Spotted Fever Group *Rickettsia* in Small Rodents from Areas of Low Endemicity for Brazilian Spotted Fever in the Eastern Region of Minas Gerais State, Brazil

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Abstract. We investigated the humoral immune response against different species of *Rickettsia* in serum samples from small rodents collected in two areas of a silent focus for Brazilian spotted fever in the eastern region of Minas Gerais State, Brazil. Sera samples were analyzed by indirect immunofluorescence assay using antigens from *Rickettsia* species of the spotted fever, ancestral, and transition groups. Titers $\geq 1:64$ were considered positive. In Santa Cruz do Escalvado, 94% (30 of 32) of the samples collected from *Rattus rattus*, 22% (5 of 23) from *Nectomys squamipes*, and 80% (4 of 5) from *Akodon* sp., reacted by indirect immunofluorescence assay with *Rickettsia* antigens of the spotted fever group. In the municipality of Pingo D'Água, 84% (26 of 31) of the samples collected from *R. rattus*, 86% (6 of 7) of the samples from *Oryzomys subflavus*, 86% (6 of 7) from *N. squamipes*, and 100% (1 of 1) from *Bolomys* sp. contained antibodies that reacted with rickettsial antigens of the spotted fever group. These results demonstrated the previous exposure of small rodents to spotted fever group *Rickettsia*, suggesting the participation of these animals in the natural history of these rickettsiae in this region.

INTRODUCTION

Rickettsial diseases are of worldwide importance.¹ Generally, the primary focus involves mainly small sylvan mammals of class *Rodentia* as hosts for vector arthropods that transmit infections of domestic and synanthropic animals, which may play a role in the maintenance of *Rickettsia* in nature.² Experimental studies with some small rodents have demonstrated that they are susceptible to rickettsial infection and develop rickettsemia at levels high enough to infect their ectoparasites during blood feeding.³ Those mammals have a limited dispersion and short lifespan and thus can serve as a good indicator of the circulation of rickettsial agents in a particular area by natural environmental dispersion.

In Minas Gerais State, Brazil, the cited municipalities are located at the biome Mata Atlântica, one of the last of a kind of rain forest in the southwest region of Brazil. Pingo D'Água is located on Rio Doce Valley. This municipality was selected with the aim of investigating the presence of *Rickettsia* organisms because of recent cases of Brazilian spotted fever (BSF) in that region. Santa Cruz do Escalvado, located in the Piranga Valley, had a history of occurrence of BSF cases, but this municipality has been considered as a silent focus of the disease during the past 20 years. In the beginning of the last decade, this municipality passed through several modifications in its natural landscape because of the building of a small hydroelectric power plant. We evaluated the current situation to understand the possibility of *Rickettsia* spread and the potential risk for a new BSF outbreak. In this investigation, we determined the occurrence of antibodies against different species of *Rickettsia* in serum samples of small sylvan rodents captured in these two municipalities.

MATERIALS AND METHODS

Locations. Santa Cruz do Escalvado occupies an area of 258.34 km² with an estimated population of 5,321 inhabitants,

and 80% of those living in the rural area dedicated especially to farming activities (Brazilian Institute of Geography and Statistics, 2009). Rural dwellings in Minas Gerais countryside are characteristically made of wattle and daub, which favors the periodomestic presence of vectors of diseases and small rodent reservoirs of such diseases. In Pingo D'Água, which has an area of 67 km² and an estimated population of 4,420, 90% of the inhabitants reside in an urban area.

Animals. Rodents were captured during a period of two years (2005–2007) in a three-month frequency. Approximately 100 live-traps were placed near dwellings, including garages, storage facilities, corn fields, bamboo fields, landfills, and around dwellings. The rodents were anesthetized with halothane and then blood samples were collected.

Serum sample collection. Serum samples were analyzed by indirect immunofluorescence assay (IFA), according to the protocol described by Walker and others⁴ using *Rickettsia* antigens of the spotted fever group (*R. rickettsii*, *R. parkeri*, *R. rhipicephali*, and *R. amblyommii*), an ancestral group (*R. bellii*), and a transitional group (*R. felis*).⁴ Serum samples that reacted at the screening dilution of 1:64 were then tested in serial two-fold dilutions to determine the endpoint titer. As a positive control serum, we used serum collected from a laboratory-reared water rat (*Nectomys squamipes*) 21 days after it was experimentally inoculated with *R. rickettsii* strain Taiaçu. In all reactions, we used a fluorescein isothiocyanate-labeled goat anti-rat IgG (Sigma, St. Louis, MO) as secondary antibody.

RESULTS

In Santa Cruz do Escalvado, 62 rodents were trapped, of which 23 were identified as *N. squamipes*, 2 as *Oryzomys subflavus*, 32 as *Rattus rattus*, and five as *Akodon* sp. Of these 62 rodents, 39 (63%) had antibody titers $\geq 1:64$ by IFA against *R. rickettsii*, *R. parkeri*, *R. rhipicephali*, and *R. amblyommii* antigens (Table 1). The highest titer (1:16,384) was found in serum of *R. rattus* using *R. rickettsii* antigen. None of the serum samples contained antibodies against *R. felis* or *R. bellii* antigens.

