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Serological survey in persons occupationally exposed to tick-borne pathogens in cases of co-infections with *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, *Bartonella* spp. and *Babesia microti*

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Abstract

Sera of 39 farmers, 119 foresters and 32 blood donors were investigated for the presence of antibodies against *B. burgdorferi*, *A. phagocytophilum*, *B. microti and Bartonella* spp. Semi-quantitative indirect immunofluorescence test was used to measure titers of anti-*A. phagocytophilum*, *B. microti* and *Bartonella* spp. IgG. ELISA test was used to measure titers of anti-*B. burgdorferi* IgM and IgG. *B. burgdorferi* was the most frequently observed among all the examined pathogens. 27.7% of farmers, 23.1% of forestry workers and 37.5% of control group were infected with *Bartonella* spp. Anti-*A. phagocytophilum* and anti-*B. microti* reactions were observed rarely. Sera of persons with single infection dominated in farmers and forestry workers. Co-infection with 2 pathogens was observed more frequently in forestry workers and farmers than in the control group. Co-infections with 3-4 pathogens were observed only in forestry workers. Among the observed co-infections, the most frequent were: *B. burgdorferi* with *Bartonella* spp. and *B. burgdorferi* with *A. phagocytophilum*. Moreover, in forestry workers, triple coinfections with *B. burgdorferi*, *Bartonella* spp. and *A. phagocytophilum* and one quadruple coinfection were observed. Persons with occupational risk of tick bites, especially forestry workers, more often have anti-*B. burgdorferi* antibodies and are more often co-infected with various tick-borne pathogens than the persons from the control group. It seems that more often coinfections in persons with occupational risk of tick bites are a consequence of the higher incidence of infection with *B. burgdorferi*, as anti-*B. microti*, *A. phagocytophilum* and *Bartonella* spp. antibodies are not more commonly prevalent in persons with occupational risk of tick bites than in healthy volunteers.

Key words

Borrelia burgdorferi, *Anaplasma phagocytophilum*, *Babesia microti, Bartonella* spp., tick-borne, co-infection, occupationally exposed, seroepidemiology

INTRODUCTION

Among the tick-borne diseases the most serious problem is caused by Lyme disease (LD), and although it is under epidemiological supervision it still causes many diagnostic and therapeutical problems. It is considered to be endemic in Poland, especially in the forestry and agricultural regions [1, 2]. Ticks belonging to *Ixodes ricinus,* depending on the region of Poland, may be infected with *Borrelia burgdorferi* within the range between a few to several dozen percent [3].

LD is observed in almost all European countries; however, recently the problem of co-infections with various tick-borne pathogens has come under investigation [4]. The presented study on the occurrence of *B. burgdorferi*, *Anaplasma phagocytophilum*, *Bartonella* spp. and *Babesia microti* in ticks in the Lublin Region of southeastern Poland indicates real threat for people working in forestry or agriculture. The frequency of infected ticks in various areas of the Lublin Region is differentiated: *B. burgdorferi* (5.3%-13.3%), *A. phagocytophilum* (3.5%-40%) and *Babesia* spp. (0.5%-5.4%) [5, 6, 7]. The variance of occurrence is proof of geographical distribution of LD, human granulocytic anaplasmosis, babesiosis and bartonellosis, and the possibility of co-infection in humans.

The objective of the presented study was to evaluate the prevalence of infection with *B. burgdorferi*, *A. phagocytophilum*, *B. microti and Bartonella* spp. in people occupationally exposed to tick bites.

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MATERIALS AND METHOD

Sera of 39 farmers living in 3 neighbouring communities: Jabłonna, Bychawka and Piotrowice, located south of the

city of Lublin (eastern Poland), were chosen from the sera of farmers examined in 2008, during a comprehensive study at the Institute of Rural Health in Lublin conducted on Lyme disease in farmers and ticks from this region. This resulted in 15 clinical cases being described. Infection rate of *Ixodes ricinus* ticks with *Borrelia burgdorferi sensu lato* in the study area was 13.1% [8]. The second group comprised 119 occupationally exposed foresters (mean age 35 years) employed in three forest districts of the Lublin Region: Krasnystaw, Radzyń Podlaski and Mircze. The control group (CG) consisted of 32 healthy blood donors (mean age 29 years) residing in the city of Lublin. Almost all those occupationally exposed reported biting by ticks when interviewed epidemiologically.

