Full Article

Rev. Bras. Parasitol. Vet., Jaboticabal, v. 22, n. 3, p. 367-372, jul.-set. 2013
ISSN 0103-846X (impresso) / ISSN 1984-2961 (eletrônico)

Environmental infestation and rickettsial infection in ticks in an area endemic for Brazilian spotted fever

Infestação ambiental e infecção por rickéttsias em carrapatos de área endêmica para Febre Maculosa Brasileira

José Brites-Neto[1]*; Fernanda Aparecida Nieri-Bastos[2]; Jardel Brasil[1]; Keila Maria Roncato Duarte[3]; Thiago Fernandes Martins[2]; Cecília José Veríssimo[3]; Amália Regina Mar Barbieri[2]; Marcelo Bahia Labruna[2]

[1]Tick Surveillance and Control Program, Municipal Health Department, Americana, SP, Brazil

[2]Department of Preventive Veterinary Medicine and Animal Health, School of Veterinary Medicine, University of São Paulo – USP, São Paulo, SP, Brazil

[3]Institute of Animal Science, Nova Odessa, SP, Brazil

Received March 6, 2013
Accepted April 8, 2013

Abstract

Brazilian spotted fever (BSF), caused by *Rickettsia rickettsii,* is endemic in the municipality of Americana, southeastern Brazil, where the disease is transmitted by the tick *Amblyomma cajennense.* This study evaluated the tick fauna and rickettsial infection in free-living ticks that were captured monthly using dry ice traps in areas endemic for BSF in Americana, from July 2009 to June 2010. Two tick species were captured: *A. cajennense* (6,122 larvae; 4,265 nymphs; 2,355 adults) and *Amblyomma dubitatum* (7,814 larvae; 3,364 nymphs; 1,193 adults). The immature stages of *A. cajennense* and *A. dubitatum* had similar distribution through the 12-month period, with larvae of both species collected in highest numbers between April and July, and nymphs between June and October. The highest numbers of *A. cajennense* adults were collected between October and December, whereas *A. dubitatum* adults were collected in relatively similar numbers throughout the 12-month period. Rickettsial infection was evaluated by means of PCR in 1,157 *A. cajennense* and 1,040 *A. dubitatum* ticks; only 41 (3.9%) *A. dubitatum* were found to be infected by *Rickettsia bellii*. The present study showed that the areas of Americana that are endemic for BSF are characterized by high environmental burdens of *A. cajennense* and *A. dubitatum*.

Keywords: *Amblyomma cajennense*, *Amblyomma dubitatum*, *Rickettsia bellii*, Brazil.

Resumo

A Febre Maculosa Brasileira (FMB) é uma antropozoonose endêmica no município de Americana/SP, causada pela bactéria *Rickettsia rickettsii* e transmitida pelo carrapato *Amblyomma cajennense*. Este estudo avaliou a fauna de carrapatos e a infecção por riquétsias em carrapatos de vida livre capturados mensalmente com armadilhas de CO_2, em áreas de risco para FMB de Americana, de julho de 2009 a junho de 2010. Duas espécies foram capturadas, *A. cajennense* (6.122 larvas; 4.265 ninfas; 2.355 adultos) e *Amblyomma dubitatum* (7.814 larvas; 3.364 ninfas; 1.193 adultos). Os estágios imaturos de *A. cajennense* e *A. dubitatum* apresentaram uma distribuição anual semelhante, com larvas de ambas as espécies sendo coletadas em maior número no período de abril a julho e ninfas de junho a outubro. Maior número de adultos de *A. cajennense* foi coletado de outubro a dezembro, enquanto que os adultos de *A. dubitatum* foram coletados em número relativamente semelhante durante todo o ano. A infecção por *Rickettsia* foi avaliada pela PCR em 1157 carrapatos *A. cajennense* e 1040 *A. dubitatum*, com apenas 41 (3,9%) *A. dubitatum* infectados com *Rickettsia bellii*. Este estudo demonstrou que as áreas de risco para FMB de Americana são caracterizadas por elevadas infestações ambientais de *A. cajennense* e *A. dubitatum*.

Palavras-chave: *Amblyomma cajennense*, *Amblyomma dubitatum*, *Rickettsia bellii*, Brasil.

