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PART I. DISEASES AND PROBLEMS DISTINGUISHED BY WHO AND FAO
DZIAŁ I. CHOROBY I PROBLEMY WYRÓŻNIONE PRZEZ WHO I FAO

SEROLOGICAL STATUS OF HUNTERS AND THEIR FAMILY MEMBERS IN RELATION TO RISK OF HEV INFECTIONS

STATUS SEROLOGICZNY OSÓB ZAJMUJĄCYCH SIĘ ŁOWIECTWEM I CZŁONKÓW ICH RODZIN W ODNIESIENIU DO RYZYKA ZAKAŻEŃ WIRUSEM HEV

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Summary

Background. The aim of the study was to assess the HEV serological status in inhabitants of the Lublin Province, Poland, including subjects involved in hunting activities and their family members.

Material and methods. The study group comprised 594 subjects from the Lublin Province, i.e. hunters (23%), members of hunters' families (19%) and subjects (58%) that are not engaged in hunting activities. The determination of the presence of anti-HEV IgG antibodies in the serum was carried out in two stages (ELISA, Western blot).

Results. The presence of anti-HEV IgG antibodies was confirmed in 9.7% of the subjects. In the individual groups, positive anti-HEV IgG results were obtained in 8.1% of the hunters (8.1%), 9.5% of the members of hunters' families, and in 10.5% of those not involved in hunting. A relationship was found between the anti-HEV IgG positive results and participation in the processing of game meat declared by the members of hunters' families.

Conclusions. Participation in the processing of game meat may be an important factor in human exposure to HEV infection. Dietary habits, such as game meat consumption, are also of great importance in this regard. For this reason, it is important to raise the awareness of zoonoses, HEV transmission routes and food safety in subjects from the risk groups and in the general population.

Keywords: game meat, HEV, IgG antibodies, food safety, hunters

Streszczenie

Wprowadzenie. Celem pracy była ocena statusu serologicznego w odniesieniu do wirusa HEV u mieszkańców z terenu województwa lubelskiego, w tym osób zajmujących się łowiectwem oraz członków rodzin myśliwych.

Materiał i metody. Grupę badaną stanowiło 594 osoby z terenu województwa lubelskiego w tym: myśliwi (23%), osoby z rodzin myśliwych (19%) oraz osoby nie zajmujące się łowiectwem (58%). Badania w kierunku określenia obecności w surowicy przeciwciał IgG anty-HEV prowadzono dwuetapowo (test ELISA, test Western blot).

Wyniki. Obecność przeciwciał IgG anty-HEV stwierdzono u 9.7% ogółu badanych. W poszczególnych grupach pozytywne wyniki IgG anty-HEV uzyskano u 8.1% myśliwych, 9.4% osób z rodzin myśliwych i 10.5% osób nie zajmujących się łowiectwem. Wykazano związek między istnieniem wyników pozytywnych w kierunku IgG anty-HEV, a deklarowaną obróbką dziczyzny u osób z rodzin myśliwych.

Wnioski. Istotnym czynnikiem narażenia ludzi na ryzyko zakażenia wirusem HEV może być udział w obróbce mięsa dzikich zwierząt. Nawyki żywieniowe, jak spożywanie dziczyzny, mają w tym względzie również duże znaczenie. Dlatego też ważne jest kreowanie świadomości w zakresie chorób odzwierzęcych, dróg transmisji wirusa HEV i bezpieczeństwa żywności wśród osób z grup narażenia, ale również populacji ogólnej.

Słowa kluczowe: dziczyzna, HEV, przeciwciała IgG, bezpieczeństwo żywności, myśliwi

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Introduction

Infections caused by the hepatitis E virus (HEV) are one of the new public health problems currently encountered in many countries [1,2]. The highest prevalence of HEV infection occurs in Africa, Asia, Europe, North America, and South America [3]. However, current data on HEV seroprevalence among different population groups is lacking in many countries (e.g. in Latin America). HEV infection occurs frequently in Uruguay, with a seroprevalence rate of 10% among blood donors [4,5]. The seroprevalence in the general European population ranges from 6.1% to 52.5%, which depends on e.g. occupational hygiene, nutritional habits, and diagnostic methods [1].

