International Journal of Advanced Engineering Research and Science (IJAERS)
Peer-Reviewed Journal
ISSN: 2349-6495(P) | 2456-1908(O)
Vol-8, Issue-6; Jun, 2021
Journal Home Page Available: https://ijaers.com/
Article DOI:https://dx.doi.org/10.22161/ijaers.86.22

Seroprevalence for *Rickettsia* spp. and *Borrelia* spp. in horses from non-endemic areas at the Southeastern Brazil

Elaine Santana Gonçalves[1], Matheus Dias Cordeiro[2], Adivaldo Henrique da Fonseca[2], Thays Figueiroa[2], Izabela Mesquita Araújo[2], Marcelo Bahia Labruna[3], Elizângela Guedes[1]

[1]Programa de Pós-graduação em Reprodução, Sanidade e Bem-Estar Animal, Universidade José do Rosário Vellano (UNIFENAS), Alfenas, MG, Brasil.
[2]Laboratório de Doenças Parasitárias, Universidade Federal Rural do Rio de Janeiro (UFRRJ), Seropédica, RJ, Brasil.
[3]Laboratório de Doenças Parasitárias, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo (USP), São Paulo, SP, Brasil.

Received: 03 Apr 2021;
Received in revised form:
11 May 2021;
Accepted: 03 Jun 2021;
Available online: 17 Jun 2021

***Keywords*—Baggio-Yoshinari Syndrome, Brazilian Spotted Fever, arthropod, zoonosis, serology.**

***Abstract*—** *Spotted Fever Group (SFGR) and Baggio-Yoshinari Syndrome (BYS) are described as important tick-borne zoonosis. Horses do not participate directly in the cycle of these diseases, but they work as sentinels of epidemiological studies. We analyzed the distribution of Rickettsia spp. and Borrelia spp. in horses and ticks of two non-endemic areas from Southeastern Brazil. Blood serum from 102 horses of different ages (> 12 months) and breeds were analyzed by the indirect immunofluorescence reaction (IFR) with the aid of specific antigens for R. rickettsii, R. parkeri, R. rhipicephali, R. amblyommatis and R. bellii, besides the indirect immunoadsorption assay (ELISA) aiming to detect homologous IgG antibodies against B. burgdorferi (American strain G39/40). Free-living and parasitic ticks were collected for PCR and Nested-PCR tests to detect both Rickettsia spp. (citrate synthase gene) and Borrelia spp. (flagellin gene). The data showed 51.96% (53/102) of seropositive horses at least in one of the five tested Rickettsia antigens, and 10.78% (11/102) were considered serum-specific for R. parkeri. Besides that, a total seroprevalence of 13.73% (14/102) for immunoreactive antibodies of the IgG class against B. burgdorferi were obtained from the indirect ELISA. Three hundred and fifty-three ticks were collected, all identified as Amblyomma sculptum and negative for PCR and Nested-PCR. The obtained results suggest the circulation of SFGR and Borrelia spp. in a non-endemic area of Brazil, added to a large occurrence of vector ticks. This scenario deserves attention for the possibility of a zoonotic cycle in the region.*

I. INTRODUCTION

Rickettsioses from the Spotted Fever Group (RSFG) and the Baggio-Yoshinari Syndrome (BYS) are emergent diseases transmitted to men through the bite of infected ticks. Thereby, domestic and wild animals are important for both epidemiology and spreading of these diseases [1,2].

Brazilian Spotted Fever (BSF) caused by the bacteria *Rickettsia rickettsii* is the most important among those

from the RSFG, with great lethality. However, the 'Mata Atlântica' strain from *Rickettsia parkeri*, and the *Rickettsia parkeri sensu stricto* (*s.s.*) have been described in some regions of Brazil, but without reports of seriousness. Generally, the RSFG have an endemic nature in many regions of the country, and they have been reported both in rural and urban areas, with many cases in the Southeastern Region [3–6].

The complex *Borrelia burgdorferi sensu lato (s.l.)* comprises a group with a large number of spirochetes that cause diseases as Lyme Disease (LD), mainly in the USA and Europe. In Brazil, there is a suspicious that the BYS is regarded to the *Borrelia* species, and its occurrence was described both in humans and animals through serological and molecular techniques [7–9].

The *Amblyomma* genus has been reported as the main vector for RSFG in Brazil [3,10]. Nevertheless, vectors of BYS are not well-described yet. Considering that, it is suggested that the wild cycle can occur among species from the *Ixodes* genus [11], while the domestic cycle occurs by ticks from *Amblyomma* and *Rhipicephalus* genus [1,9].

About this context, as horses are ticks' hosts, mainly of *Amblyomma sculptum*, besides they are often used for work or leisure in rural areas, these animals can be important dispersers of infected ticks [2, 12].

