

Rocky Mountain Spotted Fever in an Endemic Area in Minas Gerais, Brazil

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Only one species of spotted fever-group rickettsiae that is pathogenic for humans has been isolated in Brazil, where few physicians are familiar with this disease. In order to obtain information on tick-borne rickettsiosis, a study was performed in the County of Santa Cruz do Escalvado, State of Minas Gerais, Brazil, where a fatal clinical case confirmed by specific immunofluorescence had been reported.

Serum samples obtained from 679 humans and 96 dogs were tested by indirect immunofluorescence for detectable antibodies to spotted fever-group rickettsiae, the criterion for a positive result being a titer $\geq 1:64$. Seropositivity was detected in 7.14% of the humans sera examined and 13.68% of the dogs.

We discuss the significance of these findings and formulate some questions, emphasizing the need for further investigation.

Key words: spotted fever - Brazil - humans - dogs

Rocky Mountain spotted fever is an endemic febrile disease with an acute course, caused by *Rickettsia rickettsii*, a microorganism belonging to the Rickettsiaceae family (Weiss & Moulder 1984). Transmission to humans occurs accidentally through tick bites, leading to a clinical spectrum ranging from asymptomatic cases to a classical picture with suggestive exanthem and death (Cox 1952, Brezina et al. 1973). The disease has been known to be found in Brazil since the beginning of this century, mainly in the States of Minas Gerais, Rio de Janeiro, and São Paulo (Cox 1952, Magalhães 1952). In 1930, Salles Gomes isolated the rickettsia for the first time in Brazil in a hospitalized patient in São Paulo, followed by Octávio Magalhães, who isolated the microorganism in 1933 in Minas Gerais (Magalhães 1952). Studies carried out by the 1950's confirmed the similarity between the Brazilian and North American spotted fevers, both in clinical-epidemiological and anatomopathological aspects, as well as immunological and experimental behavior (Monteiro 1931, Dias & Martins 1939, Travassos & Dias 1939).

Following the emergence of effective antibiotics against rickettsiae and the use of insecticides in tick control, together with a certain increase in knowledge about the disease, in addition to other

factors, there was a worldwide decline in the morbidity and mortality of tick-borne rickettsioses (WHO Working Group on Rickettsial Diseases 1982, Mcdade & Newhouse 1986).

Since the 1970's, however, an increase has been observed in the incidence of spotted fever-group rickettsioses in various countries such as the United States, France, Italy, Japan, and Israel, among others (D'Angelo et al. 1978, CDC 1986, 1987, Mansueto et al. 1986, Salgo et al. 1988). Studies resumed in the United States in particular led to knowledge concerning the ecology of *R. rickettsii* and recognition of other, correlated rickettsiae, considered nonpathogenic for humans, like *R. belli*, *R. montana*, and *R. riphicephali* (Peters 1971, Burgdorfer et al. 1975, Philip & Casper 1981, Philip et al. 1981, Weiss & Moulder 1984, Mcdade & Newhouse 1986).

In Brazil, since the 1960's, occurrence of Rocky Mountain spotted fever came to be subject to doubt. In addition to the lack of reliable data on the disease's situation, in most cases the lack of specialized diagnostic resources only allowed for reporting of clinical cases (Gonçalves et al. 1981, Sampaio et al. 1988, Souza et al. 1991, Dietz et al. 1992, Melles et al. 1992). The State of Minas Gerais currently has the highest prevalence of Rocky Mountain spotted fever in Brazilian territory; there, several dozen cases have been reported to the State Health Department in spite of the lack of specialized diagnostic procedures.

MATERIALS AND METHODS

In the State of Minas Gerais, where the majority of cases of Rocky Mountain spotted fever in Brazil have occurred, the County of Santa Cruz do Escalvado was chosen for this study for the following reasons: (a) at the beginning of this century it was an endemic area for the disease; (b) an index case confirmed by necropsy occurred there. A five year old girl with a clear history of the tick bite died after becoming ill with fever, chills, generalized maculopapular rash and encephalopathy; (c) there was presumptive serological evidence obtained in 10 samples out of 30 sera selected at random from the region and stored at the Department of Tropical Medicine at the Oswaldo Cruz Institute; (d) it was certain that some inhabitants of the region were familiar with the disease, as well as the presence of *Amblyomma cajennense*, the prevailing tick species, indicated through a pilot study; (e) there was easy access to the region, since the County is part of a study area for the Department.

