

Experience report

INVESTIGAÇÃO EPIDEMIOLÓGICA EM FOCO ENDÊMICO DE FEBRE MACULOSA BRASILEIRA EM MINAS GERAIS, BRASIL

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Resumo

A febre maculosa brasileira (FMB) é uma doença zoonótica transmitida por carrapatos, causada pela bactéria *Rickettsia rickettsii*, endêmica em várias regiões do sudeste do Brasil. Uma investigação epidemiológica foi realizada em um novo foco de FMB no município de Florestal-MG, onde ocorreu um caso fatal de FMB em 2017. O local foi visitado em agosto de 2018, quando carrapatos e soro foram coletados de três equinos e três cães, todos com livre acesso a matas ciliares habitadas por capivaras. O soro também foi coletado de três humanos. Os carrapatos foram identificados em quatro espécies: *Amblyomma sculptum*, *Dermacentor nitens*, *Rhipicephalus sanguineus* sensu lato e *Rhipicephalus microplus*. Todos os equinos e cães foram sororreativos a *R. rickettsii* com títulos variando de 512 a 2048; os três humanos foram soronegativos. Estes resultados suportam o status endêmico do local para FMB, uma vez que todos os cavalos e cães foram sororreativos para *R. rickettsii* com altos títulos, indicando exposição recente a rickettsias do grupo da febre maculosa. Apesar dos resultados sorológicos negativos dos três humanos, a endemicidade da FMB é caracterizada por baixa incidência. Portanto, as autoridades de saúde devem estar cientes dos riscos iminentes de novos casos de FMB na área de estudo.

Palavras-chave: *Rickettsia rickettsii*; sorologia; carrapato

AN EPIDEMIOLOGICAL INVESTIGATION OF A BRAZILIAN SPOTTED FEVER- ENDEMIC FOCUS IN MINAS GERAIS, SOUTHEASTERN BRAZIL

Abstract

Brazilian spotted fever (BSF) is a zoonotic tick-borne disease caused by the bacterium *Rickettsia rickettsii*, which is endemic in many areas of southeastern Brazil. Herein an epidemiological investigation was performed in a new BSF focus of Florestal municipality-MG, where a fatal BSF case occurred in 2017. The site was visited in August 2018, when tick and serum were collected from three horses and three dogs, all with free access to riparian forests inhabited by capybaras. Blood was also collected from three humans. Ticks were identified into four species: *Amblyomma sculptum*, *Dermacentor nitens*, *Rhipicephalus sanguineus* sensu lato and *Rhipicephalus microplus*. All horses and dogs were seroreactive to *R. rickettsii* with endpoint titers varying from 512 to 2048; the three humans were seronegative. These results support the endemic status of the site for BSF, since all horses and dogs were seroreactive to *R. rickettsii* with high endpoint titers, indicating recent exposure to spotted fever group rickettsiae. Despite of the serologically negative results of the three humans, BSF endemicity is characterized by low incidence. Therefore, health authorities must be aware of the imminent risks of new BSF cases in the study area.

Keywords: *Rickettsia rickettsii*; serology; tick

INVESTIGACIÓN EPIDEMIOLÓGICA SOBRE EL FOCO ENDÊMICO DE LA FIEBRE MACULOSA BRASILEÑA EN MINAS GERAIS, BRASIL

Resumen

La fiebre maculosa de Brasil (BSF) es una enfermedad zoonótica transmitida por garrapatas y causada por la bacteria *Rickettsia rickettsii*, endémica en muchas áreas del sureste de Brasil. Aquí se realizó una investigación epidemiológica en un nuevo foco de BSF en el municipio de Florestal-MG, donde ocurrió un caso fatal de BSF en 2017. El lugar fue visitado en agosto de 2018, cuando se tomaron garrapatas y suero de tres caballos y tres perros, todos con libre acceso a los bosques ribereños habitados por capivaras. También se recogió sangre de tres

humanos. Las garrapatas se identificaron en cuatro especies: *Amblyomma sculptum*, *Dermacentor nitens*, *Rhipicephalus sanguineus* sensu lato y *Rhipicephalus microplus*. Todos los caballos y perros fueron seroreactivos a *R. rickettsii* con títulos finales que varían de 512 a 2048; los tres humanos eran seronegativos. Estos resultados apoyan el estado endémico de el lugar para BSF, ya que todos los caballos y perros fueron seroreactivos a *R. rickettsii* con títulos altos de punto final, lo que indica una exposición reciente a rickettsias. A pesar de los resultados serológicamente negativos de los tres humanos, la endemidad de BSF se caracteriza por una baja incidencia. Por lo tanto, las autoridades sanitarias deben ser conscientes de los riesgos inminentes de los nuevos casos de BSF en el área de estudio.

Palabras clave: *Rickettsia rickettsii*; serología; garrapatas

Brazilian spotted fever (BSF) is a zoonotic tick-borne disease caused by the bacterium *Rickettsia rickettsii*, which has been endemic in several areas of the state of Minas Gerais, southeastern Brazil, since the 1920s^{1,2,3,4,5,6}. Until now, the tick *Amblyomma sculptum* (formerly *Amblyomma cajennense*) has been incriminated as the only possible vector of *R. rickettsii* to humans in Minas Gerais, with several detections/isolations of this bacterium from field-collected *A. sculptum* ticks^{1,3,7,6}.