Many serological and molecular studies have been carried out at areas with notification of human cases, or at endemic areas for RSFG or BYS. Conversely, there are few studies about non-endemic regions. Thereby, the knowledge about the epidemiology in regions with the biotic potential to develop these vector diseases is essential to prevent new outbreaks. In this present study, we analyzed the distribution of *Rickettsia* spp. and *Borrelia* spp. in both horses and ticks of two non-endemic areas from Southeastern Brazil.

II. METHODOLOGY

The study was carried out at municipalities of Guaxupé, Minas Gerais state (21° 18' 18" S 46° 42' 46" W) and Tapiratiba, São Paulo state (21°27'20"S 46°43'31"W) Brazil, from May to November 2018. These municipalities did not have notification of BSF and BYS. The samplings occurred in six farms chosen according to the availability of animals, besides their similarities regarding the morphoclimatic characteristics, presenting favorable epidemiological conditions to maintain the cycle of diseases transmitted by ticks. For example, fragments of tropical forests, pastures, water collections, humans and animals living together, besides the presence of capybaras and domestic animals were conditions found out in both municipalities.

Blood samples were collected from adult horses (with different breeds, aged over 12 months) through jugular venocentesis. The blood serum was obtained by centrifugation at 3000 rpm and 10 minutes, following by freezing into polypropylene tubes at -20°C until the analysis. These samples were identified according to each animal and farm. Aliquots of 15 µL from each diluted serum (buffer phosphate – PBS pH 7.2) were submitted to the indirect immunofluorescence reaction (IFR) [13] Antigens from the five species of *Rickettsia* found out in Brazil were submitted to the IFR: *R. rickettsii* strain Taiaçu, *R. parkeri* strain At24, *R. amblyommatis* strain Ac37, *R. rhipicephali* strain HJ5 and *R. bellii* strain Mogi. The serum of a naturally infected animal, confirmed as positive, was used as the positive control, and a serum sample of a previously tested animal, stored at -20 °C, was used as the negative control. Samples with reaction at dilutions over 1:64 were considered positive ones to the final titration, and tested until present themselves as negatives in series. All samples with titres at least four times greater than the other ones were considered homologous for the greatest titre, for each species of *Rickettsia* [14].

The indirect Enzyme-Linked Immunosorbent Assay (ELISA) was used to analyze the antibodies of the IgG class against the crude antigen of *B. burgdorferi* strain G 39/40 [15]. The serum of a healthy young animal, which was vaccinated with the crude antigen of *B. burgdorferi*, was used as the positive control. Negative controls were made of ten serum samples obtained from healthy animals, without historical affection by ticks. The assay cut-off was defined by the arithmetic average of optical density values from the negative controls added to three times their standard deviation [16] . The optical density index was calculated based on the formula: DO × 100/cut-off, for each sample.

Living-free ticks were collected from pastures owned to the farms through the methodology of CO_2 chemical traps [10,17], and the flannel dragging [18], for the assessment of ectoparasites population. The complete scraping of the animal's body surface was made to collect the ticks at parasite stage. All ticks were preserved with the aid of isopropyl alcohol. Thereafter, they were identified [19,20] and individually submitted to DNA extraction, according to the boil protocol[21] for non-engorged larvae and nymphs, besides the phenol-chloroform protocol for engorged adults and nymphs [22]. The extracted DNA was tested by PCR using the primers CS-239 and CS-1069, which amplified a fragment with 834 pb from the *citrate synthase* (*gltA*) gene, found out in all species of *Rickettsia* genus

[23,24]. For the DNA detection of *Borrelia* spp., the Nested-PCR was used with primers that amplified parts of the *flagellin B* (*flaB*) gene found out in *Borrelia* spp. [24]. For the primary reaction, the primers *FlaLL* and *FlaRL* were used, while for the Nested reaction, the used primers were *FlaLS* and *FlaRS*.

The research project was approved by the Comitê de Ética em Pesquisa em Animais / UNIFENAS, under the 10A/2018 endorsement.

III. RESULTS

The total seroprevalence for immunoreactive antibodies (≥1:64) in the IFR, for at least one of the five *Rickettsia* antigens, was 51.96% (53/102) and titres varied from 1:64 to 1:1024 (Table 1). In Guaxupé-MG municipality there was found 36.4% (12/33) of seropositive horses for at least one of the five-tested *Rickettsia*, while in Tapiratiba-SP, 59.42% (41/102) were found out. Regarding the serum specificity of reactions, 18.63% (19/102) of all animals presented homologous serum for *R. bellii*, while 10.78% (11/102) showed it for *R. parkeri* with titres varying from 1:64 to 1:1024 for both species. It was cannot possible to identify the probably antigen from 22.55% of the reactions, and because of that, they were classified as unspecific. In Guaxupé-MG, 30.30% (10/33) of the horses were considered serum-specific for *R. bellii*, 3% (1/33) for *R. parkeri* and one reaction was classified as unspecific (3%; 1/33). In Tapiratiba-SP, 14.49% (10/69) of horses were serum-specific for *R. parkeri*, 13.04% (9/69) for *R. bellii*, and 31.88 (22/69) of the reactions were unspecific.

