Detection and Identification of Rickettsial Agents in Ticks From Domestic Mammals in Eastern Panama

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ABSTRACT Several outbreaks of Rocky Mountain spotted fever have occurred in recent years in Colombian communities close to the border with Panama. However, little is known about rickettsiae and rickettsial diseases in eastern Panamanian provinces, the Darien Province and the Kuna Yala, located north of the endemic area in Colombia. In 2007, 289 ticks were collected in several towns from dogs, horses, mules, cows, and pigs. DNA was extracted from 124 *Dermacentor nitens*, 64 *Rhipicephalus sanguineus*, 43 *Amblyomma ovale*, 35 *A. cajennense*, 10 *Boophilus microplus*, 4 *A. oblongoguttatum*, and 9 *A. cajennense* nymphs. SYBR-Green polymerase chain reaction assays targeting a fragment of the OmpA and 16S rRNA genes were used for detection of DNA of the spotted fever group rickettsiae (SFGR) and Anaplasmataceae (*Anaplasma* and *Ehrlichia*), respectively. In total, 37.4% ticks were positive for SFGR, including 20.3% *R. sanguineus*, 27.9% *A. ovale*, 25.8% *D. nitens*, 50% *B. microplus*, 50% *A. oblongoguttatum*, and 100% *A. cajennense*. The presence of *Rickettsia amblyommii* DNA was confirmed by sequencing in *A. cajennense*, *A. oblongoguttatum*, *A. ovale*, *B. microplus*, and *R. sanguineus*. DNA of *R. rickettsii* was only detected in one *D. nitens* collected from a horse in Santa Fe, Darien Province. Prevalence of Anaplasmataceae varied from 6.3% in *R. sanguineus* to 26.5% in *A. cajennense*. DNA of *Ehrlichia chaffensis* was found in three *D. nitens* and three *A. cajennense* from horses. This is the first study providing molecular characterization and prevalence information on SFGR in ticks from these areas and thus will be helpful for future evaluations of the risk of rickettsial diseases for individuals living in this region.

KEY WORDS Panama, ticks, spotted fever group rickettsiae, Anaplasmataceae

It is hard to overestimate the significance of ticks for human and veterinary medicine because of their intimate associations with a variety of pathogenic fungal, viral, parasitic, and bacterial organisms, many of which can be transmitted to mammals during acquisition of a bloodmeal (Guimaraes et al. 2001, Labruna and Machado 2006). Bacteria belonging to the order Rickettsiales, including numerous species of the genus *Rickettsia*, *Ehrlichia*, and *Anaplasma*, are tick-transmitted microorganisms whose distribution and prevalence in different endemic areas varies significantly (Acha and Zsyfres 2003).

Rickettsioses are among the most important emergent and re-emergent zoonotic diseases in the Americas. In some areas, there is a history of sustained circulation of these agents, but recent outbreaks have also occurred in several areas where disease was not known previously (Azad and Beard 1998, Parola et al. 2005). The distribution of Rocky Mountain spotted fever (or disease primarily caused by *Rickettsia rickettsii* but bearing different names outside the United States, such as Fiebre Maculosa in Brazil) is the most documented. Unfortunately, only a few countries maintain long-term and systematic epidemiological surveillance programs permitting better understanding of the real burden of these diseases in the Americas. However, contemporary information regarding the presence of multiple rickettsial agents in the same areas and the lack of specific diagnosis contributes to ambiguous epidemiological data in the region (Parola et al. 2005).

In recent years, multiple cases of Rocky Mountain spotted fever were diagnosed in several countries, including Brazil (Galvao et al. 2003a, Galvao 2004), Mexico (Zavala-Castro et al. 2006), Colombia (Acosta et al. 2006, Hidalgo et al. 2007), Panama (Estripeaut et al. 2007), and Argentina (Ripoll et al. 1999, Paddock et al. 2008). To study these outbreaks, classical serologic

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diagnostic techniques and contemporary molecular assays were used to perform detection and species identification of the rickettsial agents causing the infections (Galvao et al. 2003b).

