

Prevalence of antibodies against selected zoonotic agents in forestry workers from eastern and southern Poland

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Abstract

Introduction and objective. Due to the nature of their work, foresters are exposed to many infectious pathogens from the wildlife reservoir. The primary aim of this study was to assess their contact with these pathogens.

Material and methods. Sera and antibodies, mainly of IgG class against selected infectious factors, collected from a group of 216 employees of National Forests from eastern and southern Poland, were investigated.

Results. Antibodies to *Anaplasma phagocytophilum* were detected in 64 sera (29.6%), to *Bartonella* spp. in 133 sera (61.6%), to *Babesia microti* in 50 sera (23.1%), and to *Coxiella burnetii* in 14 sera (6.4%); 7 sera (3.2%) were positive to *Francisella tularensis*. In studies of diseases not carried by ticks positive results were found in 28 sera (12.9%) to *Toxocara canis*, 13 sera (6.0%) to *Trichinella spiralis*, and in 7 sera (3.2%) to *Echinococcus granulosus*. Antibodies to Hanta viruses were detected with antigens from Puumala and Dobrava/Hantaan strains in both IgM and IgG class. In 9 foresters, positive results were found, including one positive result to 2 antigens, 5 to Dobrava/Hantaan antigen and 3 to Puumala antigen.

Conclusion. Frequently higher percentages of positive results were found in people working outdoors, and in men when compared with women.

Key words

foresters, tick-borne diseases, zoonoses, serological diagnostics

INTRODUCTION

Foresters are exposed to various types of diseases. The main threat to professional forestry workers are infectious pathogens with reservoir in wild animals. These diseases are a serious epidemiological problem as occupational diseases among forest exploitation workers. The best known infectious health threat to professional foresters is Lyme disease – the most common tick-borne disease in Poland. It is not always timely diagnosed and treated and in the late phase of systemic infection it often requires hospitalization and long-term treatment, whereas early initiation of antibiotic treatment leads to a relatively fast recovery [1]. Another dangerous factor is tick-borne encephalitis (TBE). Foresters are usually vaccinated against TBE; therefore, the study of antibodies by ELISA serve mostly for immunity evaluation [2]. Because many of our previous studies have been carried out on Lyme disease and TBE [3], this study focuses on other diseases. The role of other tick-borne microorganisms may be very important. Ticks in Poland can transmit *Anaplasma phagocytophilum* [4], *Bartonella* spp. [5], *Francisella tularensis*

[6] and *Coxiella burnetii* [7] bacteria and *Babesia* protozoa [8]. Infections with agents which are not transmitted by ticks, and which may be of epidemiological importance in the forest work environment, are Hanta viruses, eggs of *Toxocara* spp. and *Echinococcus* spp., and larvae of *Trichinella* spp. [9,10,11]. Diagnosis of occupational infections causes many problems in medical practice. Clinical diagnosis is difficult because symptoms of many infections are similar, and the key to success may be the proper selection of diagnostic methods [12].

Objective. The primary aim of this study was to check the frequency of contact with selected biological agents with laboratory diagnosis.

MATERIALS AND METHOD

The study group consisted of 216 employees of the State Forests: 40 women (mean age 45.0 years ± 10.1) and 176 men (mean age 46.8 ± 9.4) from 8 forest divisions located in 5 provinces of Poland: northeastern (Podlaskie – Augustów), eastern (Mazowieckie – Sarnaki, Lubelskie – Radzyń Podlaski, Janów Lubelski), southeastern (Podkarpackie – Nowa Dęba, Lesko) and southern (Małopolskie – Stary Sącz, Olkusz). The study plan was approved by the Bioethical Committee at the Institute of Rural Health (Consent No. 8/2012). Prior

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to testing, each person was thoroughly informed about the purpose and progress, and the possibility to withdraw from participation in the study at every stage without suffering any consequences. Blood samples in the amount of 20 ml was collected, centrifuged, and the obtained sera secured for further study.

