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Communication

[Comunicação]

Rickettsial infection in Cerro Largo, State of Rio Grande do Sul, Brazil

[Infecção rickettsial em Cerro Largo, Rio Grande do Sul]

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Bacteria of the genus *Rickettsia* are obligate intracellular organisms primarily associated with invertebrate hosts, such as ticks, mites, fleas, lice, leeches, and insects. A few *Rickettsia* species are capable of infecting vertebrates including humans, to whom they are vectored mostly by ticks (Weinert et al., 2009). In 2005, there was the first reported case of spotted fever group (SFG) rickettsiosis in Rio Grande do Sul, in a patient from Cerro Largo municipality who developed headache, arthralgia, generalized body aches, nasal secretion, and fever 20 days after a tick bite on his back. Since only one serum sample was collected from this patient (during the acute phase), which reacted positively (titer >64) against *R. rickettsii* antigens by indirect immunofluorescence assay (IFA), this case was regarded as probable. In 2007, there was the first laboratory-confirmed case of SFG rickettsiosis in Rio Grande do Sul, in another patient from the same area, who developed general symptoms similar to the first case, with no fatality. The case was serologically confirmed by IFA on paired serum samples using *R. rickettsii* antigens; acute serum was non-reactive whereas convalescent serum gave a titer of 128 (unpublished data from the Brazilian Ministry of Health). Based on these cases, an epidemiological study that evaluated rickettsial infection in domestic animals (considered to be sentinels for rickettsiosis) and asymptomatic humans was performed in the rural area of Cerro Largo, where the two cases of SFG rickettsiosis had occurred.

The municipality of Cerro Largo has a population of 12,000 inhabitants and its economy is based on services, commerce, industry, and agropecuary (soy, wheat, corn, swine and dairy cattle). There are many small, family-employed farms. Often, these small properties raise horses and dogs in areas near native forests and ecological reserves. On the outskirts of the town, the horse and cart are common ways of transportation for many of the residents, who keep horses and dogs in natural fields that are infested with ticks.

In March 2007, blood samples were collected from 31 horses, 27 dogs, and 27 humans living in rural properties on the outskirts of Cerro Largo. Animal blood samples were collected by the jugular vein in order to obtain sera samples by centrifugation, which were stored at – 20°C until processed by serological analysis. All serum samples were individually processed by IFA using crude antigens of the following *Rickettsia* species that have been isolated from ticks in Brazil: *R. rickettsii* (strain Taiacu) and *R. parkeri* (strain At24). If a serum reacted at the 1/64 dilution to any of these two antigens, its IgG titer was titrated from 1/64 to the endpoint dilution against both, and also against the following *Rickettsia* species: *R. amblyommii* (strain Ac37), *R. rhipicephali* (strain HJ5), and *R. bellii* (strain Mogi). Antigen preparation and IFA reactions were performed as previously described (Horta et al., 2004; Labruna et al., 2007). Serum showing for a *Rickettsia* species titer at least 4-fold higher than that observed for any other *Rickettsia*

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species was considered homologous to the first *Rickettsia* species or to a very closely related species, as previously determined (Horta et al., 2004; Labruna et al., 2007; Piranda et al., 2008; Saito et al. 2008).

This study was approved by the Ethics Committee at the Universidade Federal de Santa Maria and is in accordance with the CNS Resolution 196/96 (CEP 23081.008609/2007-54/CAAE: 0091.0.243.000-07).

A total of 16 (51.6%) horses, six (22.3%) dogs, and eight (29.6%) humans were shown to contain reactive antibodies (titer ≥ 64) to *R. rickettsii* and *R. parkeri*. These sera showed endpoint titers varying from 64 to 2,048 to at least one of the five rickettsial antigens tested by titration (Tables 1-3). No serum showed higher titer to a

Rickettsia species other than those elicited to *R. parkeri* or/and *R. rickettsii*. No serum showed endpoint titer to *R. rickettsii* 4-fold higher than that to *R. parkeri*. Two canine sera (Table 2) showed titers to *R. parkeri* at least 4-fold higher than those to any of the other four antigens. The antibody titers in these two dogs were considered to have been stimulated by *R. parkeri* or a very closely related species. This result is in accordance with a recent epidemiological study performed in another part of the State of Rio Grande do Sul (Saito et al., 2008), in which at least 100 dogs showed serological evidence of infection by *R. parkeri* or a very close-related genotype, as indicated by titers anti-*R. parkeri* at least 4-fold higher than those against the same other four *Rickettsia* species tested in the present study.

