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SHORT COMMUNICATION

The Serological Surveillance of *Hepatitis E virus* among Hunters and Foresters in Eastern Poland

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Abstract

The aim of the study was the analysis of the occurrence of specific antibodies against HEV among hunters and foresters who are at risk to be exposed. The study group consisted of 210 hunters (23–80 years old) and 60 foresters (22–64 years old). Anti-HEV IgG were present in 3.81% of the samples of the hunters and in 5% of the samples of the foresters. The statistical analysis shows no significant differences in the results anti-HEV IgG between the groups of hunters and foresters ($p = 0.5278$). Significantly higher anti-HEV IgG titers were found in the older age group (> 55 years old).

Key words: anti-HEV IgG in hunters and foresters, HEV, risk of hepatitis E

Hepatitis E virus (HEV) is a ssRNA positive-strand virus, a member of the *Hepevirus* genus, *Hepeviridae* family and consists of 4 major genotypes (1, 2, 3 and 4). Genotypes 1 and 2 of HEV have been found only in humans and they are responsible for epidemics in endemic regions (HEV 1 in North America and Asia, HEV 2 in West Africa and Mexico). The transmission is primarily *via* the faecal-oral route through drinking water or food contaminated with human excreta. In developed countries those two genotypes cause travel associated infections, in Europe cases caused by HEV 1 are predominant. Genotypes 3 and 4 of HEV have been found in humans and animals (pigs, wild boars, shellfish and other), and are responsible for worldwide autochthonous infections. Zoonotic transmission to humans is the most important for HEV genotypes 3 and 4. Consumption of raw or undercooked wild-boar meat, offal or beef is significantly associated with autochthonous HEV infection. HEV infections caused by blood transfusions, mother-to-child, person-to-person, and sexual intercourses were also documented but uncommonly noted. In Europe, there is mostly genotype 3 in humans, but a few cases of HEV 4 were described (Wichmann *et al*., 2008; Lee *et al*., 2015; Pérez-Gracia *et al*., 2015). HEV-infected persons exhibit a wide clinical spectrum, ranging from asymptomatic infection through acute icteric hepatitis to fulminant hepatitis. Acute hepatitis E usually manifests with icterus, malaise, anorexia, fever, hepatomegaly, and occasionally pruritus. Certain population sub-groups are at a higher risk of severe disease following HEV infection. These include pregnant women, persons with pre-existing liver disease and persons with immunosuppression (WHO, 2014).

Contact exposure to infected animals leads to an elevated risk of HEV transmission in humans. Studies have shown that occupational groups *e.g.* swine breeders (Forgách *et al*., 2007), slaughterers (Krumbholz *et al*., 2012), veterinarians who come into close contact with pigs (Bouwknegt *et al*., 2008) run the risk of being infected with HEV. Swine workers in Spain were found to be 5.4 times more likely to be positive for anti-HEV IgG than those not exposed to swine (Galiana *et al*., 2008). Contact with swine is the most widely recognized route for occupational exposure to HEV; however, the multitude of novel strains of HEV in wildlife and other domestic animal species suggest additional mechanisms of transmission (Yugo and Meng, 2013). The aim of the study was the analysis of the occurrence of specific antibodies against HEV among hunters and foresters who are at risk to be exposed.

The study group consisted of 210 hunters aged 23–80 (54, SD 11.61): 208 men (99.05%) and 2 women (0.95%)

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Table I
The titers of anti-HEV IgG depending on the age of hunters and foresters

Group	The titers of anti-HEV IgG (RU/ml)						
	Age	Number of persons	Average	Median Me	MIN	MAX	SD
Hunters	≤ 35	17	0.68	0.26	0.20	4.00	0.96
	36–55	99	0.59	0.30	0.20	5.57	0.75
	> 55	94	0.89***	0.46	0.20	19.71	2.08
Foresters	≤ 35	13	1.61	0.22	0.20	16.73	4.55
	36–55	32	0.55	0.26	0.20	2.79	0.56
	> 55	15	2.13	0.47	0.20	24.00	6.07
All studied persons	≤ 35	30	1.08	0.25	0.20	16.73	3.05
	36–55	131	0.58	0.29	0.20	5.57	0.71
	> 55	109	1.06*/**	0.46	0.20	24.00	2.94
Total		270	0.83	0.35	0.20	24.00	2.19

Significant differences: * p = 0.004; ** p = 0.0194; *** p = 0.0119

and 60 male foresters, aged 22–64 (45.63, SD 11.81). The selection of the groups was purposeful because hunters and foresters have a diverse activity by being in the forest environment. Blood samples were collected in Lubelskie voivodship from 2014 to 2015. The groups of hunters and foresters were divided into three age groups: ≤ 35 years old, 36–55 years, > 55 years. The Bioethical Committee of Medical University of Lublin authorized the project (permission No. KE-0254/177/2014).