Altogether, 216 human sera antibodies were searched against tick-borne factors and other zoonotic factors. Indirect immunofluorescence test for IgG antibodies was performed with antigens of *Anaplasma phagocytophilum*, *Babesia microti*, *Bartonella quintana* and *Bartonella henselae*, *Coxiella burnetii* phase I and phase II (Focus Technologies, USA). Immunoenzymatic tests (Enzyme-linked immunosorbent assay, ELISA) were used for detecting IgG antibodies against *Trichinella* spp. (R-Biopharm, Germany), *Toxocara canis*, *Echinococcus granulosus* and *multilocularis* (Bordier, Switzerland), and *Francisella tularensis* (Virion/Serion, Germany). In sera with positive or equivocal result in IgG to *F. tularensis* test for specific IgM antibodies was performed (Virion/Serion, Germany). Antibodies were also tested against the Hanta virus with antigens of Puumala strain and Dobrava/Hantaan strain in both classes IgG and IgM antibody (Progen, Germany). The information about antibodies against Lyme disease was collected during interview as foresters are serologically-monitored on a regular basis. Statistical analysis was performed by chi-square test. The level of significance was established at $p < 0.05$.

RESULTS

Results of tests for tick-borne diseases. In the analyzed group of foresters, antibodies against *Anaplasma phagocytophilum* were detected in 64 persons (29.6%). Positive results were observed more frequently in males than in females (32.4% vs. 17.5%), and in the group where the predominant work was performed outdoors (50% or more), compared with others (33.8% vs. 21.2%); both of these differences were almost statistically significant in the chi-square test. In as many as 133 (61.6%) foresters, *Bartonella* spp. antibodies were detected. Similar percentages of positive results were

obtained regardless of gender and nature of the work. In the case of antibodies to *Babesia microti*, in 50 (23.1%) sera the result was positive. Males had higher percentages of positive results (26.1% to 10.0%; $p < 0.05$) as the group working mainly outdoors (25.7% to 15.2%). In the case of Q fever, 14 sera (6.4%) were positive. In the case of tularemia, 7 sera (3.2%) were positive (Tab. 1).

Analyzing the coexistence of antibodies to *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, *Babesia microti*, and *Bartonella* spp., it was found that 67 sera (31.0%) showed single infection, 88 (40.7%) double infection; in 39 sera (18.1%), 3 from 4 examined antibodies were found, and in 8 sera (3.7%), all 4 infectious factors were detected.

Results of tests for other zoonotic diseases. In the study for antibodies against *Trichinella* spp., 13 positive results (6.0%) were found. Positive results for trichinosis was found only in males (7.4%), and almost exclusively in the group where outdoor work was prominent (7.4% to 1.5%). Anti-*Toxocara canis* antibodies were found in 28 sera (12.9%). As in the case of anaplasmosis, babesiosis and trichinosis positive results were more frequent in males (14.2% to 7.5%, not significant), and in persons working mainly outdoors (16.2% to 4.5%, $p < 0.05$). The presence of specific antibodies against *Echinococcus granulosus* was observed in 7 sera (3.2%), while antibodies against *Echinococcus multilocularis* were observed in 2 sera (0.9%), but were studied only in sera with a positive IgG result against *Echinococcus granulosus*, and in 2 people who had negative result but close to the cut-off point. Foresters with positive results for *Echinococcus multilocularis* were invited to the clinic and carefully checked.

Sera of 179 foresters were negative in all 4 tests (2 strains of virus and 2 classes of antibodies) checking antibodies against Hanta viruses. In 9 sera, positive results were found, including 1 with a positive result to both antigens, 5 positive with Dobrava/Hantaan antigen, and 3 positive with antigen of Puumala. All positive results were found in those who reported 50% or more time working outdoors (6.1% to 0.0%, $p < 0.05$). There were no significant differences between the genders (Tab. 1).

