SHORT COMMUNICATION

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Serological detection of Brazilian Spotted-Fever Group Rickettsiae in horses in Brasília, Federal District, Brazil

Detecção sorológica de Rickettsiae do grupo da Febre Maculosa Brasileira em cavalos de Brasília, Distrito Federal, Brasil

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ABSTRACT: Brazilian spotted fever (BSF) is a zoonosis caused by spotted-fever group Rickettsiae that is transmitted to humans through tick bites. Dogs and horses are considered to be sentinel animals for this disease. In this study, a serological survey was carried out among horses apprehended by the governmental animal control service of the Federal District, Brazil. Serum samples were obtained from 122 horses over a one-year period. Through the indirect immunofluorescence antibody assay (IFA) for *Rickettsia rickettsii* anti-IgG antibodies, 21 samples were found to be positive, with titers ranging from 1:64 to 1:512. This demonstrates that the agent presents silent circulation in the Federal District.

KEYWORDS: Brazilian Midwestern, tick-borne diseases, zoonosis.

RESUMO: A febre maculosa brasileira (FMB) é uma zoonose causada por riquétsias do Grupo da Febre Maculosa transmitida ao homem através de picadas de carrapatos. Cães e cavalos são considerados animais sentinelas para essa doença. Neste estudo, foi realizado inquérito sorológico em cavalos apreendidos pelo serviço governamental de controle de animais do Distrito Federal, Brasil. Os soros utilizados foram obtidos de 122 cavalos, coletados por conveniência pelo período de um ano. Através do ensaio de imunofluorescência indireta (IFA) para anticorpos anti-IgG contra *Rickettsia rickettsii*, 21 amostras foram positivas para títulos variando de 1:64 a 1:512, demonstrando a circulação silenciosa do agente no Distrito Federal.

PALAVRAS-CHAVES: Centro-Oeste Brasileiro, doenças transmitidas por carrapatos, zoonoses.

INTRODUCTION

Brazilian spotted fever (BSF) is a zoonosis caused by a gram-negative obligate intracellular bacterium within the spotted-fever group of Rickettsiae. It is transmitted to humans through tick bites (SZABÓ; PINTER; LABRUNA, 2013). In humans, this bacterium can cause so-called rickettsial vasculitis and can produce clinical signs such as fever, headache, cutaneous rash, muscle pain, nausea, seizure and death (BRASIL, 2019). The disease was first described in the United States in 1909 and was called Rocky Mountain spotted fever (RICKETTS, 1909). In Brazil, it is known as Brazilian spotted fever and has been registered since 1929 (PIZA;MEYER;GOMES, 1932).

This disease has been subject to compulsory notification in Brazil since 2001 (Portaria nº 1.943, de 18 de outubro de 2001). Between 2007 and 2021, 2569 cases were confirmed and 837 deaths recorded. Most of the lethal cases occurred in the southeastern region of Brazil. In the Distrito Federal, two cases have already been confirmed,with no deaths (BRASIL,2022a; BRASIL,2022b).

The main causative agent of serious illness due to this disease in Brazil is *Rickettsia rickettsii*, which is present particularly in the southeastern region of the country. Other species have also been recorded, such as the Atlantic rainforest strain of *Rickettsia* spp., which can cause a milder form of spotted fever and is present in the south-eastern, southern and north-eastern regions (SZABÓ et al., 2013; OLIVEIRA et al., 2016).

These microorganisms are transmitted to wild and domestic hosts by hematophagous arthropods such as ticks of the genus *Amblyomma* and other suspected vectors like *Rhipicephalus sanguineus* (SZABÓ; PINTER; LABRUNA,2013). The infection

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is maintained in nature through complex vector-*Rickettsia*-host interactions in which dogs and horses play an epidemiological role as sentinel species (CARDOSO et al.,2006, MORAES-FILHO et al., 2009).This etiological agent is transmitted among horses mainly by the tick *Amblyomma sculptum*, which can infect wild animals like capybaras (*Hydrochoerus hydrochaeris*) (LEMOS et al., 1996) and is found in tropical areas like the Cerrado (tropical savanna), Atlantic rainforest, Pantanal and Caatinga biomes (BITENCOURTH et al., 2017).

