SPOTTED FEVER IN BRAZIL: A SEROEPIDEMIOLOGICAL STUDY AND DESCRIPTION OF CLINICAL CASES IN AN ENDEMIC AREA IN THE STATE OF SÃO PAULO

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Abstract. During 1985–1995, illnesses clinically and epidemiologically compatible with Brazilian spotted fever were identified in 17 patients in the county of Pedreira, in the state of São Paulo, Brazil. Spotted-fever group rickettsial infection was confirmed by serology and/or immunostaining of tissues in 10 of these patients. Immunostaining confirmed infection in a 37-year-old pregnant patient, although rickettsial antigens were not demonstrable in the tissues of the fetus. A serosurvey was conducted in four localities in the county to determine the prevalence of subclinical or asymptomatic infections with spotted fever group rickettsiae. Five hundred and twenty-five blood samples were tested by an indirect immunofluorescence assay for antibodies reactive with Rickettsia rickettsii. Twenty-two (4.2%) of these samples demonstrated titers \( \geq 1:64 \). The results indicate that Brazilian spotted fever is endemic within this region of Brazil.

INTRODUCTION

Brazilian spotted fever (BSF) is the most prevalent rickettsial disease in Brazil.\(^1,2\) As with Rocky Mountain spotted fever (RMSF) in North America, the disease is caused by Rickettsia rickettsii. In Central and South America, the Cayenne tick (Amblyomma cajennense) is the most important vector for R. rickettsii, and humans are frequently bitten by larvae and nymphs of this tick. The clinical presentation of BSF closely resembles RMSF\(^2\) and typically presents as a sudden onset of fever, malaise, myalgia, headache, chills, and conjunctival injection, followed several days later by a maculopapular rash. Before the development of antibiotics effective against rickettsiae (e.g., tetracyclines and chloramphenicol), case-fatality ratios of patients infected with R. rickettsii were as high as 25–80%.\(^2,6\) Although BSF generally occurs sporadically, geographic and family clusters of disease have been described.\(^2,7\)

Following the initial description of BSF in 1920,\(^7\) relatively few cases were reported in Brazil in the subsequent 60 years. In the last two decades, however, more than 90 confirmed cases have been reported in the southeastern region of the country.\(^8-11\) In addition, many clinically compatible but unconfirmed cases have been reported, reflecting the unavailability of laboratory diagnostic tests for this disease in many regions of Brazil.

Between 1985–1995, 17 patients with disease clinically and epidemiologically compatible with BSF, including eight fatalities, were reported from the county of Pedreira, state of São Paulo. To determine the background seroprevalence of spotted fever group rickettsial infections in this region, a serological survey was initiated among residents in the four Pedreira localities where the majority of BSF cases occurred. This paper summarizes the clinical, pathologic, and epidemiologic findings associated with cases over the 10-year study period, and the results of the serosurvey.

MATERIALS AND METHODS

Case finding for Brazilian spotted fever, 1985–1995. The study was conducted in the county of Pedreira, located in the southeast region of São Paulo, Brazil. Clinical and epidemiological data were obtained from clinical records of all patients admitted to Pedreira County Hospital, Hospital of the Campinas University, and Amparo County Hospital between 1985 and 1995. Data were summarized on all patients evaluated during this period who presented with an acute febrile illness associated with rash and a history of recent tick exposure.

Serologic information. Acute-phase serum samples were available for evaluation for 12 patients. Three patients were tested for febrile agglutins by using the Weil-Felix (WF) test. The cut-off for a positive result was considered a titer \( \geq 1:80 \). Serum specimens from nine patients were evaluated for IgM and IgG antibodies reactive with R. rickettsii by using an indirect immunofluorescence assay (IFA) as previously described.\(^1,2\) Endpoint titers were recorded as the reciprocal of the highest dilution exhibiting specific rickettsial fluorescence. Patients with titers \( \geq 1:64 \) were considered seropositive.