Table 1. Results of serological tests for selected zoonoses

| Disease | All subjects N=216 | | Outdoors ¹ N=148 | Office ² N=66 | Males N=176 | Women N=40 | |
|------------------------------------|-----------------------|----------------------|--------------------------------|-----------------------------|---------------------|---------------------|-----------|
| | Negative [n (%)] | Equivocal [n (%)] | Positive [n (%)] | Positive [n (%)] | Positive [n (%)] | Positive [n (%)] | |
| Trichinosis | 194 (89.8) | 9 (4.2) | 13 (6.0) | 11 (7.4) | 1 (1.5) | 13 (7.4) | 0 (0.0) |
| Toxocariasis | 188 (87.0) | - | 28 (13.0) | 24 (16.2)* | 3 (4.5) | 25 (14.2) | 3 (7.5) |
| Tularaemia | 202 (93.5) | 7 (3.2) | 7 (3.2) | 3 (2.0) | 4 (6.1) | 5 (2.8) | 2 (5.0) |
| HCPS/HFRS | 179 (82.9) | 28 (13.0) | 9 (4.2) | 9 (6.1)* | 0 (0.0) | 8 (4.5) | 1 (2.5) |
| Puumala strain | 204 (94.4) | 8 (3.7) | 4 (1.9) | 4 (2.7) | 0 (0.0) | 3 (1.7) | 1 (2.5) |
| Dobrava/Hantaan strain | 184 (85.2) | 26 (12.0) | 6 (2.8) | 6 (4.1) | 0 (0.0) | 6 (3.4) | 0 (0.0) |
| Human granulocytic anaplasmosis | 133 (61.6) | 19 (8.8) | 64 (29.6) | 50 (33.8) | 14 (21.2) | 57 (32.4) | 7 (17.5) |
| Babesiosis | 153 (70.8) | 13 (6.0) | 50 (23.1) | 38 (25.7) | 10 (15.2) | 46 (26.1)* | 4 (10.0) |
| Bartonellosis | 55 (25.5) | 28 (13.0) | 133 (61.6) | 90 (60.8) | 42 (63.6) | 106 (60.2) | 27 (67.5) |
| Q fever | 197 (91.2) | 5 (2.3) | 14 (6.5) | 9 (6.1) | 5 (7.6) | 11 (6.3) | 3 (7.5) |
| Echinococcosis | 209 (96.8) | - | 7 (3.2) | 6 (4.1) | 1 (1.5) | 6 (3.4) | 1 (2.5) |

* - $p < 0.05$; ¹ - 50% or more time working outdoors; ² - less than 50% time working outdoors (information about the type of work for 2 persons was missing)

DISCUSSION

Foresters are a professional group closely related to the forest environment, where they are exposed to contact with pathogens of infectious diseases which cycle in the animal reservoir, known as zoonoses. An important role in the epidemiology of these diseases is often played by ticks. Many years of research and observation has indicated the endemic presence of some tick-borne diseases in Poland, such as Lyme disease and tick-borne encephalitis. Ticks may also transmit other pathogens harmful to humans. In addition, there are infectious agents that are transmitted by other routes.

Anaplasmosis is transmitted by ticks and may coexist with other tick-borne diseases. e.g. Lyme disease, described as multiform and presenting multi-organ symptoms. In cases of co-infection, anaplasmosis may be much more difficult to diagnose, and *vice versa*, Lyme disease may not be recognized, while human infection by other bacteria, such as the course of Oligosymptomatic anaplasmosis, which may make diagnosis difficult and may lead to organ damage.

The tests for anaplasmosis performed in forestry workers employed in the Lublin province in previous studies showed the presence of antibodies against *Anaplasma phagocytophilum* in 21.0% cases [4]. Surveys among foresters in other countries have shown infection in 17.1% of foresters in Switzerland [13], in 11.4% in Sweden [14], and 24% in Slovenia [15]. The rate of infection found in the presented study is higher than those observed by Brouqui et al. [13] and Dumler et al. [14], but similar to those found by Zwoliński et al. [4] and Rojko et al. [15].

