Identifying the first case of Infection by *Rickettsia sibirica* in Portugal

Conceição Barata, Rita de Sousa, Alice Zambujalinho, Rodrigo Gusmão, Francisco Azevedo, Fátima Bacelar

Abstract

We report the first isolation of *Rickettsia sibirica* (*mongolotimonae* strain) from the blood of a patient in the Alentejo region, Portugal.

Key words: *Rickettsiae*, Mediterranean spotted fever, *Rickettsia sibirica* (*mongolotimonae* strain).

INTRODUCTION

Rickettsia sibirica (*mongolotimonae*), initially known as HA-91, was originally isolated from the tick *Hyalomma asiaticum* collected in the Alashian region of Inner Mongolia, in 1991.[1] In 1996, in France, human infection by *Rickettsia sibirica* was described for the first time.[2] Subsequently, other cases were described, in South Africa and Greece.[3,4]

In Portugal, known cases of Rickettsia are those caused by the strains of the *Rickettsia conorii* complex, and by *R. typhi,* although there are reports of isolation in Portuguese ticks of the *R. slovaca* and *R. aeschlimannii* strains, as well as *R. helvética*, the latter of undetermined pathogenicity – *Fig. 1*.[5-8] The principal vector and reservoir is the ixodide *Rhipicephalus sanguineus9* (commonly known as brown dog tick).

The first version of this article was published in Emerging Infectious Diseases, and focused more on the laboratory structure and molecular biology. The authors then decided to submit the article for publication in a Portuguese journal because, being a rare pathology, it was important to divulge the fact among us and enable the clinical case to be presented in more detail.

CLINICAL CASE

Identification

Female patient, aged 73 years, a widow, Caucasian, works in an electrical appliances store.

Reason for Hospitalization

Fever symptoms with two days of evolution, and cutaneous lesions.

Current History

Admitted to the Emergency Service of the Hospital do Espírito Santo de Evora on the 19th August 2004, due to a clinical condition with onset five days earlier, characterized by the appearance of pain, redness and heat in the 3rd toe of the right foot, worsening over the subsequent two days, when it was accompanied by fever (39-40ºC), sweating and hot flushes, myalgia, exhaustion and anorexia. On the day of admission, in the morning, erythematous cutaneous lesions were observed, which were non-pruriginous, widespread, and affecting the palms of the hands and soles of the foot, with marked worsening of all the symptoms, particularly the exhaustion.

Previous history

She was always considered a healthy person.
In the personal history, hypertension is reported, which was medicated and apparently controlled.

Epidemiological history

She lives in Reguengos de Monsaraz (village), on the 1st floor of a centrally located house, and works in an electrical appliances store on the ground floor of the same building. She is not in the habit of traveling to the countryside, and does not own a pet. She reported that she had not been being stung by any insect or tick, that she was aware of.

Objective Exam

The patient was alert, oriented in time and place, cooperating on questioning, but exhausted. Obese, with

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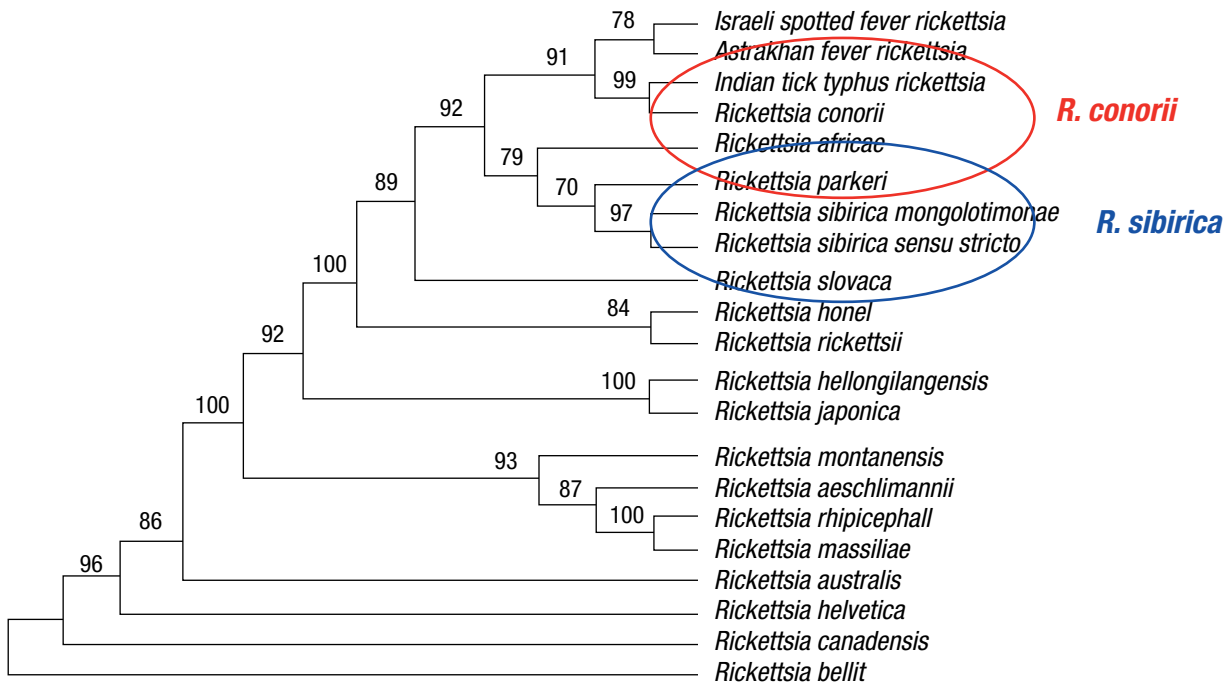

