Rickettsia sp. Strain Colombianensi (Rickettsiales: *Rickettsiaceae*): A New Proposed *Rickettsia* Detected in *Amblyomma dissimile* (Acari: Ixodidae) From Iguanas and Free-Living Larvae Ticks From Vegetation

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ABSTRACT From January to December 2009, 55 *Amblyomma dissimile* (Koch) ticks removed from iguanas in the municipality of Monteria and 3,114 ticks [458 *Amblyomma* sp. larvae, 2,636 *Rhipicephalus microplus* (Canestrini) larvae and 20 *Amblyomma* sp. nymphs] collected over vegetation in Los Cordobas were included in the study. The ticks were pooled into groups from which DNA was extracted. For initial screening of *Rickettsia* sp., each pool was analyzed by *gltA* real-time polymerase chain reaction (PCR). Positive pools were further studied using *gltA*, *ompA*, and *ompB* conventional PCR assays. Sequencing and phylogenetic analysis were also conducted. Rickettsial DNA was found in 28 pools of ticks (16 *A. dissimile* pools and 12 free-living larvae pools) out of 113 (24.7%) using real-time PCR. The same 28 pools were also positive using conventional PCR assays aimed to amplify *gltA*, *ompA*, and *ompB*. For each gene analyzed, PCR products obtained from 4/28 pools (two pools of *A. dissimile*, one pool of *Amblyomma* sp. larvae and one pool of *Rh. microplus* larvae) were randomly chosen and sequenced twice. Nucleotide sequences generated were identical to each other for each of the rickettsial genes *gltA*, *ompA*, and *ompB*, and showed 99.4, 95.6, and 96.4% identity with those of *Rickettsia tamurae*. They were deposited in the GenBank database under accession numbers JF905456, JF905458, and JF905457, respectively. In conclusion, we present the first molecular evidence of a novel *Rickettsia* (*Rickettsia* sp. strain Colombianensi) infecting *A. dissimile* ticks collected from iguanas, and also *Rh. microplus* and unspeciated *Amblyomma* larvae from vegetation in Colombia.

KEY WORDS *Rickettsia* sp. strain Colombianensi, *Amblyomma dissimile*, *Amblyomma* sp. larvae, *Rhipicephalus microplus* larvae, tick

Numerous diseases are caused by bacterial, viral, and protozoan agents transmitted by ticks. There are ≈877 tick species worldwide, and vertebrate blood is required for their survival, growth to the next developmental stage, and egg production. This close relationship between ticks and their vertebrate hosts has resulted in the emergence and maintenance of zoonotic infections that can cause severe illness or death in people and domesticated animals (Díaz 2010).

Rickettsia are gram-negative, nonmotile, nonspore forming, highly pleomorphic α-1-proteobacteria. They are obligate intracellular parasites that multiply freely in the cytosol of eukaryotic cells. They have a worldwide distribution and are mainly maintained in nature by transovarial transmission in arthropod populations, with arthropods sometimes acting as vectors of rickettsia to vertebrate hosts (Parola et al. 2005).

Recently, the availability of molecular tools to investigate rickettsial infections in arthropods and clinical samples has allowed the description of new *Rickettsia* spp. (Raoult et al. 2005, Hechemy et al. 2006).

The purpose of this study was to provide molecular evidence for the presence of a possible new species of rickettsia found in ticks collected in a township from the North of Colombia (Cordoba).

Materials and Methods

From January to December 2009, a total of 55 *Amblyomma dissimile* (Koch) ticks (42 adult and 13 nymphs) were removed from 19 iguanas (*Iguana iguana*) in the Municipality of Monteria (8° 45′01″ N. 75° 53′25″ W in the North West of Colombia). All captured iguanas were infested by ticks, and between 1 and 5 ticks were collected per animal (average of 2.8 ticks per host). Blood samples from iguanas were not obtained. Furthermore, 3,114 ticks [458 *Amblyomma* sp. larvae, 2,636 *Rhipicephalus microplus* (Canestrini) larvae and 20 *Amblyomma* sp. nymphs] were collected in the Municipality of Los Cordobas (8° 53′59″ N. 76° 21′59″ W North West of Colombia) by drag sampling.

