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RESEARCH NOTE

Serological evidence of *Rickettsia* infections in forestry rangers in north-eastern Italy

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ABSTRACT

The prevalence of antibodies to Rickettsiae and other tick-borne microrganisms in the sera of 181 forestry rangers from Friuli-Venezia-Giulia, Italy, was examined. Seven (3.9%) sera were positive for *Rickettsia conorii* and *Rickettsia helvetica*, as single or dual infections; four of these sera had been found previously to be positive for *Borrelia burgdorferi*. Antibodies to *Coxiella burnetii* were detected in five (2.8%) sera, four of which were also positive for *B. burgdorferi*. These findings indicate that patients in this north-eastern Italian region with fever subsequent to tick-bite should be investigated for *Rickettsia* and *Coxiella* infections.

Keywords Antibodies, *Borrelia burgdorferi*, *Coxiella burnetii*, forestry rangers, Rickettsiae, tick-bites

Original Submission: 6 June 2005; **Revised Submission:** 22 September 2005; **Accepted:** 7 October 2005

Clin Microbiol Infect 2006; 12: 493–495
10.1111/j.1469-0691.2006.01385.x

Tick-borne diseases caused by organisms transmitted by the tick *Ixodes ricinus*, such as Lyme borreliosis (LB), tick-borne encephalitis (TBE) and anaplasmosis (formerly ehrlichiosis), have been detected previously in the Friuli-Venezia-Giulia (FVG) region of Italy [1–3]. *Ixodes* spp. ticks have the potential to transmit several human pathogens, including members of the rickettsiales. Patients with antibodies to *Rickettsia helvetica*, and evidence of this microrganism in the ticks, have been reported in Switzerland, Sweden, France, Portugal and Japan [4], and also in an Italian region bordering FVG [5–7]. Infections concomitant with or consecutive to infections with *Coxiella burnetii* and other tick-borne organisms, such as *Rickettsia conorii*, *Rickettsia slovaca*, *Rickettsia africae* and *Francisella tularensis*, have been detected in patients from southern France, none of whom had a history of Q fever [8]. In a previous prevalence study carried out among 181 forestry rangers from FVG, Italy, seropositivity levels of 0.6% for TBE virus, 23.2% for *Borrelia burgdorferi* and 0.6% for *Anaplasma phagocytophilum* were detected, with 21 of the rangers having a previous diagnosis of LB [1]. The present study aimed to extend the previous serosurvey by investigating the presence of antibodies to Rickettsiae and other tick-borne microorganisms in the same set of sera, obtained from 181 forestry

rangers in November 2002. A questionnaire concerning the clinical history of each subject, including possible clinical manifestations of tick-borne diseases, was completed at the time of collection of the serum samples. Antibodies to *B. burgdorferi* were detected previously in these sera with the C6 ELISA IgG kit (Immunetics, Cambridge, MA, USA) [1].

The sera were assayed by microimmunofluorescence for IgM, IgG and, sometimes, IgA against a large panel of antigens, comprising *Rickettsia typhi*, *C. burnetii*, *Rickettsia felis*, *R. helvetica*, *R. conorii*, *Rickettsia israeli*, *R. africae*, *Rickettsia sibirica mongolotimonae*, *Rickettsia massiliae*, *R. slovaca*, *F. tularensis*, *Bartonella henselae*, and *Bartonella quintana*, at the WHO Collaborative Centre for Rickettsial Research (Marseille, France), as described previously [9]. When cross-reacting antibodies with a titre > 1:64 prevented identification of the infecting agent, cross-adsorption was performed using either *R. helvetica* or *R. conori* antigens. Positive sera were assayed further by western blot IgG as described previously [8].