Serum analysis also revealed a total seroprevalence of 13.72% (14/102) for immunoreactive antibodies from IgG class against *B. burgdorferi*, by the indirect ELISA. All seropositive horses owned to only one farm located at the Tapiratiba-SP municipality. This overestimated the region prevalence to 20.28%. None tested horse from Guaxupé-MG was serum-reactive.

All the 353 collected ticks were identified as *Amblyomma sculptum*. Only one adult female was captured and the other ticks were nymphs. All analyzed ticks were negative regarding all tested bacteria.

Most of all assessed farms had presence of capybaras and wild animals during the assay. In two of the six farms, capybaras were observed at the time of data collection. There were also rural communities near to these capybaras' habitats and the *A. sculptum* presence.

IV. DISCUSSION

The municipalities of Guaxupé-MG and Tapiratiba-SP have areas where rural tourism is economically important, and there are many horse stables and training centers. Furthermore, there are rivers, abundant native vegetation, and wild animals as capybaras, which can maintain many species of ticks that often are vectors of diseases like the ones mentioned here. These specific regions are non-endemic, and no notification of suspicious or confirmed cases of RSFG was reported until this moment. Moreover, there were no studies about infections in humans, horses, dogs, or other vertebrates.

In this present study, the seroprevalence of immunoreactive antibodies to *Rickettsia* in horses was 51.96% (53/102), values greater than those ones reported (25% and 27.3%) in other studies also carried out at non-endemic areas of Brazil [13,25,26]. Contrariwise, studies carried out in endemic areas, or with confirmed human cases, showed serological results near to those found in our study [13,27]. Besides that, Souza et al. [27] verified that horses largely exposed to the infection by *Rickettsia* spp. (prevalence from 6.1% to 54.7%), but with a geometrical average of titres greater in endemic areas, can suggest a possible underestimation of cases reported by the health surveillance of BSF. This fact points out the importance of sentinel animals on the diagnosis and observation of areas without human cases report.

Our survey showed 19.6% of reactive samples for *R. rickettsii*, but none can be considered serum-specific because they were reactive to other tested species, which suggests a crossed reactivity among *Rickettsia* species or a previous exposition to infection by different species. Many studies showed the occurrence of a large crossed reactivity among the RSFG, mainly between *R. rickettsii* and *R. parkeri* [27–29]. Only one sample did not present crossed reactivity for *R. rickettsii* and *R. parkeri*. Nevertheless, not all reactive samples for *R. parkeri* reacted to *R. rickettsii*. It was not possible to determine the probable antigen involved in 43.4% (23/53) of all reactions, once there were positive reactions with similar titres at least two of the studied *Rickettsia* species.

Contrasted with that, 33.33% (34/102) of all horses were reactive to *R. parkeri*, and 10.78% (11/102) were considered serum-specific with titres varying from 64 to 1024. Horta et al. [13] investigated infections by *Rickettsia* spp. in animals, humans, ticks and fleas collected in areas from São Paulo state, and verified serological reactivity for *R. parkeri* in animals, even in a non-endemic area.

In Brazil, *R. parkeri* was found out in tick species from *Amblyomma* genus [30] such as *A. tigrinum*, *A. triste*, and *A. ovale* [31,32], and most recently in the *A. sculptum* [9,33].

Previous studies experimentally demonstrated the infection by *R. parkeri* in *A. cajennense sensu lato* [25], which suggests this tick species as potential vector of Spotted Fever caused by this bacteria. Our study showed that all serum-specific horses for *R. parkeri* resided into farms with *A. sculptum* occurrence, which can point out the necessity of complementary studies to elucidate it.

Regarding the indirect ELISA with antibodies from class IgG *B. burgdorferi* strain G 39/40, 13.73% of all horses were positive, which are results near to those found by Montandon et al.[8] and Salles et al.[15]. All horses positive to indirect ELISA were from the municipality of Tapiratiba-SP, owned to only one farm that had the presence of cattle, *A. sculptum* and capybaras. This fact is relevant because some studies already indicated that the coexistence between cattle and horses allows parasitism on horses by *Rhipicephalus microplus*, the main vector of *Borrelia theileri* [34]. It is possible to have crossed reactions between different *Borrelia* agents, due to the great phylogenetic association among *Borrelia* spirochetes [35,36]. Moreover, according to Rogers et al.[35], possible crossed reactions between *B. theileri* and *B. burgdorferi* should be considered on the analysis of serological tests for *B. burgdorferi* in ruminants, mainly regarding the crude antigen. Vector aptitude of ticks from both *Amblyomma* and *Rhipicephalus* genus on the transmission of the *B. burgdorferi* was not defined yet. However, Rezende et al.[37] reported embryonic cells from *Rhipicephalus microplus* and *A. cajennense s.l* as possible substrates for the growth of *B. burgdorferi sensu stricto* strain G39 / 40. Recently, Higa et al.[9] described the first molecular evidence of *Borrelia* spp. in *A. sculptum*, which were collected in the Midwest region of Brazil.