Phylogeny of *R. sibirica mongolotimonae*.[5]

FIG. 1

rosy but dehydrated mucosa, anicteric and eupneic at rest. She had fever (39.6ºC), tachycardia (102/minute) and BP: 156/72 mm Hg. She presented a diffuse exanthema, slightly reddish, with occasional disperse nodular lesions, affecting the palms of the hands and soles of the feet. Cardiac and pulmonary auscultation did not show any alterations; abdomen with audible stomach sounds, painless to both surface and deep palpation, with palpable hepatosplenomegaly. There were no alterations in the upper limbs; the right leg presented marked redness and increased temperature below the knee, which was diffuse and homogenous with poorly defined outline. Also, a purple lesion surrounding a whitish, central lesion of around 3 mm in diameter, on the anterior and internal surface of the 3rd toe of the right foot. Neurological exam presented slow progression but without alterations, and without focal signals or meningeal signs.

Complementary Tests:

Hemogram and biochemical: in the laboratory tests on admission, the following are highlighted: Hemoglobin 14.3 g/dL with hematocrit of 42%, Leukocytes 7860 /mm3 with Neutrophils 87%, Lymphocytes 10%, Monocytes 3% and Eosinophils and Basophils 0 and CPR 18. INR 0.95, Glycemia 232 mg/dL, Urea 36 mg/dL and Creatinine 1.0 mg/dL. AST 116 UI/L, ALT 93 UI/L, GammaGT 92, Total bilirubin 0.8 mg/dL, Sodium 131 mEq/L and Potassium of 2.6 mEq/L. Glucose-6-Phosphate-Dehydrogenase and Pyruvate-Kinase levels normal.

Chest X-ray did not reveal any pleuro-parenchymatous alterations, with cardiothoracic Index > 0.5.

ECG showed sinus rhythm and left ventricular hypertrophy by voltage criteria.

Serology and isolation of the agent: In the patient's blood serum, the presence of anti-rickettsial antibodies (IgG and/or IgM) was not detected by the indirect immunofluorescence technique, probably because the disease was in the early stage. It was not possible to determine seroconversion, due to the lack of a second serum sample.

In parallel with the serology, an attempt was made to isolate the agent from a sample of the patient's

blood, which was collected in a heparinized vacutainer under antiseptic conditions, and sent to the Centro de Estudos de Vectores e Doenças Infecciosas (Center for the Study of Vectors and Infectious Diseases) of the National Health Institute Dr. Ricardo Jorge. For the isolation of the agent, buffy coat inoculation was carried out, or leukocyte concentrate, in a culture of Vero cells by the shell-vial technique[8]. The cells were incubated at 32ºC and on the 6th day, they were transferred to a 25 cm^3 bottle. At the end of 7 days, a cell control was performed; the cells were stained by the Giménez technique to detect the presence of rickettsiae in the cell culture. DNA extraction of Rickettsia-positive culture was performed, and PCR (Polymerase Chain Reaction) and sequential techniques carried out, to characterize the strain. Our strain was identified with a homology of 100% to the agent *R. sibirica mongolotimonae* with the sequences available in the GenBank database, confirming the clinical diagnosis of infection by Rickettsia.

Evolution during hospitalization

The patient was medicated with penicillin G potassium alternated with sodium, for 7 days, with a marked improvement in the cutaneous lesions below the knee, interpreted as erysipeloid – with possible entry site of the lesion on the 3rd toe of the right foot.

However, given the existence of the diffuse cutaneous exanthema affecting the palms of the hands and soles of the feet, and although there was no apparent epidemiological history, the patient was medicated with Doxycycline due to suspected rickettsiosis. Patient became apyretic after 48 hours, with the disappearance of the diffuse exanthema and circumscription of the lesions of the right lower limb, but which developed small solutions of continuity, requiring daily dressings.

