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Brief report

Risk of acquiring tick-borne infections in forestry workers from Lazio, Italy

Simona Di Renzi¹, Agnese Martini¹, Alessandra Binazzi¹, Alessandro Marinaccio¹,
Nicoletta Vonesch¹, Wanda D'Amico¹, Tommaso Moro², Cristiano Fiorentini³, Maria
Grazia Ciufolini³, Paolo Visca⁴, Paola Tomao¹✉

¹*Department of Occupational Medicine, Istituto Superiore per la Prevenzione e Sicurezza
del Lavoro, 00040 Monte Porzio Catone, Rome, Italy;*

²*Immune-Haematological and Blood Transfusion Unit, Presidio Ospedaliero “San
Camillo de’ Lellis”, 02100 Rieti, Italy;*

³*Department of Infectious, Parasitic and Immune-Mediated Diseases, Istituto Superiore di
Sanita`, 00161 Rome, Italy;*

⁴*Department of Biology, University “Roma Tre”, 00146, Rome, Italy.*

✉ Corresponding author: paola.tomao@ispesl.it

Phone: +39 0697896109

Fax: +39 0694181410.

Key-words: occupational risk; forestry workers; *Borrelia burgdorferi*; *Ixodes ricinus*; TBE
virus.

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6 **ABSTRACT**

7 The seroprevalence of antibodies to *Borrelia burgdorferi* and tick-borne
8 encephalitis (TBE) virus was evaluated in a group of forestry rangers in Lazio region
9 (Italy). A hundred and forty-five forestry rangers and 282 blood donors were examined by
10 two-tiered serological tests for *B. burgdorferi* and TBE virus. Information on occupation,
11 residence, tick bites, outdoor leisure activities and other risk factors was obtained. The
12 prevalence of IgG/IgM antibodies to *B. burgdorferi* showed no statistical difference
13 between the two groups, but there was higher occurrence of IgM antibodies. There were
14 significant differences between indoor and outdoor, urban and rural workplaces among the
15 145 exposed workers (χ^2 test: $p < 0.001$), and a higher risk for outdoor rural than urban tasks
16 was detected among the 10 Western blot-tested forestry rangers positive to *B. burgdorferi*
17 (χ^2 test: $p < 0.1$). No seropositivity was observed for the TBE virus. Forestry rangers from
18 Lazio region did not have a higher risk of *Borrelia* infection than the blood donors, though
19 an increase in the risk for outdoor tasks in a rural environment was observed.
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Lyme borreliosis (LB) and tick-borne encephalitis (TBE) are tick-borne diseases that are transmitted in Europe by hard ticks, and pose growing health problems in rural areas.

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LB is a multisystem, multistage, inflammatory disease resulting from infection by the spirochete *Borrelia burgdorferi* sensu lato (s.l.), which is transmitted by the tick *Ixodes ricinus*. It may cause skin, joint, neurological, ocular and cardiac disorders. Since forests and woods are the preferred ecosystem for maintenance of LB [1], the risk of contracting LB is greater in forestry workers, farmers, and other workers in wooded areas [2,3] which can acquire LB from infected ticks taking a blood meal.

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TBE virus (TBEV, Flaviviridae family) infection is a biphasic febrile infection, often with neurological manifestations of meningo-encephalitis. The disease is prevalent in certain European areas and, like LB, transmitted by *I. ricinus*.

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Sero-epidemiological studies suggest that LB and TBE infections are more prevalent among people exposed to tick bites, such as forestry workers [4].

To assess the occupational risk factors for tick-borne infections, the seroprevalence of antibodies to *B. burgdorferi* and TBEV was compared in 145 forestry rangers (exposed subjects) working in Central Italy and 282 blood donors (controls) from the same area (Lazio region; 17,227 Km² with ca. 22 % of wood; population 5,264,077 inhabitants). All 427 subjects were interviewed through a questionnaire and provided informed consent to the study (Supplementary text). All subjects were free of signs and symptoms of LB and TBEV infection. Both groups were age- and sex-modelled. Age was analyzed by ranking subjects in three groups with cut-off at the 33rd percentile and no significant difference was found (Supplementary table 1).

Sera were collected in Summer 2008 and stored at -80°C until used. Enzyme-linked immunosorbent assays (ELISA; recombWell *Borrelia* IgG and recombWell *Borrelia* IgM Arnika Diagnostics, Milan, IT; FSME IgG Immunozy, Progen Biotech GmbH, Heidelberg, DE) were used for the serological screening, followed by confirmatory Western blot analysis (WB; *Borrelia* “MIQ” Virablot Test kit IgG/IgM - Arnika Diagnostics; recomLine *Borrelia* IgM, Mikrogen, Neuried, DE) and haemagglutination inhibition (HI) [5] for *B. burgdorferi* and TBEV, respectively (Supplementary text). Since

