

Characterization of *Rickettsia rickettsii* in a Case of Fatal Brazilian Spotted Fever in the City of Rio de Janeiro, Brazil

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A lethal case of Brazilian spotted fever (BSF) is presented. Clinical features were initially of gastrointestinal involvement and evolved with progression to septic shock, meningoencephalitis and death on the 6th day of illness. Indirect immunofluorescence assay (IFA) for spotted fever group rickettsia (SFGR) was non-reactive. Diagnosis was confirmed by the polymerase chain reaction (PCR) and the nucleotide sequencing of a fragment of the ompA gene showed 100% homology to *Rickettsia rickettsii*. BSF has not been reported in the city of Rio de Janeiro in the last three decades, and the present description should alert the clinicians to its presence in urban Rio de Janeiro, and to the differential diagnosis with dengue fever, gastroenteritis, leptospirosis and bacterial septic shock, among others. Key-Words: Brazilian spotted fever, spotted fever group rickettsia, *Rickettsia rickettsii*, lethal case, Rio de Janeiro city, indirect immunofluorescence, polymerase chain reaction, central nervous system involvement.

Brazilian spotted fever (BSF) is a systemic disease caused by *Rickettsia rickettsii*, a bacterium transmitted by the horse tick *Amblyomma cajennense*. It is endemic in the Southeast of Brazil (Rio de Janeiro, São Paulo, Minas Gerais and Espírito Santo states) and affects exposed children and adults [1-4]. Case presentation mimics several conditions which are endemic in the area, such as dengue fever, leptospirosis, gastroenteritis, meningococcal meningitis and severe sepsis [1-4]. This paper reports a fatal case of BSF, occurring in July, in the metropolitan area of Rio de Janeiro, with prominent sepsis, rash and neurological and cerebrospinal fluid (CSF) findings.

Case Report

A 48 year-old white male presented, six days prior to hospital admission, with acute onset of high-grade fever, myalgia, headache, nausea, vomiting and diarrhea. He was a heavy smoker, but past medical history was otherwise unremarkable. He sought medical attention and was treated symptomatically. Two days later his symptoms persisted and he developed jaundice: he was given sulfamethoxazole-trimethoprim and was sent home. The following day he sought the Emergency ward because of hematemesis and melena; he was oliguric. He was transferred to an infectious diseases reference hospital with the presumptive diagnosis of leptospirosis. He was a porter in the north area of Rio de Janeiro, and had frequent contact with rodents. He had been bitten by ticks in Campo Grande (west area of Rio Janeiro) two weeks previously, while visiting his brother, who was a horse cart driver. On arrival he was jaundiced, in deep coma with no neck stiffness, systolic

blood pressure was 60 mmHg, heart rate =128 bpm, and a generalized purpuric rash was noted. He was intubated, mechanically ventilated, given rapid intravenous fluid, noradrenaline, ceftriaxone, oxacillin and chloramphenicol. Myoclonus was observed, and two hours after admission he presented a generalized tonic-clonic seizure. Initial investigation showed leukocytosis (18,200 cells/mm³, differential count: 58% polymorphs, 14% band forms), platelet count 90,000/mm³, hemoglobin = 13.9 g/dL, glucose 129 mg/dL, creatinine 4.4 mg/dL, AST = 314 IU, ALT = 124 IU; blood gas analysis showed metabolic acidosis.

He died six hours after admission; post mortem lumbar puncture was performed. Cerebrospinal fluid (CSF) analysis showed 213 cells per 100 powerfields, with 70% of polymorphs, protein > 1 g/dL, glucose = 11 g/dL. Gram and Indian ink stains were normal. Latex tests for *Cryptococcus neoformans*, *Haemophilus influenzae*, *Neisseria meningitidis* and *Streptococcus agalactiae* were negative. CSF culture was negative at 48 hours; blood cultures were negative after seven days incubation.

Indirect immunofluorescence assays (IFA) for spotted fever group rickettsia (SFGR) (Panbio[®])-specific immunoglobulin G (IgG) antibodies were performed on serum and CSF.

Polymerase chain reaction (PCR) was performed on whole blood and serum using four previously described oligonucleotide primer pairs as shown in Table 1 and Figure 1 [5,6].

The sequence data of the PCR products were analyzed using the BLAST 2.0 program (National Center for Biotechnology Information) for homology search. The determined sequences were then analyzed for phylogenetic relationships with other sequences registered in the GenBank.

Family members of the index case, who lived where exposure to horse ticks occurred, were recalled for history, physical examination and serology for SFGR two months after the patient's death.

Ticks were captured from grass and garden near the house; they were identified taxonomically, and individually frozen at

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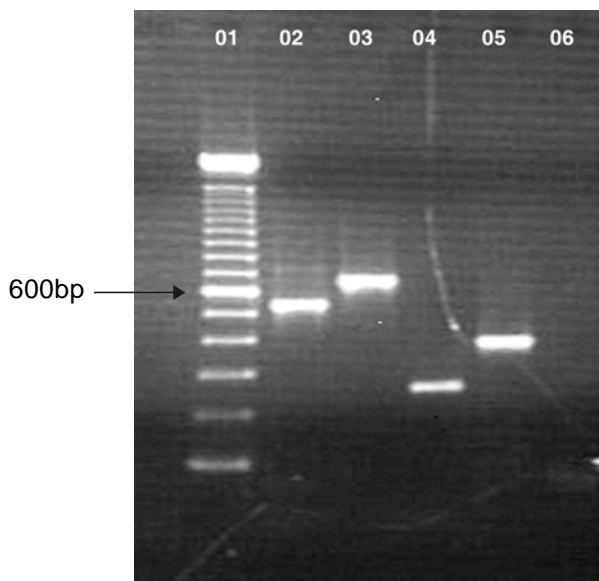
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Table 1. Primers used for detection of spotted fever group rickettsia in a PCR assay [5,6]