Introduction

The bacterium *Rickettsia rickettsii* is the etiological agent for the deadliest form of rickettsiosis in the world, namely Brazilian spotted fever (BSF) (LABRUNA, 2009). This disease is endemic in southeastern Brazil, especially in the state of São Paulo, where 555 laboratory-confirmed cases occurred from 1985 to 2012, with a 40% case-fatality rate (official data from the São Paulo State Health Office available at http://www.cve.saude.sp.gov.br/). BSF is transmitted by ticks. In Brazil, *Amblyomma cajennense* is the most important vector, since it is incriminated in transmitting *R. rickettsii* to humans in most of the endemic areas, including

*Corresponding author: José Brites-Neto
Programa de Vigilância e Controle de Carrapatos – PVCC, Rua Fernando de Camargo, 876, Centro, CEP 13465-020, Americana, SP, Brasil
e-mail: samevet@yahoo.com.br

rural areas in the interior of the state of São Paulo (LABRUNA, 2009; PINTER et al., 2011). In these endemic areas, capybaras (*Hydrochoerus hydrochaeris*) and domestic horses are the main hosts for all parasitic stages of *A. cajennense.* In addition, capybaras are also considered to be amplifier hosts for *R. rickettsii,* which means that they are responsible for generating new lineages of infected ticks in endemic areas (SOUZA et al., 2009; LABRUNA, 2009; SOARES et al., 2012). Besides *R. rickettsii,* a number of other *Rickettsia* species have been reported in Brazil, mostly infecting only ticks, as is the case of *Rickettsia bellii,* the most common species of the genus in Brazilian ticks. Until now, *R. bellii* has been reported infecting 11 different tick species in Brazil; however, this *Rickettsia* species is considered to be non-pathogenic (LABRUNA et al., 2011).

Americana is a municipality located in the eastern part of the state of São Paulo. It has a population of 212,791 inhabitants, mostly living in a 92 km^2 urban area surrounded and crossed by water courses, namely the Salto Grande reservoir (9.3 km^2) and four rivers: Piracicaba, Jaguari, Atibaia and Ribeirão Quilombo (FELICIANO, 2012). From 2004 to 2012, there were ten confirmed cases of BSF with a 60% fatality rate in Americana, thus indicating endemicity for BSF. All these cases were related to tick bites acquired along the water courses of the municipality, where there are established populations of capybaras (BRITES-NETO, 2011). Therefore, the present study aimed to evaluate the tick fauna of these risk areas, and to investigate rickettsial infection in these ticks.

Materials and Methods

From July 2009 to June 2010, free-living ticks were collected by means of dry ice traps, as previously described (WILSON et al., 1972; OLIVEIRA et al., 2000), in the following areas within the municipality of Americana, state of São Paulo, Brazil: 1- Sobrado Velho (22°41'16"S and 47° 15' 09" W, 516 m); 2- Carioba (22° 41' 75" S and 47° 19' 30" W, 496 m); 3- Bosque das Nascentes (22° 41' 94" S and 47° 17' 93" W, 534 m); 4- Fazenda Palmeiras (22° 45' 01" S and 47° 16' 82" W, 558 m); 5- Museu Histórico (22° 41' 60" S and 47° 17' 42" W, 508 m); and 6- Ribeirão Quilombo (22° 43' 39" S and 47° 19' 66" W, 528 m). All of these six areas (1 to 6) were inhabited by capybaras, whereas horses were present only in areas 1, 2, and 4. Human parasitism by ticks had frequently been reported in these six areas. Confirmed cases of BSF have been reported in area 1 (5 cases; 4 deaths) and area 2 (1 case) during the last nine years.

In each area, 10 to 13 traps were mounted every month for 12 consecutive months, totaling 830 traps for the whole study. The ticks collected were counted and taken to the laboratory, where they were kept frozen at –20 °C until further testing. The adult ticks were taxonomically identified in accordance with Onofrio et al. (2006), whereas nymphs were identified as described by Martins et al. (2010). Larvae were identified to species level by means of morphological comparisons with laboratory-reared larvae.

The frozen adult ticks were thawed and their salivary glands were separated through dissection under a stereomicroscope, as previously described (EDWARDS et al., 2009). The salivary glands of each tick were subjected to DNA extraction using the guanidine isothiocyanate-phenol technique, as previously described (SANGIONI et al., 2005). For every 10 individual ticks, a blank tube was included in the DNA extraction. Samples were tested individually by means of *Rickettsia* genus-specific PCR, targeting a 401-bp fragment of the rickettsial gene *gltA*, as previously described (LABRUNA et al., 2004).

