



Confirming *Rickettsia rickettsii* as the etiological agent of lethal spotted fever group rickettsiosis in human patients from Espírito Santo state, Brazil

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ABSTRACT

Although Espírito Santo state is considered an endemic area for Brazilian spotted fever (BSF) with related lethal cases, it also constitutes the only state of southeastern Brazil that currently lacks a specific confirmation of the specific rickettsial agent. In an attempt to a species level confirmation of the etiological agent of fatal rickettsiosis cases in Espírito Santo state, in this study we tested human sera obtained between 2015 to 2017 by means of qPCR and subsequent conventional PCR protocols targeting *gltA* (citrate synthase) and *ompA* (190-kDa outer membrane protein) rickettsial genes. All samples were found to contain rickettsial DNA through the citrate synthase qPCR protocol. By conventional PCR, rickettsial *gltA* and *ompA* specific DNA fragments were detected in 25% (one sample) and 50% (2 samples) of the screened sera, respectively. Obtained consensus for each gene partial sequences were 100% identical to *Rickettsia rickettsii* *gltA* and *ompA* genes. The present study confirms for the first time *R. rickettsii* as the etiological agent of a lethal spotted fever group rickettsiosis in human patients from Espírito Santo state.

1. Introduction

Spotted fever group (SFG) rickettsioses are tick-borne infectious diseases caused by pathogenic species of the genus *Rickettsia* (Parola et al., 2013). In Brazil, *Rickettsia rickettsii* and *Rickettsia* sp. strain Atlantic rainforest are recognized as the two main SFG species that produce human disease (de Oliveira et al., 2016). The former agent causes a serious, deadly illness known as Brazilian spotted fever (BSF), and predominantly occurs in the southeastern region of Brazil, with a case-fatality rate up to 50% (de Oliveira et al., 2016). In this region of the country, the eco-epidemiology of this disease is related to rural areas and/or leisure activities, which correspond to the main risk factors that increase the probability of contacting domestic (e.g. dogs, horses) and/or wild hosts (e.g. capybaras) that, in turn, sustain the *R. rickettsii* vector ticks, *Amblyomma sculptum* (formerly *Amblyomma cajennense*), and *Amblyomma aureolatum* (Angerami et al., 2012; de Oliveira et al., 2016). On the other hand, *Rickettsia* sp. strain Atlantic rainforest causes a less severe eschar-associated rickettsiosis, and has

mainly been reported in southern (Krawczak et al., 2016), southeastern (Spolidorio et al., 2010) and northeastern (Silva et al., 2011) Brazilian states, where its eco-epidemiology is related with the bite of *Amblyomma ovale* within preserved Atlantic rainforest areas (Szabó et al., 2013).

For the diagnosis of human SFG rickettsioses, current Brazilian reference laboratories use different approaches in order to confirm suspected cases, including indirect immunofluorescence assay (IFA), immunohistochemical staining (IHC), and isolation or real-time PCR (qPCR) of a variety of specimens (only serum for non-lethal cases, and organs, blood clot and serum in lethal cases) (dos Santos et al., 2012; Gehrke et al., 2006; Rozental et al., 2006). Unfortunately, in most cases the specific rickettsial agent has not been confirmed, since IFA and IHC tests cross-react with antigens of other SFG rickettsial species (Biggs et al., 2016), and positive qPCR results are not routinely confirmed with a conventional PCR with subsequent DNA sequencing methods.

Although Espírito Santo state is considered an endemic area for SFG rickettsioses with a related case-fatality rate of up to 20% (Barros e

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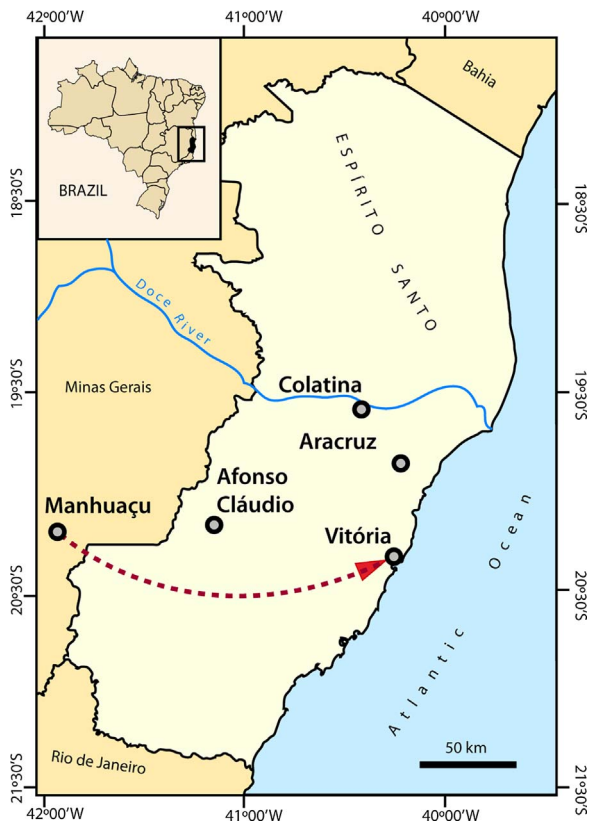