Both presence of *Borrelia* spp. and *Rickettsia* spp. were analyzed through the detection of specific DNA sequences, but all tests were negative. Negative results for PCR can be explained by the lower samples of examined ticks, besides the deleterious effect on these ticks caused by pathogenic *Rickettsia* [4,38] and spirochetes [39,40]. Even epidemiological surveys in endemic areas for RSFG in Brazil demonstrate a low frequency of DNA detection, varying from 0 to 1.28 [10,41].

V. CONCLUSION

Horses' seropositivity for RSFG, mainly for *R. parkeri* and *Borrelia* spp., added to a large occurrence of vector ticks deserve attention for the possibility of an enzootic cycle with zoonotic potential at the studied regions, once these vectors coexist with humans on the same niche. Nevertheless, the etiological agents that are responsible for the serological reactivity of horses must be well-defined yet.

Geographic amplitude added to the distribution of human communities near to the rural and native areas, and the large biodiversity from these areas make them a priority regarding the investigation of potential diseases transmitted by ticks.

ACKNOWLEDGEMENTS

"This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001".

REFERENCES

[1] Yoshinari, N. H., Mantovani, E., Bonoldi, V. L. N., Marangoni, R. G., & Gauditano, G. (2010). Doença de lyme-símile brasileira ou síndrome baggioyoshinari: zoonose exótica e emergente transmitida por carrapatos. *Revista Da Associação Médica Brasileira*, *56*(3), 363–369. https://doi.org/10.1590/s0104-42302010000300025

[2] Labruna, M. B. (2009). Ecology of rickettsia in South America. *Annals of the New York Academy of Sciences*, *1166*, 156–166. https://doi.org/10.1111/j.1749-6632.2009.04516.x

[3] Ribeiro, C. M., Costa, V. M., Carvalho, J. L. B., Mendes, R. G., Bastos, P. A. de S., Katagiri, S., & Amaku, M. (2020). Brazilian spotted fever: A spatial analysis of human cases and vectors in the state of São Paulo, Brazil. *Zoonoses and Public Health*, *67*(6), 629–636. https://doi.org/10.1111/zph.12742

[4] Weck, B., Dall'Agnol, B., Souza, U., Webster, A., Stenzel, B., Klafke, G., Martins, J. R., & Reck, J. (2016). Spotted Fever Group Rickettsia in the Pampa Biome, Brazil, 2015–2016. *Emerging Infectious Diseases*, *22*(11), 2014–2016. https://doi.org/10.3201/eid2211.160859

[5] Weck, B., Dall'Agnol, B., Souza, U., Webster, A., Stenzel, B., Klafke, G., Martins, J. R., & Reck, J. (2017). Rickettsia parkeri in Amblyomma dubitatum ticks in a spotted fever focus from the Brazilian Pampa. *Acta Tropica*, *171*, 182–185. https://doi.org/10.1016/j.actatropica.2017.03.028

[6] Weck, B., Krawczak, F. S., Costa, F. B., Dall'Agnol, B., Marcili, A., Reck, J., & Labruna, M. B. (2020). Rickettsia parkeri in the Pampa biome of southern Brazil: Isolation, molecular characterization, and serological evidence of canine infection. *Veterinary Parasitology: Regional Studies and Reports*, *22*, 100448. https://doi.org/10.1016/j.vprsr.2020.100448

[7] Cordeiro, M. D., De Meireles, G. S., Da Silva, J. B., De Souza, M. M. S., & Da Fonseca, A. H. (2012). Soroprevalência para borrelia spp. em cães no município de Seropédica, estado do Rio de Janeiro. *Revista Brasileira de Medicina Veterinaria*, *34*(3), 251–256.

