SHORT COMMUNICATION

Detection of Brazilian spotted fever infection by polymerase chain reaction in a patient from the state of São Paulo

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Brazilian spotted fever (BSF) cases have been increasing in the state of São Paulo but no genomic information about local rickettsia isolated from humans has been well documented. We recovered spotted-fever group rickettsiae from a sample of patient blood cultured in Vero cells using the shell vial technique. Rickettsial DNA fragments (glt*A*, omp*A, and,* omp*B genes) were detected, and analysis of the ompB gene base sequences showed identity with the* Rickettsia rickettsii omp*B sequence available in the GenBank.*

Key words: Brazilian spotted fever - *Rickettsia rickettsii* - *omp*B gene

Brazilian spotted fever (BSF) is a rickettsiosis very similar to the North American Rocky Mountain spotted fever (RMSF) caused by *Rickettsia rickettsii*, which is maintained in nature in a cycle involving ticks and mammals. The initial signs and symptoms of the disease include the sudden onset of fever, headache, and muscle pain, followed by the development of a rash (Lemos et al. 2001). This disease can be difficult to diagnose in its early stages, and without prompt and appropriate treatment it can be fatal.

BSF cases are known to occur in the Southeastern Brazilian states of São Paulo, Minas Gerais, Rio de Janeiro, and Espírito Santo (Sexton et al. 1993, Lemos 2002, Galvão et al. 2003). Although several Ixodid tick species of the genus *Amblyomma* may be involved in the BSF cycle, *A. cajennense* is considered the main vector species (Magalhães 1952, Lemos 2002). Isolated reports from areas of the country that are far apart, with differing ecology, suggest that the disease may be more common than is shown by existing data.

In recent years, an upsurge of cases has been recorded in the state of São Paulo, particularly in the Atibaia, Jaguari, and Camanducaia river basins, principally in the Pedreira municipality (Lemos et al. 2001). Increased pressure on the remaining forests and a population growth of the mammalian tick host, the capybara (*Hydrocherus hydrocherus*), could be causes for the apparent increase in human cases. The majority of studies of *R. rickettsii* characteristics rely on serology and immunostaining of tissues and culture, individually or in combination (Melles et al. 1999, Lemos et al. 2001), but no genomic information about local rickettsia isolated from humans has been well documented. From the ticks *A. cajennense* and *A. cooperi* collected in this focus, Spotted Fever Group (SFG) rickettsiae were isolated by culture in Vero cell and confirmed by immunofluorescent antibody assay (Lemos et al. 1996, Nascimento 2003), but none of these studies went as far as species identification.

We report the use of molecular biology methods for the identification of BSF-causing-*Rickettsia,* in this important Brazilian endemic area. The protocol utilized was approved by the Ethical Committee on Human Experimentation of the Instituto de Ciências Biomédicas (Biomedical Sciences Institute/USP).

The patient was a five-year old boy from Artur Nogueira, a municipality of the state of São Paulo, with history of recent tick exposure. During the course of the disease the patient presented fever, malaise, myalgia, headache, abdominal pain, and maculopapular rash. The blood sample was collected six days after the onset of symptoms in September 1998. The sample clot was diluted to a 10% suspension in brain heart infusion broth (BHI) and was stored at –70ºC in the Laboratory of the Adolfo Lutz Institute (IAL) until the use. For cultures, blood clot aliquots were centrifuged and the supernatant was inoculated in a confluent monolayer of Vero cells on circular slides adapted to the flat-bottomed tubes (shell vials) as previously described (Melles et al.1999). Infection of Vero cells was monitored by immunofluorescence reaction prepared with *R. rickettsii*-positive human serum, which permitted us to observe the presence of fluorescent microorganisms in form of intracellular bacteria (Marrero & Raoult 1989). Part of the remainder blood clot sample was used for rickettsial gene access. No patient serum was available for serology.

