

Aneruptive Fever Associated with Antibodies to *Rickettsia helvetica* in Europe and Thailand†

Pierre-Edouard Fournier,¹ Caroline Allombert,¹ Yupin Supputamongkol,² Giuseppe Caruso,³ Philippe Brouqui,¹ and Didier Raoult^{1*}

Unité des rickettsies, IFR 48, CNRS UMR 6020, Faculté de médecine, Université de la Méditerranée, 13385 Marseille cedex 05, France¹; Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand²; and Department of Infectious Diseases, Ospedale S. Martino, Belluno, Italy³

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We report that eight patients from France, Italy, and Thailand had serological evidence of *Rickettsia helvetica* infection. The infection presented as a mild disease in the warm season and was associated with fever, headache, and myalgia but not with a cutaneous rash. *R. helvetica* should be suspected in patients with unexplained fever, especially following a bite from an *Ixodes* sp. tick.

Rickettsia helvetica, a rickettsia from the spotted fever group, has been detected in *Ixodes* sp. ticks in Switzerland, Sweden, France, Italy, Portugal, Slovenia, and Japan (1, 2, 4–6, 18). To date, the pathogenic role of this rickettsia is unclear but has been suspected in acute perimyocarditis (12), unexplained febrile illness (7), sarcoidosis (13), and fever following an *Ixodes holocyclus* bite (8).

Using the microimmunofluorescence assay (9) and Western blotting performed on early-phase serum (21), we searched the serum from 37,945 patients with a suspected rickettsial infection for antibodies to *R. conorii*, *R. slovaca*, *R. massiliae*, *R. mongolotimonae*, *R. helvetica*, *R. felis*, *R. typhi*, *Borrelia burgdorferi*, and *Anaplasma phagocytophilum* for European patients and to *R. helvetica*, *R. felis*, *R. honei*, *R. japonica*, *R. typhi*, *Orientia tsutsugamushi*, and *Leptospira interrogans* serotype Icterohaemorrhagiae for Asian patients. Informed consent was obtained from all patients. We used microimmunofluorescence assay titers of 1:64 for immunoglobulin G (IgG) and 1:32 for IgM as cutoff values for rickettsial infections. We also searched for antibodies to the tick-borne encephalitis virus (IgM TBE ELISA kit; IBL Laboratories, Hamburg, Germany) in the sera of European patients. When cross-reacting antibodies above 1:64 prevented the identification of the infecting agent, we performed cross-adsorption by using either *R. helvetica* and *R. conorii* antigens for European patients or *R. helvetica* and *R. honei* antigens for Asian patients, followed by Western blotting, as previously described (10). To detect a specific anti-*R. helvetica* response in eight patients, i.e., three Italian, two French, and three Thai patients, we used the following diagnostic criteria: (i) a Western blot showing an antibody response only to *R. helvetica* proteins (21) and/or (ii) the presence of homologous antibodies directed against *R. helvetica* following cross-adsorption (10). For these patients, serum was the only type of specimen available. The case of one of the two French

patients has previously been reported (7). Among the five European patients, patients 1 to 3 (Table 1) exhibited IgM titers of >1:32 to *R. helvetica* in their early-phase serum; patient 4 developed an IgG titer of 1:128 and an IgM titer of 1:64 in his convalescent-phase serum; and patient 5, for whom only an early-phase sample was available, was seronegative. The Western blot demonstrated the presence of antibodies only to *R. helvetica* in patients 1 and 5 (Fig. 1a), and cross-adsorption revealed the presence of homologous antibodies to *R. helvetica* in patients 2 to 4 (Fig. 1b). All other serological tests were negative for all five patients. Among the Thai patients, patients 7 and 8 exhibited IgM titers of >1:32 to *R. helvetica* in the early-phase serum, and patient 6 had an IgG titer of 1:128. The Western blot demonstrated the presence of antibodies directed only against *R. helvetica* in patient 7, and cross-adsorption revealed the presence of homologous antibodies to *R. helvetica* in patients 6 and 8. Serological tests for *L. interrogans* were negative for all Thai patients.

