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**OCCURRENCE AND PREVALENCE OF SELECTED ZOO NOTIC AGENTS:
ECHINOCOCCUS MULTILOCULARIS, *TRICHINELLA SPIRALIS*
AND HEPATITIS E VIRUS (HEV) IN THE POPULATION OF POLISH HUNTERS
- RESULTS OF THE STUDY CONDUCTED IN 2010-2012***

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SUMMARY

In Poland the development of the knowledge concerning zoonotic pathogens, of which free-living animals are the reservoir of is gaining in importance both in epidemiological aspect as well as in the context of prevention for improving public health. Dietary habits such as the consumption of forest undergrowth products and wild game meat, and the way of those products being prepared (in the process of barbecuing) pose a risk factors of infection with the foodborne pathogens such as *Echinococcus multilocularis*, *Trichinella spp.*, and HEV.

AIM. The aim of this study is to estimate the prevalence of infections caused by *Trichinella spp.*, *Echinococcus multilocularis*, and HEV in the population of Polish hunters, describing their geographical distribution in Poland, and to try to define basic factors, which may contribute to their occurrence.

MATERIAL AND METHODS. In 2010-2012 a cross-sectional study was carried out among Polish hunters. A blood samples were collected as well as a survey of 1027 participants recruited in the 16 provinces was also carried out. Serological tests were performed for the presence of specific antibodies against *Echinococcus multilocularis*, *Trichinella spp.* and HEV using commercial or “in home”ELISA tests. In case of positive result for *Echinococcus*, an Em2plus ELISA or/and western blot test were carried out, and for positive results for IgM for HEV a recomLine HEV IgM test was carried out.

RESULTS. In the studied population a total number of 2 cases of *Echinococcus multilocularis* infection were found. Moreover in 47 (4,6%) participants presence of antibodies against *Trichinella spp.* were found, including 17 positive and 30 borderline results. In 206 persons (25%) IgG anti-HEV antibodies were found (by ELISA test). Geographical diversity in prevalence of both, the *Trichinella spp.* and HEV cases was observed.

SUMMARY AND CONCLUSIONS. The study confirmed presence of zoonotic infections such as *Echinococcus multilocularis*, *Trichinella spp.*, and hepatitis E (HEV) among Polish hunters. In the case of *Echinococcus multilocularis*, and *Trichinella spp.*, it appears that these infections are more common in the studied sample than in the general population. Additionally results on the prevalence of HEV infections indicate a need for further research on the occurrence of the virus in the country.

Key words: zoonoses, *Echinococcus multilocularis*, *Trichinella spiralis*, Hepatitis E virus (HEV), prevalence, hunters, Poland

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INTRODUCTION

Wild animals are a reservoir for many diseases with zoonotic potential, including emerging infectious diseases. Rapid progress of civilization and changes it brings facilitate not only emerging of new diseases but also the spread of already prevalent pathogens which may have been circulating for some time in natural environment, but had less impact on public health. Regions inhabited by wild animals are also the regions of higher risk of acquiring many infectious diseases which those animals are the reservoir of. Among other diseases trichinellosis, echinococcosis and Hepatitis E may be included.

Alveolar echinococcosis (alveococcosis). *Echinococcus multilocularis* is one of the most dangerous human parasites and is the most dangerous parasitic agent in Poland. Alveococcosis develops slowly, during couple to over a dozen years, remaining for a long period of time without the development of symptoms, so the final diagnosis is made late. Among infected persons, who were not treated, mortality exceeds 90% within 10 years from the diagnosis. Members of *Canidae* family are the definitive host of this parasite. In Poland those would be red fox (*Vulpes vulpes*), raccoon (*Nyctereutes procyonoides*) and wolf (*Canis lupus*). In endemic areas dogs may be counted among definitive hosts. In recent years in many regions of Poland much higher frequency of infection among foxes was found than in previous decades. Studies from the years 2001-2008 showed widespread infestation by this parasite among foxes in Warmińsko-mazurskie (around 40% of infected specimens), Podkarpackie (37%), Małopolskie (20%), Lubelskie (around 19%) and Mazowieckie (14%) province. The fact of no symptoms developing for many years often makes it impossible to determine the source of infection, which in this case might have been the direct contact with infested animal or with contaminated (with their feces) forest undergrowth, water, fruits or vegetables from the plantations, to which they have an access to. (1-4)