Samples that yielded visible amplicons of the expected size from this gltA-PCR were further tested by two other PCR assays: (i) one assay targeting spotted fever group *Rickettsia*, using the primers Rr190.70p and Rr190.602n, which amplified a 532-bp fragment of the rickettsial gene *ompA* (REGNERY et al., 1991); and (ii) another assay specific to *Rickettsia bellii,* using the primers 5′-ATCCTGATTTGCTGAATTTTTT-3′ (forward) and 5′-TGCAATACCAGTACTGACG-3′) (reverse), which amplified a 338-bp fragment of the *R. bellii gltA* gene (SZABÓ et al., 2013). A random sample of amplicons generated by the *Rickettsia* genus-specific PCR protocol was subjected to direct DNA sequencing using an automated ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The BLAST software (National Center for Biotechnology Information, Bethesda, MD, USA) was used to determine the similarities of the partial rickettsial sequences generated in the current study. In each PCR run, two negative control tubes containing water were included, and also a positive control tube containing *R. parkeri* DNA.

The proportions of the numbers of adult ticks of the species *A. cajennense* and *A. dubitatum* collected among the six areas were compared, taking the null hypothesis to be that these ticks were represented by equal proportions (50% each) in each sampled area. Analyses were performed using the Minitab software, release 16.

Results

Over the 12-month period, a total of 3,548 adult ticks (1,649 males and 1,899 females) and 21,565 immature ticks (7,629 nymphs and 13,936 larvae) were collected in the 830 dry ice traps mounted in the six sampled areas. Two tick species, *A. cajennense* and *A. dubitatum,* were collected in all sampled areas (Table 1). Adults of *A. cajennense* and *A. dubitatum* were collected in similar numbers, except in areas 2 and 4, where significantly more *A. cajennense* were collected. Taxonomic differentiation of unfed larvae of *A. cajennense* and *A. dubitatum* under a stereoscope microscope relied basically on the idiosome size, which was visually larger in *A. dubitatum* (Figure 1). The numbers of ticks collected in the six areas were pooled and are presented in Figure 2. Overall, the immature stages of *A. cajennense* and *A. dubitatum* had similar distribution throughout the 12-month period, with larvae of both species collected in highest numbers during the autumn and early winter (April to July), and nymphs during late autumn, winter and early spring (June to October). On the other hand, the highest numbers of *A. cajennense* adult ticks were collected during the spring and early summer months (October to December), whereas *A. dubitatum* adult ticks were collected in relatively similar numbers throughout the 12-month period (Figure 2).

The salivary glands of 2,197 adult ticks were subjected to DNA extraction and PCR. Among the 1,157 *A. cajennense* ticks, none was positive according to the initial *gltA*-PCR protocol. Among

Table 1. Numbers of free-living ticks collected by means of dry ice traps in six areas of the municipality of Americana from July 2009 to June 2010.

Area	*Amblyomma cajennense*			*Amblyomma dubitatum*		
	Larvae	Nymphs	Adults*	Larvae	Nymphs	Adults*
1	31	262	96 [a]	76	253	107 [a]
2	388	1,508	1,324 [a]	611	1,356	496 [b]
3	31	369	46 [a]	87	264	56 [a]
4	2,513	628	346 [a]	1,708	351	39 [b]
5	152	347	100 [a]	173	253	77 [a]
6	3,007	1,151	443 [a]	5,159	887	418 [a]
Total	6,122	4,265	2,355	7,814	3,364	1,193
%+	43.9	55.9	66.4	56.1	44.1	33.6

*different superscript italic letters in the same line mean significantly different proportions of *A. cajennense* and *A. dubitatum* adult ticks in the area. +refers to the proportion (%) of each tick species according to the total number of larvae, nymphs or adults.