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The area of Los Cordobas, mostly used for agriculture and livestock, is a tropical dry forest with temperature >24°C and from 20 to 100 m above sea level.

Ticks (including larvae) were individually identified by taxonomic keys (Barros-Battesti et al. 2006, Cooley 1946) with the collaboration of an expert (Prof. Marcelo B. Labruna, Universidad de Sao Paulo, Brazil). Specimens were grouped into 113 pools: 30 pools containing 1–4 *A. dissimile* individuals (adults or nymphs) from the same host, and 83 pools containing up to 30 *Amblyomma* sp. larvae, up to 50 *Rh. microplus* larvae and five *Amblyomma* sp. nymphs. DNA from pools was extracted by using a QIAamp DNA Mini-Kit (QIAGEN, Valencia, CA) and eluted in a final volume of 100 μl. To ensure there was no contamination, a blank tube was included for DNA extraction every 15 pools. Purified DNA was stored at −20°C until used as template for polymerase chain reaction (PCR). For initial screening of rickettsial DNA, 5 μl of each pooled tick DNA template were used for real-time PCR (RT-PCR). RT-PCR was performed using a LightCycler rapid thermal cycler system (Roche Diagnostics, Somerville, NJ). Primers CS-5 (forward) and CS-6 (reverse), targeting a 147-bp fragment of the citrate synthase (*gltA*) gene of *Rickettsia* spp., and a hydrolysis probe 6-FAM–CATTGTGCCATCCAGCCT-ACGGT-BHQ-1 (TIB MOLBIOL, LLC, Adelphia, NJ) were used (Labruna et al. 2004a). This assay included an internal control for each reaction which amplifies a portion of genome of Phage Lambda (265 pb). Primers Lambda F (forward)–ATGCCACGTAAGC-GAAACA-, Lambda R (reverse) GCATAAAC-GAAGCAGTCGAGT and hydrolysis probe Lambda TM DYXL-CGTCGCTTTTTGCTGTCCCAC-BBQ (TIB MOLBIOL, LLC). The hydrolysis probe assays contained a FastStart *Taq*DNA Polymerase (Roche) 4 μl, 1 μl of each primer at 10 μM, 0,2 μl of each probe at 20 μM, 2 μl of Phage Lambda DNA and 4,6 μl of molecular-grade water. Real-time PCR cycling conditions were as follows: 1 cycle at 95°C for 10 min, followed by 40 cycles of 10 s at 95°C, 15 s at 55°C, and 15 s at 70°C. For each reaction, both positive (*Rickettsia amblyommii* DNA) and negative (water) controls were included. Positive samples were further studied using *gltA* (401 bp), *ompA* (631 bp), and *ompB* (811 bp) PCR assays and sequencing (Labruna et al. 2004a, Roux et al. 1996, Roux and Raoult 2000). To minimize the risk of contamination, the work was carried out by experienced people. In addition, PCR-mix and DNA were added in different areas of the laboratory. Reactions were performed in automated MJ Research PTC-100TM thermal cyclers. For conventional PCR, a recombinant *Taq*DNA Polymerase (Invitrogen, Brazil) was used. PCR products were separated by electrophoresis on a 1.5% agarose gel, stained with ethidium bromide and examined using an ultraviolet (UV) transilluminator (ImageQuant 100, Uppsala, Sweden). The PCR products were cleaned using a PureLink Quick Gel Extraction kit (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions, and both strands of each fragment gene were directly sequenced in an automatic sequencer (model ABI-PRISM 3130XL, Applied Biosystems, Foster City, CA). Generated sequences were submitted to Basic Local Alignment Sequence Tool (BLAST) analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Phylogenetic analysis was conducted with MEGA4 (Tamura et al. 2007). GenBank accession numbers for nucleotide sequences from validly published *Rickettsia* species used to generate the phylogenetic tree are shown in Table 1.