Serological analysis. The presence of anti-HEV IgG was detected by ELISA (Anti-*Hepatitis E virus* (HEV) ELISA IgG, Euroimmun). Microtiter wells were coated with mixed recombinant antigens of *Hepatitis E virus* genotypes 1 and 3. The results above or equal 2.2 relative units/ml (RU/ml) were considered as positive, below 1.6 RU/ml as negative, whereas borderline results were ≥ 1.60 and < 2.2 RU/ml. The test was carried out and the results were interpreted according to the manufacturer's instructions.

The obtained data were analysed statistically using Statistica v. 10 software. The Chi-square test was performed for nominal features in order to detect statistically significant dependence. For data expressed numerically nonparametric Mann-Whitney *U* and Kraskala-Wallisa test was performed. The assumptive level of significance was p = 0.05.

The results for anti-HEV IgG in hunters group were as follows: 8 positive samples (3.81%), 10 borderline (4.76%) and 192 negative (91.43%). Anti-HEV IgG in the foresters group were detected in 3 samples (5%) at positive level and in 1 sample (1.67%) at borderline level. Anti-HEV antibodies were not reported among 56 (93.33%) foresters. The statistical analysis shows no significant differences in the titers anti-HEV IgG between the groups of hunters and foresters (p = 0.5278). The titers of anti-HEV IgG were presented in Table I.

Significant statistical differences (p = 0.004) in titers of anti-HEV IgG between the age group 36–55 (0.58, SD 0.71), and the age group > 55 (1.06, SD 2.94) for all the tested subjects were found. There were also significant statistical differences (p = 0.0194) in IgG titers between the age group of ≤ 35 (1.08, SD 3.05) and the age group > 55 (1.06, SD 2.94) for all the tested subjects. Significant statistical differences (p = 0.0119) in titers of anti-HEV IgG between the age group 36–55 (0.59, SD 0.75) and the age group > 55 (0.89, SD 2.08) for the hunters were found.

In the study of Sadowska-Todys *et al.* (2015) performed in Poland among 1027 hunters (17 to 85 years of age), in 206 persons (25%) anti-HEV IgG were found by means of ELISA test (positive and borderline result). Anti-HEV IgM were confirmed in 3 persons. The study showed that the percentage of persons with anti-HEV IgG is the highest in the age group ≥ 70 and the difference is statistically significant comparing to other age groups. No relation between the percentage of seropositive persons and the duration of hunting activities was found.

In our study 210 hunters aged 23–80 were examined. At the beginning of the experiment we assumed that the forester group, without the direct contact with animals, would be the control group of the study. Positive results anti-HEV IgG were present in 3.81% of the samples of the hunters group and in 5% of the samples of the foresters. The statistical analysis in our study showed no significant differences in the results anti-HEV IgG between the groups of hunters and foresters. The higher titers anti-HEV IgG was in the older age groups, significantly higher antibody titers demonstrated in the age group > 55 years.

It has already been described by Carpentier *et al.* (2012) in France, which forestry workers, particularly woodcutters are group endangered with HEV infection. French forestry workers (n = 593) were tested for anti-HEV antibodies. One hundred eighty five subjects had anti-HEV antibodies (31.2%) with prevalence increasing steadily from 15.7% in 15–34-year-olds to 46.2 in subjects older than 55 years of age. Among the 135 controls with no direct contact with the forest environment (drivers, gardeners, traders) 26 (19.2%) had anti-HEV antibodies with the prevalence increasing from 4.5% in subjects aged 15 to 34 to 39% in elders. In the study of Montegnaro *et al.* (2015) performed in central Italy (Latium region) HEV seroprevelance was noted among 25% of the examined hunters. In Italy several authors have demonstrated an appreciable frequency of HEV infection, generally in travellers returning from endemic areas, however autochthonous cases have also been documented pointing to a possible autochthonous circulation of the virus in human population, sustained by the presence of an animal reservoir of HEV. In the study of Schielke *et al.* (2015) on 126 hunters (median age 55; 94% male) 21% tested were positive for anti-HEV IgG antibodies. Anti-HEV prevalence was the highest in the age group of the 70–79 (67%). Hepatitis E has been observed in Germany since 2001. Since then, the number of recorded cases has been increasing steadily each year. Men between 50–69 years of age are the most affected group with 0.9 cases per 100,000 populations. Underreporting is expected due to asymptomatic and underdiagnosed infections. In forest workers, anti-HEV prevalence of 18% in Germany was reported (Dremsek *et al.*, 2012). Our study confirmed the higher exposure in older age groups, significantly higher anti-HEV titer demonstrated in the age group > 55.

In the study of Bura *et al.* (2015) 182 patients (101 men and 81 women), aged 19–85 (47.2 ± 14.2; half of the patients were under 48), hospitalized for different reasons in Wielkopolska Region (infectious and non-infectious liver diseases, diarrheal illnesses, herpes zoster, HIV infection, meningitis and erysipelas) were examined. Anti-HEV were found in 29 patients (15.9%).

In conclusion, the zoonotic risk of HEV is well established but seroprevelance in humans varies drastically between studies and countries. Numerous animal species seropositive for IgG anti-HEV, contaminated food, water and environment must be considered as potential sources of HEV infections in humans.

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