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Case Report

Rickettsia sibirica mongolitimonae infection, Sri Lanka

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Abstract

Introduction. *Rickettsia sibirica mongolitimonae* was recently reported as a common rickettsiosis in France. Current serological evidence suggests the presence of scrub typhus and spotted fever group rickettsiosis in Sri Lanka. We detected a human case of *R. sibirica mongolitimonae* in Sri Lanka.

Methodology. A skin biopsy of the eschar was tested for the presence of *Rickettsia* spp. using qPCR assay targeting a 109-bp fragment of a hypothetical protein and by PCR amplification and sequencing targeting the *ompA* gene.

Results. A 30-year-old woman who had just returned from travel to a jungle in Sri Lanka was evaluated as an outpatient for fever. Examination revealed an enlarged axillary lymph node, a maculopapular rash and an eschar at her left flank and a skin biopsy of the eschar was performed. The skin biopsy was positive for the presence of *Rickettsia* spp. by qPCR and PCR amplification and sequencing targeting the *ompA* gene revealed *R. sibirica mongolitimonae*. Immunofluorescence assay on an acute serum sample for spotted fever group rickettsial antigens (*Rickettsia conorii conorii*, *R. sibirica mongolitimonae*, *Rickettsia felis*) and typhus group rickettsiae (*Rickettsia typhi*) was negative. The patient was treated by oral doxycycline (200 mg/day) for one week.

Conclusions. *R. sibirica mongolitimonae* should be considered in the differential diagnosis of patients with suspected rickettsiosis in, or returning from, Sri Lanka.

Key words: *Rickettsia sibirica mongolitimonae*; skin biopsy; rope-like lymphangitis-associated rickettsiosis.

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Introduction

Tick-borne rickettsioses are zoonoses caused by spotted fever group (SFG) *Rickettsia* spp. [1]. SFG rickettsioses share characteristic clinical features, including an inoculation eschar (at the site of the tick bite), fever, local adenopathy, and rash, although some variability can be found depending on the *Rickettsia* species [2]. The first human case of infection with *R. sibirica mongolitimonae* was reported in France in 1996 in patient with rope-like lymphangitis extending from the eschar to the draining lymph node [3]. Since then, other cases of this infection have been described in the literature with or without rope-like lymphangitis [4]. For years, *R. sibirica mongolitimonae* was considered a rare pathogen, and few cases had been reported in Europe and in Africa [5]. However, *R. sibirica mongolitimonae* was recently reported as a common rickettsiosis in France, probably even more than *R. conorii*, which for decades has been considered as the most common *Rickettsia* sp. in this area [5].

Human cases of *R. sibirica mongolitimonae* infection have been only described in the Mediterranean area and in South Africa [6]. Although *R. sibirica mongolitimonae* was firstly isolated from *Hyalomma asiaticum* collected in China [7], to date, human cases of *R. sibirica mongolitimonae* infection have been never described in Asia. Current serological evidence suggests the presence of scrub typhus and SFG rickettsiosis including *Rickettsia conorii* [8] in Sri Lanka [9] (Table 1). However, serology makes difficult to identify the infecting rickettsial species because of cross-reactivity among antigens of pathogens within the same genus. As a result, studies have not characterized the SFG species involved in this country. In our laboratory we routinely use skin biopsies and cutaneous swabs for the early diagnosis of SFG rickettsiosis, as these agents are successfully detected by molecular assays and by culture in these samples [10]. Indeed molecular and culture diagnostic tools had importantly decreased the time-to-diagnosis of rickettsioses as

compared to serology [10]. Based on this, we detected a human case of *R. sibirica mongolitimonae* in Asia and most particularly in Sri Lanka in a patient with fever and eschar after a tick bite.

Methodology

Molecular methods for the detection of *Rickettsia* species

Total genomic DNA was extracted from the skin biopsy of the eschar using a QIAamp tissue kit (Qiagen, Hilden, Germany). Samples were handled under sterile conditions to avoid cross-contamination. Genomic DNA was stored at 4°C and used as a template in PCR assays. Samples were screened for the presence of *Rickettsia* spp. using a previously developed qPCR assay targeting a 109-bp fragment of a hypothetical protein as previously described [11]. If a positive result was obtained, PCR amplification and sequencing targeting the *ompA* gene were used as previously described [12]. A negative control (sterile water and DNA from a sterile biopsy specimen) and a positive control (DNA from *R. montanensis*) were included in each test. Finally, the quality of DNA handling and extraction was verified by qPCR for a housekeeping gene encoding beta-actin [13].

Culture

Sample was cultured in human embryonic lung (HEL) fibroblasts using the centrifugation-shell vial technique (Sterilin-Felthan-England, 3.7 ml) using 12-mm round coverslips seeded with 1 ml of medium containing 50,000 cells and incubated in a 5% CO₂ incubator at 37°C for three days to obtain a confluent monolayer [14]. Cultures were surveyed for eight weeks, and bacterial growth was assessed every seven days on cover slips directly inside the shell vial using Gimenez and immunofluorescence staining.