Fig. 1. Municipalities of the state of Espírito Santo, southeastern Brazil, where fatal cases of *Rickettsia rickettsii* rickettsiosis were confirmed by molecular methods. Patient from Vitória was considered to have acquired the infection in the municipality of Manhuaçu (state of Minas Gerais); however, clinical manifestations occurred in Vitória.

Silva et al., 2014), it also constitutes the only state of southeastern Brazil that currently lacks accurate confirmation of the etiological agent associated with lethal cases of human rickettsioses (Galvão et al., 2003; Gehrke et al., 2006; Rozental et al., 2006). Nonetheless, reports based on serological, immunohistochemical, and epidemiological methods have pointed *R. rickettsii* as the probable agent involved in a lethal outbreak in 1990 and 1991 (Sexton et al., 1993).

Taking into account the above-presented evidence, the aim of the present study was to confirm the species-specific etiological agent of SFG rickettsiosis in human lethal cases from Espírito Santo state by means of molecular tools.

2. Materials and methods

2.1. Clinical samples

A total of four single-serum samples from fatal human cases were collected on the day of death from patients admitted at various medical centers in Espírito Santo state during 2015–2017. The patients were from four different municipalities of Espírito Santo (Fig. 1), presented with symptoms compatible with an acute febrile severe syndrome, and died within 5–6 days after illness onset (Table 1). Upon clinical suspicion of BSF, the serum samples were sent to the Fundação Ezequiel Dias (Minas Gerais state), the regional reference center for rickettsial diseases diagnosis.

2.2. Molecular analyses

DNA for qPCR analyses was extracted from 200 µl of each serum sample using PureLink Viral RNA/DNA Mini Kit (Invitrogen, California, USA) according to the manufacturer's instructions. The integrity of the

Table 1
Information about confirmed fatal cases of *Rickettsia rickettsii* rickettsiosis in Espírito Santo state, Brazil, 2015–2017.^a

Patient code	Municipality of Espírito Santo ^a	Age (years)	Sex	Clinical findings	Epidemiologically significant exposure factors	Date of Symptoms onset	Death	Sample collected ^c	qPCR result (gltA)		Conventional PCR results ^d	
									gltA	ompA	gltA	ompA (heminested)
1	Colatina	50	M	Fever, headache, seizures, abdominal pain, diarrhea, septic shock	Ticks and capybaras	17 Oct. 2015	22 Oct. 2015	22 Oct. 2015	Positive	Positive	Positive	ND
2	Afonso Claudio	51	M	Fever, vomit, conjunctival suffusion, headache, petechial rash, diarrhea, myalgia, jaundice	Ticks, capybaras, dogs, cats, cattle, horses	12 Jun. 2017	17 Jun. 2017	17 Jun. 2017	Positive	Negative	Positive (weak band)	Positive
3	Vitória ^b	72	F	Fever, myalgia, ARDS, septic shock, oliguria	Fishing	05 May. 2017	11 May. 2017	11 May. 2017	Positive	Negative	Negative	Negative
4	Aracruz	3	M	Fever, headache, petechial rash, myalgia, hepatosplenomegaly, septic shock	Ticks	04 Oct. 2016	09 Oct. 2016	09 Oct. 2016	Positive	Negative	Negative	Negative

M, Male; F, Female; ARDS, Acute respiratory distress syndrome; qPCR, quantitative polymerase chain reaction; gltA, rickettsial citrate synthase gene; ompA, rickettsial outer membrane protein A gene; ND, not done.

^a Probable location of infection.

^b Report in Vitória; nevertheless Manhuaçu municipality (Minas Gerais state) was the probable location of infection.

^c Serum sample.

^d All PCR amplicons were sequenced and confirmed a 100% identity with *Rickettsia rickettsii*.

^e Clinical and epidemiological data were retrieved from the “Official Brazilian spotted fever case form” (one for each patient), as an official document provided by the Brazilian Ministry of Health.

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