Trichinellosis (lat. *Trichinosis*, *trichinellosis*). The incidence of trichinellosis in Poland is one of the highest in the EU. Outbreaks, sometimes involving couple of hundred exposed persons are a heavy burden for the country, which covers the costs of diagnostics, medical therapy as well as hospitalization. Wild boar meat is considered as the main source of infection for people from 1995. *Trichinella* spp. larvae digested with the undercooked or raw meat are the cause of the disease. Trichinellosis may develop in mild form, but consumption of large amount of contaminated food and personal susceptibility may result in severe disease and even death. The role of wild animals as the reservoir for

trichinellosis is increasing in significance as the role of domestic pigs was almost reduced completely (5-8).

Viral Hepatitis E (HEV). Wild boar meat might be the source of viral hepatitis E. HEV was primary classified as a member of *Calciviridae* family, but now is included in *Hepesvirus* genus of *Hepeviridae* family. However much variability in isolated strains was discovered and recent classification system includes 5 main genotypes: 1,2,3,4,5. Genotype 1 and 2 are responsible for epidemics in endemic regions (which are India, Pakistan, Somalia, Egypt, China and Japan). In European countries indigenous cases are reported from Holland, Great Britain, France, Spain, Hungary, Finland and Denmark and Sweden. All of the indigenous cases from countries listed above were caused by genotype 3. Genotype 5 was isolated only from birds. The natural reservoir of genotype 1 and 2 is human who sheds the virus with feces. For HEV genotype 3 and 4 pigs and wild boars are recognized to be the source of infection. The presence of HEV was discovered in wild boar population in such countries like Germany, Italy or Spain. There were studies providing the direct proofs of hepatitis E being zoonosis, describing infection development after consumption of wild boar and deer meat. In those studies strains of HEV isolated from the sick patients were identical with those from the animals. Studies carried among persons who, due to their profession, were linked with animals (veterinary physicians and pig breeders) showed that (in comparison to persons who had not had contact with animals) that the frequency of specific anti HEV antibodies was higher in the first group. That, as well, provides the proof of hepatitis E being a zoonosis. (9-18)

Aim. The aim of the study was to estimate the prevalence of infection with *Trichinella* spp., *Echinococcus multilocularis* and HEV among the population of Polish hunters, and to define its geographical distribution throughout the country as well as to establish the basic risk factors concerning the occurrence of these diseases.

The aim of this study is to estimate the prevalence of infections caused by *Trichinella* spp., *Echinococcus multilocularis*, and HEV in the population of Polish hunters, describing their geographical distribution in Poland, and to try to define basic factors, which may contribute to their occurrence.

MATERIALS AND METHODS

Studied population. Between 2010 and 2012 a cross-sectional study was conducted in the population of Polish hunters, which was preceded by the Bioethical Commission's approval. The information concerning the study was dispersed by the Board of the Polish Hunting Association (PHA) which forwarded it to regional

units of Polish Hunting Association. In addition some short articles were printed in hunting magazines "Lowiec Polski" and "Brac Lowiecka". Study participants were recruited during hunting meetings, hunters fairs, conventions, shooting competitions etc. moreover some of the participants reported directly to NIPH- NIH by themselves. To every person participating in the study, we provided information concerning the study and a short information about the diseases for which they were tested. Every person gave his/hers written approval for participating in the study.

Material for testing. Every person involved in the study had his or her blood sample taken as well as a questionnaire was conducted concerning a hunting activity, the possibility of other exposures. The health status data and some basic demographical data were also collected. In total blood samples were taken from 1027 hunters, from 16 provinces (from Lubuskie province samples were taken from 3 persons only).

