Exposure to hepatitis E virus, hepatitis A virus and *Borrelia* spp. infections in forest rangers from a single forest district in western Poland

Maciej Bura[1,A–F], Alicja Bukowska[2,A,B,E], Michał Michalak[3,C,D], Aleksandra Bura[4,B–D], Mariusz J. Nawrocki[5,6,B,D], Marek Karczewski[7,A,C,E], Iwona Mozer-Lisewska[1,E,F]

[1] Department of Infectious Diseases, Hepatology and Acquired Immunodeficiencies, Poznan University of Medical Sciences, Poland
[2] Regional Blood Center in Poznań, Poland
[3] Department of Computer Science and Statistics, Poznan University of Medical Sciences, Poland
[4] Department of Infectious Diseases, Jozef Strus Multidisciplinary Municipal Hospital in Poznań, Poland
[5] Department of Biochemistry and Molecular Biology, Poznan University of Medical Sciences, Poland
[6] Department of Anatomy, Poznan University of Medical Sciences, Poland
[7] Department of General and Transplant Surgery, Poznan University of Medical Sciences, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

Advances in Clinical and Experimental Medicine, ISSN 1899–5276 (print), ISSN 2451–2680 (online) *Adv Clin Exp Med*. 2018;27(3):351–355

Address for correspondence
Maciej Bura
E-mail: mbura@umed.poznan.pl

Funding sources
This research was funded by Poznan University of Medical Sciences, Poland (No. of funds 502-01-02205314-04519) and the Regional Blood Center in Poznań, Poland

Conflict of interest
None declared

Acknowledgements
We would like to thank Dr Hanna Skalisz from the Regional Blood Center in Poznań and forest rangers from the Międzychód forest division for their support in the organization of the study, and Dr Michał Chojnicki for rapid transportation of the BDs' samples.

Received on July 14, 2016
Reviewed on July 26, 2016
Accepted on October 12, 2016

DOI
10.17219/acem/65787

Abstract

Background. Hepatitis E virus (HEV) infection is an emerging problem in developed countries. At least 2 zoonotic genotypes of the virus (HEV-3 and HEV-4) infect human beings. There are some data suggesting that forest rangers (FRs) can be at a higher risk of contact with HEV.

Objectives. The aim of this study was to assess the prevalence of HEV exposure markers in FRs from a single forest district in Greater Poland in relation to anti-HAV (hepatitis A virus) IgG, and anti-*Borrelia* spp. IgM and IgG antibodies.

Material and methods. In total, 138 participants (48 FRs and 90 blood donors – BDs) were tested for anti-HEV IgM and IgG (EUROIMMUN Medizinische Labordiagnostika AG, Luebeck, Germany) and 96 individuals (48 FRs and 48 BDs) were tested for anti-HAV IgG (ARCHITECT immunoassays, Abbott Laboratories, Wiesbaden, Germany); anti-*Borrelia* IgM and IgG (EUROIMMUN kits) were assessed in FRs only.

Results. Anti-HEV markers were detected in 3 participants (2.2%; IgM in 1 FR, IgG in 2 BDs), less frequently than anti-HAV (16 out of 96 individuals, about 17%; FRs 19% vs BDs 15%) or anti-*Borrelia* antibodies (18 out of 48 individuals, 37.5%) ($p < 0.0001$ for both). Older study participants (≥45 years of age) were more frequently HAV-seropositive (29% vs 4% of the younger individuals; $p = 0.0012$).

Conclusions. We failed to unequivocally prove HEV exposure in FRs. The HAV seroprevalence in this study paralleled the situation in the general population. Exposure to *Borrelia* spp. in FRs was common.

