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EVIDENCE OF SPOTTED FEVER GROUP RICKETTSIAE IN STATE OF RIO DE JANEIRO, BRAZIL

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SUMMARY

Ticks were obtained from dogs from February to September of 1999 at weekly intervals, in the County of Piraí, State of Rio de Janeiro. Four hundred seventy four ixodids were taxonomically identified, 103 *Amblyomma cajennense*, seven *Amblyomma ovale*, 209 *Rhipicephalus sanguineus*, and 155 *Amblyomma sp.* An hemolymph test associated with Giemsa's stain revealed two specimens in 163 ticks tested (*R. sanguineus* and *Amblyomma sp.*), containing rickettsia-like organisms. Direct immunofluorescence verified the presence of spotted fever group rickettsia in one specimen of *R. sanguineus*. Considering the limited information on rickettsiosis in Brazil, principally in relation to the vectors involved in perpetuating it *in foci*, these preliminary results give us an idea on the importance of infection in ticks, allowing to expand our knowledge on this zoonosis.

KEYWORDS: Spotted fever; Ticks; Hemolymph test; Brazil

INTRODUCTION

Brazilian spotted fever (BSF), caused by *Rickettsia rickettsii*, is the most significant tick-borne disease in Brazil^{33,46}.

The Cayenne tick^{9,17,47} (*Amblyomma cajennense*) is the most important vector of *R. rickettsii*, and humans are frequently bitten by larvae and nymphs of this arthropod.

Other groups of ixodids have also been implicated as transmitters and/or reservoir of this zoonosis such as *Amblyomma ovale*, *Amblyomma braziliensis*, *Amblyomma cooperi*, *Rhipicephalus sanguineus*^{14,23,29,30,31,32,36,45}.

Although most cases occurred in the Brazilian Southeast region, these ticks are distributed throughout Brazil^{15,19,20,21,28,40,41,42,43}. The absence of officially reported cases of BSF in other regions may be due in part to the lack of confirmatory diagnostic tests and clinical suspicion.

This paper provides preliminary results on rickettsia-infected ticks in an area within the State of Rio de Janeiro, where sporadic cases of BSF has been confirmed since 1970'.

MATERIALS AND METHODS

The County of Piraí was chosen for this study based on three cases of BSF (two in 1977 and one in 1997) which have been confirmed in a neighbouring county, Barra do Piraí, and for the support offered by the County's Health Department to collaborate.

The County of Piraí is located in the Medio Paraíba Industrial Region in the State of Rio de Janeiro (22°37'44"S, 43°53'59"W) that consists of

five districts: Piraí, Monumento, Arrozal, Pinheiral, and Santanésia^{12,16}. Ticks were collected in two neighborhoods, Cacaraia and Serra do Matoso, in the District of Monumento (Fig. 1).

With the help of a veterinarian and two employees of the county's health office, 578 ticks were captured between February and September 1999. The arthropods were collected weekly from dogs and taxonomically identified^{1,2,39} at the Department of Entomology, Laboratory of Ixodids, Oswaldo Cruz Institute. After entomologic analysis, the ticks were submitted to investigation for rickettsiae.

The ticks were disinfected with 10% formaldehyde solution, 70% alcohol and washed in sterile distilled water for five minutes.

For each live tick, two slides were prepared with the hemolymph, fixed in alcohol and acetone for 15 minutes⁵. The first slide was used for investigation of rickettsia-like organisms using Giemsa staining, and the second for the direct immunofluorescence test with *R. rickettsii* positive human serum and fluorescein.

RESULTS

Of the 578 ixodids collected from 129 dogs, 474 ticks were taxonomically identified (Table 1): 103 *A. cajennense*, seven *A. ovale*, 209 *R. sanguineus*, and 155 *Amblyomma sp.* (Table 2).

