Notice of Retraction

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Title page

First human case of *Rickettsia sibirica mongolotimonae* infection in Northern Greece in a teenager from Thrace.

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Summary

Spotted fever group (SFG) rickettsioses usually present with high fever, a maculopapular rash, and frequently an inoculation eschar at the site of tick bite. Among the SFG species, *Rickettsia sibirica mongolitimonae* is the etiologic agent of lymphangitis-associated rickettsiosis that has been described in France, Spain, Portugal and Crete. We describe herein a male adolescent from Thrace, Northern Greece with clinical and serological evidence of infection due to this rickettsia species, who recovered uneventfully with appropriate therapy. The patient got infected in the island of Samothraki, the most northern island of the Aegean Sea, during a recent trip. Although *R. sibirica mongolitimonae* was neither cultivated nor was a PCR performed in a cutaneous specimen from the inoculation eschar, serological testing along with the typical clinical picture makes the diagnosis highly probable. *Rickettsia sibirica mongolitimonae* appears to be an emerging pathogen in both southern and northern Greece. Physicians caring for patients who have traveled to endemic areas should consider *R. sibirica mongolitimonae* in the differential diagnosis of any febrile illness associated with a maculopapular rash and an inoculation eschar.

Rickettsiae are Gram negative, non-spore-forming and highly pleomorphic bacteria, divided into the Spotted Fever Group (SFG), the Typhus Group and the Scrub Typhus Group.

SFG rickettsioses typically present with high fever, headache, a maculopapular rash, and frequently an inoculation eschar at the site of tick bite. The presenting symptoms are relatively non-specific, making the timely diagnosis difficult, especially for physicians that are unfamiliar with the disease.

In Greece, six SFG *Rickettsia* species have been detected in ticks and in humans, i.e., *R. conorii* (1, 2), *R. massiliae* (1, 3), *R. sibirica mongolitimonae* (4), *R. aeschlimannii* (5), *R. rhipicephali* (6) and *R. slovaca* (7, 8). So far, 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), so far recorded in Greece in the island of Crete only (4). We report herein the first human case of *R. sibirica mongolitimonae* in Northern Greece, in a male adolescent from Alexandroupolis, Thrace.

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 up to 39.5°C along with chills over the last three days, dizziness and weakness. Fever was spiking approximately every 6 hours. The patient had normal blood pressure (the lowest recorded BP was 102/65mmHg). His heart rate was up to 120/min. He didn't show intense tachycardia, likely due to the fact that he was a fit and well-trained teenager. The patient reported a trip to Samothraki, the most northern island of the Aegean Sea (Figure 1), 10 days ago, with 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 3,410/ μ l with 59% neutrophils, 10% bands, 20% lymphocytes and 11% monocytes, hemoglobin 15 g/dl and platelets 154,000/ μ l. No atypical lymphocytes were recorded. The patient showed intense left shift with 10% bands and the polymorphonuclear granulocytes demonstrated toxic granulation. 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 2,410/ μ l), intense left shift (25% bands) and thrombocytopenia (platelets 68,000/ μ l). A bone marrow aspirate showed good cellularity with abundant

megakaryocytes with no evidence of hemophagocytosis and no *Leishmania* parasites. An abdominal ultrasonogram confirmed the presence of mild splenomegaly. The patient was started on intravenous ceftriaxone along with oral rifampicin and oral doxycycline. Serum ALT and AST picked at 171 U/l and 226 IU/l, respectively, on the third day of hospitalisation. 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. The patient defervesced within 48 hours after starting antibiotics, while he continued receiving 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 left-over scar at the site of the chest eschar. Two serum samples along with whole blood, one sample 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 by using the QIAamp DNA blood mini Kit (QIAGEN, Hilden, Germany) according to manufacturer's instructions. Real-time 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, California, USA). Sera were also tested by IFA for the presence of IgM and IgG antibodies against *A. phagocytophilum* (Focus Diagnostics, California, 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 was doing well >9.5 months after the described events, although a left-over scar at the site of the tick bite remained on the chest wall (Figure 2).

Rickettsia sibirica mongolitimonae, was first isolated from *Hyalomma asiaticum* ticks in Mongolia in 1991 (9). In Europe, the agent has been detected and/or isolated in *Hyaloma anatolicum excavatum* in Greece (4) and in *Rhipicephalus pusillus* in Portugal (10) and France (11).

The first human case was described in France in 1996 (12). Since then, human cases have been described in Greece, Portugal, France, and Spain (4, 10, 11, 13). 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. The frequent presence of lymphangitis along with ≥ 1 inoculation eschars is considered characteristic of *R. sibirica mongolitimonae*. Recently, Ramos et al 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°C-39.5°C), myalgia, headache, and a single inoculation eschar, five (83%) had enlarged regional lymph nodes, and three (50%) had regional lymphangitis (14).