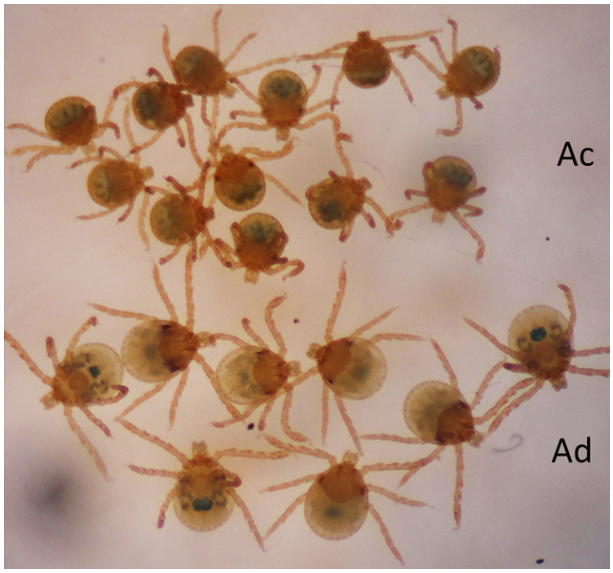

Figure 1. Unfed larvae of *Amblyomma cajennense* (Ac) and *Amblyomma dubitatum* (Ad) under a stereoscope microscope. Note larger size of *A. dubitatum* larvae.

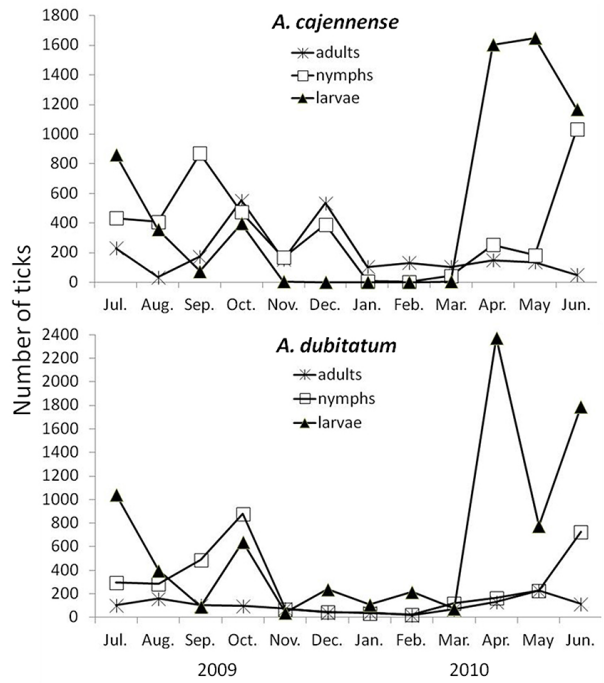

Figure 2. Numbers of *Amblyomma cajennense* and *Amblyomma dubitatum* free-living ticks collected monthly by means of dry ice traps in the municipality of Americana, state of São Paulo, from July 2009 to June 2010.

Table 2. Ticks tested by means of PCR for rickettsial infection in the present study.

Area	Number of PCR-positive ticks/Number of tested ticks (% positive*)	
	Amblyomma cajennense	*Amblyomma dubitatum*
1	0/87 (0)	9/106 (8.5)
2	0/508 (0)	19/462 (4.1)
3	0/46 (0)	1/56 (1.8)
4	0/157 (0)	0/37 (0)
5	0/94 (0)	1/76 (1.3)
6	0/265 (0)	11/303 (3.6)
Total	0/1,157 (0)	41/1,040 (3.9)

*All PCR-positive ticks were infected by *Rickettsia bellii.*

the 1,040 *A. dubitatum* ticks, 41 (3.9%) were positive according to the initial *gltA*-PCR, negative according to the *ompA*-PCR and positive according to the *R. bellii*-specific PCR protocol. These PCR positive samples were found in 5 out of the 6 sampled areas, with infection rates varying from 1.3 to 8.5% (Table 2). PCR products were randomly selected from 10 of these ticks, and were subjected to DNA sequencing. The 10 ticks generated sequences that were 100% identical to the corresponding sequence of *R. bellii* in GenBank (accession number CP000087).