Other wild animals such as birds, tapirs, rodents, snakes, fish, bats and opossums were also described as hosts of rickettsial diseases. These animals are usually involved in wild cycles, but could integrate domestic cycles. Serologic response against rickettsiae was already verified in serum samples from opossums in south-eastern region, positive dogs and horses were verified in the same region highlighting the importance of wild animals in maintaining wild cycles but also the possible integration with the domestic cycle (MILAGRES et al., 2010).

According to Oliveira et al. (2016), 17, 4% of confirmed human cases of BSF were correlated with horses. In this regard, serological surveys have been conducted among brazilian equids in different states and regions, such as Paraná, São Paulo, Rio de Janeiro, Goiás, Bahia and others, with the aim of characterizing the circulation of *Rickettsia* spp. in rural and/or urban environments (FREITAS et al., 2010; CUNHA et al., 2014;; MARTINS et al., 2016; SOUZA et al., 2016; OLIVEIRA et al., 2019). With the same motivation, we aimed to detect antibodies to *Rickettsia* spp., in serum samples from horses and to make inferences about the silent circulation of these bioagents in the region of the Federal District of Brazil.

MATERIAL E METHODS

This study was conducted in the Federal District (15°47′ S and 47°56′ W), which is in the central-western region of Brazil. It has a territorial area of 5,769 km^2 and an estimated population of 3,094,325 inhabitants (IBGE, 2021).

Blood samples were collected from horses that had been seized by the Secretary of Agriculture and Rural Development of Federal District (SEAGRI). Most of the animals came from seizures of stray animals or related to cases of abuse. The animals that were sampled were selected according to convenience, and this sampling was performed weekly between September 2012 and September 2013. The weekly sampling was adopted because of the dynamics of animal movements into and out of the shed. Samples were collected from at least 33% of the animals present, whether or not they presented parasitism with ticks.

A total of 122 samples of volumes between 5 Ml and 10 Ml were aseptically obtained by means of jugular venipuncture. The samples were collected in BD Vacutainer® tubes containing clot activators. These tubes were then centrifuged at 2500 rpm for five minutes to separate the serum. Each serum sample was identified with a number and was stored by freezing at -20°C. All the procedures were performed under approval from the Ethics Committee for Animal Research (CEUA) of the University of Brasília (approval number 2563-1, dated November 2011).

The indirect immunofluorescence assay (IFA) is the gold-standard serological technique for making the laboratory diagnosis of BSF Rickettsiae. Presence of IgG immunoglobulin was investigated. This is detectable from the seventh to the tenth day after infection and its presence signifies that the animal has chronic contact with the microorganism (GALVÃO et al., 2005; BRASIL, 2019). The IFA was performed at the Laboratory of Riquetsioses and Hantaviruses at Fundação Ezequiel Dias (FUNED), in Belo Horizonte, Minas Gerais, Brazil with the aim of detecting *Rickettsia rickettsii* IgG antibodies. The samples were diluted 1:64 in phosphate-buffered saline (PBS; Ph 7.4) (Sigma Diagnostics, St Louis, MO, USA) and were homogenized in a vortex stirrer.

From the diluted samples, 20 Ml per well were inoculated onto immunofluorescence slides containing the antigen (*Rickettsia rickettsii* antigen slides, Scimedx Corporation, Dover, DE, USA). This part of the sample was then incubated in a humid chamber at 37°C for 30 minutes. Following this, the slides were removed from the incubator, the excess reagent was removed using distilled water and then the slides were washed in 0.01 M PBS (Ph 7.4) in a shaker chamber at low speed for five minutes. After drying the slides, each well was covered with 20 Ml of anti-*Rickettsia* IgG conjugate. The incubation and washing procedure was repeated as described above. After the last incubation and washing, the wells were covered with one drop of buffered glycerin, covered with a coverslip and observed under a immunofluorescence microscope.