Semi-quantitative indirect immunofluorescence test (IFT) was used (Focus Technologies, Cypress, California, USA) to measure titers of anti-*A. phagocytophilum*, *B. microti* and *Bartonella* spp. IgG. ELISA test (*Borrelia* IgM recombinant, Bellco Biomedica, Austria; *Borrelia* IgG recombinant, Bellco Biomedica, Vienna, Austria) was used to measure titers of anti-*B. burgdorferi* IgM and IgG.

Statistical analysis was performed using test χ^2 (chi-square). The study was approved by the Bioethical Commission of the Institute of Rural Health in Lublin (No. 03/2007).

RESULTS

B. burgdorferi was the most frequently observed among all the examined pathogens in occupationally exposed groups (farmers – 38.5%, forestry workers – 47.9%, mean in both groups: 45.6%, in CG – 12.5%) and the differences in rates between the control group and examined groups were statistically significant (Fig. 1). 27.7% of farmers, 23.1% of forestry workers and 37.5% of CG were infected with *Bartonella* spp. (Fig. 1). Antibodies against *A. phagocytophilum* and *B. microti* were rarely observed and were reported in farmers: 5.1% and 2.6%, respectively; in forestry workers: 11.8% and 5%, respectively; in CG: 9.4% and 9%, respectively. Differences in rates between the examined groups were not statistically significant in cases of *Bartonella* spp., *A. phagocytophilum*, *B. microti* (Fig. 1).

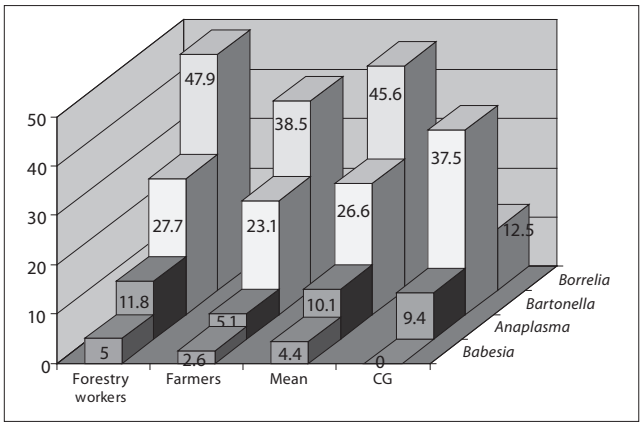

Figure 1. Results of seroepidemiologic survey of *Borrelia, Bartonella, Anaplasma* and *Babesia* antibodies prevalence in occupationally exposed people and control group (CG). Statistically significant were differences between seropositive results in cases of borreliosis in forestry workers and control group (p<0.001), between all with occupational risk and control group (p<0.001) and between farmers and control group (p<0.05)

Sera of persons with a different number of co-infections were divided into 5 groups: 0-4 coinfections. No antibodies were found in 41% of farmers, in 31.8% of forestry workers (mean from both occupationally exposed groups: 34.2%) and in CG – 43.8% (Fig. 2). Sera of persons with a single infection dominated: in farmers: 48.7% and in forestry workers 47.1% (mean from both occupationally exposed groups: 47.5%), in CG – 53.1% (Fig. 2).

