

Study on tick-borne rickettsiae in eastern Poland. II. Serological response of occupationally exposed populations

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Abstract

A group of 150 persons living in the Lublin province of eastern Poland and occupationally exposed to tick bite were examined by the immunoenzymatic ELISA test for the presence of antibodies against tick-borne Spotted Fever Group (SFG) rickettsiae. The group consisted of 75 forestry workers employed in 3 forest inspectorates and 75 agricultural workers living in 2 villages. As a control group, 43 urban dwellers living in the city of Lublin and not occupationally exposed to tick bite were examined. Among 150 persons occupationally exposed to tick bite, the presence of antibodies against SFG rickettsiae was found in 54 (36.0% of the total). In the control group, the frequency of positive findings was only 4.7%, being significantly smaller compared to the exposed group ($p=0.0001$). Within the exposed group, the percentage of positive results in forestry workers (50.7%) was greater than in agricultural workers (21.3%); the difference was statistically significant ($p=0.0002$). Also within this group, the frequency of positive findings in males (46.5%) was significantly greater than in females (21.9%) ($p=0.0029$). In the exposed group, the positive results tended to increase with the age of the examined persons. However, a significant relationship between age and positive findings was found only in forestry workers ($\chi^2=14.207$, $p=0.00264$), but not in agricultural workers and total exposed workers. The frequencies of positive results in forestry workers varied significantly depending on place of work ($\chi^2=11.271$, $p=0.00357$). Similarly, the difference between the positive reactions in agricultural workers living in 2 villages proved to be significant (34.2% vs. 8.1%; $p=0.0074$). The obtained results indicate that people occupationally exposed to tick bite and living in the area of eastern Poland where over half of *Dermacentor reticulatus* ticks harbour SFG rickettsiae, are under significantly increased risk of infection with these rickettsiae.

Key words

Spotted Fever Group Rickettsiae, serology, ELISA, forestry workers, agricultural workers, eastern Poland

INTRODUCTION

Spotted Fever Group (SFG) rickettsiae comprise more than 20 species of Gram-negative, obligatory intracellular bacteria transmitted to vertebrates by ticks. They occur worldwide and their distribution largely depends on the presence of a specific tick vector [1, 2]. Apart from the well-known pathogenic species *Rickettsia rickettsii* and *Rickettsia conorii*, SFG includes species revealed during last 20 years (*R. japonica*, *R. africae*, *R. slovaca*, *R. sibirica*, *R. helvetica*, *R. raoultii*, *R. massiliae*, *R. aeschlimannii*), described as emerging pathogens [2, 3, 4]. SFG rickettsiae may evoke in humans clinical symptoms such as fever, rash, eschar and tick-borne lymphadenopathy (TIBOLA), also called Dermacentor-borne necrosis erythema, and lymphadenopathy (DEBONEL) [5, 6].

People employed in forestry and agriculture are suspected of being under elevated risk of tick bite and infection with SFG rickettsiae [7, 8, 9]. To study this relationship on the territory of eastern Poland, groups of foresters, agricultural workers and people occupationally not exposed to ticks (firemen, employees of a research institute) were examined.

MATERIALS AND METHODS

Examined population. A total of 150 persons (86 males, 64 females; mean age 46.4 ± 9.5 years) occupationally exposed to tick bite were examined. The group comprised 75 forestry workers (17 females, 58 males; mean age 45.8 ± 9.3 years) and 75 agricultural workers (47 females, 28 males; mean age 47.0 ± 9.7 years). Forestry workers were employed in the forest inspectorates in Puławy, Kraśnik and Świdnik, while agricultural workers lived in the villages of Wilków and Strzyżewice, all on the territory of Lublin province of eastern Poland. As a control group, 43 urban dwellers (20 males, 23 females; mean age 35.8 ± 9.2 years) were examined. Working as firemen or employees of a research institute and living in the city of Lublin, they were not occupationally exposed to tick bite. Blood samples were taken by venipuncture and sera separated by centrifugation.

Serological test. Sera were tested for the presence of IgG antibodies against SFG rickettsiae by the immunoenzymatic test ELISA, using the Spotted Fever Group Rickettsia EIA IgG Antibody Kit (Fuller Laboratories, Fullerton, CA, USA). The test was carried out according manufacturer's instructions. The absorbance values of tested samples were divided by the mean absorbance values of Cutoff Calibrator. The obtained index values below 0.8 were considered negative, between 0.8–1.2 as weak positive (equivocal) and above 1.2 as

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distinctly positive. For statistical analysis, the weak positive results were included into positive group.