Table 1. GenBank accession numbers for nucleotide sequences from validly published *Rickettsia* species used to generate the phylogenetic tree of *Rickettsia* sp. strain Colombianensi

Rickettsia spp.	GenBank accession no. for PCR target genes		
	gltA	*ompA*	*ompB*
R. sibirica sibirica	U59734	U43807	AF123722
R. sibirica mongolitimonae	U59731	U43796	AF123715
R. parkeri	U59732	U43802	AF123717
R. africae	U59733	U43790	AF123706
R. conorii caspia	U59728	U43791	AF123708
R. conorii israelensis	U59727	U43797	AF123712
R. conorii indica	U59730	U43794	AF123726
R. conorii conorii	U59730	U43806	AF123721
R. slovaca	U59725	U43808	AF123723
R. honei	U59726	U43809	AF123724
R. rickettsii	U59729	U43804	X16353
R. japonica	U59724	U43795	AF123713
R. heilonjgiangensis	AF178034	AF179362	AY260451
R. montanensis	U74756	U43801	AF123716
R. raoultii	DQ365804	DQ365801	DQ365798
R. aechlimannii	U59722	U43800	AF123705
R. massilliae	U59719	U43799	AF123714
R. rhipicephali	U59721	U43803	AF123719
R. tamurae	AF394896	DQ103259	DQ113910
R. australis	U59718	AF149108	AF123709
R. monacensis	DQ100163	DQ100169	EF380356
R. felis	AF210692	AF210694	AF210695

Results

Rickettsial DNA was detected in 28 out of 113 (24.8%) pools of ticks by RT-PCR for *gltA* gene. These 28 positive pools were subsequently subjected to conventional PCR protocols targeting *gltA*, *ompA*, and *ompB*. Sixteen *A. dissimile* pools corresponding to 14 iguanas as well as 12 free-living larvae pools (six *Amblyomma* sp. larvae pools and six *Rh. microplus* larvae pools) were positive using this three conventional PCR assays. As expected, all negative controls gave negative results. The prevalence of *Rickettsia* in ticks expressed as percentage and minimum infection rate (MIR) or the minimum percentage of ticks in a pool with detectable *Rickettsia* is shown in Table 2. This is based on the assumption that a PCR-positive pool contains at least one positive tick (Labruna et al. 2004b). Subsequently, *gltA*, *ompA*, and *ompB* amplicons from two pools of *A. dissimile* adult ticks removed from iguanas in Monteria, and from two pools of free-living larvae collected in Los Cordobas (one pool of *Amblyomma* sp. larvae and one pool of *Rh. microplus* larvae) were sequenced. These four pools were randomly chosen from the 28 positive pools. Generated sequences were edited and assembled, and primer

Table 2. Prevalence of *Rickettsia* in ticks expressed as the min. percentage of ticks in a pool with detectable *Rickettsia* and MIR

Municipality of Colombia	Tick pools	No. ticks	No. positive pools/ no. tested pools	% positive pools	MIR[a]
Monteria	*Amblyomma dissimile* pools	55 (42A, 13N)[b]	16/30	53.3	16/55 (29%)
Los Cordobas	*Amblyomma* sp. larvae pools	458	6/15	40	6/458 (1.3%)
Los Cordobas	*Rh. microplus* larvae pools	2,636	6/63	9.5	6/2,636 (0.2%)
Los Cordobas	*Amblyomma* sp. nymphs pools	20	0/5	0	0/20 (0%)
Monteria and Los Cordobas	Total pools	3,169	28/113	24.7	28/3,169 (0.9%)

[a] MIR, minimum infection rate.
[b] A, adult ticks; N, nymphs.

sequences were removed from the edges. For each fragment gene analyzed, identical nucleotide sequences were obtained for all four pools. As for the closest valid *Rickettsia* species, *gltA* and *ompA* nucleotide sequences generated from this study showed 99.4 and 95.6% identity with *Rickettsia tamurae* (AF394896 and DQ103259, respectively). *ompB* nucleotide sequences showed maximum identity (97.6%) with *Rickettsia monacensis* (EF380356), and were 96.4% identical to *R. tamurae* (DQ113910).