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 (15). In fact, early administration of antibiotic therapy for patients with an inoculation

eschar and/or rash, before whole blood sampling, may jeopardize the PCR amplifications. That is why we have recently introduced an IFA screening test for antibodies against six rickettsial species, i.e., the ones identified in ticks during various surveys conducted in Greece, in every clinical case where a tick bite is reported. As a result of this approach > 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 neither cultivated nor was a PCR performed in a cutaneous swab and/or skin biopsy specimen from the inoculation eschar. Since serology detects cross reactive antibodies, like in our case, shared among different SFG rickettsiae, the diagnosis is highly probable rather than proven. However, the patient's clinical picture with 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. In the absence of a sample suitable for PCR analysis and/or of a positive PCR, serological testing can still be very useful for diagnosis. *Rickettsia sibirica mongolitimonae* appears to be an emerging pathogen in both southern and northern Greece.

Conflict of interest

The authors have no funding or conflicts of interest to disclose.

References

1. Babalis T, Tselentis Y, Roux V, Psaroulaki A, Raoult D. Isolation and identification of a rickettsial strain related to *Rickettsia massiliae* in Greek ticks. *Am J Trop Med Hyg.* 1994 Mar;50(3):365-72.
2. Psaroulaki A, Spyridaki I, Ioannidis A, Babalis T, Gikas A, Tselentis Y. First isolation and identification of *Rickettsia conorii* from ticks collected in the region of Fokida in Central Greece. *J Clin Microbiol.* 2003 Jul;41(7):3317-9.
3. Chochlakis D, Bongiorno C, Partalis N, Tselentis Y, Psaroulaki A. Possible *Rickettsia massiliae* infection in Greece: an imported case. *Jpn J Infect Dis.* 2015 Sep 11.
4. Psaroulaki A, Germanakis A, Gikas A, Scoulica E, Tselentis Y. Simultaneous detection of "*Rickettsia mongolotimonae*" in a patient and in a tick in Greece. *J Clin Microbiol.* 2005 Jul;43(7):3558-9.
5. Germanakis A, Chochlakis D, Angelakis E, Tselentis Y, Psaroulaki A. *Rickettsia aeschlimannii* infection in a man, Greece. *Emerg Infect Dis.* 2013 Jul;19(7):1176-7.
6. Psaroulaki A, Ragiadakou D, Kouris G, Papadopoulos B, Chaniotis B, Tselentis Y. Ticks, tick-borne rickettsiae, and *Coxiella burnetii* in the Greek Island of Cephalonia. *Ann N Y Acad Sci.* 2006 Oct;1078:389-99.
7. Kachrimanidou M, Souliou E, Pavlidou V, Antoniadis A, Papa A. First detection of *Rickettsia slovaca* in Greece. *Exp Appl Acarol.* 2010 Jan;50(1):93-6.
8. Kostopoulou V, Chochlakis D, Kanta C, Katsanou A, Rossiou K, Rammos A, et al. A case of human infection by *Rickettsia slovaca* in Greece. *Jpn J Infect Dis.* 2015 Sep 11.
9. Yu XJ, Jin Y, Fan MY, Xu GM, Liu QH, Raoult D. Genotypic and Antigenic Identification of 2 New Strains of Spotted-Fever Group *Rickettsiae* Isolated from China. *Journal of Clinical Microbiology.* 1993 Jan;31(1):83-8.

10. de Sousa R, Barata C, Vitorino L, Santos-Silva M, Carrapato C, Torgal J, et al. *Rickettsia sibirica* isolation from a patient and detection in ticks, Portugal. *Emerg Infect Dis*. 2006 Jul;12(7):1103-8.
11. Edouard S, Parola P, Socolovschi C, Davoust B, La Scola B, Raoult D. Clustered cases of *Rickettsia sibirica mongolitimonae* infection, France. *Emerg Infect Dis*. 2013 Feb;19(2):337-8.
12. Raoult D, Brouqui P, Roux V. A new spotted-fever-group rickettsiosis. *Lancet*. 1996 Aug 10;348(9024):412.
13. Aguirrebengoa K, Portillo A, Santibanez S, Marin JJ, Montejo M, Oteo JA. Human *Rickettsia sibirica mongolitimonae* infection, Spain. *Emerg Infect Dis*. 2008 Mar;14(3):528-9.
14. Ramos JM, Jado I, Padilla S, Masia M, Anda P, Gutierrez F. Human infection with *Rickettsia sibirica mongolitimonae*, Spain, 2007-2011. *Emerg Infect Dis*. 2013 Feb;19(2):267-9.
15. Renvoise A, Rolain JM, Socolovschi C, Raoult D. Widespread use of real-time PCR for rickettsial diagnosis. *FEMS Immunol Med Microbiol*. 2012 Feb;64(1):126-9.

Figures

Figure 1: Geophysical map of Greece with a focus on Northern Greece, including the island of Samothraki.

Figure 2: A left-over scar at the site of the tick bite remains on the patient's anterior chest wall.

Retracted

<https://www.google.com/maps/d/viewer?mid=1Exiiu19REOQPIycQK8CcYPXjqQI&hl=en>

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