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DOI: http://dx.doi.org/10.3201/eid1804.111419

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Rickettsia monacensis as Cause of Mediterranean Spotted Fever–like Illness, Italy

To the Editor: *Rickettsia conorii*, the etiologic agent of Mediterranean spotted fever (MSF), is transmitted to humans by the brown dog tick (*Rhipicephalus sanguineus*). MSF is endemic to Italy; incidence is highest in the south and on the islands of Sardinia and Sicily (*1*). Recently, the use of molecular methods has enabled identification of other rickettsiae of the spotted fever group (SFG) from *Ixodes ricinus* ticks in northeastern Italy and in other areas of Europe (*2*–*6*). *R. monacensis* was identified as an etiologic agent of MSF-like illness in Spain (*7*).

We report a case of MSF-like illness in a 28-year-old man from Sassari in northwestern Sardinia who was admitted to the Infectious Disease Unit of the University of Sassari Hospital in April 2011. At admission, he reported fever (38.2°C) and headache of 2 days' duration. At physical examination, he had a crusty skin lesion surrounded by edema and erythema, which was compatible with inoculation eschar, on the left calf. He had no rash. Laboratory results showed a slight leukocyte increase, hypocromic and microcytic anemia (hemoglobin 10.6 g/dL [reference range 13.1–17.1 g/dL], mean corpuscular volume 67.7 fL [reference range 81–88 fL], mean corpuscular hemoglobin concentration 29.6 g/dL [reference range 33–35 g/dL]), hyperbilirubinemia (total bilirubin 1.36 mg/dL [reference range 0.2–1.3 mg/dL], direct bilirubin 0.49 mg/dL [reference range 0.0–0.6 mg/dL]), and erythrocyte sedimentation rate 37 mm/h (reference range 0–25 mm/h). The remaining parameters

were within reference ranges. A small skin sample taken from the inoculation eschar and whole blood were stored at –30°C. The patient immediately started taking doxycycline 100 mg every 12 hours. Serologic tests were negative for *R. conorii* IgM and IgG (ELISA) and positive for SFG *Rickettsia* spp. IgG on indirect immunofluorescence with a titer of 128. After 24 hours of antimicrobial drug therapy, he was afebrile; he was discharged on day 3. He completed a 7-day course of doxycycline at home and recovered completely.

The skin biopsy sample, collected in phosphate-buffered saline, and whole blood were obtained before antimicrobial therapy began and were subjected to DNA extraction. Bacterial detection and identification were conducted by using molecular methods based on real-time PCR, classical PCR, and nucleotide sequencing (Table).

A set of primers for *glt*A gene that encodes the citrate synthase enzyme (*8*) was used to determine that the organism belonged to the genus *Rickettsia*, which includes the SFG and typhus group. Each real-time PCR reaction was performed by QuantiTect SYBR Green PCR kit (QIAGEN, Hilden, Germany) by using 20 ng of purified DNA. *R. conorii* and *R. typhii* were used as positive controls for SFG and typhus group, and *Anaplasma phagocytophilum*, *Bartonella henselae*, *Ehrlichia chaffeensis*, and *Coxiella burnetii* (*Bartonellaceae* and *Coxiellaceae* members) served as negative controls. Results were checked for the specific molecular length by electrophoresis on a 3% (wt/vol) agarose gel.

The skin biopsy specimen of the inoculation eschar was positive for *Rickettsia* spp. The whole blood sample was negative for *Rickettsia* spp.

These results were confirmed by amplification of the *ompA* gene by using the ompA–F and ompA–R primers (*9*) and by the sequencing of the PCR amplicon. The nucleotide sequence analyzed by using the BLAST search tool (www.ncbi.n/m.nib.gov/blast) showed 100% identity with the *R. monacensis* isolate N72 (GenBank accession no. FJ919650.1). We identified *R. monacensis* as cause of MSF-like illness in the patient reported here.

Our results have several clinical and microbiological implications. Although MSF-like illness is highly endemic to Sardinia, to our knowledge no pathogens other than *R. conorii* had ever been identified. Antibodies against *R. monacensis* were not detected by the *R. conorii* ELISA commonly used in hospital laboratories. In contrast, indirect immunofluorescence, which cannot distinguish between rickettsial species because of cross-reactivity, was positive. Therefore, the cocirculation of *R. monacensis* and, possibly, of other SFG rickettsiae, could lead to misdiagnosis and therapeutic delay. Furthermore, in consideration of the negative result in whole blood, a small skin sample from the eschar might improve the diagnostic sensitivity of PCR.

We did not perform entomologic studies. However, *I. ricinus* ticks, which are considered vectors of *R. monacensis*, are widely distributed in Italy and have been found in Sardinia, although less often than other tick species (*10*). Moreover, it is not excluded that other ticks might act as vectors for *R. monacensis* in Sardinia, where ticks of the genus *Rhipicephalus* are prominent. Molecular investigations of ticks could better clarify the extent of circulation of SFG rickettsiae in Sardinia.