HEV represents the family *Hepaviridae* and subfamily *Orthohepevirinae,* which comprises four genera: *Paslahepevirus, Avihepevirus, Rocahepevirus*, and *Chirohepevirus*. Eight genotypes (from HEV-1 to HEV-8) have been distinguished in the *Paslahepevirus balayani* species, which belongs to the genus *Paslahepevirus* [6,7]. Genotypes HEV-1 and HEV-2 are spread among humans via the fecal-oral route, most frequently through consumption of contaminated water and food. Genotypes HEV-3 and HEV-4 are zoonotic pathogens infecting humans and some species of wild mammals (mostly wild boars and deer) and are transmitted to farm animals [6,8]. Humans can be infected by these genotypes through consumption of raw meat (usually pork, wild boar and deer meat) or feces-contaminated water and through direct contact with the blood of infected animals [1,9-11]. Genotypes HEV-5 and HEV-6 have been detected in wild boars, while dromedaries have been reported to be carriers of HEV-7. In turn, HEV-8 has been detected in Bactrian camels and rabbits [6]. Pigs, wild boars, roe deer, deer, rabbits, ferrets, mongooses, rats, cattle, sheep, goats, dogs, cats, and horses, as well as crustaceans, mussels, and oysters, are reservoirs of genotypes HEV-3 and HEV-4 [8,12-17]. These animals can therefore be regarded as potential sources of this type of virus transmitted to humans. It has also been proved that it is possible to detect the virus in the feces of asymptomatic animals and in the milk of infected cows [11].

The HEV virus is regarded as an etiological agent of hepatitis E. Most infections are asymptomatic, which may result in underdiagnosis in the European population [1,11]. However, 5%-30% of HEV-infected subjects develop acute disease with such symptoms as abdominal pain, nausea, vomiting, fever, jaundice, and dark urine. It is assumed that HEV-1 and HEV-2 cause hepatitis with a more severe course than HEV-3 and HEV-4 [11]. Nevertheless, elderly people with chronic liver disease may develop acute infection regardless of the virus genotype. It has been shown that HEV infection in patients with chronic HBV hepatitis may exacerbate the disease, worsen symptoms, and increase the risk of mortality in cirrhosis patients. Hemodialyzed and immunosuppressed patients are in the group of high HEV infection risk. The infection is especially dangerous to pregnant women and is associated with high morbidity and even a risk of death. A subclinical course of HEV infection in blood donors has also been described [1,6,10,11,18].

In terms of the HEV transmission routes, farmers, veterinarians, and slaughterhouse workers may be at higher risk of infection than the general population [11]. The group with an increased risk of infection also comprises subjects engaged in hunting, which is associated with direct contact with wild animals [2,6]. Hunters and their families consume game meat more frequently than the general population. As reported in some studies, the consumption of wild boar sausages and pork that has not been properly cooked may have been a risk factor in confirmed cases of hepatitis E [1].

Aim of the work

The aim of the study was to assess the HEV serological status in subjects involved in hunting activities in the Lublin Province, Poland, and their family members, as they have frequent contact with game animals or the raw meat of culled animals and consume game meat.

Material and methods

The study group comprised 594 subjects from the Lublin Province, i.e. 135 hunters (23%), 116 members of hunters' families (19%), and 343 subjects (58%) that are not engaged in hunting activities (control group). Detailed data on the sex and age of the subjects is presented in Table 1.

Table 1. Characteristics of the study group

Parameters		Population group			Total
		Hunters	Members of hunters' families	General population	
Number of people N (%)		135 (23)	116 (19)	343 (58)	594 (100)
Sex	Female N (%)	4 (3.0)	91 (78.4)	226 (65.9)	321 (54.0)
	Male N (%)	131 (97.0)	25 (21.6)	117 (34.1)	273 (46.0)
Age (years)	Mean value	55	50	53	53
	Min	19	18	18	18
	Max	81	74	84	84
	SD	13.8	13.9	13.8	13.8

Venous blood collected from January 2022 to May 2023 was the material for the analyses. It was drawn by qualified personnel in compliance with laboratory diagnostic procedures and sanitary and epidemiological standards.