All sera were negative for *B. henselae*, *B. quintana* and *F. tularensis*, but seven (3.9%) sera were positive for *R. conorii* and *R. helvetica* by microimmunofluorescence (IgG titres > 1/64), and four of these sera cross-reacted with different rickettsial antigens. Western blot revealed a band-pattern typical of *R. helvetica* for serum 1. Three sera were cross-adsorbed with either *R. conorii* or *R. helvetica* antigens, and were assayed further by western blot to identify the species-specific antibodies. Serum 2 maintained reactivity with *R. conorii* following adsorption with *R. helvetica*, while serum 3 showed a residual band with *R. helvetica* following adsorption with *R. conorii*; this indicates that serum 2 had antibodies to *R. conorii* and that serum 3 had antibodies to *R. helvetica*. Serum 4 was positive for both rickettsial antigens with an IgG titre of 1/1024 and an IgM titre of 1/128; a dual infection with *R. helvetica* and *R. conorii* has been shown with a higher absorption. Since no history of Boutonneuse Fever or other Spotted Fever group rickettsial diseases had been reported by the forestry rangers, these antibodies were considered to be elicited by repeated or concomitant asymptomatic infections. Four of the seven sera had been found previously to be positive for *B. burgdorferi* [1].

Five (2.8%) sera were positive for *C. burnetii*. Antibody titres were high, and both phases of *C. burnetii* were identified by microimmunofluorescence (Table 1). On the basis of antibody titres (IgG and IgM) obtained during phase I and/or II, the patterns of sera 1, 2, 3 and 5 indicated chronic infections, while the pattern of serum 4 indicated an acute infection. No clinical history of Q fever, particularly symptoms associated with atypical pneumonia, hepatitis or endocarditis, was obtained for these five forestry rangers, four of whom had been found previously to be seropositive for *B. burgdorferi* [1]. Interestingly, of the 42 forestry rangers seropositive for *B. burgdorferi*, four (9.5%) were also positive for *C. burnetii*, whereas only one (0.7%) of 139 forestry rangers seronegative for *B. burgdorferi* was positive for *C. burnetii* (chi-square test, $p < 0.01$).

Table 1. Serological findings for *Borellia burgdorferi* among the five forestry rangers who were seropositive for *Coxiella burnetii*[a]

Serum No	*C. burnetii*, phase I IgG/IgM/IgA	*C. burnetii*, phase II IgG/IgM/IgA	*B. burgdorferi* (C6 ELISA)[b]
1	6400/0/100	3200/0/50	+ (1121)
2	3200/100/1600	1600/50/800	+ (221)
3	3200/200/400	1600/100/200	+ (202)
4	100/200/0	50/100/0	–
5	1600/50/0	800/25/0	+ (415)

[a]Titres obtained by microimmunofluorescence.
[b]OD values (cut-off value = 183).

More than 40 tick species, including *Ixodes*, *Rhipicephalus* and *Amblyomma*, are infected naturally with *C. burnetii* [10]. However, arthropod-borne transmission of the Q fever agent in humans is reported rarely. According to the present findings, the significant association between *C. burnetii* and *B. burgdorferi* seropositivity, undoubtedly transmitted through *I. ricinus* bites, suggests that this tick may also transmit *C. burnetii* in some areas of FVG. Q fever has been detected in almost every country, but its incidence is probably underestimated because of poor surveillance of the disease [10]. Indeed, only a few sporadic cases have been notified in Italy since 1970 [11]. More recently, two outbreaks of Q fever occurred in Vicenza and Como, northern Italy, involving 54 and 133 patients, respectively [12,13]. In both outbreaks, indirect exposure to migrating flocks of sheep turned out to be the only risk-factor for acquisition of *C. burnetii* infection. This infection is usually asymptomatic or manifests as a mild flu-like illness in humans. Among symptomatic patients, most experience a self-limited febrile illness, atypical pneumonia or hepatitis,

with < 1% suffering from chronic Q fever, including endocarditis [10]. Thus, *C. burnetii* infection is usually not detected unless serological testing is performed.

To our knowledge, this is the first report to demonstrate the presence of *R. helvetica* antibodies, as well as co-infection with *R. conorii*, in FVG. The findings suggest that *C. burnetii* and other rickettsial tick-transmitted agents should be considered in the differential diagnosis of febrile patients with a recent history of tick bite in this north-eastern region of Italy.

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