Our *gltA* and *ompA* sequences showed similar or even higher percentages of identity (99.4 and 97.5%, respectively) with those of a *Rickettsia* endosymbiont of *Amblyomma dubitatum* recently found in Brazil (JN676158 and JN676159). *ompB* sequences for this unclassified rickettsial strain (designated as *Rickettsia* sp. strain Pampulha) are not available. The nucleotide sequences of *gltA*, *ompA*, and *ompB* genes generated from this study have been deposited in the GenBank database under the accession numbers JF905456, JF905458, and JF905457, respectively. This new proposed spotted fever group *Rickettsia* detected in Colombia belongs to the same lineage of *R. tamurae* and *R. monacensis*, under 100% bootstrap support (Fig. 1). We proposed the name *Rickettsia* sp. strain Colombianensi.

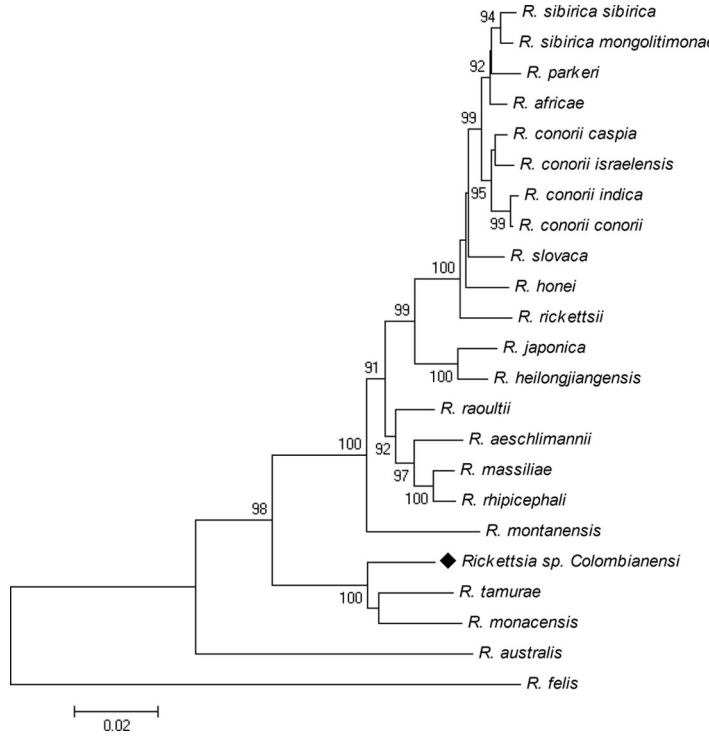

Fig. 1. The phylogenetic position of *Rickettsia* sp. strain Colombianensi is shown. The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 0.54610104 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown next to the branches; only bootstrap values ≥80 are shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset. Three genes were concatenated (*gltA-ompA-ompB*), and a total of 1,672 positions were analyzed. Phylogenetic analyses were conducted in MEGA4 (Tamura et al. 2007).

Discussion

In Latin America, several *Rickettsia* species (belonging mostly to the spotted fever group) pathogenic for humans or with unknown pathogenicity have been reported. *Rickettsia rickettsii*, the etiological agent of the most severe spotted fever in the world, has been reported in *Amblyomma cajennense* ticks in Brazil, Colombia, Panama, and Mexico (Guedes et al. 2005, Patiño et al. 2006, De Rodaniche 1953) and in *Amblyomma aureolatum* ticks in Brazil (Pinter and Labruna 2006). More recently, other *Rickettsia* species, such as *Rickettsia parkeri* has been reported in Uruguay (Venzal et al. 2004); and *Rickettsia* strain COOPERI (closely related to *R. parkeri*) has been isolated in Brazil (Labruna et al. 2004a). *Rickettsia massiliae* has been detected in Argentina (García-García et al. 2010), and *Rickettsia bellii* in many ticks in Brazil (Horta et al. 2006; Labruna et al. 2004b, 2007a; Pinter and Labruna 2006) and Argentina (Labruna et al. 2007a). *Rickettsia amblyommii* has been also found in Brazil (Labruna et al. 2004b) and Argentina (Labruna et al. 2007a). "*Candidatus* Rickettsia andeanae" was reported in Peru (Blair et al. 2004) and *Rickettsia rhipicephali*, in Brazil (Labruna et al. 2005, 2007b). In addition, *R. prowazekii* was reported in *A. cajennense* or *A. imitator* ticks in Mexico (Medina-Sánchez et al. 2005). These studies reflect the increasing interest on rickettsial diseases in Latin America during the last years.

