First Human Case of *Rickettsia sibirica mongolotimonae* Infection in Northern Greece

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Rickettsiae are Gram-negative, non-spore-forming and highly pleomorphic bacteria. On the basis of antigenic and genetic information, *Rickettsiae* customarily are divided into the spotted fever group (SFG), the typhus group and the scrub typhus group. The etiologic agent of the latter is *Orientia tsutsugamushi*, which belongs to the genus *Orientia* within the Rickettsiales group. SFG rickettsioses typically present with high fever, headache, a maculopapular rash, and frequently an inoculation eschar at the site of the tick bite [1]. The presenting symptoms are relatively non-specific, making timely diagnosis difficult, especially for physicians who are unfamiliar with the disease.

In Greece, *six* SFG *Rickettsia* species have been *detected in ticks and in humans*, namely, *R. conorii* [2,3], *R. massiliae* [2,4], *R. sibirica mongolitimonae* [5], *R. aeschlimannii* [6], *R. rhipicephali* [7] and *R. slovaca* [8,9]. To date, three SFG *Rickettsia* species have been implicated in human diseases in the same country, i.e., *R. conorii, R. aeschlimannii* and *R. sibirica mongolitimonae,* the latter being the etiologic agent of lymphangitis-associated rickettsiosis (LAR), recorded in Greece in the island of Crete only [5]. We report here the first human case of *R. sibirica mongolitimonae* in northern Greece, in a male adolescent from Alexandroupolis, Thrace.

PATIENT DESCRIPTION

A 13 year old previously healthy boy presented to the emergency room of the University Hospital of Alexandroupolis in May 2013 due to intermittent high fever of up to 39.7°C, with chill, dizziness and weakness during the previous 3 days. The patient reported a trip to Samothraki, the most northern island of the Aegean Sea, 10 days earlier for vacation. During this trip he had eaten a home-made dairy product and taken frequent leisurely walks to the woods.

Physical examination on admission showed a 1 cm healing eschar on the right upper chest, consistent with recent tick bite, along with regional lymphangitis, a palpable spleen tip just below the left costal margin, and a generalized faint maculopapular rash. A complete hemogram showed leukocytes 3410/μl with 59% neutrophils, 10% bands, 20% lymphocytes and 11% monocytes, hemoglobin 15 g/dl and platelets 154,000/μl. Biochemical tests showed normal electrolytes, blood urea nitrogen and creatinine, minimal elevation of transaminases, and C-reactive protein 3.1 mg/dl (normal < 0.5). A chest radiograph was normal.

A new hemogram conducted the next morning showed worsening leukopenia (leukocytes 2410/μl), intense left shift (25% bands) and thrombocytopenia (platelets 68,000/μl). A bone marrow aspirate showed good cellularity with abundant megakaryocytes and no *Leishmania* parasites, while an abdominal ultrasonogram confirmed the presence of mild splenomegaly. Based on the epidemiological history and the initial biochemical and hematological studies, the patient was started on intravenous ceftriaxone 2 g twice a day together with oral rifampicin 300 mg twice a day and oral doxycycline 100 mg twice a day to cover brucellosis and rickettsioses. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels increased on the third day of hospitalization (171 U/L and 226 IU/L, respectively). An IgM, IgG enzyme immunoassay test (Delta Biologicals, Rome, Italy) for *Leishmania infantum* and an indirect immunofluorescent test for IgG antibodies against *L. infantum* (Vircell, Granada, Spain) were negative. Serological testing for brucellosis with a commercially available qualitative card agglutination test (Brucelloslide-test, bioMerieux, Marcy-l'Etoile, France) was also negative.

The patient defervesced within 48 hours after starting antibiotics and continued this therapy for the next 10 days. All blood and bone marrow cultures were negative for pathogens, while the clinical, biochemical and hematological abnormalities resolved except for a leftover scar at the site of the chest eschar. Two serum samples and whole blood samples, one obtained 24