The purpose of this study is to determine the identity and prevalence of rickettsial agents found in ticks in Eastern Panama to assess the potential risk they pose to human and animal health in several communities along the Panamanian–Colombian border. Because an outbreak of fatal Rocky Mountain spotted fever occurred at the adjacent province in Colombia at Necoclí, Antioquia, in February 2006 (Acosta et al. 2006), the concern was raised if the Panamanian population in this area may have an increased risk of exposure to *R. rickettsii.*

Materials and Methods

Tick Collection. Ticks were collected in towns along the Panamanian–Colombian border in Darien and Kuna Yala provinces. Specifically, ticks were collected in and around the towns of Yaviza (4–9 June 2007), Meteti-Santa Fe (16–22 July 2007), Jaque (28 September to 2 October 2007), and Puerto Obaldia (7–14 December 2007). The populations of these towns are composed of indigenous peoples, people from other Panamanian provinces, and people from Colombia who live in close contact with the largest rainforest in Panama. The ticks were collected from domestic animals and transported live to the Medical Entomology Laboratory of the Gorgas Memorial Institute, where adults were identified using the taxonomic keys of Fairchild et al. (1966) and Onofrio et al. (2006). The ticks were stored in 100% ethanol and sent to the Centers for Disease Control and Prevention (CDC) in Atlanta, GA, for screening. The species identification of *Amblyomma* nymphs was determined by sequencing ITS2 fragment as described by Marrelli et al. (2007).

Tick Processing and DNA Extraction. On arrival at the CDC, the ticks were separated and placed individually in sterile 1.7-ml polypropylene microtubes (Axygen Scientific, Union City, CA). The ticks were next disinfected by washing with 10% bleach for 10 min, followed by a 5-min wash in 70% ethanol. The ticks were rinsed three times with distilled water to remove any remaining ethanol. After the last wash, all remaining water was removed, and the tubes containing the ticks were frozen by placing in liquid nitrogen. The ticks were crushed using Kontes pestles (Kimble-Kontes, Vineland, NJ), resuspended in 220 μl lysis buffer consisting of 160 μl Nuclei lysis solution (Promega, Madison, WI), 40 μl 0.5 M EDTA, and 20 μl 20 mg/ml Proteinase K (Qiagen, Valencia, CA), and incubated overnight at 56°C. DNA was extracted using the Wizard SV 96 Genomic DNA purification system (Promega) and a Biomek 2000 Robotic Workstation (Beckman Coulter, Fullerton, CA), and was eluted with 120 μl sterile nuclease free water.

PCR Testing of DNA Samples. The tick DNA samples were tested for the presence of rickettsial DNA using a SYBR Green–based quantitative polymerase chain reaction (qPCR) assay targeting a 155-bp fragment of the OmpA gene as previously described Eremeeva et al. (2003). Briefly, 4 μl of the tick DNA sample was used as a template in a 20-μl reaction consisting of 0.2 nM each primers Rr190.547 and Rr190.701, 3.75 mM $MgCl_2$, 625 nM dNTPs, 1× SYBR Green reaction buffer, and 0.625 U AmpliTaq Gold polymerase using the SYBR Green core reagent kit (ABI, Foster City, CA). Each sample was run in duplicate. Genomic DNA of both *R. slovaca* and *R. sibirica* were run on every plate as positive controls, and four no template controls were run on each plate to ensure there was no contamination of reagents. Reactions were run in an iCycler (Bio-Rad, Hercules, CA) with the following conditions: an initial 3-min denaturation at 95°C, followed by 50 cycles of 95°C for 20 s, 57°C for 30 s, and 65°C for 30 s. A final 5-min extension at 72°C was followed by melting curve analysis. A seminested PCR was performed to obtain 532 bp of *omp*A for species identification of spotted fever group rickettsiae as previously described (Estripeaut et al. 2007).