In Colombia, Rocky Mountain Spotted Fever (RMSF) was first reported in 1937 by Patiño. It was named Tobia fever because of the village where these cases occurred. The disease remained forgotten until 2003, when two fatal cases were identified and reported in Villeta (a locality near Tobia) (Patiño et al. 2006, Hidalgo et al. 2011). More recently, three outbreaks of RMSF have occurred in the country: In 2006 among military personnel in Necocli (Antioquia) (Acosta et al. 2006), in 2007 in a township of Los Cordobas (Córdoba) (Hidalgo et al. 2011) and in 2008 in Altos de Mulatos (Antioquia) (Pacheco et al. 2008). These reports defined the reemergence of the disease in Colombia and alerted the systems of surveillance across the country. No other tick-borne rickettsia species pathogenic or with unknown pathogenicity have been reported in Colombia.

According to Raoult et al., 2005, to be classified as a potential new *Rickettsia* species, a bacterium should not exhibit more than one of the following percentages of nucleotide identity: >99.8, >99.9, >98.8, >99.2, and >99.3 for *rrs*, *gltA*, *ompA*, *ompB*, and *sca4*, respectively, with a validated *Rickettsia* species. In our cases, the nucleotide sequences corresponding to *gltA* and *ompA* exhibited 99.4 and 95.6% identity with *R. tamurae* as the nearest validly published species. The *ompB* sequence generated was 97.6% identical to the corresponding sequence of *R. monacensis* (and 96.4% identical to that of *R. tamurae*). These results suggest that a new genotype of the spotted fever group rickettsiae is present in our samples, and based on the nucleotide sequences found for *gltA*, *ompA*, and *ompB*, a novel *Rickettsia*, designated as *Rickettsia* sp. strain Colombianensi, is proposed.

Over the last few decades, several 'new' rickettsia species, with unknown pathogenicity, have been identified in many species of arthropods and some leeches and amoebae (Ogrzewalska et al. 2009). No evidence of human pathogenicity is presented herein for *Rickettsia* sp. strain Colombianensi, and there is no evidence to suggest that this rickettsia is transmissible to humans. Our finding of *A. dissimile* ticks on cattle in areas where no iguanas were found may explain the identical rickettsial sequences occurring in *A. dissimile* and *Rh. microplus* larvae, suggesting that this *Rickettsia* sp. is infective for both iguanas and cattle.

The new genotype of rickettsial organism detected in this work was closely related to *R. tamurae* and *R. monacensis.* In 1993, *R. tamurae* was isolated from *Amblyomma testudinarium* ticks in Japan. Later on it was formally identified as a novel *Rickettsia* species (Fournier et al. 2006). Detection of *R. tamurae* DNA in a person that had no spotted fever clinical signs, besides a local inflammation at the tick-bite site has been recently reported in Japan (Imaoka et al. 2011). *R. monacensis* was first isolated and characterized from *Ixodes ricinus* collected in Germany in 2002 (Simser et al. 2002). This rickettsia species has been reported as one of the etiological agents of Mediterranean spotted fever-like (Jado et al. 2007).

In addition, this is the first report of *A. dissimile* on iguanas in Cordoba. This tick species is distributed from the United States to Argentina. The adult stage has a preference to parasitize reptiles (snakes, lizards, and iguanas, but less often caimans and tortoises) and amphibians (toads). However, this species has also been recorded on mammals, such as cattle and rodents of the families Hidrochaeridae (*Hydrochaeris hydrochaeris*) and Echimyidae (*Proechimys semispinosus* and *Peromyscus gossypinus*) (Freitas et al. 2004). *A. dissimile* could play an important role in the enzootic cycle of rickettsial transmission between animals.

In conclusion, we report the detection of a new rickettsial agent (strain Colombianensi) infecting *Amblyomma dissimile*, *Rh. microplus*, and possibly other *Amblyomma* species in Colombia. More studies are needed to a better characterization, to know the distribution, epidemiology and the possible role of this rickettsia as human or animal pathogen.