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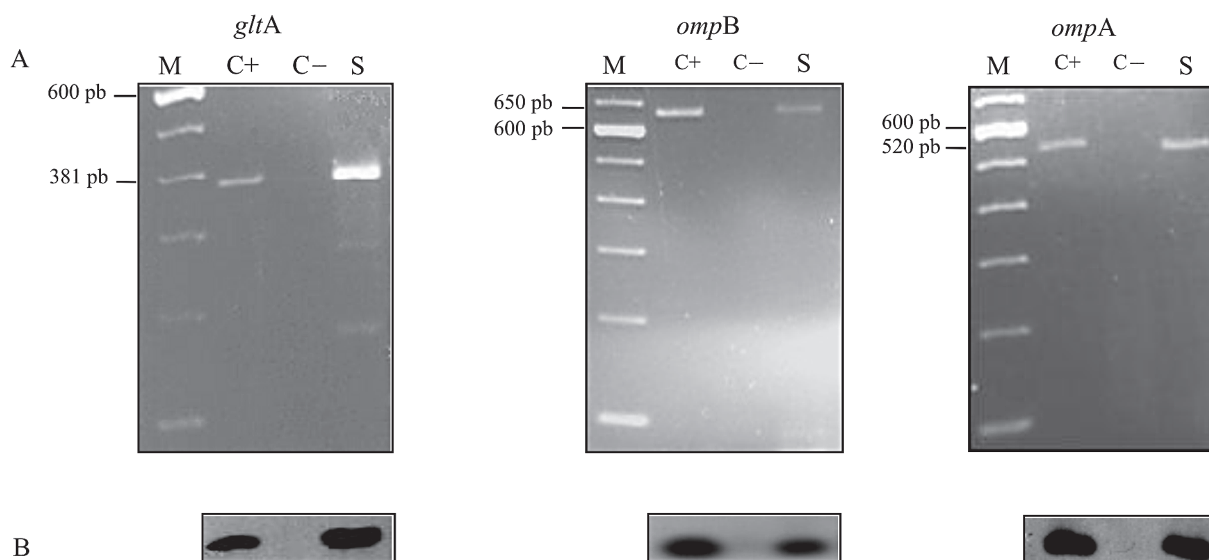
Prior to DNA extraction through the use of phenol/phenol-chloroform, the frozen blood clot was incubated at 56ºC for 2 h for rickettsiae inactivation (Tzianabos et al. 1989). Rickettsial DNA was detected by polymerase chain reaction (PCR) using previously described conditions (Regnery et al. 1991) and the three sets of primers (GIBCO BRL), *Rp*Cs.877p (5'- GGGGGCCTGCTCACGGCGG) and *Rp*Cs.1258n (5'- ATTGCAAAAAGTACAGTGAACA) to amplify a 381-bp fragment of the citrate synthase gene (*glt*A) of *Rickettsia* species (Wood et al. 1987); *Rr*190.70p (5'-ATGGCGAATATTTCTCCAAAA) and *Rr*190.602n (5'-AGTGCAGCATTCGCTCCCCCT) for a 532-bp fragment of the 190-kDa surface protein gene (o*mp*A) of SFG rickettsia (Regnery et al. 1991); and BG1-21 (5'-GGCAATTAATATCGCTGACGG) and BG2-20 (5'- GCA TCTGCACTAGCACTTTC) for a 650-bp fragment of the 120 kDa surface protein gene (*omp*B) of SFG and Typho Group rickettsiae (Eremeeva et al. 1994). PCR products to be sequenced were cloned into plasmid vector pGEMT-Easy (Promega). *Escherichia coli* strain DH5 α cells were transformed by the method described by Sambrook et al. (1989). The transformants carrying the vector with the DNA insert were screened by color on plates containing X-Gal, IPTG and ampicilin. The clones were digested with the restriction endonuclease *Eco* RI. The sequence analysis was carried out using the Thermo Sequenase Fluorescent Labelled Primer Cycle Sequencing kit (Amersham Pharmacia) and an ALF express automatic sequencer (Amersham Pharmacia) with ALFwin 2.1 software. The sequence of both DNA strands was determined twice.

For Southern blot analysis, 10 µl of PCR product was separated by electrophoresis on a 2% agarose gel and transferred to a positively charged nylon membrane (Hybond N^+, Amersham-Pharmacia Biotech) (Sambrook et al. 1989). Hybridization and signal detection were performed using the Gene Images labelling and detection kit (Amersham-Pharmacia-Biotech).

The PCR amplification of 381 bp, 650 bp, and 532 bp fragments respectively for *glt*A, *omp*B, and *omp*A genes, and the positive hybridization shown in the Southern blot (Figure) confirms the SFG rickettsia propagation through cell Vero culture. The sequence analysis of *omp*B gene fragment showed 98% identity with *R. rickettsii* (GenBank access number Gi46939). The GenBank accession number for the partial *omp*B reported in this communication is AY751299 (*R. rickettsii* strain Brazil).

Recently, molecular techniques were used to detect *Rickettsia* in blood sucking arthropods collected in the BSF transmission foci in the state of São Paulo. The rickettsial *glt*A gene fragments were detected in *A. cooperi*, by PCR, and the sequence analyzed showed homology with *R. belli*, a non-pathogenic rickettsia species (Horta 2002, Estrada 2003). The *omp*A gene of SFG rickettsia was accessed in *A. cajennense* collected from different localities (Nascimento 2003). *R. felis*, a pathogenic SFG rickettsia, was detected in *Ctenocephalides felis* collected from local domestic dogs (Horta 2002). In addition, *R. felis* was detected in *Ctenocephalides* fleas (Oliveira et al. 2002) in the state of Minas Gerais, Brazil, employing these same molecular techniques, collected in an area where patients with *R. felis* rickettiosis were reported (Raoult et al. 2001, Galvão et al. 2004). These findings suggest that, although *R. rickettsii* is accepted as a BSF causing agent other rickettsiae species or different *R. rickettsii* variants may be involved, playing roles as yet to be determined.

Based on our present result, we can say that *R. rickettsii* can be one of the etiological agents of BSF in the state of São Paulo and the use of PCR amplification of the *omp*B gene coupled with partial DNA sequencing can be used as a valuable technique for establishing a definitive diagnosis of BSF during the critical stage. For a better characterization of the BSF agent sequence analysis of the amplified *omp*A and *glt*A fragments is in progress.



A: polymerase chain reaction of *glt*A, *omp*B, and *omp*A genes from Brazilian patient blood. M: weight molecular 600 base pair; C+: positive control *Rickettsia rickettsii,* Sheila Smith strain; C–: negative control water; and S: human blood. B: Southern blot analysis of the PCR products, using the fluorescein labelled fragment of *glt*A, *omp*B, and *omp*A genes as probes to their respective blots.

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