Identification of *R. monacensis* as a cause of MSF-like illness in Sardinia expands the list of pathogenic rickettsiae circulating in Italy. It also highlights the need for further investigation in humans and vectors to understand infection dynamics and improve diagnosis and treatment of this potentially life-threatening disease.

This study was supported by a Centro Nazionale per il Controllo e la Prevenzione delle Malattie Project of the Italian Health Ministry.

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DOI: http://dx.doi.org/10.3201/eid1804.111583

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Table. Selected inner primers used to amplify rickettsial *glt*A and *omp*A genes*

Rickettsial groups	Gene	Primer	Nucleotide sequence, 5′ → 3′	Product size, bp	Reference
Rickettsiae spotted fever group plus typhus group	*gtlA*	gltA–F	TCGCAAATGTTCACGGTACTTT	74	(*8*)
		gltA–R	TCGTGCATTTCTTTCCATTGTG		
Rickettsiae ompA	*ompA*	ompA–F	ATGGCGAATATTTCTCCAAAA	632	(*9*)
		ompA–R	GTTCCGTTAATGGCAGCATCT		

**gltA*, citrate synthase; *ompA*, outer membrane protein A.

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Leishmania Resistance to Miltefosine Associated with Genetic Marker

To the Editor: During 2000–2010, serial *Leishmania* isolates obtained from an HIV-infected patient who was not responding to treatment showed a gradual decrease in in vitro miltefosine susceptibility. We performed *L. donovani* miltefosine transporter (*Ldmt*) gene analysis to identify an association between miltefosine resistance of reference *L. donovani* lines and variability in miltefosine response of *L. infantum* isolates. A new single-nucleotide polymorphism (SNP), L832F, was identified, which might be a marker of miltefosine resistance in leishmaniasis.

The patient, a 46-year-old woman, had lived in France since 1994 but regularly returned to Algeria, her country of birth. HIV-1 infection was diagnosed in 1991. Antiretroviral therapy was initiated in 1993, leading to undetectable viral load and a CD4+ T-cell count of 185 cells/mm^3 (reference >450/mm^3). Concurrent conditions were thoracic herpes zoster in 1996, hairy leukoplakia of tongue, oropharyngeal candidiasis, and chronic renal failure of unknown cause since 2000.

Visceral leishmaniasis was diagnosed in 1998 by culture of a bone marrow smear, which showed intracellular amastigotes. Use of meglumine antimonate (Glucantime; Sanofi, Paris, France), a drug of choice for the treatment of leishmaniasis, was contraindicated because of pancreatitis in the patient and in vitro isolate susceptibility variation; therefore, induction therapy consisted of liposomal amphotericin B (AmpB [AmBisome; Astellas Pharma US, Deerfield, IL, USA]) at a dose of 3 mg/kg/d for 5 consecutive days, then 1× week for 5 weeks (total dose 30 mg/kg) during 1998–2000 (Table). The same medication was administered for relapses at 4 mg/kg/d for 5 days, then 4 mg/kg 1× week for 5 weeks (total dose 40 mg/kg) during 2001–2010. Given the adverse effects of AmpB and the availability of oral miltefosine (Impavido; AEterna Zentaris Inc., Quebec City, Quebec, Canada), the latter drug was used for maintenance treatment during 2001–2007 at 50 mg 2×/d. Leishmaniasis was monitored by leukocytoconcentration and culture of blood samples on Novy-Nicolle-McNeal medium.

When signs of biological and clinical relapse appeared, bone marrow was aspirated for parasite detection. After culture of the aspirate and isoenzyme determination, the strain was identified as *L. infantum*, zymodeme MON-24. Eleven relapses were documented; all were confirmed by positive direct examination of bone marrow or blood, but cultures of only 7 samples yielded positive results (Table).

The susceptibility of 4 cryopreserved isolates (S_1, S_3, S_4, and S_6; Table) to AmpB and to miltefosine was studied in the in vitro promastigote and axenic amastigote form by determining the concentrations inhibiting parasite growth by 50% (*1*,*2*). The 50% inhibitory concentration (IC_{50}) was determined in parallel for the following reference *L. donovani* lines: a wild-type *L. donovani* LV9 (MHOM/ET/67/HU3) line (LV9 WT), a wild-type *L. donovani* DD8 (MHOM/IN/80/DD8) line (DD8 WT), a laboratory miltefosine-resistant line obtained from LV9 WT (LV9 miltefosine-R, resistant to 90 μmol/L miltefosine), and the laboratory AmB-resistant line obtained from DD8 WT (DD8 AmB-R, resistant to 1.4 μmol/L AmB) on promastigote and axenic amastigote forms (*3*,*4*).

The AmB susceptibility of the isolates did not change notably over time; IC_{50} values ranged from 0.09