Serological surveillance of vector-borne and zoonotic diseases among hunters in eastern Poland

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ABSTRACT

Background & objectives: Many etiological agents of zoonoses are considered as significant biological hazard to people visiting forested areas frequently, for instance, hunters. They may be exposed to ticks, rodents, and birds as well as excreta/secretions of wild animals or contaminated water and soil. Hence, this population is at risk of contracting infection with pathogens such as Borrelia burgdorferi sensu lato (s.l.), Anaplasma phagocytophilum, Babesia spp., tick-borne encephalitis virus, Bartonella spp., Francisella tularensis, Echinococcus spp., or hantaviruses. The aim of the study was to assess the seroprevalence of zoonotic agents, viz. A. phagocytophilum, hantaviruses, and Echinococcus spp., with special regard to B. burgdorferi s.l., among hunters in Lubelskie Voivodeship (eastern Poland).

Methods: Serum samples collected from 134 hunters from Lubelskie Voivodeship were analyzed with the use of immunological techniques (enzyme-linked immunosorbent assay, line immunoblot test, and indirect fluorescence assay) for the presence of antibodies against the agents.

Results: Specific antibodies were detected in 66% of the tested individuals. Antibodies against B. burgdorferi s.l. (39%), A. phagocytophilum (30%), hantaviruses (9%), and Echinococcus spp. (8%) were detected individually or as mixed results.

Interpretation & conclusion: The results confirm that there is a risk of exposure to different pathogens in the forested areas in eastern Poland and that hunters are highly vulnerable to infection with the examined zoonotic agents. A significant proportion of co-occurring antibodies against different pathogens was noticed. Thus, hunters have to take special care of their health status evaluation and mitigate the exposure risk by using adequate prophylaxis measures.

Key words Anaplasma phagocytophilum; Borrelia burgdorferi s.l.; eastern Poland; Echinococcus spp.; hantaviruses; hunters; seroprevalence

INTRODUCTION

Hunters, who spend time in forested and wild areas, may have contact with many causative agents of zoonoses through exposure to ticks, dead rodents, and birds, as well as excreta/secretions of wild animals or contaminated food, water, and soil¹. Hence, this population is at significant risk of contracting infection with different pathogens like Borrelia burgdorferi sensu lato (s.l.), Anaplasma phagocytophilum, Babesia spp., tick-borne encephalitis virus, Bartonella spp., Francisella tularensis, Echinococcus spp., or hantaviruses², which are etiologic agents of diseases associated with severe health and socioeconomic consequences¹.

Ticks are important vectors of zoonoses, among which Lyme borreliosis is the most widespread zoonotic disease in the northern hemisphere. Spirochaetes from the B. burgdorferi s.l. complex cause over 10,000 cases of Lyme borreliosis per year in Poland [12,759 cases in 2013, incidence per 100,000 (CI: 33.1); and 13,875 cases in 2014 (CI: 36)]³. The infection risk depends on the density of vectors, contribution of infected ticks in the local tick population, and the number of animals that are hosts for the vectors and reservoirs of the bacteria. The disease is a significant epidemiological problem due to its long-lasting asymptomatic course, multisystem involvement, and serious consequences of untreated infection¹.

Another important tick-borne disease is human granulocytic anaplasmosis caused by the bacterium A. phagocytophilum, which is an obligate parasite of neutrophil granulocytes. The disease manifestations in humans are not specific (influenza-like illness symptoms such as headache, fever, and muscle pains). The course of the disease is usually mild and self-limiting or even...
asymptomatic, thus specific diagnosis is rare and the number of reported cases is limited. In rare severe cases, liver malfunction, respiratory failure, and neurological complications may develop².