Histopathological study. For patients with clinically and epidemiologically compatible disease identified by case finding, a review of pathology files from the three hospitals was conducted to identify formalin-fixed, paraffin-embedded biopsy and autopsy specimens obtained between 1985 and 1995 from suspect BSF patients. Tissues from six patients with presumed BSF were evaluated by using routine hematoxylin and eosin (HE) stain and by specific immunofluorescence and immunohistochemical stains.

Indirect immunofluorescence (IF) staining for spotted fever group rickettsiae was performed by using a previously described method,\(^1,3,11\) with some modifications. Briefly, after a blocking step using 10% bovine serum albumin for 20 min at room temperature, sections were incubated with human
antibody obtained from a seropositive patient (IFA titer of ≥ 1:1,024) diluted 1:2 in PBS, pH 7.4 for 90 min at room temperature. After being washed, slides were incubated at room temperature in fluorescein-labeled rabbit antihuman IgG (Sanofi Diagnostic Pasteur, Paris, France) diluted 1:5, for 60 min. For each test, a series of three controls were run in parallel with the patient tissue: a) omission of the human antibody; b) omission of the fluorescein-labeled rabbit anti-human IgG conjugate; and c) substitution of normal human sera (inactivated at 56°C for 30 min) for the primary antibody. As an additional control, paraffin-embedded tissues from a patient with fatal, culture-confirmed meningiococcal meningitis were evaluated with each run.

Patient tissues were additionally evaluated at the Centers for Disease Control and Prevention (CDC) by using an indirect immunofluorescence (IHF) stain for spotted fever group rickettsiae, as described previously. Briefly, tissue sections were digested with proteinase-K and then incubated with a polyclonal rabbit anti- R. rickettsii antibody. This step was followed by incubation in biotinylated swine anti-mouse and anti-rabbit antibody, followed by an incubation in alkaline phosphatase-conjugated streptavidin. Rickettsial antigens were detected using a naphthol/fast red substrate.

Serosurvey. During 1994, whole blood samples were collected from apparently healthy volunteers at four study sites selected for the survey because of their proximity to the locations of the majority of identified BSF cases (Figure 1). These included The Workers’ Colony of Nadir Figueiredo Industry (NF), Fortaleza Farm (FF), Jaguari Farm (JF), and the village of São Nilo (SN), located along Jaguari River, 3 km from Pedreira City. Serum samples were collected from a total of 525 persons in NF, FF, and JF and were represented by 93% (379), 95% (20), and 82% (18) of the total inhabitants from each study site, respectively. In the village of São Nilo serum specimens were collected from 108 (23%) of 470 total inhabitants who agreed to participate in this study. Serum samples were separated by centrifugation and stored at −20°C until tested. Serum was evaluated by using IFA, as previously described.

RESULTS

Case findings of BSF patients, 1985–1995. Seventeen patients identified from a review of hospital records demonstrated clinical and epidemiological findings compatible with BSF. A laboratory diagnosis of spotted fever group rickettsial infection was available for 10 patients, including 3 by Weil-Felix assay, 5 by IFA, and/or 5 by IF or IHC staining of tissues (Tables 1 and 2). Except for a single patient (patient 13), who was a visitor to the area, all patients resided in the county of Pedreira. When a population denominator obtained by averaging the population of Pedreira in the census between 1980 and 199116 was used, the median annual rate of occurrence of confirmed cases of BSF was 3.68/100,000/year, from 1985 through 1995. When all the cases (probable and confirmed) were considered, the median annual rate of occurrence of BSF was 6.07/100,000/year with the highest rate in males who showed an annual rate of 11.41/100,000/year. Only three patients were female (annual rate of occurrence of 2.45/100,000/year). All the patients were white and 16 (94%) of the patients became ill between June and October. Patients ranged in age from 3 to 59 years (median of 28 years).