To screen for *Anaplasma* and *Ehrlichia* (Anaplasmataceae) DNA, another SYBR Green qPCR assay was used as previously described (Eremeeva et al. 2007) that targets a ~150-bp region of the 16S ribosomal RNA gene. Once again, a 4-μl tick DNA sample was screened in a 20-μl reaction consisting of 0.2 nM each primer (ECSYBR-F and ECSYBR-R), 3.75 mM $MgCl_2$, 625 nM dNTPs, 1× SYBR Green reaction buffer, and 0.625 U AmpliTaq Gold polymerase using the SYBR Green core reagent kit (ABI). Reactions were run in an iCycler (Bio-Rad) with the following conditions: an initial 10-min denaturation at 95°C followed by 50 cycles of 15 s at 95°C and 1 min at 60°C. Finally, a melting curve analysis was run according to the manufacturer's instructions. A recombinant plasmid containing a homologous fragment of the 16S rRNA gene of *E. ewingii* was used as a positive control.

Melting curves were analyzed for each amplicon; the amplicons with *Tm*s similar to the *Tm* of the positive control were further resolved on a 1% agarose gel to detect the presence of a product of the expected size.

DNA Sequencing and Analysis. The amplicons were purified using the Wizard SV gel and PCR clean-up system (Promega) and sequenced using the BigDye Terminator v3.1 cycle sequencing kit (ABI) according to the manufacturer's recommendations on an ABI 3130*xl* genetic analyzer. Sequencing reads were assembled and analyzed using Sequencher 4.8 (Gene Codes, Ann Arbor, MI).

Results

A total of 289 ticks, including 280 adults and 9 nymphs, were collected from animals in the Darien and Kuna Yala provinces of Panama between June and December 2007; host association and species identification of each specimen are provided in Tables 1 and 2. All ticks collected were tested for the presence of spotted fever group (SFG) rickettsiae

Table 1. Prevalence and identity of DNA of SFG rickettsiae and Anaplasmataceae in ticks from different sites in Darien and Kuna Yala Provinces

Town	Total ticks tested	Spotted fever group rickettsiae			Anaplasmataceae	
		No. ticks positive for SFGR DNA	No. samples with *R. rickettsii* DNA sequenced	No. samples with *R. amblyommii* DNA sequenced	No. ticks positive for Anaplasmataceae DNA	No. samples with *E. chaffeensis* DNA sequenced
Darien Province						
Santa Fe	78	32 (41.0%)	1 (1.3%)	20 (25.6%)	13 (16.7%)	2 (2.6%)
Mete	107	48 (44.9%)	0	33 (30.8%)	13 (12.1%)	4 (3.7%)
Kuna Yala Province						
Puerto Obalda	48	11 (22.9%)	0	2 (4.2%)	1 (2.1%)	0
La Miel	24	7 (29.2%)	0	0	0	0
Mulatupo	4	0	0	0	0	0
Armila	28	10 (35.7%)	0	0	1 (3.6%)	0
Total	289	108 (37.4%)	1 (0.35%)	55 (19.0%)	28 (9.7%)	6 (2.1%)

and Anaplasmataceae using SYBR Green–based qPCR assays as described previously (Eremeeva et al. 2003, 2007). Of these, 108 (37.4%) ticks tested positive for the presence of SFG rickettsial DNA, of which only 56 positive specimens could be identified to a species by sequencing. One sample (0.35%) was found to contain DNA from *R. rickettsii*, whereas the other 55 samples (19.0%) had *Rickettsia amblyommii* DNA through sequencing of the *omp*A amplicons. The other PCR-positive samples could not be sequenced because of low rickettsial DNA copy numbers. Additionally, 28 samples (9.7%) tested positive for the presence of Anaplasmataceae DNA, and 6 (2.1%) of these had DNA from *Ehrlichia chaffeensis* based on DNA sequencing. Seventeen ticks (5.9%) were positive for the presence of both SFG and Anaplasmataceae DNA.

Both SFG rickettsiae and Anaplasmataceae were more prevalent in ticks ($n = 185$) in the Darien Province, with 43.2 and 14.1% being positive, respectively, compard with Kuna Yala ($n = 104$), with 26.9 and 1.9% positive, respectively. *E. chaffeensis* DNA was detected only in both sites sampled in Darien province (Table 1).