Hantaviruses from the family *Bunyaviridae* are causative agents of rodent-borne diseases that are newly reported in Poland³. European species can cause infections with a varied course: from completely asymptomatic, through mild to severe, including a life-threatening course. They are carried by a specific asymptomatically infected rodent host and transmitted to humans through aerosolized excreta of infected rodents, direct contact, or bites⁴. The endemic focus of hantavirus diseases has recently been described in southeastern Poland⁵ and the occurrence of hantaviruses in local wild rodents has also been confirmed⁶, but the viruses have been found in other regions of Poland as well⁷-⁸.

Another important disease associated with the forest environment is echinococcosis caused by parasites from the genus *Echinococcus*. In Europe, *Echinococcus granulosus*, *E. ortleppi*, *E. canadensis*, and *E. intermedius* species cause hydatid echinococcosis and *E. multilocularis* causes alveolar echinococcosis⁹-¹⁰. The disease is reported rather rarely (39 cases in 2013, CI: 0.10; 48 cases in 2014, CI: 0.12)³. These parasites circulate among the intermediate hosts (sheep, cattle, moose, camels, pigs, etc) and definitive hosts (dogs, wolves, foxes, raccoon dogs, and other canidae). Infections of humans, who are rare intermediate hosts, are generally food-borne¹⁰. The symptoms usually occur after a few years affecting different organs—Liver, brain, lungs, or other organs. Moreover, in the case of alveolar echinococcosis, the infection symptoms are similar to those related to malignant liver tumor development¹¹.

All these pathogens may develop apparent, clinically distinguishable symptoms, but cases with a mild course may be neglected and not reported. Hunters seem to be an underexamined population, which has not been studied in Poland so far. This social group is heterogeneous in terms of social provenance, experience in hunting, and average time spent in the forest environment. The aim of the study was to analyze the occurrence of specific antibodies against selected pathogens among hunters, who are at risk to be exposed. The results may reveal the scale of contact with these pathogens and emphasize the necessity of conducting regular individual check ups of their health status and applying appropriate prophylaxis and mitigation measures.

Laboratory diagnosis of Lyme borreliosis is a complex process, which basically comprises serological detection of various anti-*B. burgdorferi* s.l. antibodies. An additional aim of the study was to assess the usefulness of different bacterial antigens in diagnostic tests.

**MATERIAL & METHODS**

**Study group**

Blood samples were collected in Lubelskie Voivodeship, eastern Poland from October 2014 to April 2015. Subsequently, serum was obtained for further analysis. The study group consisted of 134 hunters aged between 23 and 80 yr (average age 53 yr, SD 11.4), which included 124 men (93%) and 10 women (7%).

**Serological analysis**

All serological assays were carried out and results were interpreted according to the manufacturers’ instructions. The ELISA test (anti-*Borrelia* ELISA IgM and anti-*Borrelia* plus VlsE ELISA IgG, Euroimmun, Germany) was applied for general screening of anti-*B. burgdorferi* s.l. IgM and IgG. The wells were coated with mixed antigens of *B. burgdorferi* s.s., *B. afzelii*, and *B. garinii* and recombinant protein VlsE. Results above 22 relative units/ml (RU/ml) were considered as positive, below 16 RU/ml as negative, and between 16 and 22 RU/ml as borderline.

Western blot (Wb) (anti-*Borrelia* Euroline-WB IgM/IgG; Euroimmun, Germany) was used for confirmatory diagnosis of Lyme borreliosis for the positive and borderline results. The test stripes comprised immobilized antigens of *B. afzelii* (p83, p41, p39/BmpA, p31/OspA, p30, p25/OspC, p21, p19 and p17/DbpA) as well as a chip with recombinant antigen VlsE. EuroLinescan (Euroimmun, Germany) software was used for readout of the results. Additionally, antibodies against an extended pool of *B. burgdorferi* antigens (BBA36, BBO323, CRASP3 and pG) were analyzed by *Borrelia* Line IgG Line Immunoblot (Sekisui Virotech, USA).

