Short Communication

A Case of Human Infection by *Rickettsia slovaca* in Greece

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SUMMARY: Although tick-borne rickettsiosis is endemic in Greece, until recently, human samples arriving at the National Reference Centre under suspicion of rickettsial infection were routinely tested only for *Rickettsia typhi* and *R. conorii*. However, identification of additional rickettsia species in ticks prompted revision of the protocol in 2010. Until that year, all human samples received by the laboratory were tested for antibodies against *R. conorii* and *R. typhi* only. Now, tests for *R. slovaca*, *R. felis*, and *R. mongolotimonae* are all included in routine analysis. The current description of a human *R. slovaca* case is possible as a result of these changes in routine testing.

A 42-year-old woman from the island of Corfu, Greece, was hospitalized in November 2011 for high-grade fever (up to 39.5°C) and malaise. The patient had visited a physician 2 days before admission and was administered clarithromycin (500 mg twice daily) with no improvement. Routine blood tests revealed moderately elevated transaminases: AST: 116 IU/L (NR < 33 IU/L), ALT: 99 IU/L (NR < 31 IU/L), γ GT: 143 IU/L (NR < 32 IU/L), ALP: 141 IU/L (NR < 125 IU/L), LDH: 532 IU/L (NR < 480 IU/L) (Table 1). The patient reported living in a rural area of the island, but no contact with animals. A careful clinical examination did not reveal any signs of a tick bite such as an eschar or lymphadenopathy; however, 2 days after admission the patient developed a maculopapular rash on the face, thorax, and extremities, including the palms and soles. In addition, she complained of myalgia, arthralgia, and a non-productive cough. On clinical examination, conjunctivitis was also noted. A summary of the patient's clinical data is shown in Table 2. New blood tests 4 days after admission showed mild leucopenia (WBC: 3.3×10^6 /L) and further increase of transaminase levels (AST: 210 IU/L, ALT: 243 IU/L, γ GT: 300 IU/L, ALP: 296 IU/L, LDH: 829 IU/L) (Table 1). The patient received doxycycline (100 mg twice daily for 7 days) as soon as the rash developed. One week later, she became afebrile and the rash faded, although malaise persisted. The patient returned home and at one month follow-up there was complete resolution of symptoms and normalization of blood tests.

Blood samples, whole and serum, were drawn 10 days after hospitalization. DNA was extracted using the QIAamp DNA blood mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions.

Real-time PCR targeting the *gltA* gene of *Rickettsia* spp. was performed as previously described (1). As confirmation, the *ompA* gene was also amplified by PCR (2). Additionally, real-time PCR was used to test for the DNA of *Anaplasma phagocytophilum*, *Borrelia* spp., and *Bartonella* spp. IFA was used to test serum for presence of antibodies against *Rickettsia* spp., using a slide that tested for *R. conorii*, *R. siberica*, *R. slovaca*, *R. felis*, *R. massiliae*, and *R. typhi* as individual antigens (Fuller laboratories, Fullerton, CA, USA). IFA was also conducted for antibodies against *A. phagocytophilum* (Focus Diagnostics, Cypress, CA, USA), *B. henselae*, and *B. quintana* (Focus Diagnostics). Western blot analysis was used to test for antibodies against *Borrelia* spp. (Mikrogen Diagnostik, Neuried, Germany). All analyses were performed at the Regional Laboratory of Public Health of Crete, Greece.

IgM antibodies against *R. slovaca* were detected (1/512). No antibodies were detected against any other *Rickettsia* spp., *Borrelia* spp., *Bartonella* spp., or *A. phagocytophilum*. Both real-time PCR and PCR targeting the *ompA* gene of *Rickettsia* spp. gave positive results. All real-time PCR analyses for other pathogens were negative. The PCR product corresponding to the *ompA* gene was sequenced, revealing a 100% similarity to an already *R. slovaca* published sequence (U43808). This sequence is also identical to a strain detected in ticks in Northern Greece (3).