Discussion

This study was conducted in six areas of the municipality of Americana, which has been considered to be an area endemic for BSF since 2004 (PINTER et al., 2011). It was found that all the active stages of *A. cajennense* and *A. dubitatum* were abundant in the six sampled areas, which were also all inhabited by free-ranging capybaras. Indeed, the environmental tick burdens found in the present study are directly related to capybaras, which are primary hosts for all the parasitic stages of both *A. cajennense* and *A. dubitatum* (PEREZ et al., 2008; NAVA et al., 2010; LABRUNA, 2013). Horses, another primary host species for all parasitic stages of *A. cajennense* (LABRUNA et al., 2002), were also present in areas 1, 2 and 4. Interestingly, areas 2 and 4 were the only ones where significantly greater numbers of *A. cajennense* than of *A. dubitatum* were collected. It is possible that this higher *A. cajennense* burden was related to higher host availability, namely horses and capybaras. Unfortunately, we could not quantify the populations of capybaras or horses in the present study, thus

precluding any comparative analysis of host density and tick burdens. Interestingly, the six sampled areas were interconnected by water courses, in which capybaras were present and to which they had free access (data not shown). Therefore, it is possible that constant gene (and pathogen) exchange exists between ticks in the six sampled areas. Nonetheless, the present study corroborates a number of previous studies that have reported the presence of capybaras associated with the ticks *A. cajennense* and *A. dubitatum* in southeastern Brazil, in both BSF-endemic and non-endemic areas (GUEDES et al., 2005; PEREZ et al., 2008; TOLEDO et al., 2008; PACHECO et al., 2009; QUEIROGAS et al., 2012).

The seasonal dynamics of *A. cajennense* has been well studied in southeastern Brazil, where this tick completes one generation per year, with larvae predominating in autumn, nymphs in winter and adults during spring and summer (OLIVEIRA et al., 2000, 2003; LABRUNA et al., 2002). This seasonal pattern is determined by the behavioral diapause of unfed larvae, as regulated by photoperiod and ground temperature (LABRUNA et al., 2003; CABRERA; LABRUNA, 2009). In the present study, albeit encompassing only a 12 month period, the highest peaks of larvae, nymphs and adults of *A. cajennense* followed the well known seasonal dynamics of this tick in southeastern Brazil.

Three previous studies evaluated the seasonal dynamics of free-living *A. cajennense* and *A. dubitatum* for two consecutive years in areas ecologically similar to the present study. i.e. with capybaras sustaining simultaneous populations of *A. cajennense* and *A. dubitatum.* In Jaguariúna, state of São Paulo, Souza et al. (2006) observed *A. dubitatum* and *A. cajennense* adults peaking during spring and summer; immature stages were not identified to species level. On the border between the states of São Paulo and Mato Grosso do Sul, Szabó et al. (2007) observed *A. dubitatum* and *A. cajennense* nymphs during winter and spring, and adults peaking during spring to autumn. Only three larval clusters were identified, thus precluding any seasonal inferences for this stage. In the state of Minas Gerais, Guedes and Leite (2008) observed *A. dubitatum* and *A. cajennense* adults peaking during spring and summer; again, immature stages were not identified to species level. On the other hand, although the present study only encompassed a 12-month period, it quantified not only adults but also larvae and nymphs of both *A. dubitatum* and *A. cajennense* ticks. In this, we observed that the larval and nymphal peaks of *A. dubitatum* were congruent with those of immature states of *A. cajennense*, i.e. larvae peaking in autumn and nymphs in winter. Therefore, our results, together with those previous studies, suggest that the seasonal dynamics of *A. dubitatum* are similar to those of *A. cajennense* in southeastern Brazil.

While no *Rickettsia* species was found infecting *A. cajennense* ticks in the present study, *R. bellii* was found infecting nearly 4% of *A. dubitatum* adult ticks. At first sight, this result is congruent with an extensive study (PACHECO et al., 2009) that evaluated 3,545 *A. cajennense* and 2,666 *A. dubitatum* ticks from 16 municipalities (some endemic for BSF) in the state of São Paulo, in which none of the *A. cajennense* specimens were found to be infected by *Rickettsia,* and 634 (23.8%) *A. dubitatum* ticks were infected by *R. bellii,* with infection rates per municipality ranging from 6.1 to 44.9%. However, looking at the data more closely, the present study reports a relatively low infection rate for *R. bellii*, compared with the results of Pacheco et al. (2009). In contrast with Pacheco et al. (2009), who extracted DNA from the whole tick body, here we performed DNA extraction directly from the tick salivary glands. This procedure has the advantage of eliminating PCR inhibitors (e.g. hemoglobin derivatives) that are potentially present in the tick gut contents (KREADER, 1996), and at the same time, it provides a more reliable result relating to rickettsial infection, since we excluded the possibility of rickettsial DNA remnants in the tick gut, derived from a previous blood meal. On the other hand, we would have missed any *Rickettsia* species that did not infect the salivary glands and hemolymph, as is the case of infection by *Rickettsia peacockii* in *Dermacentor andersoni* ticks in the United States (NIEBYLSKI et al., 1997). *R. bellii* is known to infect *A. dubitatum* hemolymph (LABRUNA et al., 2004), but it is not known whether it infects the salivary glands. If it does not infect the salivary glands, then our findings of PCR-positive ticks would have resulted from hemolymph residues that were inevitably collected with the salivary glands. Thus, our low *R. bellii*-infection rate may have been related to lower tropism of *R. bellii* to tick salivary glands, which has yet to be demonstrated.