Amblyomma cajennense from horses ($n = 34$) and from a dog ($n = 1$) had the highest infection rate of SFG rickettsiae, with all 35 adult ticks tested being positive for SFG DNA; 29 of these samples, including the tick from the dog, were confirmed to contain DNA of *R. amblyommii*. All the *A. cajennense* nymphs ($n = 7$) from a pig and a dog ($n = 2$) contained *R. amblyommi* DNA. The rates and levels of detection of SFG rickettsial DNA were lower in *A. ovale* (12 of 43) and *A. oblongoguttatum* (2 of 4), and only one *A. ovale* could be shown to have *R. amblyommii* DNA. *R. sanguineus* had the lowest prevalence of SFG rickettsial DNA, with only 20.3% of the ticks being positive. The presence of *R. rickettsii* DNA was detected in only one tick, a *Dermacentor nitens* ($n = 124$) taken from a horse in Santa Fe, Darien Province. *A. cajennense* also had the highest prevalence of Anaplasmataceae DNA, with 26.5% of ticks being positive. Anaplasmataceae DNA was not detected in *A. ovale* ($n = 45$), *A. ob-*

Table 2. Prevalence and identity of DNA of SFG rickettsiae and Anaplasmataceae in ticks collected from different animals in Darien and Kuna Yala Provinces

Animal host and tick collected	No. ticks tested	Spotted fever group rickettsiae			Anaplasmataceae	
		No. ticks positive for SFGR DNA	No. samples with *R. rickettsii* DNA sequenced	No. samples with *R. amblyommii* DNA sequenced	No. ticks positive for Anaplasmataceae DNA	No. samples with *E. chaffeensis* DNA sequenced
Cow						
Boophilus microplus	10	5 (50.0%)	0	0	0	0
Dog						
Amblyomma cajennense	1	1 (100%)	0	1 (100%)	0	0
Amblyomma ovale	42	12 (28.6%)	0	1 (2.4%)	0	0
Amblyomma oblongoguttatum	4	2 (50.0%)	0	0	0	0
Rhipicephalus sanguineus	64	13 (20.3%)	0	2 (3.1%)	4 (6.3%)	0
A. cajennense nymph	2	2 (100%)	0	2 (100%)	0	0
Horse						
A. cajennense	34	34 (100%)	0	28 (82.4%)	9 (26.5%)	3 (8.8%)
Dermacentor nitens	115	30 (26.1%)	1 (0.87%)	14 (12.2%)	15 (13.0%)	3 (2.6%)
Mule						
D. nitens	9	2 (22.2%)	0	0	0	0
Pig						
A. ovale	1	0	0	0	0	0
A. cajennense nymph	7	7 (100%)	0	7 (100%)	0	0
Total	289	108 (37.4%)	1 (0.35%)	55 (19.0%)	28 (9.7%)	6 (2.1%)

longoguttatum ($n = 4$), or *Boophilus microplus* ($n = 10$; Table 2).

Discussion

To our knowledge, this study summarizes the first detailed assessment of the presence and prevalence of rickettsial agents in ticks from multiple peridomestic sites along the Panamanian–Colombian border. An outbreak of fatal Rocky Mountain spotted fever (5 fatalities of 14 cases identified) occurred in the adjacent province in Colombia at Necoclí, Antioquia, in February 2006 (Acosta et al. 2006).