The presence of anti-*Echinococcus* IgG was detected by ELISA (NovaLisa *Echinococcus* IgG, NovaTec Immunodiagnostica, Germany). Microtiter wells were coated with *Echinococcus* antigens. Samples were considered as positive, if the absorbance value was higher than 10% over the cut-off or as negative if the absorbance value was lower than 10% over the cut-off. Samples with an absorbance value of 10% above or below the cut-off were interpreted as borderline.

The samples were tested for the presence of anti-*A. phagocytophilum* IgG by immunofluorescence antibody (IFA) test (*A. phagocytophilum* IFA IgG antibody kit,
Fuller Laboratories, USA). Solid-phase antigens containing fixed THP-1 cells were infected with *A. phagocytophilum*. Patients’ sera were diluted to 1:80. A positive reaction was seen as sharply defined green fluorescent inclusions in the cytoplasm of infected cells. The fluorescence intensity of the positive control diluted to 1:640 was considered as the cut-off value.

The samples were also tested for the presence of anti-hantavirus IgG against Hantaan virus (HNTV), Puumala virus (PUUV), Dobrava-Belgrade virus (DOBV), Seoul virus (SEOV), and Saaremaa virus (SAAV) by IFA (IIFT Hantavirus Mosaic 2: Eurasia IgG, Euroimmun, Germany). Based on the intensity of fluorescence, the scores were considered as positive (++, +), borderline (±) or negative (−). Positive and borderline samples were subsequently tested by qualitative ELISA (anti-Hantavirus Pool 1 “Eurasia” ELISA IgG, Euroimmun, Germany) for HNTV, DOBV, and PUUV. Wells were coated with recombinant viral antigens. Results >1.1 were considered as positive, <0.8 were negative, and results between 0.8 and 1.1 as borderline.

The Bioethical Committee of Medical University of Lublin authorized the project (permission No. KE-0254/177/2014). Free and informed consent was obtained from the participants. The data obtained were analyzed statistically using Statistica v.10 software.

**RESULTS**

**General screening:** The screening revealed that there were 89 (66%) seropositive samples (84 men and 5 women, average age 54 yr, SD 12) out of the 134 examined hunters while 45 (34%) patients from the study group (40 men and 5 women, average age 54 yr, SD 9.2) were negative.

The *B. burgdorferi* screening ELISA test revealed 51 (38%) negative samples for anti-*B. burgdorferi* s.l. IgG, whereas 83 (62%) samples were positive or borderline. The results for IgM were as follows: 119 negative (89%) and 15 borderline or positive samples (11%). The Wb test, which was performed for the positive and borderline samples, showed positive or borderline results for 51 (38%) of the IgG samples and 3 (2%) of the IgM samples. The detailed results about the classes of the detected antibodies are presented in Figs. 1 and 2.

The anti-*B. burgdorferi* s.l. IgM antibodies detected by the Wb test were against p25/OspC, whereas the anti-*B. burgdorferi* s.l. IgG antibodies found in the examined samples were against various proteins. For four samples (8% of the positive or borderline samples), positive results were determined on the basis of the presence of only anti-VlsE IgG, whereas the other 32 (60%) positive serum samples contained IgG against two or more antigens. The most common IgGs were as follows: anti-p17 (26 samples, 50% of the positive or borderline IgG samples), anti-VlsE (21 samples, 40%) anti-p19 (19 samples, 37%), anti-p83 (18 samples, 35%), and anti-p39 (14 samples, 27%). The presence of IgG for all the antigens from the extended pool (BBA36, BBO323, Crasp3 and pG) was confirmed for 16 samples (30%) by an additional Wb test; however, all of these samples were previously determined as positive in the first test.

Antibodies (IgG), only against *A. phagocytophilum* were detected in 21 samples (16%) and while 19 (14%) samples were detected as mixed results. Anti-hantavirus antibodies (IgG) were detected in 44 (33%) samples by IFA (positive or borderline results), but only 9 (7%) and 3 (2%) of them were confirmed by ELISA as positive and as borderline results, respectively. There were eight single results and four mixed results. *Anti-Echinococcus*
The presented results of the investigations conducted on the hunters’ population in Poland can be useful for assessment of the risk posed to them.