In two recent studies in an area endemic for BSF in the state of Minas Gerais, Guedes et al. (2005, 2011) found that the proportions of *A. cajennense* ticks infected with *R. rickettsii* were 1.28% (1/78) and 0.5% (2/400), respectively. Even though Americana is an area endemic for BSF where *R. rickettsii* is presumably transmitted by *A. cajennense* (PINTER et al., 2011), we failed to encounter any *R. rickettsii*-infected ticks. However, this finding is not totally unexpected, since the prevalence of this pathogen among *A. cajennense* ticks can be very low due to the low efficiency of transovarial and transstadial transmission of *R. rickettsii* in *A. cajennense* ticks (SOARES et al., 2012). Similarly to the present study, Sangioni et al. (2005) was unsuccessful in finding any infected ticks among 810 *A. cajennense* adult ticks collected from areas endemic for BSF in the state of São Paulo.

In conclusion, the present study showed that the areas at risk of BSF in Americana are characterized by high environmental burdens of *A. cajennense* and *A. dubitatum,* which are primarily sustained by capybaras. It was shown for the first time that larvae and nymphs of *A. dubitatum* are active during the same periods as the corresponding stage of *A. cajennense.* While *R. bellii* was found infecting *A. dubitatum* ticks, infection by *R. rickettsii* among *A. cajennense* ticks was not found, thus indicating that it probably has a very low infection rate, as also seen in other areas endemic for BSF in the interior of the state of São Paulo.

Acknowledgements

We wish to thank the officials of the Americana Vector Control Office, Amarildo Azarias, Jesus Tendor and Ubirajara Pedroso Jesuíno, who actively participated in the field work conducted in this research.

References

Brites-Neto J. *Diagnóstico epidemiológico de infectividade para Rickettsia rickettsii em Amblyomma spp. no Município de Americana,SP* [Dissertação]. Nova Odessa: Instituto de Zootecnia; 2011.

Cabrera RR, Labruna MB. Influence of photoperiod and temperature on the larval behavioral diapause of *Amblyomma cajennense* (Acari: Ixodidae). *J Med Entomol* 2009; 46(6): 1303-1309. http://dx.doi.org/10.1603/033.046.0608

Edwards KT, Goddard J, Varela-Stokes AS. Examination of the Internal Morphology of the Ixodid Tick, *Amblyomma maculatum* Koch, (Acari: Ixodidae); a "How-to" Pictorial Dissection Guide. *Midsouth Entomol* 2009; 2(1): 28-39.

Feliciano MAM. (Coord.) *Informativo Sócio-Econômico do Município de Americana-SP n° 28 - ano base 2011*. Americana: SEPLAN – Unidade de Estatística e Análise Sócio-Econômica; 2012. Available from: http://www.americana.sp.gov.br/americanaV5/download/planejamento/pl_02_Informativo_SocioEconomico_2012.pdf.

Guedes E, Leite RC. Dinâmica sazonal de estádios de vida livre de *Amblyomma cajennense* e *Amblyomma dubitatum* (Acari: Ixodidae) numa área endêmica para febre maculosa, na região de Coronel Pacheco, Minas Gerais. *Rev Bras Parasitol Vet* 2008; 17(S1): 78-82.