days following the onset of the febrile illness and a convalescent one 12 weeks later, were tested for tick-borne pathogens. DNA was extracted from whole blood using the QIAamp DNA blood mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Real-time polymerase chain reaction (PCR) targeting the *glta* gene of *Rickettsia* spp. was performed as previously described. Furthermore, DNA extract was tested for *Anaplasma phagocytophilum* and *Borrelia* spp. by real-time PCR. Sera were tested by immunofluorescent assay (IFA) test for the presence of IgM and IgG antibodies against *Rickettsia* spp. using a slide that could test for *R. conorii, R. sibirica mongolitimonae, R. slovaca, R. felis, R. massiliae and R. typhi* as individual antigens (Fuller Laboratories, CA, USA). Sera were also tested by IFA for the presence of IgM and IgG antibodies against *A. phagocytophilum* (Focus Diagnostics, CA, USA). IgM and IgG antibodies against *Borrelia* spp. were tested by Western blot analysis (Mikrogen Diagnostik, Neuried, Germany). The patient's acute-phase serum was positive for IgM antibodies (titer 1/1600) and negative for IgG antibodies against *R. sibirica mongolitimonae*. Low cross-reacting IgM titers were detected against *R. typhi* (1/400), *R. conorii* (1/200), and *R. slovaca* (1/400). The convalescent serum revealed IgG antibodies (1/480), and a dropping IgM titer (1/200) against *R. sibirica mongolitimonae*, while the cross-reacting IgM antibodies to the other *Rickettsiae* spp. persisted in low titers (*R. typhi* 1/400, *R. conorii* 1/200 and *R. slovaca* 1/100). Antibodies against *A. phagocytophilum* and *Borrelia* spp. were negative. All real-time PCR amplifications were negative against all the pathogens tested. The patient has been doing well for the last 10 months of follow-up, although a leftover scar at the site of the tick bite remains on the chest wall [Figure 1].

Figure 1. A leftover scar at the site of the tick bite remains on the patient's anterior chest wall

COMMENT

Rickettsia sibirica mongolitimonae, the etiological agent of LAR, was first isolated from *Hyalomma asiaticum* ticks in Mongolia in 1991 [10]. In Europe, the agent has been detected and/or isolated in *Hyaloma anatolicum excavatum* in Greece [5] and in *Rhipicephalus pusillus* in Portugal [1,11] and France [12].

The first human case of tick-borne spotted fever due to *R. sibirica mongolitimonae* was described in France in 1996 in a patient with an eschar who developed fever and a maculopapular rash [13]. Since then, human cases have been described in Greece, Portugal, France and Spain [5,11,12,14]. The acronym LAR was proposed due to the frequent presence of lymphangitis; the presence of one or more inoculation eschars has also been recorded in most cases described to date. In fact, the frequent presence of lymphangitis along with at least one eschar inoculation is considered characteristic of *R. sibirica mongolitimonae*. The cases of a patient who suffered from septic shock and of a pregnant woman with ocular vasculitis due to *R. sibirica mongolitimonae* have also been reported [15,16]. Recently, Ramos et al. [17] described six additional cases of *R. sibirica mongolitimonae* infections that occurred in the Mediterranean coast region of Spain during the period 2007–2011. All patients had fever (38.5–39.5°C), myalgia, headache, and a single inoculation eschar, five (83%) had enlarged regional lymph nodes, and three (50%) had regional lymphangitis [17]. Our patient had lymphangitis, a single inoculation eschar, and in all likelihood became infected during his recent trip to Samothraki since it was the only place he could have been exposed to ticks while walking outdoors.

The widespread use of real-time PCR in cutaneous swabs and biopsies has been proven useful for the diagnosis of rickettsial diseases, even in cases with negative blood molecular studies [18]. In fact, early administration of antibiotic therapy for patients with an inoculation eshcar and/or rash, before whole blood sampling, may jeopardize the PCR amplifications. That is why during the past 4 years we have introduced an IFA screening test for antibodies against six rickettsial species, i.e., the ones identified in ticks during various surveys in Greece and in every clinical case where a tick bite is reported. As a result of this approach more than 10 cases of rickettsial infections caused by species other than *R. typhi* and *R. conorii* have been diagnosed (unpublished data).

Our report is limited by the fact that *R. sibirica mongolitimonae* was not cultivated and a PCR was not performed in a cutaneous swab and/or skin biopsy specimen from the inoculation eschar. Since serology detects cross-reactive antibodies, as in our case, shared among different SFG rickettsiae, the diagnosis is highly probable rather than proven. However, the patient's clinical picture including the presence of lymphangitis is totally consistent with the presumed serological diagnosis.

In conclusion, pediatricians treating children and adolescents with unexplained fever, tick-bite history, with or without

a rash, and an inoculation eschar should consider rickettsioses in the differential diagnosis and obtain appropriate whole blood, cutaneous swabs and/or skin biopsies for real-time PCR testing. In the absence of a sample suitable for PCR analysis and/or a positive PCR, serological testing can still be useful for diagnosis, even though in rickettsial infections cross-reactivity among different species is the rule. Finally, *R. sibirica mongolitimonae* appears to be an emerging pathogen in both southern and northern Greece. For this reason, we plan in the future (mainly in spring) to vigorously search for *R. sibirica mongolitimonae* in our region, aiming to better define the epidemiology of this uncommon rickettsiosis.

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