Tick-borne rickettsiosis is endemic in Greece; various *Rickettsia* species have been identified in humans and ticks in the past 10 years (4–10). Recently, *R. slovaca* infection was detected for the first time in ticks in Northern Greece (3).

The pathogen was first isolated in 1968 from *Dermacentor marginatus* in Slovakia (11). The first proven case of infection was reported in France in 1997 (12). In recent years, human *R. slovaca* infection has been reported with increasing frequency in France, Hungary, Spain, Italy, Germany, and Bulgaria (13–16). Clinical signs of infection consist of a skin lesion (eschar) at the site of inoculation, usually the scalp, and regional lymphadenopathy. This recently described syndrome is

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Table 1. Summary of laboratory data

Variable	Reference range, adult	On admission	3rd day	6th day	10th day
Hematocrit	36.0–46.0	43.4	38.2	38.0	34.6
Hemoglobin	12.0–16.0	14.5	13.0	13.0	11.8
White-blood-cell count (per mL)	4,500–11,000	6,150	3,390	6,080	5,720
Differential count (%)					
Neutrophils	40–70	78.3	39.0	41.5	64.9
Lymphocytes	22–44	16.5	26.1	47.1	26.4
Monocytes	4–11	4.8	34.7	9.8	7.35
Eosinophils	0–8	0.2	0.2	0.0	0.6
Basophils	0–3	0.2	0.0	0.9	0.7
Platelet count (per mm ³)	150,000–400,000	149,000	160,000	249,000	321,000
Erythrocyte sedimentation rate (mm/h)	0–15		30		30
Sodium (mmol/L)	135–145	137	139	144	141
Potassium (mmol/L)	3.4–4.8	4.4	3.8	3.5	3.5
Urea nitrogen (mg/dL)	15–45	18	11	21	19
Creatinine (mg/dL)	0.60–1.30	0.9	0.8	0.7	0.6
Glucose (mg/dL)	70–110	79	92	80	86
Alkaline phosphatase (U/L)	45–125	141	296	179	130
Aspartate aminotransferase (U/L)	5–33	116	210	168	65
Alanine aminotransferase (U/L)	5–31	99	243	275	176
Lactate dehydrogenase (U/L)	170–480	532	829	679	451
Creatine kinase (U/L)	26–140	43	51	25	21
γ -glutamyltransferase (U/L)	7–32	143	300	223	165
Bilirubin (mg/dL)					
Total	0.0–1.0	0.7	0.5		
Direct	0.0–0.4	0.3	0.2		

Table 2. Summary of patient's clinical data

Symptom and sign	3 days before admission	On admission	2nd day	6th day	10th day
Fever	+	+	+	+	–
Malaise	+	+	+	+	+
Eschar	–	–	–	–	–
Lymphadenopathy	–	–	–	–	–
Conjunctivitis	–	+	+	–	–
Maculopapular rash	–	–	+	+	+/-
Myalgias	–	–	+	+	+/-
Arthralgias	–	–	+	+	+/-
Cough	–	–	+	+	+

known as SENLAT (scalp eschar associated with neck lymphadenopathy after a tick bite), or TIBOLA/DEBONEL (tick-borne lymphadenopathy/*Dermacentor*-borne necrosis, erythema, lymphadenopathy). Often caused by *R. slovaca*, it is characterized as a local infection controlled by the immune system, which is neither pathogen- nor vector-specific (17,18). Fever and rash are not as common as in other rickettsial infections. In the current case, the clinical presentation included high-grade fever and a maculopapular rash, whereas other main signs (eschar, lymphadenopathy) were missing. There are previous reports of *R. slovaca* infections without skin lesions (19), or that present a maculopapular rash (14), indicating that *R. slovaca* can be associated with other clinical forms. Moreover, our patient presented with moderately elevated trans-

aminase levels, indicating hepatic involvement in the course of infection.

To our knowledge, this is the first reported case of human infection by *R. slovaca* in Greece. The case was also associated with atypical clinical manifestations. Clinicians should bear in mind the possibility of infection by pathogens other than the well-known *R. conorii* and *R. typhi*.

Conflict of interest None to declare.