Key words: hepatitis A virus, hepatitis E virus, *Borrelia*, seroprevalence, Poland

Introduction

Hepatitis E virus (HEV) is an important etiologic agent of enterically transmitted hepatitis worldwide.[1] In developed European countries, this infection was previously considered only in persons returning from highly endemic areas – some parts of Asia and Africa. However, awareness of its presence in industrialized parts of the world has significantly increased in recent years.[2–4]

The virus belongs to the *Hepeviridae* family, *Orthohepevirus* genus.[5] Its virions are small (27–34 nm), non-enveloped and icosahedral particles containing positive-sense single-stranded RNA, approx. 7.2 kb in length. Four HEV genotypes representing 1 serotype have been identified as a cause of human infections, all of which are classified as members of the *Orthohepevirus* A species. Genotypes 1 and 2 (HEV-1 and HEV-2) are present in developing areas of the world (Asia and Africa) and can induce large waterborne outbreaks. Genotype 3 (HEV-3), which has worldwide distribution (including Europe), and genotype 4 (HEV-4), predominant in Asia, cause zoonotic infections – pigs, wild boars and deer represent recognized reservoir animals for these variants of the virus. Recently, a single case of HEV-7-related disease resulting from contact with dromedaries has also been reported.[6] A broad range of clinical presentations may be related to HEV infection, from an asymptomatic course to severe hepatitis.[7,8]

It is recognized that contact with HEV reservoir animals may be related to occupational exposure to this virus.[9–11] A few reports have suggested that forest rangers (FRs) can be one of the populations at risk of HEV infection.[12–16]

The aim of the present study was to assess the seroprevalence of HEV exposure markers among FRs from a single forest district in western Poland in relation to anti-HAV (hepatitis A virus) IgG and anti-*Borrelia* spp. antibodies.

Material and methods

The study involved 48 out of 52 FRs from a single forest division in western Poland (Międzychód forest division) who were screened for anti-*Borrelia* antibodies in the Laboratory of the Department of Infectious Diseases, Jozef Strus Multidisciplinary Municipal Hospital in Poznań in December, 2014, and agreed to participate in this analysis.

Additionally, we recruited 90 unpaid voluntary healthy blood donors (BDs) from the Regional Blood Center in Poznań (west-central Poland) to form the control group.

All the study participants were asked to complete a simple short questionnaire on their demographic, travel and culinary habits, and medical history.

Serologic testing

Anti-*Borrelia* IgM and IgG detection was performed in a 2-step procedure. First, ELISA tests were used (anti-*Borrelia* ELISA [IgM] and anti-*Borrelia* plus VlsE ELISA [IgG]); next, for sera that were positive in this initial screening, confirmation line-blot tests were performed using Anti-*Borrelia* EUROLINE-RN-AT-adv (IgM) or Anti-*Borrelia* EUROLINE-RN-AT (IgG) (EUROIMMUN Medizinische Labordiagnostika AG, Luebeck, Germany). Positive results were defined as the presence of the appropriate antibodies (IgM and/or IgG) detected by both screening and confirmatory testing. Anti-*Borrelia* testing was performed in FRs only (n = 48).

For the HAV seroprevalence assessment (anti-HAV) in 96 individuals (48 FRs and 48 BDs), we used a chemiluminescent microparticle immunoassay, ARCHITECT HAVAb-IgG kits (Abbott Laboratories, Wiesbaden, Germany).

HEV exposure was assessed in all the study participants (n = 138) with IgM and IgG antibody enzyme immunoassay tests (Anti-Hepatitis E Virus ELISA [IgM] and Anti-Hepatitis E Virus ELISA [IgG]; EUROIMMUN Medizinische Labordiagnostika AG, Luebeck, Germany). Additionally, other kits were also used for the detection of anti-HEV IgM in the FRs (MP Diagnostics ASSURE® HEV IgM Rapid Test, MP Biomedicals Asia Pacific Pte. Ltd., Singapore).

All the serologic tests were carried out according to the manufacturers' instructions.

Hepatitis E virus RNA testing

It was planned that the search for HEV RNA would be performed only in anti-HEV IgM-positive participants of the study.