The determination of the presence of anti-HEV IgG antibodies in the serum was carried out in two stages. In the first stage of the study, ELISA (Euroimmun) assays were used to detect anti-HEV IgG antibodies. The microplate had reaction wells coated with recombinant HEV antigens derived from recombinant genotype 1 and 3 HEV structural proteins. The anti-HEV IgG antibodies were quantified at a lower detection limit of 0.1 RU/ml (relative unit/ml) and a cut-off of 1 RU/ml. The sensitivity of the test was 95.5%, and the specificity was 100%. The results were interpreted in accordance with the manufacturer's instructions: <0.8 RU/ml – negative results, ≥0.8 RU/mL to <1.1 RU/mL – borderline results, and ≥1.1 RU/mL – positive results.

A photometric evaluation of the color intensity was carried out within 30 minutes of stopping the reaction at a wavelength of 450 nm and a reference wavelength of 620-650 nm. A negative serological test does not rule out infection. Especially in the early stages of infection, antibodies may not be present in the serum or are present in amounts so small that they are practically undetectable. A positive serological test indicates contact with the pathogen.

Samples with borderline or positive results in the ELISA assays were subjected to a Western blot test (stage II) intended for qualitative *in vitro* evaluation of human anti-HEV IgG antibodies (Euroimmun). The Western blot test was performed on the same blood samples as the ELISA assay. The test strips were coated with HEV antigens from Open Reading Frame 2 (ORF2) of genotypes 1, 2, 3, and 4. EUROLineScan software (Euroimmun) was used to evaluate the incubated strips. The test system is validated for the determination of specific antibodies against hepatitis E virus antigens from the ORF2 of the genotypes 1 to 4 in human serum or plasma only. The pipetting volumes, incubation times, temperatures, and preparation steps given in the instruction for use were adhered to in order to prevent possible incorrect results. A correctly performed incubation was indicated by a positive reaction of the control band and a positive reaction of the IgG band on the conjugate control chip. The results of the Western blot test Anti-Hepatitis E Virus (IgG) were divided into negative and positive results. The presence of fewer than two positive antigen bands was interpreted as a negative result, whereas the presence of at least two positive antigen bands with specific GT1 ORF2, GT2 ORF2, GT3 ORF2, and GT4 ORF2 antigens was regarded as a positive result.

The subjects analyzed in the study provided information on their participation in the processing of game meat, consumption of venison, and species of game animals whose meat was harvested and consumed.

Statistical analysis of the data was performed using STATISTICA version 13.0 by StatSoft Polska. Nominal data was described with the use of distribution series, in which variants of traits were specified in terms of their abundance and frequency in the entire analyzed population. Pearson's Chi-square test was used for statistical analysis of the data. Statistical inference was carried out at a standardized significance level of α=0.05. The following interpretation rules were adopted: p<0.05 – statistically significant relationship, p<0.01 – highly statistically significant relationship, and p<0.001 – very highly statistically significant relationship.

Results

The ELISA test detected the presence of anti-HEV IgG antibodies in the range of values qualified as a positive result in 43/594 subjects (7.2%) from the entire analyzed group. Borderline and negative results were exhibited by samples from 28/594 (4.7%) and 523/594 (88.1%) subjects, respectively. It is recommended that the borderline results be verified by other diagnostic methods or that the next sample taken after a certain period of time be serologically tested. In our studies, we used the first variant of the Western blot test to confirm the presence of IgG anti-HEV antibodies. The positive and borderline ELISA results were confirmed with the use of the Western blot test. Finally, the presence of anti-HEV IgG antibodies was confirmed in 58/594 subjects (9.7%). In the individual groups, positive anti-HEV IgG results were obtained in 11/135 hunters (8.1%), 11/116 members of hunters' families (9.4%), and 36/343 samples from the control group (10.5%). Detailed results obtained in the diagnostic process are presented in Table 2.

Table 2. Detailed ELISA and Western blot results of the presence of anti-HEV IgG antibodies

Population group N (%)	anti-HEV IgG			
	ELISA n (%)		Western blot n (%)	
Hunters N=135 (23)	-	124 (91.9)	x	
	+	5 (3.7)	+	5 (3.7)
	-/+	6 (4.4)	+	6 (4.4)
Members of hunters' families N=116 (19)	-	102 (87.9)	x	
	+	10 (8.6)	+	9 (7.7)
			-	1 (0.9)
	-/+	4 (3.4)	+	2 (1.7)
			-	2 (1.7)
General population (control group) N=343 (58)	-	297 (86.6)	x	
	+	28 (8.1)	+	20 (5.8)
			-	8 (2.3)
	-/+	18 (5.2)	+	16 (4.7)
			-	2 (0.6)
Total N=594 (100)	-	523 (88.1)	-	536 (90)
	+	43 (7.2)		
	-/+	28 (4.7)	+	58 (9.7)

Notes: - a negative result; -/+ a borderline result; + a positive result; x the test was not performed.