[8] Montandon, C. E., Yoshinari, N. H., Milagres, B. S., Mazioli, R., Gomes, G. G., Moreira, H. N., Padilha, A. de

F., Wanderley, G. G., Mantovani, E., Galvão, M. A. M., Langoni, H., Mafra, C. (2014). Evidence of Borrelia in wild and domestic mammals from the state of Minas Gerais, Brazil. *Revista Brasileira de Parasitologia Veterinária*, *23*(2), 287–290. https://doi.org/10.1590/S1984-29612014040

[9] Higa, L. de O. S., Csordas, B. G., Garcia, M. V., Oshiro, L. M., Duarte, P. O., Barros, J. C., & Andreotti, R. (2020). Spotted fever group Rickettsia and Borrelia sp. cooccurrence in Amblyomma sculptum in the Midwest region of Brazil. *Experimental and Applied Acarology*, *81*(3), 441–455. https://doi.org/10.1007/s10493-020-00513-2

[10] Guedes, E., Leite, R. C., Prata, M. C. A., Pacheco, R. C., Walker, D. H., & Labruna, M. B. (2005). Detection of Rickettsia rickettsii in the tick Amblyomma cajennense in a new Brazilian spotted fever-endemic area in the state of Minas Gerais. *Memorias Do Instituto Oswaldo Cruz*, *100*(8), 841–845. https://doi.org/10.1590/S0074-02762005000800004

[11] Muñoz-Leal, S., Ramirez, D. G., Luz, H. R., Faccini, J. L. H., & Labruna, M. B. (2020). "Candidatus Borrelia ibitipoquensis," a Borrelia valaisiana–Related Genospecies Characterized from Ixodes paranaensis in Brazil. *Microbial Ecology*, *80*(3), 682–689. https://doi.org/10.1007/s00248-020-01512-x

[12] Pacheco, R. C., Moraes-Filho, J., Guedes, E., Silveira, I., Richtzenhain, L. J., Leite, R. C., & Labruna, M. B. (2010). Rickettsial infections of dogs , horses and ticks in Juiz de Fora , southeastern Brazil , and isolation of Rickettsia rickettsii from Rhipicephalus sanguineus ticks. *Medical and Veterinary Entomology*, *25*(2), 1–8. https://doi.org/10.1111/j.1365-2915.2010.00915.x

[13] Horta, M. C., Labruna, M. B., Sangioni, L. A., Vianna, M. C. B., Gennari, S. M., Galvão, M. A. M., Mafra, C. L., Vidotto, O., Schumaker, T. T. S., & Walker, D. H. (2004). Prevalence of antibodies to spotted fever group rickettsiae in humans and domestic animals in a Brazilian spotted fever-endemic area in the state of São Paulo, Brazil: Serologic evidence for infection by Rickettsia rickettsii and another spotted fever gro. *American Journal of Tropical Medicine and Hygiene*, *71*(1), 93–97. https://doi.org/10.4269/ajtmh.2004.71.93

[14] Pacheco, R. C., Horta, M. C., Moraes-Filho, J., Ataliba, A. C., Pinter, A., Labruna, M. B. (2007). Infección por rickettsia en capibaras (Hydrochoerus hydrochaeris) de São Paulo, Brasil: evidencia serológica de infección por Rickettsia bellii y Rickettsia parkeri. *Biomédica*, *27*(3), 364–371.

[15] Salles, R. S., Fonseca, A. H., Scofield, A., Madureira, R. C., & Yoshinari, N. H. (2002). Sorologia para Borrelia burgdorferi latu sensu em eqüinos no estado do Rio de Janeiro. *Hora Vet*, 46–49.

[16] Frey, A., Di Canzio, J., & Zurakowski, D. (1998). A statistically defined endpoint titer determination method for immunoassays. *Journal of Immunological Methods*, *221*(1–2), 35–41. https://doi.org/10.1016/S0022-1759(98)00170-7

[17] Cançado, P. H. D., Piranda, E. M., Mourão, G. M., & Faccini, J. L. H. (2008). Spatial distribution and impact of cattle-raising on ticks in the Pantanal region of Brazil by using the CO2 tick trap. *Parasitology Research*, *103*(2), 371–377. https://doi.org/10.1007/s00436-008-0982-8

[18] Oliveira, P. R., Borges, L. M. F., Lopes, C. M. L., & Leite, R. C. (2000). Population dynamics of the free-living stages of Amblyomma cajennense (Fabricius, 1787) (Acari: Ixodidae) on pastures of Pedro Leopoldo, Minas Gerais State, Brazil. *Veterinary Parasitology*, *92*(4), 295–301. https://doi.org/10.1016/S0304-4017(00)00322-8

[19] Battesti, Darci Moraes Barros; Arzua, Márcia; Bechara, G. H. (2006). *Carrapatos de importância médico-veterinária da região neotropical: um guia ilustrado para identificação de espécies*. Butantan.