RNA extraction and reverse transcription

Total RNA was extracted with the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) from 140 µL of serum according to the manufacturer's instructions, and then stored at –80°C for downstream applications. For reverse transcription (RT), 8.25 µL samples of the extracted RNA were used. First, the RNA was incubated with 1.75 µM oligo d(T)23, 1.25 µM random primers pd(N)6 and 0.5 mM dNTP mix at 70°C for 5 min, then it was reverse-transcribed into cDNA using Invitrogen Moloney Murine Leukemia Virus (M-MLV) Reverse Transcriptase (Thermo Fisher Scientific, Carlsbad, USA). The final volume of 20 µL RT mix contained 4 µL of 5X First Strand Buffer, 10 U of RNaseOUT™ Recombinant Ribonuclease Inhibitor and 100 U M-MLV Reverse Transcriptase (all ingredients by Thermo Fisher Scientific, Carlsbad, USA). The cycling parameters were: 10 min at 25°C, 60 min at 37°C, 15 min at 75°C, followed by a 4°C hold.

Table 1. Baseline characteristics of the study participants (n = 138)

Parameter	FRs (n = 48)	Control group, BDs (n = 90)	p-value
Age, mean ±SD (range); median [years]	45.0 ±9.6 (29–65); 44.5	44.1 ±6.5 (29–58); 43.5	0.5349
Men, n (%)	34 (70.8%)	66 (73.3%)	0.7542
Consumption of raw/undercooked meat	38 (79.2%)	34 (38.2%)	<0.0001
Consumption of seafood	27 (56.2%)	42 (47.2%)	0.3117
Travel abroad	47 (97.9%)	72 (80.0%)	0.0036

Real-time polymerase chain reaction assay

Detection of HEV RNA was performed in a LightCycler® 480 instrument (Roche Molecular Diagnostics, Pleasanton, USA) using the TaqMan® approach. Primers and probes for real-time polymerase chain reaction (RT-PCR) were synthesized based on the highly conserved region of the different HEV genotypes in the ORF3 region, where the primers and probe anneal. The forward primer (JVHEVF; 5'-GGTGGTTTCTGGGGTGAC-3'), reverse primer (JVHEVR; 5'-AGGGGTTGGTTGGATGAA-3') and probe (JVHEVP; 5'-TGATTCTCAGCCCTTCGC-3') described by Jothikumar et al. were employed for HEV detection by RT-PCR.[17] The TaqMan® probe was labeled with a 6-carboxy fluorescein fluorophore (6-FAM) at the 5' end and a Black Hole Quencher-1 (BHQ-1) at the 3' end (both primers and probes were provided by DNA Sequencing and Oligonucleotides Synthesis Laboratory, Polish Academy of Science, Warsaw).

The 10 µL reaction mixture contained 5 µL of 2 × LightCycler® 480 Probes Master (Roche Molecular Diagnostics, Basel, Switzerland), 500 nM of primers, 200 nM of probe, and finally 1 µL of cDNA added as a template. Cycling conditions were optimized to 1 cycle of initial denaturation for 10 min at 95°C, followed by 55 amplification cycles at 95°C (10 s), 55°C (25 s) and 72°C (10 s). The products from the TaqMan® RT-PCR were analyzed on 2% agarose gels.

Ethical issues

Informed consent was signed by all the FRs and BDs. The study was approved by the Bioethics Committee of Poznan University of Medical Sciences (reference No. 155/15).

Statistical analysis

Numerical data were presented as mean values and standard deviations. The comparison of age was performed by Student's t-test. The assumption of normal distribution of the data was checked using the Shapiro-Wilk test. The homogeneity of variances was verified by Levene's test. Nominal data were presented as numbers and percentages. The comparison was done using the χ^2 test of independence. The statistical analysis was performed with STATISTICA v. 12 software (StatSoft Inc., Tulsa, USA). Results were considered significant at $p < 0.05$.

Results

The baseline characteristics of all the study participants are presented in Table 1.