Of 289 ticks collected from pets and domestic animals, 37.43% of the ticks were positive for spotted fever group rickettsiae. Sequencing of an OmpA gene amplicon (155 or 532 nt from the 70- to 701-nt region) was successful from ≈50% of SYBR Green PCR–positive tick DNA specimens; *R. amblyommii* DNA was identified in 52 of 53 amplicons sequenced, whereas the other was from *R. rickettsii.* The majority of *R. amblyommii*–positive ticks were *A. cajennnese* and *D. nitens* collected from horses. The presence of spotted fever group rickettsia DNA was also detected in *R. sanguineus*, *A. ovale*, and *A. oblongoguttatum;* however, the speciation of these rickettsiae could not be completed because of the low rickettsial DNA copy number found in those ticks. Our pilot tick surveillance project conducted in other sites of the Darien province and Cocle province in 2007 also established the presence of spotted fever group rickettsia DNA in 61% of ticks analyzed (Eremeeva et al. 2009). However, DNA from *R. amblyommii* was also detected in *Boophilus*, *Amblyomma*, and *D. nitens* collected from different animals. It is possible that this agent may be shared by co-feeding ticks. Similarly, the widespread presence of *R. amblyommii* in Panamanian ticks is very likely based on results of isolation attempts with ticks from other regions in guinea pig experiments conducted in the 1970s (Yunker et al. 1975).

Rickettsia amblyommii seems to have a very wide distribution in the western hemisphere, and it has been found in the United States in *A. americanum* with a prevalence reaching as high as 84% at some sites (Mixson et al. 2006). In Brazil, both *R. amblyommii* and *R. amblyommii*-like rickettsiae were detected in *Amblyomma longirostre* collected from the Brazilian porcupine, *Coendou prehensilis*, passerine birds (Labruna et al. 2004a, Ogrzewalska et al. 2008), and questing *A. cajennense* and *Amblyomma coelebs* from Brazil (Labruna et al. 2004b). The prevalence of *R. amblyommii* in *A. cajennense* seems to be lower in Brazil. *R. amblyommii* or a related spotted fever group rickettsiae was also found in *Amblyomma neumanni* from Argentina (Labruna et al. 2007) and in *A. coelebs* collected from white-lipped peccaries in French Guyana (Parola et al. 2007).

A role of *R. amblyommii* as a human or animal pathogen has not yet been directly shown; however, serologic evidence suggests that this rickettsia may cause a self-limiting mild infection in humans and be responsible for the high rates of seropositivity to SFG rickettsiae in areas infested with *Amblyomma* ticks (Sanchez et al. 1992; Dasch et al. 1993, 2001; Parola et al. 2005). Several publications suggest that *R. amblyommii* might be an etiologic agent of southern tick–associated rash illness (STARI), which occurs after an *A. americanum* bite (Billeter et al. 2007, Apperson et al. 2008, Nicholson et al. 2009, Stromdahl et al. 2008). All three stages of *A. americanum* and *A. cajennense* can bite humans, providing multiple routes for transmission of this rickettsia, which is maintained transtadially and transovarially throughout the tick life cycle (Guglielmone et al. 2006).

Although *A. cajennense* is often considered one of the primary vectors of *R. rickettsii* in Central and South America (Parola et al. 2005), DNA of *R. rickettsii* was not detected in *Amblyomma* sp. ticks available for this study. In contrast, DNA of *R. rickettsii* was detected in one *D. nitens* collected from a horse. To our knowledge, this is the first molecular detection of such an association, although the presence of rickettsia-like microorganisms in *D. nitens* from Brazil was previously shown using the Gimenez stain (Lemos et al. 1997). *D. nitens* typically feed on equines and episodically may be found on cattle, goats, and other mammals, but it typically does not bite people. It cannot be excluded that these ticks may acquire *R. rickettsii* during co-feeding with *A. cajennense* on horses. The potential of *D. nitens* for maintenance and transmission of *R. rickettsii* requires further evaluation. A role for horses as a putative reservoir or passive co-feeding transmission station for *R. rickettsii* needs better understanding, because horses are regarded as a possible source of *R. rickettsii* infection transmitted with a bite of *A. cajennense* in Argentina and Colombia (Ripoll et al. 1999, Acosta et al. 2006).