The County of Santa Cruz do Escalvado is located in the Mata de Ponte Nova microregion, and the County seat is located at 20° 13' 36" latitude South and 42° 49' 24" longitude West, 412m above sea level, 212 km from the State capital (Fig. 1). It has a mesothermic tropical climate with two distinct seasons. The rainy season ranges from October to March and the other, moderately dry season runs from March through September. Dogs, horses, and cattle are generally infested with ticks in various places in the county, mainly during the dry season. The majority of the population has contact with domestic animals and has a history of ticks bites. The field study was carried out at intervals from March 1989 to November 1990.

Serum sampling - Serum samples were taken at nine locations with different geographic characteristics, in addition to differences in soil management and population density. Blood samples were taken from 679 apparently healthy humans (Table I). In the village of Patrimônio, where the index case came from, the study universe was the whole population, whereas in the other eight locations approximately 25% of the population were included in the study.

Blood samples were also taken from 95 dogs, seven horses, and six cattle. All of the sera were transported to the laboratory in styrofoam containers with ice and were stored at -20°C until the time testing.

Serological testing - All human and animal sera were examined using indirect immunofluorescence with antigens obtained from yolk sacs infected with *R. rickettsii* (Sheila Smith strain) and subsequently inactivated by gamma-irradiation. The antigens