In the group with positive Western blot results, anti-HEV-3 IgG antibodies were the dominant antibodies (56/58 subjects; 96.5%). Anti-HEV-1 IgG (53/58 subjects; 91.2%) and IgG anti-HEV-4 (51/58; 87.9%) were often detected, whereas anti-HEV-2 IgG antibodies (47/58; 81.0%) were found less frequently.

There was no significant effect of hunting activities undertaken by the hunters or the affiliation to hunters' families on the frequency of positive HEV results compared to the control group.

In the study group, 26.2% of the subjects (156/594) declared that they participated in the processing of game meat, and 47.9% (285/594) declared consumption of this type of meat. A relationship was found between the anti-HEV IgG positive results and the participation in game meat processing declared by the members of hunters' families (41/116; 35.3%; Pearson's χ^2 4.256; p=0.039). In the same group, there was a close-to-significant relationship between the anti-HEV IgG positive results and the consumption of game meat declared by the respondents (90/116; 77.5%; Pearson's χ^2 3.510; p=0.060). Details of the processing of game meat and consumption of game meat in population groups are listed in Table 3. In the group of hunters, a relationship was found between the presence of anti-HEV IgG antibodies and the declared ownership of a dog (94/135; 69.6%; Pearson's χ^2 10.160; p=0.001).

Table 3. Processing of game meat and consumption of game meat in study group

Parameters	Population group N (%)			
	Hunters N=135 (23)	Members of hunters' families N=116 (19)	General population (control group) N=343 (58)	Total N=594 (100)
Processing of game meat	110 (81.4)	41 (35.3)	5 (1.5)	156 (26.2)
Wild boars	11 (8.1)	3 (2.6)	2 (0.6)	16 (2.7)
Wild boars, deer	18 (13.3)	12 (10.3)	3 (0.9)	33 (5.6)
Wild boars, roe deer	5 (3.7)	2 (1.7)	-	7 (1.2)
Wild boars, roe deer, deer	46 (34.1)	13 (11.2)	-	59 (9.9)
Wild boars, roe deer, deer, wild birds (pheasant, goose, duck)	18 (13.3)	3 (2.6)	-	21 (3.5)
Other game animals	12 (8.9)	8 (6.9)	-	20 (3.3)
Consumption of game meat	126 (93.3)	90 (77.6)	71 (20.7)	287 (48.3)
Wild boars	7 (5.2)	6 (5.2)	28 (8.2)	41 (6.9)
Wild boars, deer	30 (22.2)	27 (23.3)	9 (2.6)	66 (11.1)
Wild boars, roe deer	3 (2.2)	11 (9.5)	16 (4.7)	30 (5.1)
Wild boars, roe deer, deer	48 (35.5)	22 (18.9)	5 (1.4)	75 (12.6)
Wild boars, roe deer, deer, wild birds (pheasant, goose, duck)	17 (12.6)	5 (4.3)	3 (0.9)	25 (4.2)
Other game animals	21 (15.6)	19 (16.4)	10 (2.9)	50 (8.4)

Discussion

In Europe, the incidence of HEV infection varies depending on the region. Some areas with high seroprevalence are defined as hyperendemic for HEV-3, e.g. south-western France (>50%) [11]. Other studies have reported the presence of anti-HEV IgG in 52.35% of the general population in France [19]. As shown by De Sabato et al., the seroprevalence in some areas of Italy ranges from 10% to >22% and is 8.7% among blood donors [1].