Our study also provided additional evidence that *E. chaffeensis*, the agent of the human monocytic ehrlichiosis, is present in Panama, as we previously detected it in ticks from cattle and horses in Chucanti Hill and El Valle, respectively (Eremeeva et al. 2009). Subsequently, human cases of human monocytic ehrlichiosis may occur among patients with fever of unknown origin in Darien Province. Because antibodies against *E. chaffeensis* were also found in sera of healthy donors and febrile patients in Argentina (Ripoll et al. 1999) and a human case of *E. chaffeensis* was recently diagnosed in Venezuela (Martínez et al. 2008), this pathogen may have a wide distribution throughout the Americas and may be a common cause of tick-transmitted diseases in individuals working in rural occupations. It seems that different *Amblyomma* species may be involved in maintenance and transmission of *E. chaffeensis* (Tomassone et al. 2008, Eremeeva et al. 2009).

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References Cited

Acha, P., and B. Zsyfres. 2003. Zoonosis y enfermedades transmisibles comunes al hombre y a los animales, vol. II. Clamidiosis, rickettsiosis, virosis, 3rd ed. OPS/OMS, Washington, DC.

Acosta, J., L. Urquijo, A. Díaz, M. Sepúlveda, G. Mantilla, D. Heredia, M. Gonzalez, E. Parra, G. Rey, G. Munera, et al. 2006. Outbreak of fatal Rocky Mountain spotted fever in Necoclí, Antioquia, Colombia, pp. 111. *In* Proceedings of the 20th Meeting of the American Society for Rickettsiology in conjunction with the 5th International Conference on *Bartonella* as Emerging Pathogens, 2–7 September 2006, Asilomar Conference Grounds, Pacific Grove, CA.

Apperson, C. S., B. Engber, W. L. Nicholson, D. G. Mead, J. Engel, M. J. Yabsley, K. Dail, J. Johnson, and D. W. Watson. 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: 1–10.

Azad, A. F., and C. B. Beard. 1998. Rickettsial pathogens and their arthropod vectors. Emer. Infect. Dis. 4: 179–186.

Billeter, S., H. Blanton, S. Little, M. Levy, and E. Breitshwerdt. 2007. Detection of "*Rickettsia amblyommii*" in association with a tick bite rash. Vector. Borne. Zoonotic. Dis. 7: 607–610.

Dasch, G. A., D. J. Kelly, A. L. Richards, J. L. Sanchez, and C. C. Rives. 1993. Western blotting analysis of sera from military personnel exhibiting serological reactivity to spotted fever group rickettsiae. Am. J. Trop. Med. Hyg. (Suppl.) 49(3): 220.

Dasch, G. A., A. Highbaugh, and W. L. Nicholson. 2001. Direct binding and competition enzyme-linked immunosorbent assays for identifying the etiologic agents of spotted fever group, pp. 22. *In* Proceedings of the American Society for Rickettsiology-Bartonella as an Emerging Pathogen Group, 17–22 August 2001, 2001 Joint Conference, Big Sky, MT. American Society for Rickettsiology, Tampa, FL.

Eremeeva, M. E., G. A. Dasch, and D. J. Silverman. 2003. Evaluation of SQ-PCR, an assay for quantitation of *Rickettsia rickettsii* and closely-related spotted fever group rickettsiae. J. Clin. Microbiol. 41: 5466–5472.

Eremeeva, M. E., A. Oliveira, J. Moriarity, J. Robinson, N. K. Tokarevich, L. P. Antyukova, V. A. Pyanyh, O. N. Emeljanova, V. N. Ignatjeva, R. Buzinov, V. Pyankova, and G. A. Dasch. 2007. Detection and identification of bacterial agents in *Ixodes persulcatus* Schulze ticks from the North Western region of Russia. Vector-Borne Zoon. Dis. 7: 426–436.

Eremeeva, M. E., S. E. Karpathy, M. L. Levin, C. M. Caballero, S. E. Bermudez, G. A. Dasch and J. Motta. 2009. Spotted fever rickettsiae, *Ehrlichia* and *Anaplasma* in ticks from peridomestic environments in Panama. Clin. Micro. Infect. (in press).

Estripeaut, D., M.-G. Aramburú, X. Saéz-Llórens, H. A. Thompson, G. A. Dasch, C. D. Paddock, S. Zaki, and M. E. Eremeeva. 2007. Rocky Mountain spotted fever, Panama. Emer. Infect. Dis. 13: 1763–1765.