Guedes E, Leite RC, Pacheco RC, Silveira I, Labruna MB. *Rickettsia* species infecting *Amblyomma* ticks from an area endemic for Brazilian spotted fever in Brazil. *Rev Bras Parasitol Vet* 2011; 20(4): 308-311. http://dx.doi.org/10.1590/S1984-29612011000400009

Guedes E, Leite RC, Prata MCA, Pacheco RC, Walker DH, Labruna MB. 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* 2005; 100(8): 841-845. http://dx.doi.org/10.1590/S0074-02762005000800004

Kreader CA. Relief of amplification inhibition in PCR with bovine serum albumin or T4 gene 32 protein. *Appl Environ Microbiol* 1996; 62(3): 1102-1106.

Labruna MB. Ecology of *Rickettsia* in South America. *Ann N Y Acad Sci* 2009; 1166: 156-166. http://dx.doi.org/10.1111/j.1749-6632.2009.04516.x

Labruna MB. Brazilian Spotted Fever: The Role of Capybaras. In: Moreira JR, Ferraz KMPMB, Herrera EA, MacDonald DW. *Capybara: Biology, Use and Conservation of an Exceptional Neotropical Species*. New York: Springer Science Business Media; 2013. p. 371-383.

Labruna MB, Amaku M, Metzner JA, Pinter A, Ferreira F. Larval behavioral diapause regulates life cycle of *Amblyomma cajennense* (Acari: Ixodidae) in Southeast Brazil. *J Med Entomol* 2003; 40(2): 170-178. http://dx.doi.org/10.1603/0022-2585-40.2.170

Labruna MB, Kasai N, Ferreira F, Faccini JLH, Gennari SM. Seasonal dynamics of ticks (Acari: Ixodidae) on horses in the state of São Paulo, Brazil. *Vet Parasitol* 2002; 105(1): 65-77. http://dx.doi.org/10.1016/S0304-4017(01)00649-5

Labruna MB, Mattar S, Nava S, Bermudez S, Venzal JM, Dolz G, et al. Rickettsioses in Latin America, Caribbean, Spain and Portugal. *Rev MVZ Cordoba* 2011; 16(2): 2435-2457.

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

Martins TF, Onofrio VC, Barros-Battesti DM, Labruna MB. Nymphs of the genus *Amblyomma* (Acari: Ixodidae) of Brazil: descriptions, redescriptions, and identification key. *Ticks Tick Borne Dis* 2010; 1(2): 75-99. http://dx.doi.org/10.1016/j.ttbdis.2010.03.002

Nava S, Venzal JM, Labruna MB, Mastropaolo M, González EM, Mangold AJ, et al. Hosts, distribution and genetic divergence (16S rDNA) of *Amblyomma dubitatum* (Acari: Ixodidae). *Exp Appl Acarol* 2010; 51(4): 335-351. http://dx.doi.org/10.1007/s10493-009-9331-6

Niebylski ML, Schrumpf ME, Burgdorfer W, Fischer ER, Gage KL, Schwan TG. *Rickettsia peacockii* sp. nov., a new species infecting wood ticks, *Dermacentor andersoni*, in western Montana. *Int J Syst Bacteriol* 1997; 47(2): 446-452. http://dx.doi.org/10.1099/00207713-47-2-446

Oliveira PR, Borges LMF, Lopes CML, Leite RC. Population dynamics of the free-living stages of *Amblyomma cajennense* (Fabricius, 1787) (Acari: Ixodidae) on pastures of Pedro Leopoldo, Minas Gerais State, Brazil. *Vet Parasitol* 2000; 92(4): 295-301. http://dx.doi.org/10.1016/S0304-4017(00)00322-8

Oliveira PR, Borges LM, Leite RC, Freitas CM. Seasonal dynamics of the Cayenne tick, *Amblyomma cajennense* on horses in Brazil. *Med Vet Entomol* 2003; 17(4): 412-416. http://dx.doi.org/10.1111/j.1365-2915.2003.00459.x

Onofrio VC, Labruna MB, Pinter A, Giacomin FG, Barros-Battesti DM. Comentários e chaves para as espécies do gênero *Amblyomma.* In: Barros-Battesti DM, Arzua M, Bechara GH. *Carrapatos de Importância Médico-Veterinária da Região Neotropical: Um guia ilustrado para identificação de espécies*. São Paulo: Vox/ICTTD-3/Butantan; 2006. p. 53-71.