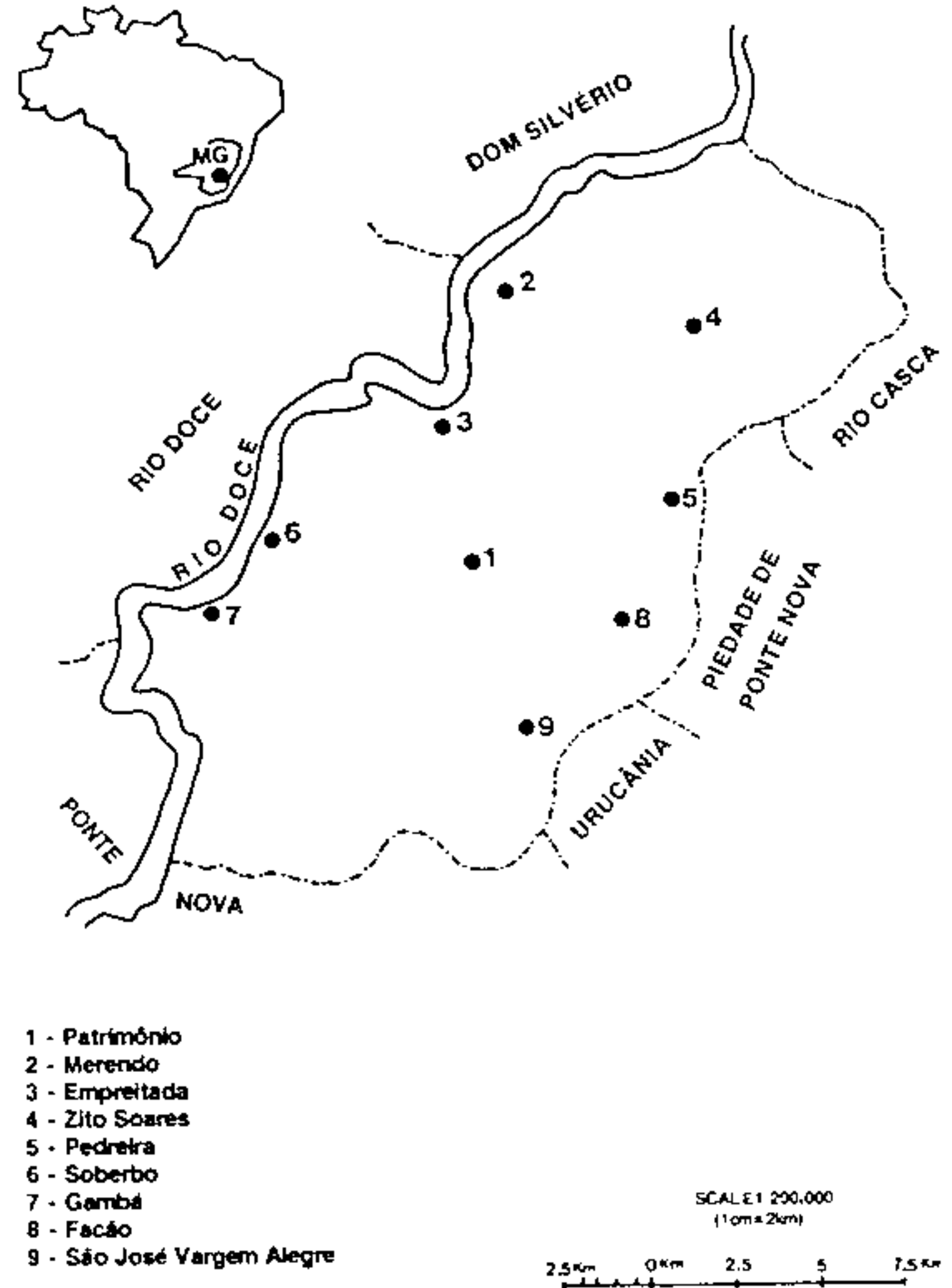


Fig.1: Santa Cruz do Escalvado, country limits, and locations studied.

TABLE I

Prevalence of infection with SFG rickettsiae in human sera by indirect fluorescent antibody in Santa Cruz do Escalvado, Minas Gerais (1989 - 1990)

Localities	No. of sera tested	No. reagent (%)
Patrimônio	305	26(8.52)
Empreitada	14	00(0.00)
Merengo	97	04(4.12)
Z. Soares	71	07(9.85)
Pedreira	16	00(0.00)
Soberbo	59	03(5.08)
Gambá	27	02(7.40)
S. José	54	06(11.11)
Facão	29	00(0.00)
Total	672	48(7.14)

SFG: spotted fever group rickettsiae

were prepared at the Centers for Disease Control, Atlanta, Georgia. Sera with antibody titers ≥ 1:64 were considered positive.

Histopathological study of the index case - Fragments of spleen, lung, liver, and brain tissue were stained with hematoxylin/eosin for exami-

nation by microscopy. Next, due to the limited amount of reagent for specific diagnosis, only sections of brain tissue were selected for study by immunofluorescence, since the brain was the organ that presented the most alterations under microscopy. Immunofluorescence was conducted according to the method proposed by Huang et al. (1970) and Walker and Cain (1978). The same procedure was carried out in fragments of brain tissue used as a negative control.

RESULTS

Seroprevalence - In the human population, the overall seroprevalence for infection was 7.14%, with 48 reactive serum samples (Tables I, II).

Overall seroprevalence for spotted fever-group rickettsioses in dogs was 13.68% (Table III).

In spite of the small number of blood samples from cattle and horses, it is worth of note that one of the six cattle and two of the seven horses submitted to indirect immunofluorescence presented seroreactivity with low titers.

Histopathological results of the index case - All the material examined by hematoxylin/eosin staining presented alterations suggestive of rickettsiosis, with the presence of interstitial pneumonia with vasculitis and perivasculitis. The spleen showed major histiocytosis, while the brain tissue showed acute multifocal encephalitis with an inflammatory infiltrate of mononuclears in scattered

TABLE II

Prevalence of infection with SFG rickettsiae in human sera by indirect fluorescent antibody, by age groups, in Santa Cruz do Escalvado, Minas Gerais (1989 - 1990)

Age group	Masculine			Feminine			Total		
	S	R	(%)	S	R	(%)	S	R	(%)
< 1	1	0	0.00	3	0	0.00	4	0	0.00
1 - 10	104	9	8.65	88	6	6.81	192	15	7.81
11 - 20	75	4	5.33	86	2	2.32	161	6	3.72
21 - 30	30	3	10.00	43	3	6.97	73	6	8.21
31 - 40	31	4	12.90	42	3	7.14	73	7	9.58
41 - 50	35	4	11.42	28	3	10.71	63	7	11.11
> 50	38	4	10.52	68	3	4.41	106	7	6.60
Total	314	28	8.91	358	20	5.58	672	48	7.14

SFG: spotted fever group rickettsiae
S: number of sample
R: number of reagent

TABLE III

Prevalence of infection with SFG rickettsiae in canine sera by indirect fluorescent antibody in Santa Cruz do Escalvado, Minas Gerais (1989 - 1990)

Localities	No. of sera tested	No. of reagent		%
		1:64	1:128	
Patrimônio	25	1	5	24.00
Morengo	15	0	0	0.00
Empreitada	04	0	0	0.00
Z. Soares	15	3	0	20.00
Pedreira	05	1	0	20.00
Soberbo	06	0	0	0.00
Gambá	06	1	0	16.66
S. José	10	1	0	10.00
Facão	09	1	0	11.11
Total	95	8	5	13.68

SFG: spotted fever group rickettsiae

foci in addition to perivasculitis with vasculitis associated with moderate cerebral edema.