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References Cited

Acosta, J., L. Urquijo, A. Díaz, M. Sepúlveda, G. Mantilla, D. Heredia, G. González, E. Parra, G. Rey, M. Hidalgo, et al. 2006. Brote de rickettsiosis en Necoclí, Antioquia, febrero-marzo de 2006. Inf. Quinc. Epidemiol. Nac. 11: 177–192.

Barros-Battesti, D. M., M. Arzua, and G. H. Bechara. 2006. Carrapatos de Importância Médico-Veterinária da Região Neotropical: um guia ilustrado para identificação de espécies. São Paulo, Vox/ICTTD-3/Butantan.

Blair, P. J., J. Jiang, G. B. Schoeler, C. Moron, E. Anaya, M. Cespedes, C. Cruz, V. Felices, C. Guevara, L. Mendoza, et al. 2004. Characterization of spotted fever group rickettsiae in flea and tick specimens from northern Peru. J. Clin. Microbiol. 42: 4961–4967.

Cooley, R. A. 1946. The genera *Boophilus, Rhipicephalus* and *Haemaphysalis* (Ixodidae) of the New World. Nat. Inst. Health, Washington, DC.

De Rodaniche, E. C. 1953. Natural infection of the tick, *Amblyomma cajennense*, with *Rickettsia rickettsii* in Panama. Am J Trop Med Hyg. 2: 696–699.

Díaz, J. H. 2010. Ticks, including tick paralysis, pp. 3649–3662. *In* G. L. Mandell, J. E. Bennett, and R. Dolin (eds.), Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. Churchill Livingstone Elsevier, Philadelphia, PA.

Fournier, P. E., N. Takada, H. Fujita, and D. Raoult. 2006. *Rickettsia tamurae* sp. nov., isolated from *Amblyomma testudinarium* ticks. Int. J. Syst. Evol. Microbiol. 56: 1673–1675.

Freitas, L.H.T., J.L.H. Faccini, E. Daemon, M.C.A. Prata, and D. M. Barros-Battesti. 2004. Experimental infestation with the immatures of *Amblyomma dissimile* Koch, 1844 (Acari:Ixodidae) on *Tropidurus torquatus* (Lacertilia: Iguanidae) and *Oryctolagus cuniculus.* Arq. Bras. Med. Vet. Zootec. 56: 126–129.

García-García, J. C., A. Portillo, M. J. Núñez, S. Santibáñez, B. Castro, and J. A. Oteo. 2010. A patient from Argentina infected with *Rickettsia massiliae.* Am. J. Trop. Med. Hyg. 82: 691–692.

Guedes, E., R. C. Leite, M. C. Prata, R. C. Pacheco, D. H. Walker, and M. B. Labruna. 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.

Hechemy, K. E., J. A. Oteo, D. Raoult, D. J. Silverman, and J. R. Blanco. 2006. A century of rickettsiology: emerging, reemerging rickettsioses, clinical, epidemiologic, and molecular diagnostic aspects and emerging veterinary rickettsioses: an overview. Ann. N. Y. Acad. Sci. 1078: 1–14.

Hidalgo, M., J. Miranda, D. Heredia, P. Zambrano, J. F. Vesga, D. Lizarazo, S. Mattar, and G. Valbuena. 2011. Outbreak of Rocky Mountain spotted fever in Cordoba, Colombia. Mem. Inst. Oswaldo Cruz. 106: 117–118.

Horta, M. C., A. Pinter, T. T. Schumaker, and M. B. Labruna. 2006. Natural infection, transovarial transmission, and transstadial survival of *Rickettsia bellii* in the tick *Ixodes loricatus* (Acari: Ixodidae) from Brazil. Ann. N. Y. Acad. Sci. 1078: 285–290.

Imaoka, K., S. Kaneko, K. Tabara, K. Kusatake, and E. Morita. 2011. The first human case of *Rickettsia tamurae* infection in Japan. Case Rep. Dermatol. 3: 68–73.

Jado, I., J. A. Oteo, M. Aldámiz, H. Gil, R. Escudero, V. Ibarra, J. Portu, A. Portillo, M. J. Lezaun, C. García-Amil, I. Rodríguez-Moreno, and P. Anda. 2007. *Rickettsia monacensis* and human disease, Spain. Emerg. Infect. Dis. 13: 1405–1407.

