR, et al. Rocky Mountain spotted fever in Argentina. *Am J Trop Med Hyg* 2008; 78(4): 687-692. PMID:18385370.

- Parola P, Paddock CD, Socolovschi C, Labruna MB, Mediannikov O, Kernif T, et al. Update on tick-borne rickettsioses around the world: a geographic approach. *Clin Microbiol Rev* 2013; 26(4): 657-702. <http://dx.doi.org/10.1128/CMR.00032-13>. PMID:24092850.
- Romer Y, Nava S, Govedic F, Cicuttin G, Denison AM, Singleton J, et al. *Rickettsia parkeri* rickettsiosis in different ecological regions of Argentina and its association with *Amblyomma tigrinum* as a potential vector. *Am J Trop Med Hyg* 2014; 91(6): 1156-1160. <http://dx.doi.org/10.4269/ajtmh.14-0334>. PMID:25349376.
- Romer Y, Seijo AC, Crudo F, Nicholson WL, Varela-Stokes A, Lash RR, et al. *Rickettsia parkeri* Rickettsiosis, Argentina. *Emerg Infect Dis* 2011; 17(7): 1169-1173. <http://dx.doi.org/10.3201/eid1707.101857>. PMID:21762568.
- Roux V, Fournier PE, Raoult D. Differentiation of spotted fever group rickettsiae by sequencing and analysis of restriction fragment length polymorphism of PCR-amplified DNA of the gene encoding the protein rOmpA. *J Clin Microbiol* 1996; 34(9): 2058-2065. PMID:8862558.
- Saracho Bottero MN, Tarragona EL, Nava S. Spotted fever group rickettsiae in *Amblyomma* ticks likely to infest humans in rural areas from northwestern Argentina. *Medicina (B Aires)* 2015; 75(6): 391-395. PMID:26707663.
- Tarragona EL, Cicuttin GL, Mangold AJ, Mastropaolo M, Nazarena De Salvo M, Nava S. *Rickettsia* infection in *Amblyomma tonelliae*, a tick species from the *Amblyomma cajennense* complex. *Ticks Tick Borne Dis* 2015; 6(2): 173-177. <http://dx.doi.org/10.1016/j.ttbdis.2014.11.010>. PMID:25544308.
- Tomassone L, Conte V, Parrilla G, De Meneghi D. Rickettsia infection in dogs and *Rickettsia parkeri* in *Amblyomma tigrinum* ticks, Cochabamba department, Bolivia. *Vector Borne Zoonotic Dis* 2010; 10(10): 953-958. <http://dx.doi.org/10.1089/vbz.2009.0126>. PMID:20426684.