Anti-HEV antibodies were detected in 3 participants (2.2%). In the FR group there was a positive anti-HEV result in a single individual: a 43-year-old man with anti-HEV IgM antibodies only (which were found with both IgM-detecting tests), but no anti-HEV IgG or HEV-RNA were detected. He had never had an icteric disease and was anti-HAV negative, but tested positive for anti-*Borrelia* IgG antibodies. In the BDs only, anti-HEV IgG was found in 2 men (aged 42 and 55 years) out of 90 persons (2.2%); anti-HAV were not detected in either of them.

The results of the assessment for *Borrelia* antibodies indicated exposure to these bacteria in 37.5% of the FRs (Table 2). In this group, anti-HEV results were positive much less frequently than anti-*Borrelia* ($p < 0.0001$).

Anti-HAV testing was performed in 96 individuals: all the FRs and 48 sex-matched BDs. It was positive in 16 persons (16.7%): 9 of the FRs (18.7%) and 7 of the BDs (14.6%) ($p = 0.5839$). Overall, HAV seroprevalence was significantly higher in comparison to HEV seroprevalence ($p = 0.0001$) and lower than *Borrelia* seroprevalence ($p = 0.0055$).

The anti-HAV positive individuals were older (51.9 ±7.8 years) than the HAV-seronegative study participants (44.0 ±7.9 years; $p = 0.0004$); this was also true when the 2 groups were analyzed separately (among the FRs: 52.6 ±9.1 years and 43.3 ±9.0 years, respectively, $p = 0.0078$; among the BDs: 51.1 ±6.3 years and 44.6 ±6.8 years, respectively, $p = 0.0221$). Susceptibility to HAV infection (as expressed by a lack of anti-HAV IgG) was more frequent among individuals under 45 years of age (46 out of 48 individuals, 95.8%) than in older individuals (34 out of 48 individuals, 70.8%; $p = 0.001$).

Table 2. Exposure to *Borrelia* spp. in serological assessment among FRs (n = 48)

Anti-*Borrelia* antibodies testing method(s)	IgM(+)	IgG(+)
ELISA, n (%)	4* (8.3%)	24 (50.0%)
ELISA + blot, n (%)	4* (8.3%)	18 (37.5%)

* All ELISA IgM-positive patients were also blot-IgM, ELISA-IgG and blot IgG-positive.

In the study, 6 FRs mentioned having had an icteric disease in the past. Viral hepatitis was recognized in 2 of them, while the diagnostic conclusions were not known in the remaining cases; all but 1 were HAV IgG-seropositives. The BDs had no jaundice in their medical histories.

In total, 9 of the study participants (3 FRs and 6 BDs) declared that they had had vaccinations against hepatitis A; anti-HAV antibodies were found in only 4 of them (2 FRs, including 1 with a history of icteric hepatitis, and 2 BDs).

Discussion

In this study, HEV exposure markers (as expressed by anti-HEV positivity) were found in only a few participants (2.2%), less frequently than anti-HAV and anti-*Borrelia* antibodies. Moreover, in spite of the detection of anti-HEV IgM in 1 FR (confirmed by 2 different assays), further tests for anti-HEV IgG and HEV-RNA in this asymptomatic individual proved to be negative. The lack of clinical symptoms characteristic of acute hepatitis does not exclude the possibility of infection, because contact with HEV is usually subclinical.[7]

On the other hand, it is also possible that this FR had a primary infection with Epstein-Barr virus (EBV) or cytomegalovirus (CMV) at the time of the HEV testing. It has been shown that the polyclonal stimulation of B cells caused by these viruses can be a source of false anti-HEV IgM positivity; due to the limited volume of available serum, the further investigation of this possibility was impossible.[18] For this reason, we postulate that the correlation between the presence of IgM antibodies and a recent HEV infection in this case is arguable – it could be a false positive result.

We are going to discuss the results of this study in the context of the particular study population and in view of the available knowledge regarding HEV seroprevalence in Poland.

