Review paper (After Acceptance of EiC)

Hepatits E virus (HEV) as a new challenge in Poland based on narrative review of epidemiological and clinical data

Keywords
diagnostics, Poland, treatment, epidemiology, clinical symptoms, HEV, hepatitis E virus

Abstract
Manuscripts on hepatitis E virus (HEV) epidemiology in Poland listed in Pubmed were analyzed in the context of current clinical and biological knowladge and epidemiological data from other regions of Europe and the world.
Analyzed data indicates a high frequency of IgG HEV in Poland compared to other European countries and parts of the world, on average it exceeds 40%. In some regions of the country, even near 60% of the population has been passed infection. It has been estimated based on the frequency of acute phase markers, that at least 113,000 residents may be infected each year.
Presented results encourage to improve availability of HEV diagnostics, to increase of physicians' awareness and taking into account HEV infection as an etiological factor of numerous diseases. Preventive measures, especially in risk groups and research on the clinical significance of HEV and on effective therapeutic management should be considered.

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Short title: hepatitis E virus in Poland

Słowa kluczowe: wirus zapalenia wątroby typu E, HEV, epidemiologia, Polska, diagnostyka, leczenie

Key words: hepatitis E virus, HEV, epidemiology, Poland, diagnostics, treatment

Introduction

Hepatitis E virus (HEV) was discovered in 1983 by Soviet virologist Mikhail Balayan~~, who was sent to Afghanistan to explain the etiology of the disease spreading among Soviet soldiers. In patients, the symptoms were mainly related to the gastrointestinal tract and the symptoms resembled viral hepatitis A (HAV), but markers of HAV infection were not detected in the patients. The Soviet scientist demonstrated the role of the infectious agent in the etiology of the newly observed disease by consuming a suspension of feces from a sick soldier, causing symptoms of the disease in himself, and then he was the first to observe virions of a previously unknown virus in an electron microscope~~ [1, 2]. Initially, it seemed that the virus occurred locally, in regions with poorly developed sanitary infrastructure - in Central Asia, Central America and Africa, but at the end of the last century, a change in the perspective on the epidemiology of HEV was brought by the identification of a new genotype occurring in developed countries around the world [3].

HEV is currently considered one of the most common causes of acute hepatitis in humans worldwide [4]. The epidemiological and clinical significance of HEV infections in Europe is growing - in just 5 years, a 10-fold increase in the number of infections reported to epidemiological surveillance has been observed, which reflects two aspects - increased access to diagnostics, but also a higher incidence of infections, which was confirmed in countries with greater availability of HEV tests [5].

In current paper we review literature on HEV epidemiology in Poland, in both the environment and human population, including patients and blood donors. We analyzed manuscripts listed in Pubmed matching phrases HEV (hepatis E virus) and Poland. Data for Poland were compared to other regions of the world and discussed in the context current knowladge on clinical significance, transmission routes and biology of the virus crucial for understangin effctivenes of infection prevention and natural course of infection, and in view on optimal diagnostic and therapeutic procedures.

Taxonomic classification and biology

HEV was initially classified into the *Hepeviridae* family, genus *Orthohepevirus*, within which four species *Orthohepevirus A-D* were distinguished. According to the latest taxonomic division, viruses infecting humans have been classified into the genus *Paslahepevirus* [6].

Within the species *Orthohepevirus A* seven genotypes (1-7) were distinguished [7]. It is known, that genotypes 1-4 infect humans, however recent observations indicate, that other genotypes within *Orthohepevirus A* and species of *Orthohepeviruses* also have a significant zoonotic potential - passinging the interspecies barrier and causing hepatitc manifestations - there are documented symptomatic human cases of infection with genotype 7, previously detected exclusevely in camels [8], in Africa, and in Spain and in Hong Kong infections with *Orthohepevirus C*, previously identified only in rats [9,10]. Genotypes 1 and 2 are human-specific pathogens, responsible for the spread of large epidemics in developing countries - mainly in Asia, Africa and Central America. The spread of infections is facilitated by poor sanitary conditions, contaminated water and, to a lesser extent, contaminated food. Infections with genotypes 3 and 4 are recorded mainly in developed countries in North and South America and in Europe and are most often a consequence of meat consumption from infected animals - pork and game and in some proportion of infected persons result in hepatic or extrahepatic symptoms [11].

The biological properties of HEV, similar to HAV, determine the course of infection and also explain resistance to external factors and infectivity through blood and transfusion and often wide spread of infections in humans, in some part of the world. HEV is a relatively small (27 - 34 nm in diameter), spherical virus, whose genetic material is in the form of short RNA (approx. 6.4 - 7.2 kb). The virus genome includes three partially overlapping open reading frames - ORF1 encodes non-structural genes: helicase, protease and RNA - dependent RNA polymerase. ORF2 encodes the capsid protein, and ORF 3 encodes a small phosphoprotein, that binds to the cytoskeleton [3, 10].

It has been shown, that the virus isolated from plasma, in contrast to virions from feces, is not neutralized by immune serum containing antibodies against ORF2 protein, and has higher density and diameter. These differences are related to the presence of enveloped virions in the blood, while in feces - non-enveloped ones. Some authors describe the structure of HEV virions as quasi-enveloped and suggest different routes of entry of the mentioned morphological forms of the virus into cell [12, 13].