All the patients were febrile. Headache, rash, and myalgias were reported for 16 (94.1%), 15 (88%), and 10 (77%) of patients, respectively (Table 1). Three surviving patients (cases 1, 11, and 16) had illnesses complicated by pneumonia, digital and scrotal gangrene, and acute renal failure. One or more findings of encephalopathy (e.g., headache, seizures, confusion, and/or coma) were observed in 16 (94%) patients. Six patients had leukocytosis (leukocyte count > 10×10⁹/L, median 14.3×10⁹/L). Three of seven patients tested demonstrated thrombocytopenia (platelets < 150×10⁹/L, median 41.3×10⁹/L). One patient (patient 16) showed marked elevations in hepatic enzyme levels, including an aspartate aminotransferase level of > 800 U. Of nine patients treated with chloramphenicol, eight became afebrile within the first five days of treatment and one patient (case 13), who received therapy on the tenth day of illness, died. None
of them was treated with tetracyclines. A total of eight deaths occurred.

Weil-Felix agglutinin titers of 1:200 (range 200–1,600) were observed in three patients (patients 1, 2, and 10); each of these patients received chloramphenicol and recovered. Serum samples from five of nine patients tested by IFA had antibody reactive with *R. rickettsii* in the endothelium and smooth muscle of dermal blood vessels (patients 8, 11, 16). One biopsy (case 13) showed no specific immunofluorescence. Indirect immunofluorescence staining was positive in the walls of blood vessels and was interpreted as inconclusive. Indirect immunofluorescence staining was detected in tissues of three patients (cases 8, 11, 13; Tables 1 and 2). Specific IF staining was detected in autopsy tissues in two patients, case 6 (Figure 2A) and case 13 (Figure 2B), and negative in one patient (case 6). Indirect immunofluorescence staining of tissues from case 13 showed diffuse immunofluorescence in the walls of blood vessels and was interpreted as inconclusive. Indirect immunofluorescence staining was positive in autopsy tissues in two patients, case 6 (Figure 2A) and case 13 (Figure 2B), and negative in one patient (case 5). Indirect immunofluorescence and IHC stains for spotted fever rickettsiae were discordant for three patients (cases 8, 11, 13; Tables 1 and 2).

**Table 1**
Epidemiology and clinical features of Brazilian spotted fever from County of Pedreira, São Paulo, 1985–1995

<table>
<thead>
<tr>
<th>UPN/Ag/Sex</th>
<th>date of onset</th>
<th>skin rash</th>
<th>myalgia</th>
<th>splenomegaly</th>
<th>edema</th>
<th>jaundice</th>
<th>coma/shock</th>
<th>IF</th>
<th>WF</th>
<th>IHC</th>
<th>outcome</th>
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<tbody>
<tr>
<td>01/35/M</td>
<td>09/85</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>NA</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>02/27/M</td>
<td>09/85</td>
<td>+</td>
<td>+</td>
<td>NA</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>+</td>
<td>NA</td>
<td>R</td>
</tr>
<tr>
<td>03/57/M</td>
<td>09/85</td>
<td>+</td>
<td>+</td>
<td>NA</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>NA</td>
<td>D</td>
<td></td>
</tr>
<tr>
<td>04/11/M</td>
<td>06/86</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>05/12/M</td>
<td>08/86</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
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<td>–</td>
<td>–</td>
<td>D</td>
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<tr>
<td>06/27/M</td>
<td>08/86</td>
<td>+</td>
<td>NA</td>
<td>+</td>
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<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>D</td>
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<tr>
<td>07/57/M</td>
<td>08/86</td>
<td>+</td>
<td>NA</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>NA</td>
<td>R</td>
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<tr>
<td>08/56/M</td>
<td>06/87</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>+</td>
<td>ND</td>
<td>–</td>
<td>NA</td>
<td>R</td>
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<tr>
<td>10/03/F</td>
<td>10/88</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>–</td>
<td>–</td>
<td>ND</td>
<td>+</td>
<td>R</td>
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<tr>
<td>11/29/M</td>
<td>07/93</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>NA</td>
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<tr>
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<td>08/93</td>
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<td>ND</td>
<td>D</td>
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<tr>
<td>13/37/F</td>
<td>09/93</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>ND</td>
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</tr>
<tr>
<td>14/28/M</td>
<td>09/93</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
<td>NA</td>
<td>D</td>
<td></td>
</tr>
<tr>
<td>15/20/M</td>
<td>09/94</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>–</td>
<td>D</td>
<td></td>
</tr>
<tr>
<td>16/44/M</td>
<td>04/95</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>R</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>17/04/M</td>
<td>10/95</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
<td>NA</td>
<td>R</td>
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</tbody>
</table>