The risk of exposure to pathogens transmitted by ticks, e.g. *B. burgdorferi* s.l. or *A. phagocytophilum*, depends on the density of population of infected ticks and their hosts. The presence of anti-*B. burgdorferi* s.l. antibodies among forestry workers has frequently been studied. The antibodies were detected among 5–23% of forestry workers in Italy, 14–20% in France, 8–43% in Germany, 24% in Slovenia, 9% in Romania, 11% in Turkey, and 37% in Hungary. In turn, this value was as high as 20–60% of examined forestry workers in Poland. The results of the study (40% of seropositive individuals among the examined hunters) seem to be coherent with results obtained from farmers, i.e. another exposed group in Lubelskie Voivodeship (38.5%); however, the results are substantially higher than those in the less exposed populations, e.g. blood donors (12.5%) from the region. Thus, the study reconfirmed that occupational or environmental conditions have an impact on exposure to pathogens and human infections.

Recommendations for laboratory medical diagnosis of Lyme borreliosis issued by the National Chamber of Medical Diagnostic in Poland comprise two-stage tests: quantitative ELISA (II or III generation) and subsequent qualitative Wb for both classes of antibodies (IgM–early phase or IgG–late phase). In the case of borderline results, the tests have to be repeated in near future. Positive laboratory results are required for notification of a confirmed case of Lyme borreliosis in the late phase; however, laboratory findings only are not sufficient to start borreliosis treatment—they have to be supplemented with the clinical picture of the disease. In this study, 43 of the 98 ELISA positive or borderline samples (44%) were not confirmed by the Wb tests. Other researchers have also reported that results of ELISA assays were not confirmed by the Wb test in 68% of the clinically suspected cases. The double stage approach seems to improve the disease diagnosis and limit false positive results, which leads to application of unnecessary antibiotic therapy.

The European Union Concerted Action on Lyme Borreliosis (EUCALB) analysis has revealed the usefulness of the following antigens in laboratory diagnosis of *B. burgdorferi* s.l.: OspC and p41 for IgM and p83/100, p58, p41, p39, OspC, p17/DbpA, and VlsE for IgG. They are important markers of immunological response in a specific disease phase. For example, anti-OspC IgM (with lack of IgG) is significant in the diagnosis of the early stage of Lyme borreliosis with manifestation of promi-
Hantaviruses are newly emerging pathogens in Poland related to a wild rodents’ reservoir. Detailed research done in Subcarpathia between 2004 and 2011 among human population and animal reservoir proved that this area was endemic for hantavirus diseases (HVD). The occurrence of HVD has been reported for a few years by the National Institute of Public Health in Warsaw (13 cases in 2007, 7 in 2008, 5 in 2009, 6 in 2010, 8 in 2011, 3 in 2012, 8 in 2013, 54 in 2014, and 6 in 2015). Therefore, hantaviruses need to be monitored in the country. However, only selected populations have been studied so far in Poland. The seropositivity among forestry workers in Lubelskie Voivodeship was reported to range from 1.9 to 6.5%. The overall seroprevalence of hantaviruses in another European country—Latvia rates 4.2%. The results of this study (9%) are coherent with these data and support the thesis that hantaviruses are an important environmental threat to social groups associated with the forest environment in Poland.

It was assumed that samples should be examined by more than one method due to possibility of false positive results. Indeed, in this study, 44 positive samples were determined by IFA but only 12 of them were confirmed by ELISA. Moreover, a seroneutralization assay is required for final confirmation of the etiological agent species, due to significant cross-reactivity among antibodies raised against various hantavirus species.