Data from a few existing reports suggest that FRs constitute an increased-risk group for contact with HEV. Dremsek et al. proved the presence of anti-HEV IgG in an average of 17.8–21.4% (range: 5.6–28%) of FRs from eastern Germany, depending on the diagnostic test used (commercial vs in-house, respectively), compared to 11.1–12.3% ($p < 0.01$) in the control group.[13] Even higher values (in control populations as well) were found by French researchers: Carpentier et al. reported HEV seroprevalence of 31.2% (compared to 19% in the control group) and Chaussade et al. reported 36.4% (compared to 26.1% in the control group).[14,15] Similar data were quoted by Yoon et al. (31.3% in a mixed population of skilled agricultural, forestry, and fishery workers, odds ratio 6.6) in a South Korean analysis.[16] On the other hand, lower seroprevalence was observed in FRs from Iowa, USA (5.7% vs 0% in the control group).[12] It is believed that higher HEV seroprevalence in FRs may be caused by the following factors: contact with reservoir animals, some culinary habits more common in this professional group (eating raw/undercooked meat, including game meat) and common membership in hunting communities.

Significantly, in spite of much more frequent consumption of food containing raw meat (including game meat) by FRs than by the control group, a high percentage of study participants who had travelled abroad (all but 1 person) and considerable exposure to the forest environment (anti-*Borrelia* IgG antibody seropositivity of 37.5%, coinciding with other Polish data on this subject), our study was unable to unequivocally establish the features characteristic of contact with HEV (anti-HEV IgG) in this professional group.[19]

In a recent publication on the HEV exposure of 1027 hunters from all over the country, the presence of anti-HEV IgG was confirmed in 20.3% of cases.[20] According to 2 other reports, HEV seroprevalence among the patients of the Department of Infectious Diseases in Poznań (n = 182), and the Department of Internal Medicine in Łódź, central Poland (n = 212), as well as HCV-positive patients (n = 149) from the Department of Infectious Diseases and Hepatology in Łódź was 15.9%, 7.5% and 10%, respectively.[21,22]

In the context of these data, the low values in the present study are surprising. In our opinion, there are at least 2 possible causes of these findings. Firstly, the available serologic tests have variable and, unfortunately, imperfect accuracy in the detection of anti-HEV markers. Despite the fact that the EUROIMMUN tests used in this analysis were compared with the assays of other manufacturers, knowledge about the diagnostic performance of these tests is very limited.[23,24] Moreover, in our recent investigation of the seroprevalence of anti-HEV IgG among 105 HIV patients and 105 age- and sex-matched BDs, using the EUROIMMUN assay, we reported similar low rates of this HEV exposure marker: 0.95% and 3.8%, respectively.[25] Additionally, it should be stressed that anti-HEV IgG tests in general have suboptimal sensitivity.[26] Secondly, exposure to HEV can differ depending on the geographical region (even within the same country) and related elements, such as environmental factors, climate, socioeconomic status, hygienic and sanitary conditions, culinary habits, and agricultural traditions (especially related to livestock husbandry). For example, in Cornwall (United Kingdom), a coastal clustering of hepatitis E cases was observed.[27] In a large study among French BDs, HEV seroprevalence varied significantly – from 8% to 86% – depending on the area.[28] Similar conclusions were drawn in the previously mentioned study by Sadkowska-Todys et al., according to which HEV seroprevalence differed significantly depending on the region: from 3.85% in the Kuyavia-Pomerania region (mid-northern Poland) to 41.7% in the Opole Province (south-western Poland).[20] Unfortunately, values for other provinces were not given. Additionally, these considerations are complicated by the fact that anti-HEV IgG prevalence in wild boars (recognized reservoir animals) in the aforementioned regions of Poland was inversely proportional to values for hunters from these regions: 29% for the Opole Province and 68%

for the Kuyavia-Pomerania region (unfortunately, data for Greater Poland are not available).[29]

Determining the factors influencing these discrepancies is challenging.

The HAV seroprevalence found in the present small study reflects the epidemiological situation in countries with very low hepatitis A endemicity, which currently include Poland.[30] Moreover, there was no difference in this respect between FRs and the control group.