Primer	Sequence (5'-3')	Amplicon size
Rr190-70	ATGGCGAATATTTCTCCAAAA	532 pb
Rr190-602	AGTGCAGCATTCGCTCCCCCT	
BG1-21	GGCAATTAATATCGCTGACGG	650 pb
BG2-20	GCATCTGCACTAGCACTTTC	
RpCS877	GGGGCCTGCTCACGGCGG	381 pb
RpCS1258	ATTGCAAAAAGTACAGTGAACA	
TZ15	TTCTCAATTCGGTAAGGGC	246 pb
TZ16	ATATTGACCAGTGCTATTTC	

Figure 1. PCR analysis of whole blood of a fatal BSF case in the city of Rio de Janeiro. Agarose gel electrophoresis, with ethidium bromide, showed the 532 bp fragment amplified with the rompA-specific set of primers (lane 2). Lane 03 - 650 bp fragment amplified with the rompB-specific set of primers. Lane 04 - 246 bp fragment amplified with the 17 kDa-protein-specific set of primers. Lane 05 - 381 bp fragment amplified with the citrate synthase-specific set of primers. Lane 06 - negative control. Lane 01 - ladder 100 bp.



-70°C until processed for DNA extraction. No ticks were collected from horses as they had been applied insecticide.

IFA for SFGR was non-reactive in the patient's serum and CSF. PCR from whole blood was positive for *R. rickettsii* and sequencing of the product amplified from the ompA gene showed 100% homology to *Rickettsia rickettsii* sequence available at the GenBank.

Eight family members, all of which lived in the same neighbourhood where the patient acquired his illness, were enrolled. Their ages varied between ten and 60 years. None presented clinical illness in the preceding two months although all reported frequent tick bites. All had non-reactive SFGR IFA.

Twenty-nine ticks (five *Amblyomma cajennense*, two *Rhipicephalus sanguineus*, and 22 *Anocentor nitens*) were

collected from grass and garden near the house. All ticks showed negative PCR results for SFGR.

Discussion

This previously healthy middle aged man presented clinical features of severe BSF following tick bites in the peak of tick reproduction, when most cases of BSF are reported. Several aspects deserve attention: he had features of a gastroenteritis-like illness and was given sulfonamides, and as previously described, this probably made his outcome worse. He progressed rapidly to acute renal failure, purpuric rash, septic shock and neurological symptoms, manifested as seizure and coma. His spinal fluid analysis showed neutrophilia and low glucose levels. CSF findings in spotted fever group rickettsial disease are diverse: pleocytosis with predominant polymorphs (as in this case), predominance of eosinophils or predominance of lymphocytes. CSF may also be unremarkable [4,7-10]. The predominant cell type in CSF may be related to the timing of CSF analysis; a report on tick-borne encephalitis showed an average of 570 cells with 60% polymorphs in the first three days of illness, and a predominance of mononuclear cells from the fifth day onwards [9].

Another issue relates to jaundice: it is noted more often in BSF than in Rocky Mountain spotted fever, possibly because more adult males (with hepatitis B or hepatitis C co-infection, alcohol use or G6PD deficiency) are present in Brazilian series [3].

Chloramphenicol was started intravenously when he was admitted to hospital because there is no intravenous formulation of doxycycline in Brazil, which would be the best choice in such a severe presentation.

Another important aspect to consider is the geographic location: BSF is no longer a rural endemy, but affects inhabitants of large cities such as Rio de Janeiro and São Paulo, making it important to train doctors to think of this diagnosis, as lethality is high (over 40%) with delayed start of antibiotics. BSF is a public notifiable illness since 2001 in Brazil, and the public, including tourist agencies, need to be made aware. No cases had been reported in the city of Rio de Janeiro since 1981, although nearby towns, such as Petrópolis, sought for weekend leisure and tourism, has recently (in 2005) had an outbreak of BSF [11]. The patient's

history of tick bites was clear, but was not elicited until he was seen at a referral hospital.

The last case reported from the city of Rio de Janeiro occurred in 1977; the report [4] accounted for two cases, both involving young males (34 and 44 years old) with cutaneous vasculitis. One of them had meningoencephalitis with abnormal CSF findings. Both were cured with intravenous chloramphenicol.

A complementary search for serological *R. rickettsii* infection in household contacts (all with tick bites) and for molecular evidence of infection in ticks from the area was performed and the results were negative: this is not surprising, because family clusters of BSF occur rarely and the prevalence of rickettsia in ticks, even in endemic areas, is low [1].

Finally, although IFA is the gold standard for diagnosis, molecular diagnosis is crucial in these rapidly fatal cases as IFA may be negative since antibody detection usually takes over ten days [12-15]. The three rickettsial reference laboratories in Brazil, located in the states of Minas Gerais, Rio de Janeiro and São Paulo, are apt to perform molecular biology tests. BSF clinical aspects and the rickettsial reference labs' abilities to make molecular diagnosis ought to be made more widely known, mainly in endemic areas where other infectious diseases as leptospirosis and dengue fever may be misdiagnosed as BSF.

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