Study of the brain tissue by specific immunofluorescence led to identification of immunofluorescent rickettsiae in the endothelium and wall of blood vessels. Immunofluorescence was practically restricted to the blood vessels and was consistent with rickettsia morphology (Fig. 2).

In the fragments of normal brain tissue studied in the negative control, there was no reactivity under immunofluorescence.

DISCUSSION

In Brazil, as in practically all Latin American countries, distribution of rickettsiosis cases is not documented. The cases that have been described are virtually limited to the States of Minas Gerais, Rio de Janeiro, and São Paulo, located in areas where the original forest was subdeciduous. Ecological studies based on reliable data concerning



Fig. 2. positive immunofluorescence for spotted fever-group rickettsiae in blood vessel wall of brain tissue in patient with a disease that is clinically, epidemiologically, and anatomopathologically compatible with Rocky Mountain spotted fever (X 2000).

the disease incidence are needed to determine whether there is an association between this type of vegetation and the presence of *A. cajennense*, the predominant tick vector in Brazil.

The results of this study indicate the importance of the relationship between humans and land occupation as a determining factor for human seroprevalence in endemic areas. Some locations, such as Patrimônio, São José, and Zito Soares, presented favorable conditions for spotted fever-group rickettsioses. Abandoned farm fields serving as pasture for several animal species, heavy tick vector density, and an almost constant infestation of the human population by these arthropods are strong indicators of potential tick-borne rickettsiosis transmission.

The current study on prevalence of rickettsia infection in humans is the third held in Brazil since the 1950's, and the results indicate that 7.14% of the overall study population had undergone prior immunological stimulation by spotted fever-group rickettsia antigens. There were no elevated serological levels, and the highest titer was 1:128, observed in seven samples in Patrimônio and one sample in São José.

As occurs in many seroprevalence surveys, it is difficult to determine whether the antibodies had

been stimulated by a specific pathogenic rickettsia such as *R. rickettsii* or by a nonpathogenic spotted fever-group rickettsia, or even by cross-reaction from a non-rickettsia antigen. Use of the criterion of seroreactivity with titers equal to or greater than 1:64 in this study ensured the specificity of indirect immunofluorescence for spotted fever-group rickettsioses.

There was no association between the percentage of seroreactive individuals and documented history of the disease, and it thus appears probable that the detected serum titers are the result either of the outbreak in 1985, which culminated in the index case confirmed by necropsy, or a cross-reaction with spotted fever-group rickettsiae with low virulence, leading to subclinical infections. We are also unable to rule out the possibility of cumulative, continuous exposure to antigens from the spotted fever group.

In the recent literature, a study was held in 1990, in the County of Virginópolis, also in Minas Gerais, following an outbreak of spotted fever affecting eight individuals, with three deaths. Animal and human seroreactivity was 87.0% and 86.1%, respectively. The Weil-Felix reaction was used, which is totally unspecific, considering positive titers ranging from 1:20 to 1:160 (Souza et al. 1991).

Of the four locations with high seroprevalence, in three (Patrimônio, Zito Soares, and Gambá) the seroreactivity was more predominant in males, probably as a result of their occupational activity. A higher seroreactivity in individuals in the 21-50 year age bracket reinforces this impression.

As for the role of dogs in the spotted fever rickettsia cycle, the serological results indicated the occurrence of infection in certain locations of the County studied. In Patrimônio, for example, 24% of the dog population were seropositive, and five dogs presented titers equal to or above 1:128, which could indicate a recent infection either by *R. rickettsii* or another spotted fever rickettsia still not described in Brazil (Norment & Burgdorfer 1984, Breitschwerdt et al. 1987).

Demonstration of structures similar to rickettsiae in the vascular walls of brain tissue, shown by specific immunofluorescence, associated with suggestive pathological alterations observed under hematoxylin/eosin staining, leads us to confirm the diagnosis of Rocky Mountain spotted fever in the index case taken to necropsy.

Thus, the presence of human and canine seroreactivity associated with positive immunofluorescence for *R. rickettsii* in nerve tissue in a fatal case objectively allow us to affirm that there are active foci spotted fever-group rickettsioses in the County of Santa Cruz do Escalvado. Beyond

this affirmation, the findings allow us to formulate some questions that may serve as a point of departure for future investigation.

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