RESULTS AND DISCUSSION

At a screening dilution of 1:64 (positive cutoff), 21 samples were diagnosed as positive for *R.rickettsii*. The prevalence coefficient (PC) was calculated from this result (number of hosts infected/number of hosts examined X 100) and was determined as 17.21%. At subsequent dilutions, the following numbers and percentages of the samples were found to be positive: 1:128, eleven (9.02%); 1:256, three (0.22%); and 1:512, one (0.82%).

Through the IFA technique, presence of antibodies against the agent *R. rickettsii* in horses was demonstrated in the present study. This result is significant therefore indicated that bacteria that can cause Brazilian spotted fever were circulating in these animals in the Federal District. This locality is not considered to be an endemic area for Brazilian spotted fever, given that only two human cases (without deaths) occurred over the period 2007-2021 (BRASIL 2022a, 2022b).

Several studies have been conducted with the objective of evaluating the seroprevalence of this agent in horses, due to their importance in the epidemiological chain of the disease. However, the seroprevalences found in some of these other studies differed from what was observed in the present study. A comprehensive survey on the seroprevalence of three isolates of Rickettsiae was carried out by Souza et al. (2016) in

the Piracicaba river basin, including four endemic areas that presented human cases and four non-endemic areas. A total of 504 serum samples were tested, from which 183 horses were found to be seropositive (36.3%), of which 73 (39.9%) were living in non-endemic areas. In Goiás, the state adjacent to the Federal District, the presence of antibodies against *R. rickettsii* was described among dogs in different municipalities (NEVES et al., 2020) and in horses too. MARTINS et al. (2016) described the epidemiological factors of a human case of BSF in Quirinópolis, Goiás, where one of main factors was contact with horses. Antibodies against *R. rickettsii* were detected, with 28.5% seroprevalence among horses. The higher proportions of seroprevalence than what was found in the present study were possibly because these studies were conducted in endemic areas and in a place with a human case of BSF.

Similar to our work, Freitas et al. (2010) carried out a study among cart horses in a non-endemic area of São José dos Pinhais, Paraná, which was the area where the first case of spotted fever in this state was reported. They collected samples from 75 cart horses and observed that 9.33% of these animals had antibodies against *R. rickettsii*. Oliveira et al. (2019) conducted a study in the Atlantic Forest in the municipality of Ilhéus, state of Bahia, among dogs and horses. They investigated a basal isolate and three different Brazilian isolates of *Rickettsia* spp. Samples were collected from 69 horses and 24.6% of them showed reactions to some species of *Rickettsia*, but only four (5.8%) were seropositive for *R. rickettsii*. They concluded that the rural zone of this municipality was not endemic for spotted fever caused by *R. rickettsii*. This correlation between seroprevalence and endemicity in different areas highlights the predictive potential of using horses as sentinel animals in relation to human cases of spotted fever (SOUZA et al., 2016).

The evidence showing that *R. rickettsii* is circulating among sentinel animals in the Federal District and the central-western region emphasizes the need for epidemiological and acarological surveillance of the disease.

Most part of the horses seized by SEAGRI-DF are used primarily to haul loads or are animals released on public roads. These animals are able to travel long distances daily, covering both urban and rural areas. Thus, they can transport vectors to different locations and come into contact with wild animals such as capybaras. Knowledge of the circulation of the agent is also important for healthcare authorities, considering that a lack of information about occurrences of the disease was the main obstacle to preventive and surveillance actions that was observed by Oliveira et al. (2017).

CONCLUSIONS

The circulation of *Rickettsia* that was diagnosed through immunofluorescence among the equine serum samples of the present study highlights some epidemiological aspects of BSF in the Federal District. It is likely that BSF Rickettsiae have been present in this region for many years: this hypothesis is possible given that the host-*Rickettsia*-vector-environment interface relates mainly to specific social scenarios like haulage activities and/or ecotourism. Even though effective treatment for BSF is available, surveillance of emerging tick-borne diseases is essential in order to stimulate prevention and thus avoid human cases, because this disease has a high coefficient of lethality. Future studies about the pathogens present in ectoparasite fauna of equids of the Federal District region can contribute for a better understand of the transmission of the agent from one host to another. It is also important to generate real-time valid information for evaluating disease behavior over space and time, for use by the population and by the public health authorities.

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