According to data from the European Food Safety Authority (EFSA), 811 echinococcosis cases in total were reported in the EU in 2013, including 39 cases in Poland. Echinococcosis is a rare disease and the epidemiological situation in Poland is stable. However, the increasing number of cases of alveolar echinococcosis across Europe is alarming due to the more severe course of the infection and worse prognosis. The research conducted among Austrian hunters has shown 5% and 11% seropositivity for *E. multilocularis* and *E. granulosus*, respectively. Zukiewicz-Sobczak et al. estimated seropositivity for *E. granulosus* among Polish foresters at 3.2%. The present study results revealed a comparable prevalence of anti- *Echinococcus* antibodies. i.e. 11 samples (8%) were determined as borderline results. Kern et al. reported that gun dogs actively participating in hunting are the most important risk factor for occurrence of multilocular echinococcosis. The animals roaming around the forest may be infected as a definite host and thus they might become a source of infections for intermediate hosts—hunters and their families.

This study has confirmed the co-presence of antibodies against the studied pathogens. This phenomenon might be a result of infections that occurred in the past. The study reported 21 (16%) samples containing antibodies against two agents, mainly *B. burgdorferi* s.l. and *A. phagocytophilum*, as well as three samples with antibodies against additional agents (hantaviruses or *Echinococcus*). This phenomenon has already been reported on other populations in Poland. The most prominent correlation was found between *B. burgdorferi* s.l. and *A. phagocytophilum* in exposed populations (4.2% foresters and 2.6% farmers). An increased number of co-prevalence was reported in 7% patients suspected of infection with *B. burgdorferi* s.l. from eastern Slovakia. But results of this study indicated greater co-prevalence (13% of the analyzed samples) than that from the previous reports. This phenomenon may be important in the complex diagnosis of disease in the case of simultaneously ongoing infections. The clinical picture and course of
Lyme borreliosis in humans may be more difficult to interpret in the case of mixed infections with *A. phagocytophilum*. The difficulty arises from the complexity of host immunological response in mixed infection, which can impede diagnostic process and treatment.

The results of the present study have proved that the studied population of hunters is a group with significant “immunological history” of exposure to all the pathogens studied. These results are especially important given the fact that these zoonoses, *i.e.* Lyme borreliosis, HVD, anaplasmosis, or echinoccosis may have an asymptomatic course throughout the disease or in certain phases only. Thus, hunters have to take into account the exposure risk in the case of illnesses with non-specific symptoms or during periodical medical health status evaluations. Moreover, they should respect safety rules and principles of behaviour. These include adequate prophylaxis measures (protective clothing, repellents), general hygiene precautions, especially when forest vegetation or wild meat are used for consumption, and appropriate veterinary care over gun dogs.

Research on the seroprevalence particularly on exposed populations is very useful to evaluate the risk related to specific pathogens. These data may be used to localize regions at risk and identify emerging and re-emerging diseases. Jaenson et al.\(^{30}\) suggest that increased number of cases of tick-borne diseases, including Lyme borreliosis, anaplasmosis, and others might be expected in palearctic countries due to climatic changes, *i.e.* milder winters, longer vegetation period, and increased number of hosts.\(^{30}\) Climate changes also influence hantavirus infection—more food and shorter periods between mast years may increase the number of rodents and environmental exposure risk to humans.\(^{6}\) Studies on emerging and re-emerging pathogens in Poland are indispensable for public health.

### CONCLUSION

The study revealed that hunter population in Lubelskie Voivodeship is vulnerable to Lyme borreliosis, echinococcosis, anaplasmosis, and HVD as evident by the significant co-occurrence of multiple antibodies against tick-borne diseases and other zoonoses on the studied population. The study provided arguments for improvement of the evaluation of hunters’ health status and prophylaxis and mitigation measures.

Diagnosis of Lyme borreliosis must be based on two-steps diagnostic schemes: The results of the ELISA, and Western blot assays. Application of antigen proteins like BBA36, BBO323, Crasp3, and pG in the serological assay does not provide any additional value in the general serosurveillance studies.

### REFERENCES


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