[20] Martins, T. F., Onofrio, V. C., Barros-Battesti, D. M., & Labruna, M. B. (2010). Nymphs of the genus Amblyomma (Acari: Ixodidae) of Brazil: Descriptions, redescriptions, and identification key. *Ticks and Tick-Borne Diseases*, *1*(2), 75–99. https://doi.org/10.1016/j.ttbdis.2010.03.002

[21] Pacheco, R. C., Horta, M. C., Moraes-Filho, J., Ataliba, A. C., Pinter, A., & Labruna, M. B. (2007). Rickettsial infection in capybaras (Hydrochoerus hydrochaeris) from São Paulo, Brazil: serological evidence for infection by Rickettsia bellii and Rickettsia parkeri. *Biomedica : Revista Del Instituto Nacional de Salud*, *27*(3), 364–371.

[22] Santolin, Í., Famadas, K., & McIntosh, D. (2014). Detection and identification of rickettsia agents in ticks collected from wild birds in Brazil by polymerase chain reaction-restriction fragment length polymorphism (PCR-rflp) analysis. *Brazilian Journal of Veterinary Medicine*, *35*(Supl.2).

[23] Labruna, M. B., Whitworth, T., Horta, M. C., Bouyer, D. H., McBride, J. W., Pinter, A., Popov, V., Gennari, S. M., & Walker, D. H. (2004). Rickettsia Species Infecting Amblyomma cooperi Ticks from an Area in the State of São Paulo, Brazil, Where Brazilian Spotted Fever Is Endemic. *Journal of Clinical Microbiology*, *42*(1), 90–98. https://doi.org/10.1128/JCM.42.1.90-98.2004

[24] Stromdahl, E. Y., Williamson, P. C., Kollars, T. M., Evans, S. R., Barry, R. K., Vince, M. A., & Dobbs, N. A. (2003). Evidence of Borrelia lonestari DNA in Amblyomma americanum (Acari: Ixodidae) Removed from Humans. *Journal of Clinical Microbiology*, *41*(12), 5557–5562.https://doi.org/10.1128/JCM.41.12.5557-5562.2003

[25] Soares, R. M., Galvão, M. A. M., Schumaker, T. T. S., Ferreira, F., Vidotto, O., & Labruna, M. B. (2005). *Rickettsial Infection in Animals and Brazilian Spotted Fever Endemicity*. *11*(2).

[26] Freitas, M. C. D. de O., Grycajuk, M., Molento, M. B., Bonacin, J., Labruna, M. B., Pacheco, R. de C., Moraes-Filho, J., Deconto, I., & Biondo, A. W. (2010). Brazilian spotted fever in cart horses in a non-endemic area in Southern Brazil. *Revista Brasileira de Parasitologia Veterinaria*, *19*(2), 56–57. https://doi.org/10.4322/rbpv.01902013

[27] Souza, C. E., Camargo, L. B., Pinter, A., & Donalisio, M.

R. (2016). High seroprevalence for Rickettsia rickettsii in equines suggests risk of human infection in silent areas for the Brazilian spotted fever. *PLoS ONE*, *11*(4), 11–19. https://doi.org/10.1371/journal.pone.0153303

[28] Batista, F. G., Silva, D. M. da, Green, K. T., Tezza, L. B. de L., Vasconcelos, S. P. de, Carvalho, S. G. S. de, Silveira, I., Moraes-Filho, J., Labruna, M. B., Fortes, F. S., & Molento, M. B. (2010). Serological survey of Rickettsia sp. in horses and dogs in an non-endemic area in Brazil. *Revista Brasileira de Parasitologia Veterinária*, *19*(4), 205–209. https://doi.org/10.1590/s1984-29612010000400003

[29] Silveira, I., Pacheco, R. C., Szabó, M. P. J., Ramos, H. G. C., & Labruna, M. B. (2007). Rickettsia parkeri in Brazil. *Emerging Infectious Diseases*, *13*(7), 1111–1113. https://doi.org/10.3201/eid1307.061397

[30] Labruna, M. B., Salim Mattar, V., Nava, S., Bermudez, S., Venzal, J. M., Dolz, G., Abarca, K., Romero, L., de Sousa, R., Oteo, J., & Zavala-Castro, J. (2011). Rickettsioses in Latin America, Caribbean, Spain and Portugal. *Revista MVZ Cordoba*, *16*(2), 2435–2457.

[31] Faccini-Martínez, Á. A., Oliveira, S. V. de, Cerutti Junior, C., & Labruna, M. B. (2018). Febre Maculosa por Rickettsia parkeri no Brasil: condutas de vigilância epidemiológica, diagnóstico e tratamento. *Journal of Health & Biological Sciences*, *6*(3), 299. https://doi.org/10.12662/2317-3076jhbs.v6i3.1940.p299-312.2018

[32]Nieri-Bastos, F. A., Szabó, M. P. J., Pacheco, R. C., Soares, J. F., Soares, H. S., Moraes-Filho, J., Dias, R. A., & Labruna, M. B. (2013). Comparative evaluation of infected and noninfected Amblyomma triste ticks with Rickettsia parkeri, the agent of an emerging rickettsiosis in the new world. *BioMed Research International*, *2013*, 1–6. https://doi.org/10.1155/2013/402737