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6 sera were taken during the period of highest tick activity, both IgM- and IgG -positive
7 results were considered as markers of recent and past infection, respectively.
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9 Tests for the TBE virus by ELISA showed 3 positive and 3 borderline samples
10 (4.1%), all among exposed forestry workers, but haemagglutination did not confirm any of
11 the samples. This is in agreement with the known epidemiology of TBE in Italy, as no
12 evidence of TBE circulation has so far been provided in Lazio region.
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15 Among both the exposed subjects and controls, there were 14 positive and 6
16 borderline samples in the ELISA for IgG antibodies to *B. burgdorferi* s.l., while the IgM
17 ELISA for *B. burgdorferi* s.l. showed 39 positive and 15 borderline samples (Table).
18 Confirmatory WB was then performed for all positive or borderline IgG and IgM sera.
19 Four subjects (1 exposed and 3 controls) were positive for IgG WB, and 42 (19 exposed
20 and 23 controls) for IgM WB with the *Borrelia* “MIQ” Virablot Test (Arnika). Retesting of
21 IgM-positive sera 20 months after sampling with the more restrictive *recomLine Borrelia*
22 IgM assay (Mikrogen) confirmed seropositivity for only 28 subjects (9 exposed and 19
23 controls). This last result was confidently considered as true IgM-positive, and used for
24 statistical analyses (Table). Overall, the WB-confirmed seropositivity for IgG and IgM was
25 10/145 (6.89%) among exposed subjects and 22/282 (7.80%) among controls. No sample
26 tested WB-positive for both IgG and IgM.
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29 A test for the difference between percentages of overall confirmed positive (IgG
30 IgM WBs) in the exposed and control group was performed, but it was not statistically
31 significant ($p > 0.1$).
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34 Significant differences were observed between indoor and outdoor, urban and rural
35 workplaces among the 145 exposed subjects (χ^2 test: $p < 0.001$), and a higher risk for
36 outdoor tasks performed in a rural rather than urban environment among the 10 positive
37 exposed subjects (χ^2 test: $p = 0.075$). A multivariate regression model applied to evaluate
38 the explanatory variables for seroprevalence of antibodies against *B. burgdorferi* in the 145
39 exposed subjects (Supplementary text) showed a higher seroprevalence associated to rural
40 work environment (OR: 1.74; 95%CI: 0.25-11.86), lack of individual protective devices
41 (OR: 2.45; 95%CI: 0.37-16.08) and male sex (OR: 1.10; 95%CI: 0.14-8.49), although none
42 of these estimates reached the level of statistical significance ($p > 0.05$).
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6 While a significantly higher prevalence of anti-*B. burgdorferi* antibodies in forestry
7 workers than in the control group was not observed, the absence of significant associations
8 with major risk factors among exposed subjects suggests a similar distribution of such risk
9 factors among controls, without any difference between the two groups. Therefore the
10 seroprevalence observed for the exposed group should be explained exclusively by
11 occupational factors. In this group the analysis of specific jobs showed an increased risk
12 for outdoor tasks performed in rural rather than urban environments.
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18 The prevalence of these tick-borne zoonoses is probably underestimated because of
19 poor surveillance of these diseases. In Italy the infectious diseases reporting system is not
20 widely applied and there is no specific information for workers likely to be frequently
21 exposed to ticks. However, serological surveys in healthy populations at risk of tick-borne
22 diseases, such as LB and TBE, have reported significant proportions with antibodies
23 reactive to microorganisms transmitted by infected *Ixodes* spp. ticks [6], and higher risk of
24 occupational exposure to *B. burgdorferi* among people with outdoor activities [4,7,8]. The
25 overall frequency of *B. burgdorferi* antibodies in forestry workers from this study is
26 similar to that previously reported for another region in Central Italy [7], although IgM
27 seroprevalence is higher (6.20% vs. 3.64% in ref. [7]). Intriguingly, we observed a similar
28 pattern of seropositivity also in the control group (1.06% and 6.73 % for IgG and IgM,
29 respectively), substantially differing from that reported by us in a previous survey of a
30 neighbouring region (1.36% and 2.46 % for IgG and IgM, respectively) [7]. Nevertheless,
31 the observed seropositivity appears to be 2 to 4 fold lower than that reported for Italian
32 regions of LB endemicity [4]. Unfortunately, the lack of a second serum sample did not
33 allow to verify seroconversion in IgM-positive subjects, and hence exclude false positives
34 among those samples that resulted concordantly positive in both IgM WB assays.
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49 We conclude that Lazio is a region of moderately high endemicity for LB.
50 Therefore, the risk of acquiring LB should be considered for outdoor workers in this area,
51 and prevention efforts will need to focus on behavioral intervention to reduce individual
52 exposure by appropriate measures in outdoor environments.
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Table. Prevalence of antibodies against *B. burgdorferi* s.l. by IgG/IgM-Elisa in 145 exposed and 282 control subjects

	IgG-Elisa			IgM-Elisa		
	Exposed (%)	Controls (%)	Total (%)	Exposed (%)	Controls (%)	Total (%)
Positive	5 (3.4)	9 (3.2)	14 (3.3)	19 (13.1)	20 (7.1)	39 (9.1)
Borderline	4 (2.8)	2 (0.7)	6 (1.4)	7 (4.8)	8 (2.8)	15 (3.5)
<i>Confirmed positive by WB1</i>	<i>1 (0.69)</i>	<i>3 (1.06)</i>	<i>4 (0.94)</i>	<i>19 (13.10)</i>	<i>23 (8.16)</i>	<i>42 (9.84)</i>
<i>WB2^a</i>				<i>9 (6.20)</i>	<i>19 (6.73)</i>	<i>28 (6.55)</i>
Negative	136 (93.8)	271 (96.1)	407 (95.3)	119 (82.1)	254 (90.1)	373 (87.4)
Total	145 (100.0)	282 (100.0)	427 (100.0)	145 (100.0)	282 (100.0)	427 (100.0)

^aWB1 performed with the *Borrelia* “MIQ” Virablot Test (Arnika) on positive and borderline ELISA samples. WB2 retesting of IgM-positive sera was performed 20 months after sampling with the *recomLine Borrelia* IgM (Mikrogen) kit.