REFERENCES

1. Stenos J, Graves SR, Unsworth NB. A highly sensitive and specific real-time PCR assay for the detection of spotted fever and typhus group Rickettsiae. *Am J Trop Med Hyg.* 2005;73:1083-5.
2. Regnery RL, Spruill CL, Plikaytis BD. Genotypic identification of rickettsiae and estimation of intraspecies sequence divergence for portions of two rickettsial genes. *J Bacteriol.* 1991;173:1576-89.
3. Kachrimanidou M, Souliou E, Pavlidou V, et al. First detection of *Rickettsia slovaca* in Greece. *Exp Appl Acarol.* 2010;50:93-6.
4. Antoniou M, Economou I, Wang X, et al. Fourteen-year seroepidemiological study of zoonoses in a Greek village. *Am J Trop Med Hyg.* 2002;66:80-5.
5. Gikas A, Doukakis S, Pedititis J, et al. Murine typhus in Greece: epidemiological, clinical, and therapeutic data from 83 cases. *Trans R Soc Trop Med Hyg.* 2002;96:250-3.
6. Gikas A, Kokkini S, Tsioutis C, et al. Murine typhus in children: clinical and laboratory features from 41 cases in Crete, Greece. *Clin Microbiol Infect.* 2009;15 Suppl 2:211-2.
7. Psaroulaki A, Germanakis A, Gikas A, et al. Simultaneous detection of "*Rickettsia mongolotimonae*" in a patient and in a tick in Greece. *J Clin Microbiol.* 2005;43:3558-9.
8. Psaroulaki A, Germanakis A, Gikas A, et al. First isolation and genotypic identification of *Rickettsia conorii* Malish 7 from a

- patient in Greece. Eur J Clin Microbiol Infect Dis. 2005;24:297-8.
9. Psaroulaki A, Ragiadakou D, Kouris G, et al. Ticks, tick-borne rickettsiae, and *Coxiella burnetii* in the Greek Island of Cephalonia. Ann N Y Acad Sci. 2006;1078:389-99.
10. Psaroulaki A, Spyridaki I, Ioannidis A, et al. First isolation and identification of *Rickettsia conorii* from ticks collected in the region of Fokida in Central Greece. J Clin Microbiol. 2003;41:3317-9.
11. Řeháček J. *Rickettsia slovaca*, the organism and its ecology. Acta SC Nat Brno. 1984;18:1-50.
12. Raoult D, Berbis P, Roux V, et al. A new tick-transmitted disease due to *Rickettsia slovaca*. Lancet. 1997;350:112-3.
13. Ibarra V, Oteo JA, Portillo A, et al. *Rickettsia slovaca* infection: DEBONEL/TIBOLA. Ann N Y Acad Sci. 2006;1078:206-14.
14. Raoult D, Lakos A, Fenollar F, et al. Spotless rickettsiosis caused by *Rickettsia slovaca* and associated with Dermacentor ticks. Clin Infect Dis. 2002;34:1331-6.
15. Rieg S, Schmoldt S, Theilacker C, et al. Tick-borne lymphadenopathy (TIBOLA) acquired in Southwestern Germany. BMC Infect Dis. 2011;11:167.
16. Selmi M, Bertolotti L, Tomassone L, et al. *Rickettsia slovaca* in Dermacentor marginatus and tick-borne lymphadenopathy, Tuscany, Italy. Emerg Infect Dis. 2008;14:817-20.
17. Dubourg G, Socolovschi C, Del Giudice P, et al. Scalp eschar and neck lymphadenopathy after tick bite: an emerging syndrome with multiple causes. Eur J Clin Microbiol Infect Dis. 2014;33:1449-56.
18. Foissac M, Socolovschi C, Raoult D. [Update on SENLAT syndrome: scalp eschar and neck lymph adenopathy after a tick bite]. Ann Dermatol Venereol. 2013;140:598-609.
19. Komitova R, Lakos A, Aleksandrov A, et al. A case of tick-transmitted lymphadenopathy in Bulgaria associated with *Rickettsia slovaca*. Scand J Infect Dis. 2003;35:213.