Hemolymph test and Giemsa staining: Of the 163 ticks submitted to hemolymph test, Giemsa staining was performed in only 153 ixodids (32.2%), due to loss of material (slides). One nymph *Amblyomma sp.* displayed intracellular rickettsia-like particles in the nucleus and the

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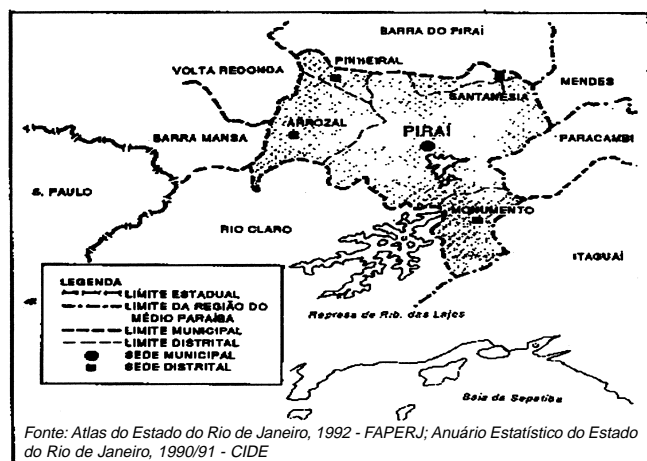
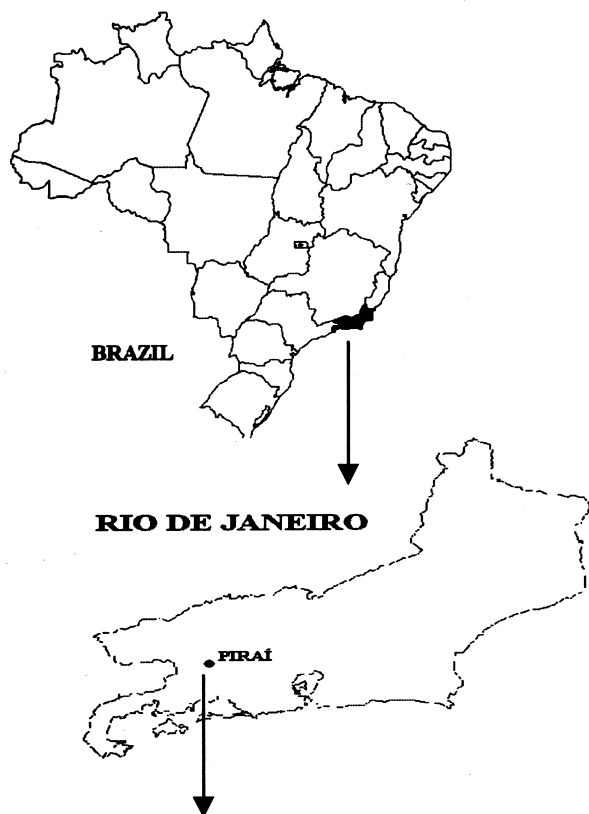


Fig. 1 - County of Pirai (Monumento District), Rio de Janeiro, Brazil.

cytoplasm of the hemocyte and one adult *R. sanguineus* only in the cytoplasm. The prevalence of infection found was 1.3%.

All positive exemplars were collected from Serra do Matoso.

Hemolymph test and direct immunofluorescence: One hundred and sixty three ticks (34.3%) were submitted to hemolymph test associated to direct immunofluorescence. The prevalence of infection to Spotted fever group rickettsiae was 0.6%, detected in one nymph *R.*

Table 1
Number of ticks collected and identified

| Localities | Total no. of ticks/ Total no. of dogs | Total no. of ticks identified (%) |
|-----------------|--|--------------------------------------|
| Cacaria | 122/33 | 121 (99.2 %) |
| Serra do Matoso | 456/96 | 353 (77.4%) |
| TOTAL | 578/129 | 474 (81.8%) |