In view of the lack of medical documentation confirming the vaccination of the study participants against hepatitis A, we believe that at least 5 out of 9 individuals who declared active HAV immunization (HAV seronegative) confused it with hepatitis B vaccinations, even though the question they had to answer was unequivocal and emphasized the differences between vaccinations against hepatitis A and B. This corresponds to what we frequently observe in daily real-life practice.

The common susceptibility to HAV infection found in this study, including among individuals over 45 years of age (70%), suggests that active hepatitis A immunoprophylaxis can also be recommended for these persons, especially when a higher risk of HAV exposure exists. For the FRs participating in the present analysis, the rationale for such an action could be travelling abroad (reported by all but 1 person in this group) and frequent consumption of seafood (56%).

Conclusions

In the present HEV seroprevalence study among FRs from a single forest district in western Poland, we failed to unequivocally prove exposure to the virus in this population. For more in-depth understanding of this issue, further research is necessary among larger populations of FRs in various regions of the country, using diagnostic tests with established reliability. The HAV seroprevalence among the FRs in this study paralleled the situation in the general population and can justify vaccination against hepatitis A in this professional group. Exposure of FRs to *Borrelia* spp. was considerable.

References

1. Rein DB, Stevens GA, Theaker J, Wittenborn JS, Wiersma ST. The global burden of hepatitis E virus genotypes 1 and 2 in 2005. *Hepatology*. 2012;55:988–997.
2. Tsang TH, Denison EK, Williams HV, Venczel LV, Ginsberg MM, Vugia DJ. Acute hepatitis E infection acquired in California. *Clin Infect Dis*. 2000;30:618–619.
3. Inoue J, Ueno Y, Nagasaki F, et al. Sporadic acute hepatitis E occurred constantly during the last decade in northeast Japan. *J Gastroenterol*. 2009;44:329–337.
4. Lapa D, Capobianchi MR, Garbuglia AR. Epidemiology of hepatitis E virus in European countries. *Int J Mol Sci*. 2015;16:25711–25743.
5. Smith DB, Simmonds P, Jameel S, et al.; International Committee on Taxonomy of Viruses Hepeviridae Study Group. Consensus proposals for classification of the family Hepeviridae. *J Gen Virol*. 2014;95:2223–2232.
6. Lee GH, Tan BH, Chi-Yuan Teo E, et al. Chronic infection with camelid hepatitis E virus in a liver transplant recipient who regularly consumes camel meat and milk. *Gastroenterology*. 2016;150:355–357.
7. Said B, Ijaz S, Kafatos G, et al.; Hepatitis E Incident Investigation Team. Hepatitis E outbreak on cruise ship. *Emerg Infect Dis*. 2009;15:1738–1744.
8. Crossan CL, Simpson KJ, Craig DG, et al. Hepatitis E virus in patients with acute severe liver injury. *World J Hepatol*. 2014;6:426–434.
9. Lewis HC, Wichmann O, Duizer E. Transmission routes and risk factors for autochthonous hepatitis E virus infection in Europe: A systematic review. *Epidemiol Infect*. 2010;138:145–166.
10. De Schryver A, De Schrijver K, François G, et al. Hepatitis E virus infection: An emerging occupational risk? *Occup Med* (*Lond*). 2015;65:667–672.
11. Ivanova A, Tefanova V, Reshetnjak I, et al. Hepatitis E virus in domestic pigs, wild boars, pig farm workers, and hunters in Estonia. *Food Environ Virol*. 2015;7:403–412.