Veterinarians, pig breeders, meat sellers, and hunters are at particularly high risk of HEV infection [13]. Studies conducted among hunters in different regions of Europe have shown significant differences in the results. The prevalence of anti-HEV IgG antibodies in hunters has been estimated at 25% in central Italy [20], 21% in

central Germany [21], 80% in the Midi-Pyrénées region in south-western France (20 of 25 hunters) [19], 29%-61% in Poland [22], 1%-24% in Croatia, up to 21% in Bulgaria, 6%-17% in Romania, 15% in Serbia, up to 10% in Greece, and 2%-10% in Albania [23]. In our research conducted in the Lublin Province (eastern Poland), anti-HEV IgG antibodies were found in 10.5% of the individuals from the control group (i.e. non-hunters and their family members) and 9.5% of members of hunters' families. These results are slightly higher than those reported by Jelicic et al. in the general population of Croatia (7.1%) [2]. In the present study, positive anti-HEV IgG results were obtained in the case of 8.1% of hunters. These are lower values than those reported by Jelicic et al. [2], who found the presence of anti-HEV IgG in 14.9% of hunters and 15.2% of veterinarians. The researchers also reported a significant increase in the seroprevalence correlated with the age of the analyzed subjects (>60 years – 23%; 50-59 years – 10.9%; <50 years – 2.9%-7.0%). However, there were no differences in the seroprevalence in relation to the frequency of consumption of game meat and pork [2].

In the present study, anti-HEV IgG antibodies were detected in 18.35% of subjects aged 51-60 and in 33.50% of those aged over 61 years. In the other age groups (18-30 years, 31-40 years, 41-50 years), anti-HEV IgG antibodies were found in 5.22%, 11.95%, and 30.98%, respectively, but there was no significant increase in seroprevalence depending on the age of the analyzed subjects.

In industrialized countries, transmission of HEV is mainly associated with the consumption of raw or undercooked meat products. In addition, the virus may be transmitted via surfaces that have been in contact with contaminated meat or excreta from infected hosts [3]. Contact with live animals and their blood, evisceration, fresh meat and offal processing, potential cross-contamination of muscle mass through bile waste dispersion, consumption of meat after insufficient heat treatment, and improper storage of raw meat may support the transfer of the HEV virus to the human organism. An additional risk factor is insufficient hygiene combined with activities that support infection, e.g. contact with animals, hunting, sheep, cattle, and goat farming, and slaughtering animals (pigs, goats, sheep and rabbits). The probability of HEV infection in subjects exposed to the above-mentioned factors may be even 50% higher than that in the general population [13]. However, in our studies, we found no significant differences in the prevalence of anti-HEV IgG between hunters and non-hunters.

It should be noted that companion animals (dogs, cats) can be zoonotic sources of HEV. The seroprevalence in these animals is estimated to be in the range of 0.9%-56.6%. A study conducted by Caballero-Gómez et al. [24] in southern Spain showed the presence of anti-HEV antibodies (HEV-3 and HEV-C1) in dogs (9.9%) and cats (2.8%). The authors highlighted the need for further research to determine the risk of HEV transmission from cats and dogs to other species and humans [24].

In the present study, we showed a relationship between the positive anti-HEV IgG results and participation in game meat processing declared by the members of hunters' families. In turn, a relationship was found between the positive anti-HEV IgG results and the ownership of a dog declared in the group of hunters. This may be important in the transfer of the virus, which is excreted in feces. Additionally, dogs participate in hunting and may thus come into contact with game animals or may be fed raw game meat and offal [24]. This issue undoubtedly requires additional investigations and verification of the information declared by the individuals of the test groups regarding hunting activity and consumption of meat from hunted animals.

In developed countries, HEV-3 is the most common genotype causing human infection. In Europe and North America, an increase in the prevalence of both HEV-3 and HEV-4 has been reported; similar to HEV-3, the latter has zoonotic potential and is considered an Asian genotype [13]. Studies carried out in Asia indicated an increasing prevalence of the HEV-4 genotype [11]. The dominance of the genotype that causes infections in humans may potentially change, as shown in China, where a study from 2019 showed that the zoonotic HEV-4 genotype started to prevail over HEV-1 [6]. In the present study, the highest prevalence was detected for IgG antibodies against the HEV-3 and HEV-4 zoonotic genotypes, i.e. in 96.5% and 87.9% of the analyzed individuals, respectively.

Conclusions

Participation in game meat processing may be an important factor in human exposure to HEV infection. Dietary factors, such as game meat consumption, are also of great importance in this regard. Thus, even as other authors indicate the relationship between contact with game animals and infection with HEV, this study does confirm this fact. For this reason, it is important to raise the awareness of zoonoses, HEV transmission routes, and food safety in subjects from the risk groups and in the general population. It is also advisable to investigate the role of dogs as companion animals in the transfer of the HEV virus.

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Artificial intelligence (AI) was not used in the creation of the manuscript.

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