Analytically, there was a regression in all the parameters of infection, but the alterations in the hepatic tests remained, and were not explained by any previous pathology. On the date of discharge, patient presented ESR 35 mm, CRP 4, INR 0.87, Glycemia 128 mg/dL, Urea 27 mg/dL and Creatinine 0.6 mg/dL. AST 137, ALT 119, Gamma GT 178, Total Bilirubin 0.54 mg/dL, Sodium 135 mEq/L and Potassium 3.6 mE/L.

The hemocultures were contaminated.

She was referred to the Internal Medicine clinic, twice missing her appointment. After being invited by telephone, she attended around eight months after discharge, due to problems related to disease and death of a family member. She reported that the cutaneous lesions with solution of continuity took around two months to cure, with no clinical worsening. Having completely recovered, she presented analytical re-evaluation without alterations.

DISCUSSION

Eschar-nodular fever is the most human rickettsiosis in Portugal,[10] with higher incidence in the months of June to September. It is a disease with compulsory notification, and together with brucellosis, it is the zoonosis that presents the highest number of notified cases.[9,11] It is estimated, however, that more than half of the cases that occur in our country are not notified. The vast majority of patients have benign evolutions, however severe forms are described in around 5% of patients. These are generally associated with alcoholism, diabetes mellitus, HIV infection, Black race, advanced age, and glucose-6-phosphate dehydrogenasis deficiencies.[9] The mortality by this anthropozoonosis is between 1.4% and 5.6%, of hospitalized cases.[9,12]

As far as we know, this is the first case of isolation of *R. sibirica* from a patient's blood, in Portugal.

The clinical symptoms and laboratory tests were, in general, similar to those of infections caused by *Rickett-*

TABLE I

Analytical Evolution

	18/08/2004	24/08/2004
Erythrocytes/Hgb/Hct	4 790 000/ 14,3/ 42%	4 730 000/ 14/ 41%
Leukocytes/Formula	7 860/ N86E0B0L10M3	9 950/ N68E0B0L26M5
INR	0,95	0,87
CRP/ESR	18,2	4,2/ 35
Ureia/Creatinine/ Na/ K	26/ 1/ 131/ 2,6	27/ 0,6/ 135/ 3,5
Glycemia	232	128
Total/direct Bilirubin	0,8/ 0,2	0,5/ 0,2
AST/ ALT/ GGT/ FA	116/ 93/ 92/ 94	137/ 119/ 178/ 118
Protein gram	P7;A3;$\alpha_1$0;$\alpha_2$1;β0,8;γ1,1	

sia. Also, the clinical case presented here has the classic inoculation eschar and typical exanthem, signs that are described in 72% and 50-97% of cases, respectively.[13]

Careful and exhaustive investigation of the epidemiological data, which corroborated the clinical diagnosis formulated, showed negative results at the time of admission (and even in the consultation).

The patient was diagnosed with eschar-nodular fever, and a blood sample was sent to the Laboratory of the Centro de Estudos de Vectores e Doenças Infecciosas de Águas de Moura, National Health Institute Dr. Ricardo Jorge (as is the custom) for serology and isolation of the agent.

It is interesting to note that the cases reported in France occurred mostly in the Spring, while the peak season for the patients in South Africa and Greece was the Winter. Our case occurred in August – during the peak period for eschar-nodular fever.

Clinically, this patient presented fever, rash, myalgias and an inoculation eschar, but without lymphangitis or adenopathies - a clinical manifestation that has been proposed as being closely related to infection by *R. sibirica mongolotimonae*.

However, of the 12 human cases published since 1996 through to the present case (eleven in Europe and 1 in South Africa), it is only described in only five patients (41.6%).[3,4,14] Would it be appropriate to propose this clinical sign as typical of this strain, when it appears in less than half of cases? It is added that Rickettsia has never been identified in the lymphatic veins, or associated with inflammatory lesions in other sites. Rickettsia has widespread involvement, which is attributed to a process of systemic vasculitis, due to their high tropism for the vascular endothelium.[15]

The alterations in the hepatic function tests, despite not being typical of this entity, have been described in some forms, both with and without hepatities.[16,17] In our patient, they had relevant expression (elevations to around 3 times normal levels), but without any ensuing complications. There is no conformation as to whether these are related to *R. sibirica*.

All rickettsioses are treated with tetracyclines or derivatives, but detection by PCR in skin biopsies (eschar) or isolation of the etiological agent, are of importance in the laboratory diagnosis when determining the causative strain. This cannot be determined by clinical criteria and has epidemiological and clinical implications, related to the severity of the disease and the control of Public Health.

Addendum: *R sibirica* has also been subsequently isolated from the vector *Rhipicephalus pusillus,* in our Country.[18,19] This fact alerts us to the fact that other rickettsia, like *R. slovaca, R. aeschlimannii* and *R. Helvética*, also isolated in Portugal, are also causative strains of rickettsioses which often go unnoticed or are masked by other pathologies, as the signals are less exuberant than in eschar-nodular fever. ■

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