**Table 2**
Immunohistochemical staining of cases of Brazilian spotted fever from County of Pedreira, São Paulo, 1985–1995

<table>
<thead>
<tr>
<th>UPN</th>
<th>Sex/age</th>
<th>Tissue examined</th>
<th>Result</th>
<th>Immunohistochemical staining</th>
<th>Tissue examined</th>
<th>Result</th>
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<tbody>
<tr>
<td>5</td>
<td>M/12</td>
<td>lung</td>
<td>negative</td>
<td>lung, nervous tissues</td>
<td></td>
<td>negative</td>
</tr>
<tr>
<td>6</td>
<td>M/27</td>
<td>nervous tissues</td>
<td>positive</td>
<td>nervous tissues</td>
<td></td>
<td>positive</td>
</tr>
<tr>
<td>8</td>
<td>M/56</td>
<td>skin*</td>
<td>positive</td>
<td>skin*</td>
<td></td>
<td>negative</td>
</tr>
<tr>
<td>11</td>
<td>M/27</td>
<td>skin*</td>
<td>positive</td>
<td>skin* kidney, pancreas, large vein, liver, and skeletal muscle</td>
<td>skin*</td>
<td>positive</td>
</tr>
<tr>
<td>13</td>
<td>F/37</td>
<td>skin* liver, and nervous tissues</td>
<td>not specific, inconclusive</td>
<td>skin* kidney, pancreas, large vein, liver, and skeletal muscle</td>
<td>skin*</td>
<td>positive</td>
</tr>
<tr>
<td>16</td>
<td>M/44</td>
<td>skin*</td>
<td>positive</td>
<td>fetal tissues</td>
<td></td>
<td>negative</td>
</tr>
</tbody>
</table>

UPN = unique patient number; * biopsy material.
FIGURE 2. A). Photomicrograph of vascular endothelium from the hippocampus of a patient with fatal Brazilian spotted fever (BSF) (Case #6), showing intracytoplasmic staining of rickettsial antigens. B). Photomicrograph of the kidney of patient with fatal BSF (Case #13) demonstrating positive staining for spotted fever group rickettsiae in a renal interstitial vessel. Both samples were stained with immunoalkaline phosphatase stain using naphthol phosphate/fast red substrate and hematoxylin counterstain, original magnification × 158.
localities combined. The majority of participants were white (94.5%); 257 (49%) were males and 268 (51%) were females. Five hundred and seven (97%) had lived in the study area for at least three years. Twenty-two (4.2%) individuals demonstrated serum antibody reactive with *R. rickettsii* at a titer of ≥ 1:64 (range 64–512). Of the 22 seropositive individuals, 19, 2, and 1 individuals were from Nadir Figueiredo, São Nilo, and Fortaleza Farm, respectively. The seroprevalence of the infection in males and females was 3.9% and 4.5%, respectively. Only one (4.5%) of 22 seropositive individuals recalled a spotted fever-like illness preceding the survey.

**DISCUSSION**

The first case of laboratory-confirmed BSF was identified in the county of Pedreira in 1985, although anecdotal evidence suggests that this rickettsiosis has existed in the region for at least 100 years. The Pedreira County Bills of Mortality from the late 1890s describe fatal disease caused by “tick typhus” and “big measles” (a colloquial name for BSF). However, BSF appears to have been unreported in this area of Brazil between the late 1930s and 1985. The absence of formally reported cases of BSF in Pedreira during this period is intriguing and may be due in part to masking of cases by the widespread use of chloramphenicol and tetracycline for acute febrile illnesses. It is also possible that cases of BSF have been missed because of waning clinical suspicion and the paucity of confirmatory diagnostic resources. Finally, the apparent emergence (or reemergence) of BSF in this region may reflect changes in the number of human exposures to naturally infected tick populations, mainly *A. cajennense*, resulting from decreases in the development of farmlands, increasing suburbanization, and increasing human incursions into tick-infested habitats.