[33] Szabó, M. P. J., Pascoal, J. O., Martins, M. M., Ramos, V. do N., Osava, C. F., Santos, A. L. Q., Yokosawa, J., Rezende, L. M., Tolesano-Pascoli, G. V., Torga, K., de Castro, M. B., Suzin, A., Barbieri, A. R. M., Werther, K., Silva, J. M. M., & Labruna, M. B. (2019). Ticks and Rickettsia on anteaters from Southeast and Central-West Brazil. *Ticks and Tick-Borne Diseases*, *10*(3), 540–545. https://doi.org/10.1016/j.ttbdis.2019.01.008

[34] Cordeiro, M. D., Bahia, M., Magalhães-Matos, P. C., Cepeda, M. B., Guterres, A., Fonseca, A. H., Cordeiro, M. D., Bahia, M., Magalhães-Matos, P. C., Cepeda, M. B., Guterres, A., & Fonseca, A. H. (2018). Morphological, molecular and phylogenetic characterization of Borrelia theileri in Rhipicephalus microplus. *Revista Brasileira de Parasitologia Veterinária*, *27*(4), 555–561. https://doi.org/10.1590/s1984-296120180083

[35] Rogers, A. B., Smith, R. D., & Kakoma, I. (1999). Serologic cross-reactivity of antibodies against Borrelia theileri, Borrelia burgdorferi, and Borrelia coriaceae in cattle. *American Journal of Veterinary Research*, *60*(6), 694–697.

[36] Soares, C. O., Ishikawa, M. M., Fonseca, A. H., & Yoshinari, N. H. (2000). Borrelioses, agentes e vetores. In *Pesquisa Veterinaria Brasileira* (Vol. 20, Issue 1, pp. 1–19). Colégio Brasileiro de Patologia Animal. https://doi.org/10.1590/S0100-736X2000000100001

[37] Rezende, J., Rangel, C. P., Cunha, N. C., & Fonseca, A. H. (2012). Primary embrionic cells of Rhipicephalus microplus and Amblyomma cajennense ticks as a substrate for the development of Borrelia burgdorferi (strain G39/40). *Brazilian Journal of Biology*, *72*(3), 577–582. https://doi.org/10.1590/S1519-69842012000300021

[38] Ogrzewalska, M., Saraiva, D. G., Moraes-Filho, J., Martins, T. F., Costa, F. B., Pinter, A., & Labruna, M. B. (2012). Epidemiology of Brazilian spotted fever in the Atlantic Forest, state of São Paulo, Brazil. *Parasitology*, *139*(10), 1283–1300. https://doi.org/10.1017/S0031182012000546

[39] Burgdorfer, W. (1956). The possible role of ticks as vectors of leptospirae: I. Transmission of Leptospira pomona by the argasid tick, Ornithodoros turicata, and the persistance of this organism in its tissues. *Experimental Parasitology*, *5*(6), 571–579. https://doi.org/10.1016/0014-4894(56)90030-3

[40] Piesman, J. (1995). Dispersal of the Lyme disease spirochete Borrelia burgdorferi to salivary glands of feeding nymphal Ixodes scapularis (Acari: Ixodidae). *Journal of Medical Entomology*, *32*(4), 519–521. https://doi.org/10.1093/jmedent/32.4.519

[41] Pinter, A., & Labruna, M. B. (2006). Isolation of Rickettsia rickettsii and Rickettsia bellii in Cell Culture from the Tick Amblyomma aureolatum in Brazil. *Annals of the New York Academy of Sciences*, *1078*(1), 523–529. https://doi.org/10.1196/annals.1374.103

Table 1 – Titres variation of antibodies for species of Rickettsia tested by the indirect immunofluorescence reaction (IFR), and probable homologous antigens by positive horses (≥1:64) and origin location (region), Brazil, 2019.