Questionnaire study. Questionnaires were conducted anonymously, and for each participant a unique id number was created. Through the questionnaire such data was gathered as: demographical data (age, sex, occupation); information concerning person's hunting activity (including duration of hunting sessions, type of hunting area, type of hunted game- mean of the numbers of animals hunted yearly, and the information on other contributing factors such as consumption of meat, products of forest undergrowth, spare time spending, contact with rodents etc. Moreover the questionnaire included some questions concerning person's medical history (previous diseases, vaccination history and specific symptoms onset).

Laboratory testing. Serological tests for the presence of antibodies against *Echinococcus multilocularis*, *Trichinella spp.* and Hepatitis E were conducted in NIPH-NIH laboratory, which was certified by the Polish Centre for Accreditation.

Parasitological tests were performed by the indirect immunoenzymatic method ELISA for the presence of specific IgG antibodies, with the use of:

- for alveococcosis- screening test Enzyme immunoassay for the diagnosis of human echinococcoses by Bordier Affinity Products SA and Enzyme immunoassay for the diagnosis of human alveolar echinococcosis by Bordier Affinity Products SA (Em2plus). In case of positive result in the screening test and negative result in the referral test, an additional western blot test was conducted, which facilitated the confirmation of potential *E. granulosus* infection confirmation;
- for *Trichinella spp.*- "in home" ELISA NIPH-NIH with the E/S *Trichinella spiralis* antigens.
- for Hepatitis E testing the following: ELISA with recombinant antigen from genotype 1 and 3 (commer-

cial set by Microgen, Germany): recomWell HEV IgG, ELISA wit recombinant antigen from genotype 1 and 3 (commercial set by Microgen, Germany): recomWell HEV IgM

All samples positive for IgM and part of positive for IgG were than subjected to confirmation test (with the aim of false positive elimination) recomWell HEV IgM or recomWell HEV IgG.

The study of the infections prevalence among the population of hunters. An association between age, sex, the size of the population and prevalence of an infection was studied by the calculation of OR along with the confidence intervals and p value.

RESULTS

The characteristics of the studied population. Study was conducted on the sample of 1027 hunters from 16 provinces: 1002 males and 25 females, ranging from 17 to 85 years of age, which accurately reflects characteristics of the whole group of Polish hunters. Moreover, it was compared to the general hunters population in terms of place of residence and the duration of performing hunting activities. Detailed characteristics of the studied group is demonstrated in table I and figure 1.

Alveococcosis. The results of the screening ELISA test for IgG turned out to be positive for 18 persons. Those results were than confirmed by two tests: western blot test (which enables the differentiation between *E. multilocularis* and *E. granulosus*) and ELISA Em2plus test (which enables the identification of the *E. multilocularis*). After conducting ELISA Em2plus test the confirmation of the infection with *E. multilocularis* was obtained for 2 persons, both males, in the age 37 and 43 respectively, from Opolskie and Zachodniopomorskie provinces.

Trichinellosis. In the same population the serological tests for trichinellosis were conducted. Positive results were obtained in 17 persons, and borderline results (which may indicate of the long gone infection) in 30 persons. Altogether (confirmed and probable) there were found 47 cases of trichinellosis in man in the age ranging from 28 till 73 (see table II for further details). In the conducted study an observation was made that the percentage of persons with the presence of specific antibodies increases with age. The univariable analysis showed that this increase is statistically significant (>70 vs. ≤ 70 OR=1,6; CI: 1,04-2,5; $p=0,034$). Moreover it was proven that the likelihood of *Trichinella* seropositivity increases with the duration of membership in PHA (table III) - >31 vs. ≤ 31 OR= 1,44; CI: 1,1-1,9; $p=0,007$).