Labruna, M. B., L. M. Camargo, E. P. Camargo, and D. H. Walker. 2005. Detection of a spotted fever group *Rickettsia* in the tick *Haemaphysalis juxtakochi* in Rondonia, Brazil. Vet. Parasitol. 127: 169–174.

Labruna, M. B., R. C. Pacheco, L. J. Richtzenhain, and M. P. Szabo. 2007b. Isolation of *Rickettsia rhipicephali* and *Rickettsia bellii* from *Haemaphysalis juxtakochi* ticks in the state of Sao Paulo, Brazil. Appl. Environ. Microbiol. 73: 869–873.

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

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

Labruna, M. B., T. Whitworth, M. C. Horta, D. H. Bouyer, J. W. McBride, A. Pinter, V. Popov, S. M. Gennari, and D. H. Walker. 2004a. *Rickettsia* species infecting *Amblyomma cooperi* ticks from an area in the state of Sao Paulo, Brazil, where Brazilian spotted fever is endemic. J. Clin. Microbiol. 42: 90–98.

Medina-Sánchez, A., D. H. Bouyer, V. Alcántara-Rodríguez, C. Mafra, J. Zavala-Castro, T. Whitworth, V. L. Popov, I. Fernández-Salas, and D. H. Walker. 2005. Detection of a typhus group *Rickettsia* in *Amblyomma* ticks in the state of Nuevo Leon, Mexico. Ann. N. Y. Acad. Sci. 1063: 327–332.

Ogrzewalska, M., R. C. Pacheco, A. Uezu, L. J. Richtzenhain, F. Ferreira, and M. B. Labruna. 2009. Rickettsial infection in *Amblyomma nodosum* ticks (Acari: Ixodidae) from Brazil. Ann. Trop. Med. Parasitol. 103: 413–425.

Pacheco, O., M. Giraldo, M. Hidalgo, A. Galeano, I. Echeverri, L. Echevarría, E. Parra, and G. Rey. 2008. Estudio de brote febril hemorrágico en el corregimiento de Alto de Mulatos: distrito Especial Portuario de Turbo, Antioquia, enero de 2008. Inf. Quinc. Epidemiol. Nac. 13: 145–160.

Parola, P., B. Davoust, and D. Raoult. 2005. Tick- and flea-borne rickettsial emerging zoonoses. Vet. Res. 36: 469–492.

Patiño, L., A. Afanador, and J. H. Paul. 2006. A spotted fever in Tobia, Colombia 1937. Biomedica 26: 178–193.

Pinter, A., and M. B. Labruna. 2006. Isolation of *Rickettsia rickettsii* and *Rickettsia bellii* in cell culture from the tick *Amblyomma aureolatum* in Brazil. Ann. N. Y. Acad. Sci. 1078: 523–529.

Raoult, D., P. E. Fournier, M. Eremeeva, S. Graves, P. J. Kelly, J. A. Oteo, Z. Sekeyova, A. Tamura, I. Tarasevich, and L. Zhang. 2005. Naming of rickettsiae and rickettsial diseases. Ann. N. Y. Acad. Sci. 1063: 1–12.

Roux, V., and D. Raoult. 2000. Phylogenetic analysis of members of the genus *Rickettsia* using the gene encoding the outer-membrane protein rOmpB (*ompB*). Int. J. Syst. Evol. Microbiol. 50: 1449–1455.

Roux, V., P. E. Fournier, and D. Raoult. 1996. 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. 34: 2058–2065.

Simser, J. A., A. T. Palmer, V. Fingerle, B. Wilske, T. J. Kurtti, and U. G. Munderloh. 2002. *Rickettsia monacensis* sp.

nov., a spotted fever group *Rickettsia*, from ticks (*Ixodes ricinus*) collected in a European city park. Appl. Environ. Microbiol. 68: 4559–4566.

Tamura, K., J. Dudley, M. Nei, and S. Kumar. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24: 1596–1599.

Venzal, J. M., A. Portillo, A. Estrada-Peña, O. Castro, P. A. Cabrera, and J. A. Oteo. 2004. *Rickettsia parkeri* in *Amblyomma triste* from Uruguay. Emerg. Infect. Dis. 10: 1493–1495.

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