Sample identification	Gender	Breed	Region	*R. rickettsii*	*R. parkeri*	*R rhipicephali*	*R. amblyommatis*	*R. bellii*	*PAIHR* *
E2	F	MP	Guaxupé - MG	-	-	64	128	512	*R. bellii*
E3	M	MP	Guaxupé - MG	-	-	64	64	1024	*R. bellii*
E12	F	MP	Guaxupé - MG	-	-	-	-	512	*R. bellii*
E13	F	MP	Guaxupé - MG	-	-	-	-	1024	*R. bellii*
E14	F	MP	Guaxupé - MG	-	-	-	-	1048	*R. bellii*
E15	F	MP	Guaxupé - MG	-				1024	*R. bellii*
E17	M	MP	Guaxupé - MG	-	-	-	-	1024	*R. bellii*
E18	M	MP	Guaxupé - MG	-	-	256	64	1024	*R. bellii*
E19	M	MP	Guaxupé - MG	-	-	-	-	1048	*R. bellii*
E28	F	QH	Guaxupé - MG	-	-	-	-	256	*R. bellii*
E30	F	MP	Guaxupé - MG	-	128	-	-	-	*R. parkeri*
E31	M	SRD	Guaxupé - MG	-	256	-	-	512	Unspecific
E93	M	SRD	Tapiratiba – SP	512	512	256	256	512	Unspecific
E95	M	SRD	Tapiratiba - SP	256	1024	512	512	1024	Unspecific
E96	M	SRD	Tapiratiba - SP	256	512	512	256	512	Unspecific
E98	M	SRD	Tapiratiba - SP	128	256	-	128	-	Unspecific
E99	M	SRD	Tapiratiba - SP	512	512	512	512	512	Unspecific
E100	M	SRD	Tapiratiba - SP	512	512	512	512	512	Unspecific
E102	M	SRD	Tapiratiba - SP	256	128	512	512	1024	Unspecific
E72	M	MP	Tapiratiba - SP	-	-	-	-	256	*R. bellii*
E73	M	MP	Tapiratiba - SP	128	256	-	-	-	Unspecific
E74	F	MP	Tapiratiba - SP	256	64	-	-	512	Unspecific
E75	F	MP	Tapiratiba - SP	64	-	-	-	128	Unspecific
E76	F	MP	Tapiratiba - SP	128	1024	-	-	64	*R. parkeri*
E78	F	MP	Tapiratiba - SP	256	512	-	-	-	Unspecific
E79	M	MP	Tapiratiba - SP	-	1024	-	-	64	*R. parkeri*
E80	F	MP	Tapiratiba - SP	-	64	-	-	-	*R. parkeri*
E81	F	MP	Tapiratiba - SP	128	256	64	-	128	Unspecific
E82	F	MP	Tapiratiba - SP	-	128	-	64	512	*R. bellii*
E84	F	MP	Tapiratiba - SP	-	-	-	-	1024	*R. bellii*
E86	M	MP	Tapiratiba - SP	-	128	-	-	64	Unspecific
E89	F	MP	Tapiratiba - SP	64	256	128	-	512	Unspecific
E90	M	QH	Tapiratiba - SP	-	128	-	-	-	*R. parkeri*
E40	M	SRD	Tapiratiba - SP	-	256	-	-	-	*R. parkeri*
E41	F	SRD	Tapiratiba - SP	64	512	-	-	64	*R. parkeri*

E42	F	SRD	Tapiratiba - SP	-	512	-	-	-	*R. parkeri*
E43	M	SRD	Tapiratiba - SP	-	256	-	-	256	Unspecific
E44	M	SRD	Tapiratiba - SP	-	256	-	-	512	Unspecific
E53	M	SRD	Tapiratiba - SP	128	512	-	-	-	*R. parkeri*
E54	M	SRD	Tapiratiba - SP	-	512	-	-	-	*R. parkeri*
E55	F	SRD	Tapiratiba - SP	128	512	64	-	256	Unspecific
E57	M	SRD	Tapiratiba - SP	256	256	-	-	-	Unspecific
E60	M	SRD	Tapiratiba - SP	-	-	-	-	128	*R. bellii*
E61	M	SRD	Tapiratiba - SP	512	512	-	-	-	Unspecific
E63	M	SRD	Tapiratiba - SP	-	256	128	-	-	Unspecific
E64	M	SRD	Tapiratiba - SP	-	256	-	-	64	*R. parkeri*
E65	M	SRD	Tapiratiba - SP	-	-	-	-	64	*R. bellii*
E67	M	SRD	Tapiratiba - SP	-	128	-	-	512	*R. bellii*
E68	M	SRD	Tapiratiba - SP	-	-	-	-	64	*R. bellii*
E20	F	MP	Tapiratiba - SP	-	-	-	-	1024	*R. bellii*
E22	M	MP	Tapiratiba - SP	-	-	-	128	64	Unspecific
E103	M	SRD	Tapiratiba - SP	256	128	-	512	1024	Unspecific
E1	F	MP	Tapiratiba - SP	-	-	-	128	1024	*R. bellii*

Abbreviations: F = female; M= male; SRD = crossbreed horses; MP = 'Mangalarga Paulista' breed; QH = Quarter Horse breed; SP = São Paulo; MG = Minas Gerais. *PAIHR* = possible antigen involved in a homologous reaction. *A homologous reaction was determined when a final titre for a *Rickettsia* species overcome at least four times the values observed for other *Rickettsia* species. In this case, the species with the greatest final titre was considered the possible antigen involved in a homologous reaction (*PAIHR*).