In Pingo D'Água, 46 rodents were trapped and 31 identified as *R. rattus*, 7 as *O. subflavus*, 7 as *N. squamipes*, and 1 as

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TABLE 1

Serum sample activity of rodents against spotted fever group *Rickettsia* antigens by indirect immunofluorescence assay, Santa Cruz do Escalvado, Minas Gerais, Brazil

Rodent species	No.	<i>Rickettsia rickettsii</i> , No. (%)	<i>R. parkeri</i> , No. (%)	<i>R. amblyommii</i> , No. (%)	<i>R. rhipicephali</i> , No. (%)
<i>Rattus rattus</i>	32	30 (94)	30 (94)	28 (88)	23 (77)
<i>Nectomys squamipes</i>	23	5 (22)	4 (17)	3 (13)	3 (13)
<i>Oryzomys subflavus</i>	2	0	0	0	0
<i>Akodon</i> sp.	5	4 (80)	4 (80)	1 (20)	1 (20)
Total	62	39 (63%)	38 (62%)	32 (52%)	27 (44%)

Bolomys sp. Of these 46 rodents, 39 (85%) contained antibodies against *R. rickettsii*, *R. parkeri*, *R. rhipicephali*, and *R. amblyommii* antigen, with titers ranging from 1:64 to 1:4,096. The highest titers were obtained against *R. rickettsii*. Two serum samples tested against *R. bellii* antigens showed positive results, and all showed negative results against *R. felis* antigen (Table 2).

The species *R. rickettsii* showed a higher percentage of positive serologic results. In Santa Cruz do Escalvado, Minas Gerais, of the 39 rodent serum samples positive for *R. rickettsii*, 11 (28.2%) sera had titers four times higher for *R. rickettsii* antigens than for other species of rickettsial antigens. In Pingo D'Água, of the 39 rodent sera positive for *R. rickettsii*, 9 (23.07%) had titers four times higher for *R. rickettsii* antigens than for other of species of rickettsial antigens. These results show this species or another closely related species stimulated the immune response, and that there was no serologic cross-reactivity. For the remaining rodent serum samples positive for rickettsial antigens, the species that triggered the immune response was not known, and cross-reactivity with other nonpathogenic species might have occurred.

DISCUSSION

The results obtained indicated exposure of small sylvan rodents to spotted fever group rickettsial organisms, suggesting the participation of these animals in the maintenance of ecologic cycle of rickettsiae in these areas in Brazil. The presence of antibodies in small rodents, such as *R. rattus*, *N. squamipes*, *Bolomys* sp., *O. subflavus*, and *Akodon* sp., indicates advantageous conditions for rickettsial development and reproduction in those animals. These animals are losing their natural predators because of alterations in the ecologic balance caused by human activities in the studied regions. Also, availability of food, such as grains found in storage structures, or the replacement of native vegetation by corn and sugar cane fields can be responsible for maintaining these species in nature.

The presence of these rodent species in the study region is consistent with the synanthropic behavior of small sylvan

rodents, which are well adapted to the residential environment.⁵⁻⁷ The percentage of seroreactivity detected in *R. rattus* samples was high, which is an important result, because *R. rattus* is the most synanthropic species among those collected. Because these rodents live near humans, they maintain their location under constant risk of infection by pathogens maintained the zoonotic cycles by other small mammals and their ectoparasite vectors. In addition, the fact that this mammalian species has a large range of distribution increases the possibility of contacts between domestic and sylvan animals, and dispersion of their ticks or other ectoparasites into the environment, which leads to infection of domestic animals and humans. Rickettsial vectors (ticks and fleas) and small rodents can serve as hosts in amplifying cycles.⁸

Laboratory studies have demonstrated that some small rodents species are competent hosts of rickettsiae and develop rickettsemia levels capable of infecting their ectoparasites, despite the short period of rickettsemia.^{3,9} There have been reports showing *R. rattus* to contain antibodies against rickettsial antigens⁸⁻¹⁰ Furthermore, our study is the first to identify a prevalent immune response in the other mammalian species by IFA using specific rickettsial antigens. Further studies are necessary to better understand the actual importance of these animals in the sylvan and domestic cycles of BSF, as well their interaction with their arthropods vectors.

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TABLE 2

Serum sample activity of rodents against spotted fever group *Rickettsia* antigens by indirect immunofluorescence assay, Pingo D'Água, Minas Gerais, Brazil

Rodent species	No.	<i>Rickettsia rickettsii</i> , No. (%)	<i>R. parkeri</i> , No. (%)	<i>R. amblyommii</i> , No. (%)	<i>R. rhipicephali</i> , No. (%)	<i>R. bellii</i> , No. (%)
<i>Rattus rattus</i>	31	26 (84)	24 (77)	18 (58)	15 (48)	1 (2)
<i>Nectomys squamipes</i>	7	6 (86)	6 (86)	5 (71)	3 (13)	0
<i>Oryzomys subflavus</i>	7	6 (86)	6 (86)	3 (43)	3 (43)	0
<i>Bolomys</i> sp.	1	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
Total	46	39 (85)	38 (83)	32 (70)	27 (59)	2 (4)

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