Recent epidemiological investigations in at least two BSF-endemic areas of Minas Gerais have implicated the capybara (*Hydrochoerus hydrochaeris*) as the main host for *A. sculptum* ticks^{3,6}. This scenario is in accordance with most of the BSF-endemic areas of the state of São Paulo (a neighbor state of Minas Gerais), where the role of capybaras as amplifying hosts of *R. rickettsii* for *A. sculptum* ticks has been demonstrated during the last few decades^{8,9}.

From 2000 to 2018, a total of 333 cases of BSF were laboratory-confirmed in the state of Minas Gerais, with an overall case-fatality rate of 36% (official data from the Brazilian Ministry of Health: <http://www.saude.gov.br/saude-de-a-z/febre-maculosa>). This number of cases and fatalities poses Minas Gerais as the state with the second highest number of BSF cases, behind only to São Paulo. Despite of that, very few epidemiological investigations have been done in the BSF focuses of Minas Gerais. In this scenario, herein an epidemiological investigation was performed in a new BSF focus of Minas Gerais, where the first case of the disease was confirmed in 2017.

The study site was a farm in Florestal municipality in the central area of Minas Gerais (19°52'46.63"S – 44°20'04.35"W). One BSF fatal case was reported in the farm during October 2017. The case was officially confirmed in the laboratory by the Fundação Ezequiel Dias (FUNED, MG), based on detection of rickettsial DNA in the patient's blood during the severe acute phase of the disease (Ana Íris de Lima Duré, personal communication). Because of this case, the farm was visited in August 2018. The farm had an area of ~200 ha, composed mainly by pastures for beef cattle and small riparian forests by the Paraopeba River (Figure 1). Although not quantified, numerous capybaras (including many young) were frequently seen by the river. During the visit, three horses and three dogs, all with free access to the entire farm area, including the riparian forests, were examined. Blood samples and ticks were collected from these animals. In addition, blood samples were collected from three humans (two residents and one worker of the farm) with no history of BSF illness. Collection of blood and ticks were underwritten informed consent.



Figure 1. General view of pastures and riparian forest by the Paraopeba River in the farm sampled in Florestal Municipality during August 2018.

In the laboratory, ticks were identified to species following Barros-Battesti et al.¹⁰ and Martins et al.^{11,12}. Blood samples were centrifuged and the resultant sera were tested by the indirect immunofluorescence assay (IFA) using crude antigens of *R. rickettsii* strain Taiacu, as previously described⁴. Briefly, sera were diluted in 2-fold increments with phosphate-buffered saline (PBS), starting from the 1:64 dilution. Slides were incubated with fluorescein isothiocyanate-labelled goat anti-horse IgG, rabbit anti-dog IgG, and goat anti-human IgG (Sigma, St Louis, MO, USA) for horse, canine, and human sera, respectively. Sera reacting at the screening dilution (1:64) were then tested in serial two-fold dilutions to determine the endpoint titer. In each slide, sera previously shown to be non-reactive (negative control) or reactive (positive control) were tested at the 1:64 dilution.

A total of 10 ticks were collected from three horses and were identified as 2 males, 2 females, and 3 nymphs of *Dermacentor nitens*, and 3 nymphs of *A. sculptum*. From the three dogs, we collected 34 ticks, which were identified as 7 males, 3 females, and 8 nymphs of *Rhipicephalus sanguineus* sensu lato, 2 males and 5 females of *Rhipicephalus microplus*, and 9 nymphs of *A. sculptum*.

By IFA, all horses and dogs were seroreactive to *R. rickettsii* with high endpoint titers. For the three dogs, the endpoint titers were 1024, 1024, and 2048. The endpoint titers of the three horses were 512, 1024 and 1024. None of the three humans were seroreactive.

The present serological results support the endemic status of the farm for BSF, since all horses and dogs were seroreactive to *R. rickettsii*. In addition, the high endpoint titers indicate recent exposure of these animals to spotted fever group rickettsiae, in accordance with the antibody dynamics of these animals after experimental infection with *R. rickettsii*^{13,14}. Results are also in accordance with previous studies in which horses and dogs were suitable sentinels for BSF endemicity when *A. sculptum* is implicated as the main vector of *R. rickettsii*^{15,4}.

While four different tick species were found infesting domestic animals in the farm, only *A. sculptum* is recognized by having high anthrophilic behavior, corroborating its vector capacity for *R. rickettsii*¹⁶. These facts, in association with the presence of numerous capybaras in the farm, suggest that the epidemiological scenario of BSF in the farm of Florestal is characterized by an *A. sculptum* population sustained by capybaras, an important amplifying host of *R. rickettsii* for *A. sculptum* in southeastern Brazil¹⁷.

Finally, the non-reactive serological status of the two human residents and the worker of the farm is in accordance with their negative historical of illness compatible with BSF.

However, it must be emphasized that BSF endemicity is characterized by low incidence, due to the very low *R. rickettsii*-infection rates of *A. sculptum* ticks, usually <1% of the tick population¹⁸. Therefore, health authorities must be aware of the imminent risks of new BSF cases in the study area. Interestingly, in August 2017 (two months before the afore-mentioned BSF-confirmed fatal case), there was another fatal case of an acute febrile illness in the same farm; however, at that time there was no suspicion of BSF and the case was not notified. It is highly possible that this previous case was also BSF.

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