Fairchild, G., G. Kohl, and V. Tipton. 1966. The ticks of Panama (Acarina: Ixodidae), pp. 188–207. *In* R. L. Wenzel and V. J. Tipton (eds.), Ectoparasites of Panama. Field Museum of Natural History, Chicago, IL.

Galvao, M. 2004. Rickettsiosis como un problema de salud pública en América del Sur, pp. 17–20. *In* OPS/OMS (ed.), Consulta OPS/OMS de expertos sobre Rickettsiosis en las Américas. OPS/OMS, Ouro Preto, Brazil.

Galvao, M., S. Dumler, C. Mafra, S. Calic, C. Chamona, G. Filho, P. Olano, and D. Walker. 2003a. Fatal spotted fever rickettsiosis, Minas Gerais, Brazil. Emerg. Infect. Dis. 9: 1402–1405.

Galvao, M., C. Mafra, C. Moron, E. Anaya, and D. Walker. 2003b. Rickettsiosis of the genus *Rickettsia* in South America. Ann. N. Y. Acad. Sci. 990: 51–61.

Guimaraes, J., E. Tucci, and D. Barros-Battesti. 2001. Ectoparasitos de importancia veterinária. Pléide/FAPESP, Sao Paulo, Brazil.

Guglielmone, A., M. P. Szabó, J. R. Martins, and A. Estrada-Peña. 2006. Diversidade e importancia de carrapatos na sanidade animal, pp. 115–138. *In* D. M. Barros-Battesti, M. Arzúa, and G. H. Bechara (eds.), Carrapatos de importancia medico-veterinária da regiao Neotropical. Instituto Butantan, ICTTD, Sao Paulo, Brazil.

Hidalgo, M., L. Orejuela, P. Fuya, P. Carrillo, J. Hernández, K. Parra, C. Keng, M. Small, J. Olano, D. Bouyer, et al. 2007. Rocky Mountain spotted fever, Colombia. Emerg. Infect. Dis. 13: 1058–1060.

Labruna, M., and R. Z. Machado. 2006. Agentes transmitidos por carrapatos na regiao Neotropical, pp. 155–164. *In* D. M. Barros-Battesti, M. Arzúa, and G. H. Bechara (eds.), Carrapatos de importancia medico-veterinária da regiao Neotropical. Instituto Butantan, ICTTD, Sao Paulo, Brazil.

Labruna, M., J. McBride, D. Bouyer, L. Camargo, E. Camargo, and D. H. Walker. 2004a. Molecular evidence for a spotted fever group *Rickettsia* in the tick *Amblyomma longirostre* in Brazil. J. Med. Entomol. 41: 533–537.

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

Labruna, M., R. Pacheco, S. Nava, P. Brandao, L. Richtzenhain, A. Guglielmone. 2007. Infection by *Rickettsia belli* and "*Candidatus*" "*Rickettsia amblyommii*" in *Amblyomma neumanni* tick from Argentina. Micr. Biol. 54: 126–133.

Lemos, E., R. Machado, F. Pires, S. Machado, L. da Costa, and L. Coura. 1997. Rickettsiae-infected ticks in a endemic area in spotted fever in the State of Minas Gerais, Brazil. Mem. Inst. Oswaldo Cruz 92: 477–481.

Marrelli, M. T., L. F. Sousa, R. C. Marques, M. B. Labruna, S. R. Matioli, A. P. Tonon, P.E.M. Ribolla, O. Marinotti, and T.T.S. Schumaker. 2007. Taxonomic and phylogenetic relationships between neotropical species of ticks from genus *Amblyomma* (Acari: Ixodidae) inferred from second internal transcribed spacer sequences of rDNA. J. Med. Entomol. 44: 222–228.

Martínez, M. C., C. N. Gutiérrez, F. Monger, J. Ruiz, A. Watts, V. M. Mijares, M. G. Rojas, and F. J. Triana-Alonso. 2008. *Ehrlichia chaffeensis* in child, Venezuela. Emerg. Infect. Dis. 14: 519–520.