In addition to *B. burgdorferi* and *A. phagocytophilum*, other infectious agents transmitted by ticks to humans and animals can also coexist, such as protozoa of the genus *Babesia* [3]. In Europe, mainly *B. divergens* infections were reported, and *B. microti* infections were observed primarily in the USA; however, a study of ticks carried out in Poland demonstrated that *B. microti* is also a threat to humans in Europe [8]. 0.5–5.4% of ticks infected with *Babesia microti* were demonstrated in the Lublin province, and 5% of seropositive results were reported in foresters [3]. A much higher proportion of positive results in the direction of *B. microti* in foresters were obtained in the presented study. This may be due to the seasonal activity of ticks as the blood was collected in early spring.

The bacteria of the genus *Bartonella*, can be transmitted by ticks, but this is not the only route of infection. In the course of bartonellosis, erythrocytes and endothelial cells are involved [5]. The research on ticks for the presence of *Bartonella* spp. in the Lublin province showed 1.1% of infected ticks. 23.1% of forestry workers had positive results in serological investigation for antibodies against *Bartonella* spp. [3]. It is difficult to interpret such a high percentage of positive results in the presented study; however, small percentages of infected ticks confirm that ticks are not the main vectors of bartonellosis.

Due to the coexistence of pathogens in ticks and humans, mixed infections are possible [16]. After the bite of a tick or multiple bites of ticks, several tick-borne micro-organisms can be found in the human body, and therefore, the symptoms caused by these microorganisms are often mixed. Clinical manifestations of the disease are usually more severe than in the case of infection with a single species, and thus can cause difficulties in diagnosing diseases caused by these microorganisms. Also, therapy may not be properly conducted and ineffective which results in a longer recovery

period. Co-infection with *Borrelia burgdorferi*, *Anaplasma phagocytophilum* and/or *Babesia microti* exacerbate the course of Lyme disease (atypical Lyme disease) and complicate diagnosis and treatment. It has been shown that infection with a pathogen stimulates the acquisition and transmission of another pathogen. Humans co-infected with *Borrelia burgdorferi* and *Babesia microti* may be related with worse course of the disease [12]. The basis of the difference in the course of co-infection, compared with a single pathogen infection, is both the direct interaction of microorganisms to target cells of the host organism, as well as (if not more) omni-stimulation of the immune system [17].

Research on 119 foresters in the Lublin province for *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, *Babesia microti*, and *Bartonella* spp. demonstrated in 56 (47.1%) of the foresters a single infection, in 22 (18.5%) – co-infection with 2 agents, in 2 persons (1.7%) – 3 agents were found, and in 1 (0.8%) – all 4 examined agents were found [3]. The results of this study showed higher rates of co-infection, mainly due to the much higher percentages of positive results in the case of *Bartonella* spp.

Cases of tularemia occur most frequently in the summer months, which is related to the high activity of ticks [6]. Epidemiological data from the years 1996–2009, issued by the National Institute of Public Health, report about 4–6 cases per year, which is about 0.01–0.02 per 100,000 [18]. Research data on occupational diseases from the Czech Republic in the years 1996–2000 showed antibodies against *F. tularensis* in 3.5% of examined persons occupationally exposed [19], which is very similar to the results obtained in the presented study. A case of arthropod-borne tularemia was recently described in Poland [20].

Although Q fever has been known as a zoonosis in Poland since 1956, albeit only fragmentarily recognized epidemiologically and occasionally diagnosed in humans, there is no doubt it is widespread in the country [7]. Italian researchers found that among foresters in north-eastern Italy, the *C. burnetii* rate of infection is about 2.8% [21]. Ticks are currently considered as a reservoir of *C. burnetii* and responsible for the spread of infection [22]. Other studies have shown that among forestry workers in the Netherlands, the prevalence ranges from 0.4 – 2.1% [23]. A high percentage of positive results obtained in the presented study may suggest a high degree of infected ticks with *C. burnetii* in the study area. However, the conclusion must be formulated very carefully, as studies have not been performed on ticks.