Table 1. Endpoint antibody titers by indirect immunofluorescence assay (IFA) to five *Rickettsia* species in horses from the rural area of Cerro Largo, State of Rio Grande do Sul, Brazil

Horse	<i>R. parkeri</i>	<i>R. rickettsii</i>	<i>R. amblyommii</i>	<i>R. bellii</i>	<i>R. rhipicephali</i>
E2	128	256	128	128	128
E3	128	256	64	nr	nr
E4	64	128	64	nr	nr
E5	64	128	nr	64	nr
E7	64	64	nr	64	nr
E9	64	128	64	nr	128
E13	256	512	512	64	512
E15	64	256	128	128	nr
E17	64	128	128	nr	64
E20	64	128	64	128	128
E21	128	256	64	64	128
E22	64	128	64	64	nr
E23	128	128	nr	nr	nr
E26	64	128	128	64	nr
E27	64	128	128	64	nr
E31	128	128	128	128	128

nr: non-reactive serum at the 1/64 dilution.

Table 2. Endpoint antibody titers by indirect immunofluorescence assay (IFA) to five *Rickettsia* species in dogs from the rural area of Cerro Largo, State of Rio Grande do Sul, Brazil

Dog	<i>R. parkeri</i>	<i>R. rickettsii</i>	<i>R. amblyommii</i>	<i>R. bellii</i>	<i>R. rhipicephali</i>
C1	512	256	256	nr	128
C2	1,024	256	256	nr	128
C3	128	64	nr	nr	nr
C4	64	64	nr	nr	nr
C5	1,024	256	128	nr	256
C19	1,024	512	128	nr	64

nr: non-reactive serum at the 1/64 dilution.

Table 3. Endpoint antibody titers by indirect immunofluorescence assay (IFA) to five *Rickettsia* species in humans from the rural area of Cerro Largo, State of Rio Grande do Sul, Brazil

Humans	<i>R. parkeri</i>	<i>R. rickettsii</i>	<i>R. amblyommii</i>	<i>R. bellii</i>	<i>R. rhipicephali</i>
H4	64	64	nr	nr	nr
H5	128	128	nr	nr	nr
H12	64	64	nr	nr	64
H14	64	64	nr	nr	nr
H15	64	64	nr	64	nr
H18	1,024	2,048	256	512	512
H19	256	512	128	256	256
H20	128	128	nr	nr	nr

nr: non-reactive serum at the 1/64 dilution.

None of the serologically positive humans of the present study recalled recent febrile illness compatible with classic spotted fever (fever, headache, and rash), and also, none of them reported to have taken antibiotics to treat a possible rickettsiosis. However, rickettsiosis can manifest with a multiplicity of general symptoms (Parola et al., 2005), making it difficult to recall previous clinical rickettsiosis with certainty. These facts also indicate that the SFG agent infecting humans in Rio Grande do Sul is potentially not lethal, in contrast to Southeastern Brazil, where *R. rickettsii* is highly pathogenic with high fatality rates in untreated cases.

The serological results indicated that horses, dogs, and humans have been in contact with SFG rickettsiae in Cerro Largo municipality. Results from at least two dogs indicated that *R. parkeri* or a close-related genotype is circulating in the region, a condition also observed in other municipalities in the State of Rio Grande do Sul (Saito et al., 2008). *R. parkeri* and its closest known species (*R. africae* and *R. sibirica*) are recognized human pathogens in other countries where they induce a milder spotted fever with no lethality reported to date (Parola et al., 2005). These facts suggest that a different rickettsial

agent, possibly related to *R. parkeri*, is the etiological agent of spotted fever in Southern Brazil (States of Rio Grande do Sul and Santa Catarina). Further studies are needed to isolate the rickettsial agent in order to determine its definitive taxonomic identification.

From 2006 to 2007, 20 ticks that were found attached or walking on humans in the rural area of Cerro Largo were collected by the local population and sent to the Laboratório Central do RS (LACEN), where they were all identified as adults of *Amblyomma ovale* (unpublished data), indicating that this species is the main human-biting tick in Cerro Largo. *A. ovale* is the main vector of *Rickettsia* sp. strain in Southeastern Brazil (Sabatini et al., 2010). Also, 17 of these ticks were tested by polymerase chain reaction targeting SFG rickettsial DNA, which was not found in any individual tick (unpublished data). Since these ticks represented only a small sample, further studies should encompass a larger sample of *A. ovale*, a potential vector of spotted fever in Southern Brazil.

Keywords: *Rickettsia*, Brazilian spotted fever, serology, Rio Grande do Sul

RESUMO

O presente estudo teve por objetivo avaliar, por imunofluorescência indireta, infecções rickettsiais em animais e humanos assintomáticos da área rural de Cerro Largo, RS. Dezesesseis (51.6%) equinos, seis (22.3%) cães e oito (29.6%) humanos mostraram anticorpos reagentes (título ≥ 64) para Rickettsia rickettsii e para Rickettsia parkeri. Os estudos sorológicos indicaram que equinos, cães e humanos entraram em contato com rickettsias do grupo da febre maculosa nesse município. Dois cães mostraram títulos de R. parkeri no mínimo quatro vezes maior que os demais agentes rickettsiais testados e sugerindo que R. parkeri ou um genótipo próximo está circulando na região.

Palavras-chave: Rickettsia, febre maculosa, sorologia, Rio Grande do Sul

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