Mixson, T. R., S. R. Campbell, J. S. Gill, H. S. Ginsberg, M. V. Reichard, T. L. Schulze, and G. A. Dasch. 2006. Prevalence of *Ehrlichia*, Borrelia, and rickettsial agents in *Amblyomma americanum* (Acari: Ixodidae) collected from nine states. J. Med. Entomol. 43: 1261–1268.

Nicholson, W. L., E. Masters, and G. Wormser. 2009. Preliminary serologic investigation of "*Rickettsia amblyommii*" in the etiology of Southern tick associated rash illness (STARI). Clin. Micro. Infect. (in press).

Onofrio, V., M. B. Labruna, A. Pinter, F. G. Giacomin, and D. M. Barros-Battesti. 2006. Comentários e chaves para as espécies do gênero *Amblyomma*, pp. 53–113. *In* D. M. Barros-Battesti, M. Arzúa, and G. H. Bechara (eds.), Carrapatos de importancia medico-veterinária da regiao Neotropical. Instituto Butantan, ICTTD, Sao Paulo, Brazil.

Ogrzewalska, M., R. C. Pacheco, A. Uezu, F. Ferreira, and M. B. Labruna. 2008. Ticks (Acari: Ixodidae) infesting wild birds in an Atlantic forest area in the state of São Paulo, Brazil, with isolation of rickettsia from the tick *Amblyomma longirostre.* J. Med. Entomol. 45: 770–774.

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

Parola, P., C. D. Paddock, and D. Raoult. 2005. Tick borne rickettsiosis around the world: emerging diseases challenging old concepts. Clin. Micro. Rev. 18: 719–756.

Parola, P., K. Matsumoto, C. Socolovschi, D. Parzy, and D. Raoult. 2007. A tick borne rickettsia of the spotted fever group similar to *Rickettsia amblyommii,* in French Guyana. Ann. Trop. Med. Parasitol. 101: 185–188.

Ripoll, C., C. E. Remondegui, G. Ordoñez, R. Arazamendi, H. Fusaro, M. J. Hyman, C. D. Paddock, S. R. Zaki, J. G. Olson, and C. A. Santos-Buch. 1999. Evidence of rickettsial spotted fever and ehrlichial infection in a subtropical territory of Jujuy, Argentina. Am. J. Trop. Med. Hyg. 61: 350–354.

Sanchez, J. L., W. H. Candler, D. B. Fishbein, C. R. Greene, T. R. Cote, D. J. Kelly, D. P. Driggers, and B. J. Johnson. 1992. A cluster of tick-borne infections: association with military training and asymptomatic infections due to *Rickettsia rickettsii.* Trans. R. Soc. Trop. Med. Hyg. 86: 321–325.

Stromdahl, E. Y., M. A. Vince, P. M. Billingsley, N. A. Dobbs, and P. C. Williamson. 2008. *Rickettsia amblyommii* infecting *Amblyomma americanum* larvae. Vector-Borne Zoon. Dis. 8: 15–24.

Tomassone, L., P. Nuñez, R. E. Gürtler, L. A. Ceballos, M. M. Orozco, U. D. Kitron, and M. Farber. 2008. Molecular detection of *Ehrlichia chaffeensis* in *Amblyomma parvum* ticks, Argentina. Emerg. Infect. Dis. 14: 1953–1955.

Yunker, C. E., J. M. Brennan, L. E. Hughes, C. B. Philip, C. M. Clifford, P. H. Peralta, and J. Vogel. 1975. Isolation of viral and rickettsial agents from Panamanian Acarina. J. Med. Entomol. 12: 250–255.

Zavala-Castro, J. E., J. E. Zavala-Velásquez, D. H. Walker, E. E. Ruiz Arcila, H. Laviada-Molina, J. P. Olano, J. A. Ruiz-Sosa, M. A. Small, and K. R. Dzul-Rosado. 2006. Fatal human infection with *Rickettsia rickettsii,* Yucatan, Mexico. Emerg. Infect. Dis. 12: 672–674.

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