The highest percentage of *Trichinella* infected was observed in Podlaskie province (more than 18%). More-

Table I. Comparison of the population of Polish hunters to the study group

Province of residence	No of members of Polish Hunting Association	% of members of Polish Hunting Association	Study group	% of study group
Poland 2012	132055	100.0	1027	0.8
Dolnośląskie	10119	7.7	59	5.7
Kujawsko- Pomorskie	7274	5.5	52	5.1
Lubelskie	7846	5.9	150	14.6
Lubuskie	5599	4.2	6	0.6
Łódzkie	6349	4.8	28	2.7
Małopolskie	7878	6.0	48	4.7
Mazowieckie	16481	12.5	117	11.4
Opolskie	3979	3.0	60	5.8
Podkarpackie	7110	5.4	34	3.3
Podlaskie	5277	4.0	44	4.3
Pomorskie	7837	5.9	44	4.3
Śląskie	7266	5.5	39	3.8
Świętokrzyskie	4000	3.0	36	3.5
Warmińsko- Mazurskie	8206	6.2	45	4.4
Wielkopolskie	13030	9.9	106	10.3
Zachodniopomorskie	9057	6.9	159	15.5
Gender				
Female	2614	2.0	25	2.4
Male	129441	98.0	1002	97.6
Age group				
≤30	9915	7.5	103	10.0
31-40	19243	14.6	183	17.8
41-50	21520	16.3	227	22.1
51-60	30154	22.8	313	30.5
61-70	21337	16.2	178	17.3
≥71	11658	8.8	37	3.6
no data	1137	0.9	-	-
Length of membership of the Polish Hunting Association (in years)				
0-3	16325	12.4	70	6.8
4-10	21993	16.7	195	19.0
11-20	26146	19.8	248	24.1
21-30	27395	20.7	280	27.3
≥ 31	22905	17.3	235	22.9
no data	200	0.2	13	1.3

Table II. Prevalence of antibodies against *Trichinella* spp. in studied hunters by age

Age group	No of people		%
	seronegative	seropositive/ borderline	
do 30	91	2	2.2
31-50	387	14	3.5
51-70	467	28	5.7
>70	35	3	7.9
Total	980	47	4.6

Table III. Prevalence of antibodies against *Trichinella* spp. in studied hunters by the length of membership of the Polish Hunting Association (PHA)

Length of membership of the PHA (in years)	No of people		%
	seronegative	positive/ borderline	
0-3	67	1	1.5
4-10	188	4	2.1
11-20	238	10	4
21-30	262	16	5.8
>30	224	16	6.7
Total	979	47	4.6

over the high percentage of persons infected was observed in Wielkopolskie (7.5%), Łódzkie (7.1%), Mazowieckie (6.6%), Kujawsko-pomorskie (5.8%), Pomorskie (4.5%) and Warmińsko-mazurskie (4.5%). In five provinces no *Trichinella* seropositive persons were found. Only 4 persons among those in whom specific antibodies were found stated in the questionnaire that they suffered from trichinellosis previously. In the subgroup of hunters in which no positive tests results were obtained, 10 persons stated having trichinellosis earlier in life.

Viral Hepatitis E. There were 33 positive results for IgM antibodies against HEV in ELISA test, among which 3 were confirmed by Western blot, which indicated the ongoing infection in those persons. All 3 new infections were discovered in men in age 48, 51 and 64, respectively. In 206 hunters (which is more than 25% of persons included in the study) IgG class antibodies were found in ELISA test. Antibodies were found in 3 women and 203 men respectively in the age between 21 and 80 (Table IV). Study also showed that the percentage of persons with antibodies is highest in the age group 70+ and the difference is statistically significant comparing to other age groups (OR=3,3; CI: 1,4-7,7). No relation between the percentage of seropositive persons and the duration of hunting activities was found (Table V).

Much differences in the frequency of infection were found throughout particular provinces. The dispersion of antibodies occurrence varies between 3.85% in Kujawsko-pomorskie to 41.7% in Opolskie.