Statistical analysis. Analysis was carried out with Student's t-test and χ^2 test for independence, using Statistica 10.0 package (StatSoft, Poland).

RESULTS

Among 150 persons occupationally exposed to tick bite, the presence of antibodies against SFG rickettsiae was found in 54 (36.0% of the total). In the control group, the frequency of positive findings was only 4.7%, being significantly smaller compared to the exposed group ($p=0.0001$) (Tab. 1).

Table 1. Serological response to Spotted Fever Group Rickettsiae antigen, in the occupational and control groups, depending on gender.

Examined group	Number of examined	Number of reactants, percent		
		Distinctly positive	Weak positive	Total positive
Agricultural workers				
Women	47	6 (12.8%)	4 (8.5%)	10 (21.3%)
Men	28	5 (17.9%)	1 (3.6%)	6 (21.4%)
Total	75	11 (14.7%)	5 (6.7%)	16 (21.3%)
Forestry workers				
Women	17	3 (17.7%)	1 (5.9%)	4 (23.5%)
Men	58	27 (46.6%)	7 (12.1%)	34 (58.6%)
Total	75	30 (40.0%)	8 (10.7%)	38 (50.7%)
Total occupationally exposed persons				
Women	64	9 (14.1%)	5 (7.8%)	14 (21.9%)
Men	86	32 (37.2%)	8 (9.3%)	40 (46.5%)
Total	150	41 (27.3%)	13 (8.7%)	54 (36.0%)
Control group				
Women	23	0 (0)	2 (8.7%)	2 (8.7%)
Men	20	0 (0)	0 (0)	0 (0)
Total	43	0 (0)	2 (4.7%)	2 (4.7%)

Within the exposed group, the percentage of positive results in forestry workers (50.7%) was greater than in agricultural workers (21.3%); the difference was statistically significant ($p=0.0002$). Separately, the frequencies of positive results both in forestry and agricultural workers were significantly greater compared to the control group ($p<0.0001$ and $p=0.0214$, respectively). Within the exposed group, the frequency of positive findings in males (46.5%) was significantly greater than in females (21.9%) ($p=0.0029$). None of the examined people showed fever and/or rash typical for the clinical form of SFG rickettsioses.

In the exposed group, the positive results tended to increase with the age of the examined persons (Tab. 2). However, the significant relationship between age and positive findings was found only in forestry workers ($\chi^2=14.207$; $p=0.00264$), but not in agricultural workers and total exposed workers. Among forestry workers, the greatest percentage of positive results was recorded in the age group 40–49 years (56.3%), and the smallest in the age group 20–29 years (25.0%).

The frequencies of positive results in forestry workers varied significantly depending on place of working ($\chi^2=11.271$, $p=0.00357$) (Tab. 3). Similarly, the difference between the positive reactions in agricultural workers living in 2 villages proved to be significant (34.2% vs. 8.1%, $p=0.0074$).

Table 2. Serological response to Spotted Fever Group Rickettsiae antigen, in the occupational and control groups, depending on age.

Examined group/ Age Intervals	Number of examined	Number of reactants, percent		
		Distinctly positive	Weak positive	Total positive
Agricultural workers				
20-29	4	0 (0)	1 (25.0%)	1 (25.0%)
30-39	10	1 (10.0%)	0 (0)	1 (10.0%)
40-49	24	4 (16.7%)	2 (8.3%)	6 (25.0%)
50-59	37	6 (16.2%)	2 (5.4%)	8 (21.6%)
Total	75	11 (14.7%)	5 (6.7%)	16 (21.3%)
Forestry workers				
20-29	4	1 (25.0%)	0 (0)	1 (25.0%)
30-39	18	8 (44.4%)	2 (11.1%)	10 (55.6%)
40-49	16	8 (50.0%)	1 (6.25%)	9 (56.3%)
50-59	37	13 (35.1%)	5 (13.5%)	18 (48.6%)
Total	75	30 (40.0%)	8 (10.7%)	38 (50.7%)
Total occupationally exposed persons				
20-29	8	1 (12.5%)	1 (7.8%)	2 (25.0%)
30-39	28	9 (32.1%)	2 (9.3%)	11 (39.3%)
40-49	40	12 (30.0%)	3 (7.5%)	15 (37.5%)
50-59	74	19 (25.7%)	7 (9.5%)	26 (35.1%)
Total	150	41 (27.3%)	13 (8.7%)	54 (36.0%)
Control group				
20-29	12	0 (0)	0 (0)	0 (0)
30-39	17	0 (0)	2 (11.8%)	2 (11.8%)
40-49	9	0 (0)	0 (0)	0 (0)
50-59	5	0 (0)	0 (0)	0 (0)
Total	43	0 (0)	2 (4.7%)	2 (4.7%)

Table 3. Serological response to Spotted Fever Group Rickettsiae antigen, in the occupational and control groups, depending on place of working and/or living.