The median age of the patients was 53 years (range, 27 to 70 years) (Table 1). Three European patients were male, and four patients reported suffering a tick bite in a forest during the period from May to August. Clinical symptoms included fever, headache, and arthralgia in all patients, myalgia in four, and an inoculation eschar in one but no cutaneous rash. An elevated leukocytosis was found in one case, thrombocytopenia was found in two cases, and elevated transaminase levels were found in three cases. None of these patients received any antibiotic, and all recovered without any sequelae. All three Thai patients were male and worked as rice farmers, and none reported any tick bite. Infection occurred from October to January. All three patients suffered from fever and headache, and two suffered from unilateral conjunctivitis but did not suffer from inoculation eschar or rash. An elevated leukocyte count was noted in two patients, and thrombocytopenia and elevated transaminase levels were noted in one. Other possible diagnoses of fever in the patients from Thailand, including malaria, viral hepatitis, and typhoid fever, had previously been eliminated. The patients received either 10 MU of penicillin G, 3 g of cefotaxime, or 200 mg of doxycycline/day and recovered without sequelae.

Overall, in the warm season eight adult patients developed a

* Corresponding author. Mailing address: CNRS UMR 6020, Faculté de médecine, Université de la Méditerranée, 27 Boulevard Jean Moulin, 13385 Marseille cedex 05, France. Phone: (33) 04 91 38 55 17. Fax: (33) 04 91 83 03 90. E-mail: didier.raoult@medecine.univ-mrs.fr.

† In memory of Giuseppe Caruso (1938–2003), a famous tick hunter, dedicated medical doctor, and friend.

TABLE 1. Epidemiological and clinical findings for 13 patients with *R. helvetica* infection

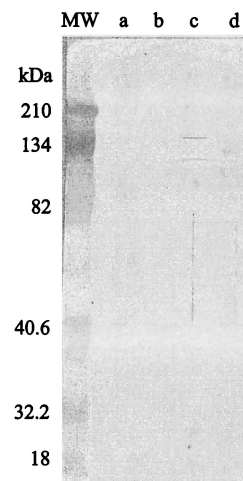
Patient	Age ^b	Sex	Geographic area	Month of onset	Fever ^a	Head-ache ^a	Myal-gia ^a	Arthral-gia ^a	Conjunc-tivitis ^a	Report of tick bite (location) ^a	Inoculation eschar (location) ^a	Cuta-neous rash ^a	Treatment ^a	Recov-ery ^a
1	70	M	Northern Italy	May	+	+	-	+	-	+ (right thigh)	+ (right thigh)	-	No treatment	-
2	58	M	Northern Italy	May	+	+	+	+	-	+ (left buttock)	-	-	No treatment	-
3	50	F	Northern Italy	May	+	+	+	+	-	+	-	-	No treatment	-
4	37	M	Northeastern France	August	+	+	+	+	-	-	-	-	No treatment	-
5	63	F	Northeastern France	June	+	+	-	+	-	+ (leg)	-	-	No treatment	-
6	27	M	Northeastern Thailand	January	+	+	+	-	-	-	-	-	Doxycycline	-
7	43	M	Northeastern Thailand	December	+	+	+	-	+	-	-	-	Cefotaxime	-
8	57	M	Northeastern Thailand	October	+	+	+	-	+	-	-	-	Doxycycline	-
Total (%)	6M, 2F				8 (100)	8 (100)	6 (75)	5 (62)	2 (25)	4 (50)	1 (12.5)	0		8 (100)

^a +, positive criterion; -, negative criterion.