The clinical characteristics of BSF seen in the study area did not differ appreciably from the clinical features of the disease described in patients from other regions of Brazil. Complications, notably renal failure, coma, and thrombocytopenia, were observed in patients who either delayed seeking treatment or who had initial diagnoses other than BSF. The majority of patients needed prolonged hospitalization, parenteral antibiotic therapy, and supportive care. The high mortality rate (47%) was associated with misdiagnosis and subsequent delays in treatment. None of the eight fatal cases received effective anti-rickettsial therapy and all died between the tenth and fourteenth day of illness. These data reaffirm the importance of early recognition of disease and prompt initiation of appropriate anti-rickettsial therapy to prevent serious complications and mortality attributable to *R. rickettsii*.

For all 17 patients, the clinical and epidemiologic findings were compatible with BSF. The BSF diagnosis was supported by one or more laboratory tests in 10 of 14 patients for whom testing was available. Only two of eight fatalities were confirmed by laboratory tests. Serum and/or tissues were unavailable for testing for three of the seven unconfirmed, and four of the seronegative patients expired relatively early in the course of infection, presumably before the development of specific antibody reactive with *R. rickettsii*. However, because many other infectious diseases mimic spotted fever infection, it is possible that one or more of the seven unconfirmed patients had diseases other than BSF.

Most cases occurred between the months of June and October, coincident with peak larval and nymphal feeding activities of *A. cajennense*. Familial clustering was observed among seven patients in two separate families; four deaths were noted in these clusters. Although three of these deaths were unconfirmed by laboratory tests, positive immunostaining for rickettsiae in autopsy tissues from one of the family members and positive serologic test in two surviving patients suggested a rickettsial etiology for all four deaths. Clusters of rickettsial spotted fever involving families have been reported in Brazil since the beginning of this century.

Sero logic survey of inhabitants from regions of presumed BSF endemicity in the county of Pedreira revealed that 4.2% of the population had prior exposure to *R. rickettsii* or to an antigenically related spotted fever group rickettsiae, indicating that spotted fever group rickettsiae are endemic in this region of Brazil.

For three patients, results of IF staining and IHC staining of skin biopsies for *R. rickettsii* did not agree. For one patient for whom the IF stain was positive and IHC stain was negative, tests were performed on separate tissue blocks. For another patient for whom IF staining was negative, yet IHC was positive, the biopsy consisted of only a scant fragment of tissue, making interpretation difficult. These discrepant results may reflect the characteristically focal distribution of rickettsial antigens observed between separate biopsies, or even within separate sections from the same biopsy.

Autopsy specimens from a woman who died from laboratory-confirmed BSF demonstrated intense positive IHC staining for spotted fever group rickettsial antigen in multiple tissues. However, rickettsiae were not observed in similarly stained tissues of the fetus. Rickettsial infections during pregnancy are rarely reported, and the direct effects of maternal *R. rickettsii* infection on the fetus are unknown. However, the findings in this study suggest that this pathogen did not initiate infection in the fetus. Although the transplacental passage of rickettsiae has been suggested, limited data from human and laboratory animal infections indicate that maternal rickettsial infections are not transmitted to the fetus.

Information obtained in this study, coupled with reports of seropositive domestic and wild animals and isolations of spotted fever group rickettsiae from ticks collected in this region, emphasize the need for increased awareness of rickettsial infections in this region of Brazil. Patients presenting with flu-like illnesses between June and October in this region and in other *R. rickettsii*-endemic regions in Brazil should be carefully evaluated for rickettsial infection. Working from a presumptive diagnosis and implementing prompt, specific anti-rickettsial therapy may reduce the high mortality observed with spotted fever cases in Brazil.

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