Pacheco RC, Horta MC, Pinter A, Moraes-Filho J, Martins TF, Nardi MS, et al. Pesquisa de *Rickettsia* spp em carrapatos *Amblyomma cajennense* e *Amblyomma dubitatum* no Estado de São Paulo. *Rev Soc Bras Med Trop* 2009; 42(3): 351-353. http://dx.doi.org/10.1590/S0037-86822009000300023

Perez CA, Almeida AF, Almeida A, Carvalho VHB, Balestrin D C, Guimarães MS, et al. Carrapatos do gênero *Amblyomma* (Acari: Ixodidae) e suas relações com os hospedeiros em área endêmica para febre maculosa no estado de São Paulo. *Rev Bras Parasitol Vet* 2008; 17(4): 210-217.

Pinter A, França AC, Souza CE, Sabbo C, Nascimento EMM, Santos FCP, et al. Febre Maculosa Brasileira. *BEPA Supl* 2011; 8(1): 1-31.

Queirogas VL, Del Claro K, Nascimento AR, Szabó MP. Capybaras and ticks in the urban areas of Uberlândia, Minas Gerais, Brazil: ecological aspects for the epidemiology of tick-borne diseases. *Exp Appl Acarol* 2012; 57(1): 75-82. http://dx.doi.org/10.1007/s10493-012-9533-1

Regnery RL, Spruill CL, Plikaytis BD. Genotypic identification of rickettsiae and estimation of intraspecies sequence divergence for portions of two rickettsial genes. *J Bacteriol* 1991; 173(5): 1576-1589.

Sangioni LA, Horta MC, Vianna MCB, Gennari SM, Soares RM, Galvão MAM, et al. Rickettsial infection in animals and Brazilian Spotted Fever endemicity. *Emerg Infect Dis* 2005; 11(2): 265-270. http://dx.doi.org/10.3201/eid1102.040656

Soares JF, Soares HS, Barbieri AM, Labruna MB. Experimental infection of the tick *Amblyomma cajennense*, Cayenne tick, with *Rickettsia rickettsii*, the agent of Rocky Mountain spotted fever. *Med Vet Entomol* 2012; 26(2): 139-151. http://dx.doi.org/10.1111/j.1365-2915.2011.00982.x

Souza SSAL, Souza CE, Rodrigues-Neto EJ, Prado AP. Dinâmica sazonal de carrapatos (Acari: Ixodidae) na mata ciliar de uma área endêmica para febre maculosa na região de Campinas, São Paulo, Brasil. *Cienc Rural* 2006; 36(3): 887-891. http://dx.doi.org/10.1590/S0103-84782006000300024

Souza CE, Moraes-Filho J, Ogrzewalska M, Uchoa FC, Horta MC, Souza SSL, et al. Experimental infection of capybaras *Hydrochoerus hydrochaeris* by *Rickettsia rickettsii* and evaluation of the transmission of the infection to ticks *Amblyomma cajennense*. *Vet Parasitol* 2009; 161(1-2): 116-121. http://dx.doi.org/10.1016/j.vetpar.2008.12.010

Szabó MPJ, Castro MB, Ramos HGC, Garcia MV, Castagnolli KC, Pinter A, et al. Species diversity and seasonality of free-living ticks (Acari: Ixodidae) in the natural habitat of wild Marsh deer (*Blastocerus dichotomus*) in Southeastern Brazil. *Vet Parasitol* 2007; 143(2): 147-154. http://dx.doi.org/10.1016/j.vetpar.2006.08.009

Szabó MP, Nieri-Bastos FA, Spolidorio MG, Martins TF, Barbieri AM, Labruna MB. *In vitro* isolation from *Amblyomma ovale* (Acari: Ixodidae) and ecological aspects of the Atlantic rainforest *Rickettsia*, the causative agent of a novel spotted fever rickettsiosis in Brazil. *Parasitology* 2013; 140(6): 719-728. http://dx.doi.org/10.1017/S0031182012002065

Toledo RS, Tamekuni K, Haydu VB, Vidotto O. Dinâmica sazonal de carrapatos do gênero *Amblyomma* (Acari: Ixodidae) em um parque urbano da cidade de Londrina, PR. *Rev Bras Parasitol Vet* 2008; 17(S1): 50-54.

Wilson JG, Kinzer DR, Sauer JR, Hair JA. Chemo-attraction in the lone star tick (Acarina: Ixodidae). I. Response of different developmental stages to carbon dioxide administered via traps. *J Med Entomol* 1972; 9(3): 245-252.