^b The mean age of the patients was 50.6 ± 14.2 years.

mild, aneuruptive febrile illness associated with headache and myalgia and, in only one case, an inoculation eschar. The lack of one or several of the classical symptoms of rickettsiosis does not eliminate this diagnosis. For example, the eschar is rare in Israeli spotted fever and Astrakhan fever and exceptional in Rocky Mountain spotted fever (19). A rash is also rare in *R. slovaca* infections (17) and present in only 50% of cases of *R. africae* infections (16). Elevated liver enzymes, or thrombocytopenia, common in rickettsioses, was observed in 75% of our patients. Determination of the causative role of *R. helvetica* in the illness in these patients relied on an array of evidence. (i) We tested our patients' sera for rickettsial species known to be endemic in their own areas and found antibodies specifically directed against *R. helvetica*. Although serological cross-reactions were observed among tested antigens (9), Western blots showing antibodies to *R. helvetica* only or cross-adsorption to remove heterologous antibodies reflected specific antibody responses, as previously demonstrated for *R. slovaca*, *R. africae*, *R. prowazekii*, and *R. typhi* infections (10, 16, 17) (Fig. 1). (ii) The causative role of *R. helvetica* was considered, as it is the main rickettsia found in *Ixodes* sp. in Europe, occurring in from 2 to 36.8% of *I. ricinus* ticks (3, 5, 11, 14, 14, 18). In Thailand, its presence is as yet unknown in *Ixodes* sp. ticks, although human cases of *R. helvetica* infection have been highly suspected in that country (15). (iii) The season of occurrence and interviews with patients suggested that the infections were caused by *Ixodes* sp. ticks. All patients were diagnosed in a warm season, which is consistent with the optimal development conditions of immature *Ixodes* sp. ticks. Although four patients from Europe reported a tick bite, the tick species was not identified. However, in Italy *I. ricinus* represents more than 90% of all ticks collected from humans during the spring (18). In Thailand, *Ixodes* sp. ticks are endemic, including *I. ovatus* (20), which is known to harbor *R. helvetica* in Japan (6). (iv)

a



b

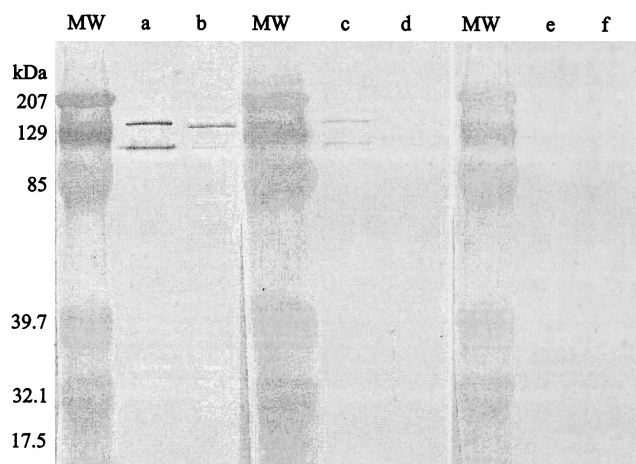


FIG. 1. (a) Western immunoblot for patient 1 showing an antibody response directed against outer membrane proteins of *R. helvetica* only. Lane a, *R. conorii*; lane b, *R. slovaca*; lane c, *R. helvetica*; lane d, *R. mongolotimonae*. (b) Western immunoblot for patient 11 before and after cross-adsorption with *R. helvetica* or *R. honeii*. Lanes a, c, and e, *R. helvetica* antigen; lanes b, d, and f, *R. honeii* antigen. Lanes a and b, untreated serum; lanes c and d, serum adsorbed with *R. honeii*; lanes e and f, serum adsorbed with *R. helvetica*. MW, molecular mass; kDa, kilodalton.

These patients tested negative against other pathogens endemic in these areas.

In our series, *R. helvetica* infection presented as a flu-like, self-limiting febrile illness without any sequelae. Further studies to confirm the causative role of *R. helvetica* in acute febrile illness should be conducted by culture or PCR amplification from clinical specimens.

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