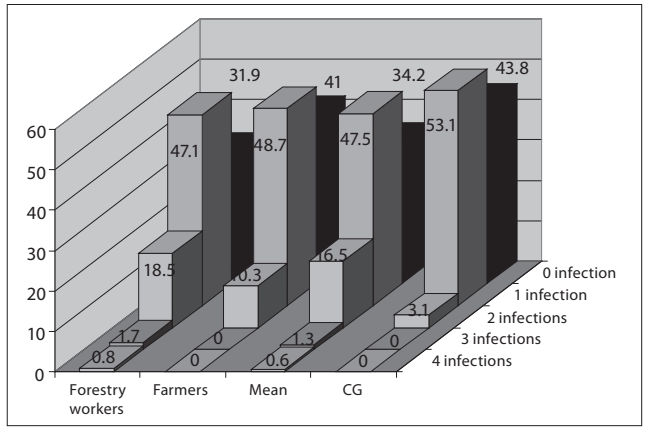

Figure 2. Percentage of co-infection in sera of forestry workers, farmers and control group (CG). Statistically significant were differences in co-infections between forestry workers and CG (p<0.05), and between all with occupational risk and control group (p<0.05)

In the control group, only one co-infection was observed. Co-infection with 2 pathogens was observed more frequently in forestry workers (18.8%) and in farmers (10.3%) than in CG (3.1%). Co-infections with 3-4 pathogens were observed only in forestry workers. Differences between forestry workers and CG were statistically significant (p<0.05). No statistical significance was observed between farmers and CG (Fig. 2).

Among the observed co-infections, the most frequent were: *B. burgdorferi* and *Bartonella* spp. (8.9% in people occupationally exposed – forestry workers – 9.2%, farmers – 7.7%) and *B. burgdorferi* and *A. phagocytophilum* (3.8% in people occupationally exposed – forestry workers – 4.2%, farmers – 2.6%) (Fig. 3). Moreover, in forestry workers triple co-infections with *B. burgdorferi*, *Bartonella* spp. and *A. phagocytophilum* (1.3%) and one quadruple co-infection (all examined pathogens) (0.6%) were observed. In CG,

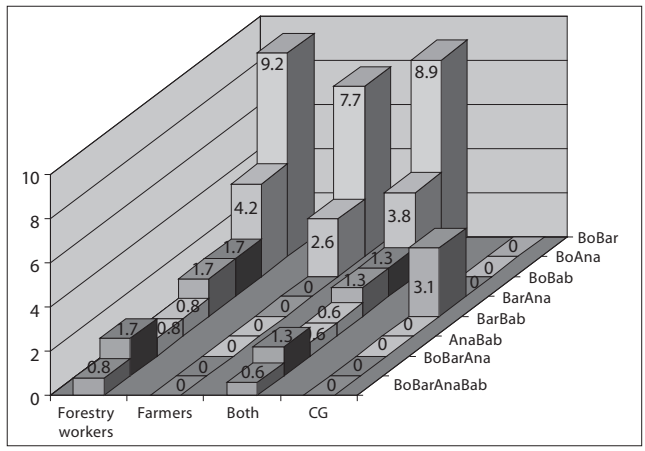

Figure 3. Percentage of co-infections with different pathogens in all analyzed groups: forestry workers, farmers and control group (CG).
CG – control group, Bo – *Borrelia burgdorferi*, Bar – *Bartonella* spp., Ana – *Anaplasma phagocytophilum*, Bab – *Babesia microti*

only one co-infection was observed (*Bartonella* spp. and *A. phagocytophilum*) (Fig. 3).