DISCUSSION

Table IV. Prevalence of antibodies against HEV in studied hunters by age

Age group	No of people		%
	seronegative	positive/ borderline	
do 30	76	17	18.3
31-50	321	73	18.5
51-70	391	100	20.4
>70	22	16	42.1
Total	810	206	20.3

Table V. Prevalence of antibodies against HEV in studied hunters by the length of membership of the Polish Hunting Association (PHA)

Length of membership of the PHA (in years)	No of people		%
	seronegative	seropositive/ borderline	
0-3	57	10	15
4-10	155	36	18.8
11-20	192	52	21.3
21-30	223	52	19
>30	182	56	23.5
Total	809	206	20.3

Conducted study of occurrence and prevalence of infection with zoonotic agents such as *Echinococcus multilocularis*, *Trichinella spp.* and HEV in the population of Polish hunters showed the presence of such in the studied population.

As for the *E. multilocularis* infections study shows that there is a high frequency of those in studied group, especially in the context of the surveillance data over the infectious diseases in Poland. The latter shows that in the period 2006 - 2011 the number of echinococcosis cases ranged between 19 to 65 yearly, and the incidence was from 0.05 to 0.17 per 100 000. In the years 1997-2011, among cases of echinococcosis with identified species of the *Echinococcus*, cystic echinococcosis (73%) was diagnosed two times more frequently than alveolar echinococcosis (27%). In 2012, the rate of recognition of these two infections did not differ so much - hydatidosis and alveococcosis were found (respectively) in 59% and 41%. It appears to be justified that in case of founding hepatic lesions in a hunter in the course of diagnostic imaging it would be reasonable to perform tests aim of which would be to confirm or to exclude echinococcosis (20).

In case of trichinellosis significant is the fact of confirming *Trichinella* infections in persons from areas where no cases were reported according to the surveillance data. This might be due to the fact that some cases are not diagnosed because of mild symptoms of the disease, frequently similar to common cold. In Poland cases of trichinellosis are reported each year. This is generally a few dozen cases a year and it is results of occurrence of small outbreaks, mostly family one. However, in some years outbreaks occurs which involved even several hundred people. Consumption of wild boar meat or its products have been implicated as a main sources of *Trichinella* outbreaks that occurred in Poland in recent years. Further studies over *Trichinella* epidemiologic situation is needed in Podlaskie province, where the percentage of the infection was the highest in the country. Comparing to Kujawsko-pomorskie and Wielkopolskie where outbreaks of trichinellosis are most frequent and from where the most cases are report-

ed in the course of routine surveillance, the percentage of infections among hunters from Podlaskie was over 50 times higher (6, 21, 22).

So far there was no information on the subject of hepatitis E among people in Poland and the above study facilitated to recognize the epidemiological situation as the first one. Obtained results indicate the big wide-spread of this disease among studied population- over 20%. In other European countries- depending on the population studied HEV seroprevalence varies from 1.3 to 52%. Genotypes 3 and 4 of HEV do not pose a threat for immuneocompetent persons, however in recent years there have been some reports of serious consequences of hepatitis E in persons after transplantations from donors infected with HEV. Results proving the widespread HEV infections may be a premise for taking under consideration the implementation of tests for hepatitis E for blood and organ donors (23-26).

CONCLUSIONS

Conducted study confirm that hunters are a risk group for infection with *E. multilocularis*, *Trichinella spp.* and HEV.

Comparing the study's results to surveillance data it might be possible that the *E. multilocularis* infection is more prevalent in the group of hunters than in the general population.

In the context of usually low intensity of *Trichinella* infection in wild boars and that the clinical manifestation of the infection in humans might be mild it is important of constantly raising awareness among hunters (concerning proper wild boar meat handling) in order to avoid the infection.

This study is one of the first conducted in Poland on HEV infection, so it is difficult to state whether such high (accounting for more than 20% of described population) prevalence of the infection reflects the prevalence in general population. HEV infections may bring some implications for transfusions and transplantations so further studies of such kind are required.

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