The infectious agents not transmitted by ticks and which may be of epidemiological importance in the work environment of foresters are: *Hanta* viruses, eggs of the parasite *Toxocara* spp. and *Echinococcus* spp., and *Trichinella* cysts. *Hantavirus* is one of 5 genera of the large *Bunyaviridae* family, and small spherical RNA virus. The groups of people particularly vulnerable to these microorganisms are foresters and staff associated with silviculture. Among the infections with *Hantavirus* one should pay particular attention to the Hantavirus cardiopulmonary syndrome (HCPS), and haemorrhagic fever with renal syndrome (HFRS). Epidemiological data from 1996–2009, issued by the National Institute of Public Health, report 6–8 cases per year or about 0.02 per 100,000 [18]. German researchers studied a population of 563 foresters from the southern regions of Germany in the direction of *Hantavirus*. Serological tests showed 9.1% positive results [24]. Grygorczuk et al. studied

a group of 69 employees of the State Forests of northeastern Poland, and showed the presence of IgG antibodies against Puumala strain and/or Hantaan strain in 6 persons (8.7%), which may suggest an unrecognized infection in the past [9]. These results are slightly higher than those obtained in the presented study carried out in the south-eastern and eastern Polish regions (4.2% of positive results).

Toxocarosis in humans is caused by ingestion of eggs of nematodes: dog worm and cat worm (*Toxocara canis*, *Toxocara cati*), which live in the small intestine of the *Canidae* family, such as dogs, wolves and foxes. The eggs of the parasite enter the soil with the faeces of definitive hosts. In the presented study, as many as 28 of the examined foresters showed positive results. A much smaller proportion of positive results concerned the prevalence of antibodies against *Trichinella* spp. Most trichinosis occurs in family outbreaks and is associated with the consumption of infected, unexamined meat – usually not well prepared, only smoked wild boar meat. Foresters are a group of people occupationally exposed to infection [10].

Alveolar echinococcosis is one of the most severe parasitological diseases. In recent years, an increased incidence of alveolar echinococcosis in humans has been noted. In recent years in Europe, the number of diagnosed alveococcosis cases have exceeded 1,000 [11], of which about 500 were in Switzerland. In Europe, the continuous increase in the population of foxes infected with *Echinococcus multilocularis* has increased the number of infections [25]. Alveococcosis has also been diagnosed in Poland, 76 cases until 2010, showing a rise in the rate of infection [11]. According to reports from 2008–2012, issued by the National Institute of Public Health in Poland, there has been an average of 28 cases of echinococcosis (both *E. granulosus* and *E. multilocularis*) per year, which is about 0.07 per 100,000 people [18].

In the presented study, 7 (3.2%) persons in the test group had antibodies against *E. granulosus*, who were also examined for *E. multilocularis*, and 2 of them had antibodies against *E. multilocularis*. They were hospitalized, but the clinical symptoms of alveococcosis were not observed. Echinococcosis develops slowly; it is therefore necessary to perform periodic medical supervision combined with ultrasound evaluation conducted by an experienced radiologist. Early detection of changes can allow avoidance of surgery, while in the advanced form of the disease, liver transplantation is the only therapy, together with treatment and prevention using anthelmintic preparations. One of the people hospitalized also had very high titers for trichinosis, believed to have been the result of cross-reaction. Such effects are quite common in the case of parasitic diseases.

CONCLUSIONS

1. The results indicate a frequent concomitant infection of foresters with other pathogens transmitted by ticks.
2. The high incidence of positive serological tests in the absence of any complaints and/or deviations from the norm in medical examination specific to particular infections, may indicate either a frequent asymptomatic infection of various pathogens, or the possibility of false positive results due to cross-reactions.
3. Interpretation of the results of some serological tests used for screening requires the use of highly specific reference tests.

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