DISCUSSION

The problem of co-infections seems to be of great importance, especially in people occupationally exposed to tick bites. It is known that more than one pathogen may co-exist in one vector. Moreover, it is known that co-infection of humans with various pathogens may be a result of a single tick bite by a tick infected with several pathogens, or as a result of multiple bites by ticks infected with one pathogen. Both situations may lead to a co-infection, often difficult to diagnose and differentiate [9, 10]. Varis *et al.* described patients simultaneously infected with the TBE virus (TBEV) and *B. burgdorferi* spirochete as a result of a single tick bite [11]. Krause *et al.* observed that among 1,156 patients with LD 10% were co-infected with *B. microti* or *A. phagocytophilum* [12]. Hermanowska-Szpakowicz *et al.* observed patients with LD (8/96 – 8.3%) and patients with TBE (4/96 – 4.1%) who were c-oinfected only with *A. phagocytophilum* but not with *B. microti* [13]. In southeastern Poland, co-infection with *Borrelia* species and *A. phagocytophilum* or *Babesia* spp. in tick-exposed individuals was observed in a low rate of coinfections (*B. burgdorferi* s. lato / *A. phagocytophilum* – 4.2%, 1/24; *B. burgdorferi* s. lato / *Babesia* spp. – 4.2%, 1/24) [14]. In northeastern Poland, a similar rate was observed of coinfections: *B. burgdorferi* s. lato / *A. phagocytophilum* – 3%, 2/67; *B. burgdorferi* s. lato / *Babesia* spp. – 3%, 2/67. A quite high rate of coinfection with TBEV and *B. burgdorferi* s. lato was observed (30% of patients with TBEV were positive for *B. burgdorferi* s. lato), which was not observed in other studies. Moreover, a high rate of *Babesia* infection was noted, which was non-symptomatic and suggested a significantly higher importance of *Babesia* infection than had been previously thought (unpublished observations).

In the presented study, a definitely higher incidence of co-infections in groups with occupational risk was observed, especially threefold and fourfold in forestry workers. It may be suppose that this is rather the result of multiple bites than a single bite by a tick infected with more than one pathogen.

Influence on the course of the disease and treatment implementation in the case of coinfections is also one of the most important issues. Clinical symptoms of the disease were not analyzed for the presented study, but Logina *et al.* analyzed 51 patients with double infection: TBEV and *B. burgdorferi* s. lato, and concluded that the clinical occurrence of both LD and TBE vary after exposure to tick bite, and the neurological manifestations of each disorder differ significantly, with an appreciable overlap [15]. Co-infections with *B. burgdorferi* and *A. phagocytophilum* exacerbate the course of Lyme disease and cause complications in the diagnostic process and treatment. Lyme disease has similar neurologic manifestations, such as human granulocytotropic anaplasmosis (HGA). Although the cause of neurologic dysfunction in HGA is not yet known, it is considered to be due to complicating opportunistic infections or concomitant co-infection with *B. burgdorferi* [16]. Anti-*B.burgdorferi* antibodies titer may be really high in cases of co-infections, compared with a single infection. Infection with one pathogen stimulates transmission of the others. Patients with co-infection with *B. burgdorferi* and *B. microti* suffer more seriously than in cases of single infection. Co-infections also decrease immunological response and cause an increase in bacteremia [17].

Another interesting observation resulting from the study is the fact of quite high seroprevalence for single pathogens infection. It has been proved that the incidence of manifestation of borreliosis in rural inhabitants in a given area does not appear to be higher than for city dwellers [18]. A high frequency of tick bites and a high rate of transmission of the bacterium is contrasted by the comparatively low rate of clinical disease [19]. This suggests that European borrelial infection may often be self-limiting. It is not known which patients might be at risk for the development of late stage disease. Also not known is the situation in cases of infection with *Bartonella* spp.; therefore, further studies are necessary.

CONCLUSIONS

1. Persons with occupational risk of tick bites, especially forestry workers, more often have anti-*B. burgdorferi* antibodies than sera of persons from the control group.
2. Anti-*B. microti*, *A. phagocytophilum* and *Bartonella* spp. antibodies are not more common prevalent in persons with occupational risk of tick bites than healthy volunteers.
3. Co-infections are more frequently seen in persons with occupational risk of tick bites.
4. It seems that more often co-infections in persons with occupational risk of tick bites are a consequence of the higher incidence of infection with *B. burgdorferi* as anti-*B. microti*, *A. phagocytophilum* and *Bartonella* spp. antibodies are not more commonly prevalent in persons with occupational risk of tick bites than in healthy volunteers.

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