Serology

An acute serum sample was tested by immunofluorescence assay (IFA) for SFG rickettsial antigens (*Rickettsia conorii conorii*, *R. sibirica mongolitimonae*, *Rickettsia felis*) and typhus group rickettsiae (*Rickettsia typhi*) as previously described [15]. IFA was considered positive for *Rickettsia* spp. infection when a single antibody titer of IgG $\geq 1/128$ combined with an IgM titer $\geq 1/64$ against one or more antigens of the tested species [15].

Results

A 30-year-old woman was evaluated as an outpatient in February 2016 for fever. The woman, who had just returned from travel to a jungle in Sri Lanka, reported several tick and mosquito bites. She mentioned that fever 40°C and a rash begun four days before her return home in France. Examination revealed an enlarged axillary lymph node, a maculopapular rash and an eschar at her left flank. A rope-like lymphangitis was not observed. Laboratory values were remarkable only for slightly elevated C-reactive protein (17 mg/L, with upper normal value at 5 mg/L). Extensive microbiological testing was done, including tests to rule out malaria, dengue, viral hepatitis, and leptospirosis; all results were negative. Blood cultures returned negative after 5 days of incubation. A skin biopsy of the eschar and an acute serum sample were sent to the National Reference Centre for *Rickettsiae* (Marseille, France) for further analysis. The skin biopsy of the eschar was positive for the presence of *Rickettsia* spp. using qPCR assay. PCR amplification and sequencing targeting the *ompA* gene revealed *R. sibirica mongolitimonae*. The skin biopsy was cultured in HEL using the centrifugation-shell vial technique but cultures remained negatives after eight weeks. The

Table 1. Previous studies identified spotted fever group rickettsiosis in patients from Sri Lanka.

Area	Population Tested	Method	Number of patients	SFG positive	Reference
Northern Sri Lanka	Military personnel	IFA	57	33	[19]
Central province of Sri Lanka	Rickettsial infection suspicion	IFA	210	134	[20]
Central hills of Sri Lanka	Patients with a rickettsial infection	IFA	371	43	[8]
Central Sri Lanka	Patients with neurological manifestations	IFA	17	10	[21]
Southern Sri Lanka	Febrile patients	IFA, ELISA	883	86	[22]
Southern Sri Lanka	Rickettsial infection suspicion	IFA	178	6	[9]
23 hospital representing 8 provinces	Rickettsial infection suspicion	IFA, ELISA	615	198	[23]
Central Sri Lanka	Rickettsial infection suspicion	IFA	118	10	[24]

SFG, spotted fever group; IFA, immunofluorescence assay.

acute serum sample was tested by IFA and was negative for SFG rickettsial antigens, typhus group rickettsiae and *Orientia tsutsugamushi*. *R. sibirica mongolitimonae* infection was considered and oral doxycycline (200 mg/day) was started for one week and the patient's condition improved.

Discussion

We describe a human case of *R. sibirica mongolitimonae* in Sri Lanka. Until recently, *R. sibirica mongolitimonae* was considered as a rare pathogen, and few cases were reported [5]. This was because rickettsioses diagnoses are based mostly on serological tests. Indeed, the sensitivity of serology in the early stages of the disease is low, and seroconversion is usually detected 7–15 days after disease onset (25–28 days for *R. africae* infection) [16]. This probably explains that the acute serum sample was negative for Rickettsiosis in this observation. Indeed, the collection of acute and convalescent phase sera separated by several weeks is necessary to confirm the disease [17]. Moreover, the cross-reactivity among SFG pathogens makes difficult the identification of the rickettsial species even by a Western blotting assay with cross-absorption techniques [17]. One of the advantages of the use of skin biopsies is that we can easily identify the infecting rickettsial species using molecular assays [10], which allowed to detect and characterize *R. sibirica mongolitimonae* as an indigenous rickettsiosis in Sri Lanka.

In agreement with previous reports [5], our patient presented fever and rash, the most common signs of *R. sibirica mongolitimonae* infection. However, she did not present with a rope-like lymphangitis extending from the eschar to the draining lymph node, that is the characteristic manifestation of this rickettsiosis. Ramos et al. proposed that the term lymphangitis-associated rickettsiosis may be unwarranted for *R. sibirica mongolitimonae* infection, as it is not present in all *R. sibirica mongolitimonae* cases, and other rickettsioses produce lymphangitis [18]. Moreover, in a recent series of patients with *R. sibirica mongolitimonae* infection, only 35% of patients presented a rope-like lymphangitis [5].

In conclusion, the importance of the recognized tick-associated rickettsial pathogens have increased dramatically during recent years, and several SFG rickettsiosis that have been considered nonpathogenic for decades are now clearly associated with human infections, making these diseases a paradigm to help understand emerging and reemerging infections. We provide evidence that *R. sibirica mongolitimonae* exists

in Sri Lanka. Clinicians should consider this *Rickettsia* in the diagnosis of patients with an eschar or a rash after a tick bite in this country. However, the epidemiology of *R. sibirica mongolitimonae* infection requires further investigation to determine how common is this SFG rickettsiosis in Sri Lanka.

Authors' Contributions

CC: wrote the manuscript, PT: wrote the manuscript, CL, conceived of the case report; MC, conceived of the case report, DR: organized the study; EA: wrote the manuscript and organized the study

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