Table 2
Number of ticks taxonomically identified collected from dogs

| Localities | Tick species | No. identified | | | Total |
|-----------------|----------------------|----------------|------------|------------|------------|
| | | Larvae | Nymph | Adult | |
| Cacaria | <i>A. cajennense</i> | - | - | 12 | 12 |
| | <i>R. sanguineus</i> | 5 | 7 | 90 | 102 |
| | <i>Amblyomma</i> sp | - | 7 | - | 7 |
| Serra do Matoso | <i>A. cajennense</i> | - | 45 | 46 | 91 |
| | <i>A. ovale</i> | - | - | 7 | 7 |
| | <i>Amblyomma</i> sp | 12 | 136 | - | 148 |
| | <i>R. sanguineus</i> | 1 | 5 | 101 | 107 |
| Total | | 18 | 200 | 256 | 474 |

sanguineus specie. This positive exemplar was also collected from Serra do Matoso.

DISCUSSION

The Giemsa staining technique showed microorganisms with shape and size compatible with rickettsia. Although this technique is unspecific, unable to distinguish from other bacterias, the presence of infection in the nucleus of some hemocytes suggest, almost certainly, spotted fever group rickettsiae¹⁰. Another exemplar tested, only one tick presented rickettsia-like organisms, with rounding shape bacillus-like organisms that differed from the short, ovoid forms compatible with *R. rickettsii*. Although significant differences in the prevalence of the infection can be observed during the year, the results showed here are similar from other investigators, that perform studies in different regions in the World^{12,5,26,27,37,38}.

The low prevalence (0.6%) observed in the direct immunofluorescence assay may have been due to the small number of exemplars studied. In spite of this, a similar finding was observed by MAGNARELLI *et al.* (1981)²⁶ in Connecticut. Beside this, various studies on infection in ticks display wide variation, with prevalences ranging from 0.14% to 13.5%^{4,6,11,13,18,24,41,44}.

As this study was performed in a population of dogs, *R. sanguineus* (known as a vector for spotted fever caused by *R. conorii* in Europe) was the most abundant specie in the ixodid fauna studied that was positive by hemolymph test. They are also important because this tick may be implicated in maintaining spotted fever group rickettsiae in nature^{7,8,36}.

Several tick species may have an important role as vector and as reservoir in ecology of spotted fever group rickettsiae^{3,7,34}. In Brazil, there are few studies about the presence of *R. rickettsii* or another spotted fever group rickettsiae although these arthropods are widely distributed throughout Brazilian territory.

Despite the small number of ticks collected and analyzed in this paper (34.3%), the results indicate the existence of circulation of spotted fever group rickettsiae, pathogenic or not, in the region. The occurrence of confirmed cases of BSF in this locality of State of Rio de Janeiro in 1977 and 1997, ratify the affirmation above.

More informations will be obtained through molecular biology³⁵ and isolation techniques^{22,35}, with the purpose of confirming the diagnosis of rickettsiosis in this region.

RESUMO

Evidência de rickettsiae do grupo da febre maculosa no Estado do Rio de Janeiro, Brasil

De fevereiro a setembro de 1999, foram realizadas, semanalmente, coletas de carrapatos de cães no Município de Pirai/RJ. Quatrocentos e setenta e quatro ixodídeos foram taxonomicamente identificados, 103 *Amblyomma cajennense*, sete *Amblyomma ovale*, 209 *Rhipicephalus sanguineus* e 155 *Amblyomma* sp.

O teste de hemolinfa associado à coloração de Giemsa revelou que duas espécies de 163 carrapatos testados (*R. sanguineus* e *A. sp.*) continham microrganismos com morfologia semelhante à rickettsia do grupo da febre maculosa.

No teste de imunofluorescência direta, mais específico, foi verificada a presença de rickettsia do grupo da febre maculosa em uma espécie de *R. sanguineus*.

Considerando que informações sobre rickettsioses no Brasil são limitadas, principalmente com relação aos vetores envolvidos na perpetuação da doença, estes resultados preliminares nos mostram a necessidade da realização deste tipo de estudo, permitindo, desta forma, aumentar nossos conhecimentos a respeito desta zoonose.

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