12. Karetnyi YV, Gilchrist MJ, Naides SJ. Hepatitis E virus infection prevalence among selected populations in Iowa. *J Clin Virol*. 1999;14:51–55.
13. Dremsek P, Wenzel JJ, Johne R, et al. Seroprevalence study in forestry workers from eastern Germany using novel genotype 3- and rat hepatitis E virus-specific immunoglobulin G ELISAs. *Med Microbiol Immunol*. 2012;201:189–200.
14. Carpentier A, Chaussade H, Rigaud E, et al. High hepatitis E virus seroprevalence in forestry workers and in wild boars in France. *J Clin Microbiol*. 2012;50:2888–2893.
15. Chaussade H, Rigaud E, Allix A, et al. Hepatitis E virus seroprevalence and risk factors for individuals in working contact with animals. *J Clin Virol*. 2013;58:504–508.
16. Yoon Y, Jeong HS, Yun H, et al. Hepatitis E virus (HEV) seroprevalence in the general population of the Republic of Korea in 2007–2009: A nationwide cross-sectional study. *BMC Infect Dis*. 2014;14:517.
17. Jothikumar N, Cromeans TL, Robertson BH, Meng XJ, Hill VR. A broadly reactive one-step real-time RT-PCR assay for rapid and sensitive detection of hepatitis E virus. *J Virol Methods*. 2006;131:65–71.
18. Fogeda M, de Ory F, Avellón A, Echevarría JM. Differential diagnosis of hepatitis E virus, cytomegalovirus and Epstein-Barr virus infection in patients with suspected hepatitis E. *J Clin Virol*. 2009;45:259–261.
19. Richard S, Oppliger A. Zoonotic occupational diseases in forestry workers – Lyme borreliosis, tularemia and leptospirosis. *Ann Agric Environ Med*. 2015;22:43–50.
20. Sadkowska-Todys M, Baumann-Popczyk A, Wnukowska N, Popczyk B, Kucharczyk B, Gołąb E. Occurrence and prevalence of selected zoonotic agents: Echinococcus multilocularis, Trichinella spiralis and hepatitis E virus (HEV) in the population of Polish hunters – Results of the study conducted in 2010–2012. *Przegl Epidemiol*. 2015;69:673–678.
21. Bura M, Michalak M, Chojnicki M, Czajka A, Kowala-Piaskowska A, Mozer-Lisewska I. Seroprevalence of anti-HEV IgG in 182 Polish patients. *Postepy Hig Med Dosw*. 2015;69:320–326.
22. Ślusarczyk J, Szemraj J, Jabłkowska-Górecka K, Juszczyk G, Białkowska J. Koinfekcja wirusem zapalenia wątroby typu E (HEV) u pacjentów zakażonych wirusem zapalenia wątroby typu C (HCV). *Przegl Epidemiol*. 2015;69(Suppl 1):13.
23. Dreier J, Juhl D. Autochthonous hepatitis E virus infections: A new transfusion-associated risk? *Transfus Med Hemother*. 2014;41:29–39.
24. Avellon A, Morago L, Garcia-Galera Del Carmen M, Munoz M, Echevarría JM. Comparative sensitivity of commercial tests for hepatitis E genotype 3 virus antibody detection. *J Med Virol*. 2015;87:1934–1939.
25. Bura M, Bukowska A, Bura A, Michalak M, Mozer-Lisewska I. Hepatitis E virus antibodies in HIV-infected patients and blood donors from western Poland: A preliminary report. *Adv Clin Exp Med*. 2017;26:577–579.
26. Khudyakov Y, Kamili S. Serological diagnostics of hepatitis E virus infection. *Virus Res*. 2011;161:84–92.
27. Hunter JG, Madden RG, Stone AM, et al. Coastal clustering of HEV; Cornwall, UK. *Eur J Gastroenterol Hepatol*. 2016;28:323–327.
28. Mansuy JM, Gallian P, Dimeglio C, et al. A nationwide survey of hepatitis E viral infection in French blood donors. *Hepatology*. 2016;63:1145–1154.
29. Larska M, Krzysiak MK, Jabłoński A, Kęsik J, Bednarski M, Rola J. Hepatitis E virus antibody prevalence in wildlife in Poland. *Zoonoses Public Health*. 2014;62:105–110.
30. Bura M, Mozer-Lisewska I. Quo vadis, HAV? *Hepatologia*. 2016;16:17–24.