Examined group/ Place of working	Number of examined	Number of reactants, percent		
		Distinct positive	Weak positive	Total positive
Agricultural workers				
Wilków	37	1 (2.7%)	2 (5.4%)	3 (8.1%)
Strzyżewice	38	10 (26.3%)	3 (7.9%)	13 (34.2%)
Total	75	11 (14.7%)	5 (6.6%)	16 (21.3%)
Forestry workers				
Puławy	36	16 (44.4%)	6 (16.7%)	22 (60.1%)
Kraśnik	29	11 (37.9%)	2 (6.9%)	13 (44.8%)
Świdnik	10	3 (30.0%)	0 (0)	3 (30.0%)
Total	75	30 (40.0%)	8 (10.7%)	38 (50.7%)
Control group				
	43	0 (0)	2 (4.7%)	2 (4.7%)

DISCUSSION

A high prevalence of the seropositive reactions to SFG rickettsiae was found among the symptomless inhabitants of eastern Poland occupationally exposed to tick bite, mostly in forestry workers. The genus-specific test did not provide the answer to the question: which SFG species evoke these reactions? The SFG rickettsiae were not known from the territory of Poland until recently when 3 species belonging to this group (*R. helvetica*, *R. slovaca*, *R. raoultii*) were isolated from the *Ixodes ricinus* (L.) and *Dermacentor reticulatus* (Fabricius) ticks [10, 11, 12]. In the first part of the presented study [13], it was found that in the *D. reticulatus* ticks collected in the study area of Lublin province, there was a high prevalence of the SFG rickettsiae mostly belonging to the species *R. raoultii*. Thus, it may be assumed that the high prevalence of seropositive reactions stated in the presented

study was probably caused by contact with the rickettsiae of *R. raoultii* complex, because the occurrence of these bacteria in ticks collected in several parts of Poland has been reported [12], and recently a case of spotted fever caused by *R. raoultii* has been described in Warsaw [6].

A very high incidence of serological reactions to SFG rickettsiae found in the work in forestry workers (50.7%) was several times greater compared to the results of analogical surveys carried earlier in this occupational group by Fournier et al. [7] in France (9.2%), by Cinco et al. [8] in Italy (3.9%), and by Podsiadły et al. [9] in Poland (14.7%).

Seropositive reactions to SFG rickettsiae have been found among populations on nearly all continents. The highest prevalence of these reactions, close to the value stated in the presented study in forestry workers was recorded by Tee et al. [14] in an occupational group of rubber estate workers in Malaysia (57.3%). A high response rate to SFG rickettsiae antigens, resembling the presented results in forestry workers, was also found by Kováčová et al. [15] among healthy populations in El Salvador (40.0%) and Iran (45.0%), and by Punda-Polić et al. [16] among healthy population in Croatia (43.7%). Lower response rates, resembling the presented results in agricultural workers, were found by the following researchers: Sekeyova et al. [17] among febrile patients in Slovakia (32%), Anstey et al. [18] among pregnant women in Tanzania (25.3%), Kováčová et al. [15] among healthy population of Guinea-Bissau (25.3%), Toledo et al. [19] among personnel of a municipal park in Brazil (20.6%), Richards et al. [20] among healthy residents of Indonesia (10.4–20.4%), Blair et al. [21] among rural inhabitants of Peru (10–19%), Lledó et al. [22] among tick-bitten people in northern Spain (16.9%), and by Tay et al. [23] among febrile patients in Malaysia (11.6%). The lowest response rates, resembling the presented results in control group, were registered by Sonnleitner et al. [24] among blood donors in the Tirol (7.7%), Lledó et al. [22] among the general population in northern Spain (3.3%), Tay et al. [23] among blood donors in Malaysia (1.7%), and by Camer et al. [25] among febrile patients in the Philippines (1.3%).

In conclusion, the results of the presented study indicate that the people occupationally exposed to tick bite in Poland, mostly forestry workers, are under serious risk of contracting SFG rickettsioses, which require application of the appropriate prevention measures, primarily in the areas of abundant occurrence of *Dermacentor reticulatus*. Also, physicians taking care of this category of people should always bear in mind the possibility of such diseases, especially when observing characteristic symptoms such as fever, rash and eschar.

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