It is believed, that the structure of virions determines the high resistance of HEV to (extreme) factors/physicochemical conditions. Disinfectants containing high concentrations of isopropanol or ethanol have been shown to be ineffective. Only the addition of phosphoric acid to alcohol improves the effectiveness of this type of disinfection [14]. The relationship between temperature and virus infectivity seems to be more limited than in the case of other viruses, especially enveloped ones - infectivity can be maintained at refrigerator temperature (4°C) for many weeks (at least 56 days); at room temperature (approx. 22°C) even for 4 weeks; only exposure to a temperature of at least 80°C almost immediately destroys the replicative potential of the virus, preventing its transmission [15].

Recent work by the research team from Catalonia indicates an even more complex biology of HEV. It has been shown, that in blood of infected donors, in addition to the enveloped form of virions, the non-enveloped form also occurs in a minority, which indicates, that the presence of this morphological form of the virus is not limited to the bile ducts and feces, as previously thought. Importantly, the share of enveloped virions fluctuates during the course of infection, increasing in seropositive individuals and those with elevated liver enzyme activity [16]. This observation seems to explain the high infectivity of HEV by transfusion, even in inactivated components.

Transmission routes

It was well documented, that HEV infection is transmitted to humans via the fecal-oral route, through the consumption of contaminated food, as well as through blood transfusion and transplantation. As far vertical transmission was reported for

genotype 1, It is not certain for genotype 3, as there is a shortage of data clearly confirming or excluding this rout of transmission for polymorphic forms authostonous in developed coutries. Sexual transmission is suspected based of population study of serological markers in MSM group, but role of this route of transmission in infection spread still require further studies [17, 18] (Figure 1). HEV transmission through drinking water contaminated with feces of infected people mainly concerns genotypes 1 and 2 of HEV and is dominant in developing countries [11]. Consumption of fruits (e.g. strawberries), vegetables watered with contaminated water, as well as seafood (e.g. oysters, mussels) is also a risk factor for HEV infection [19, 20]. HEV transmission through the consumption of infected meat concerns genotypes from animals (3 and 4), the reservoir of which are mainly domestic pigs and wild boars, as well as deer, rabbits, hares, cows and goats [11, 19, 21]. Consumption of raw or undercooked meat, such as pork or game, is particularly risky. Contact with sick animals may also be a source of HEV infection. Cases of HEV infection have been described in France as a result of frequent contact with domestic pigs, and higher seroprevalence of HEV has also been observed in Swedish, Dutch and French veterinarians or hunters in Germany and France [20]. Infection with genotype 7 HEV has also been reported in the Middle East as a result of consumption of camel meat and milk [7]. HEV transmission may occur vertically from mother to child, with the risk of death due to the development of severe liver failure being particularly high, even 20%, for genotypes 1 and 2, and not elevated for genotypes 3 and 4 [19, 22, 23].

Transmission of HEV infection by transfusion of blood and its components has been repeatedly documented [21, 24]. Based on the analysis of transfusion cases of blood and its components infected with HEV, the average risk of transmission was determined at about 42% (the highest for plasma, lower for platelet concentrates and the lowest for red blood cell concentrates) [25], and the minimum infectious dose (MID) was estimated at 3.85 log IU, although it is emphasized that transmission is more likely to occur, when the dose exceeds 4 log IU [26].

HEV transmission through transplantation has also been described, including liver [27] and kidney [28]. Transmission of infection through sexual contact seems likely, especially between men (MSM), as indicated by the higher frequency of anti-HEV IgG in the MSM population in Italy compared to the control group [29], but this route of HEV spread has not been finally confirmed so far [19].

HEV infection course and diagnosis

The infection course, taking into account appearance of infection markers, was schematically presented on Figure 2. The incubation period of HEV infection is usually 2-6 weeks. After about three weeks from infection, HEV RNA is detected in blood and feces, in which RNAemia persists for about 3-6 and 4-6 weeks, respectively. HEV RNA can be detected shortly before the onset of symptoms. The virus level doubles on average within 2.4 days, and the half-life is 1.6 days. Usually about 3 weeks after the onset of clinical symptoms, HEV RNA becomes undetectable in the blood, although it continues to be excreted in the feces for another 1-2 weeks. In studies of blood donors, HEV RNA was detected for up to 6 months [30, 31, 32].

In immunocompetent person infection usually follows similar course. Clinical symptoms may be accompanied by an increase in the activity of biochemical markers, and then antibodies - IgM and IgG - also start to appear at the same time [23]. The level of anti-HEV IgM starts to increase on average around day 33 and reaches its maximum concentration on day 36 from the detection of RNA. In most immunocompetent patients, this isotype of antibodies was no longer detectable after 6 months. The concentration of anti-HEV IgG starts to increase on average around day 32 and reaches its maximum concentration around day 53 from the appearance of RNA in blood. This isotype of antibodies was still detectable in most patients after 1 year [31, 33].

Another marker of infection, the diagnostic use of which is discussed in the literature, is the hepatitis E virus antigen (HEV-Ag) - a viral capsid protein detectable in blood during the serological window period/during the acute phase of infection, persisting for 3 to 4 weeks after the symptoms of infection have subsided [34]. Recently, it has been discovered, that HEV Ag is specifically taken up from blood by

renal cells and eliminated in urine, which is why Ag concentration is >10 times higher in urine than in blood and results in higher diagnostic sensitivity. Detectable Ag in urine was observed 6 days earlier than in serum and persisted longer than RNAemia and antigenemia in blood. In studies on rabbit models, detectability of Ag in blood showed good agreement with detectability of RNA in feces [35, 36].

In immunocompromised patients, who developed chronic infection, HEV RNA persists in blood, feces and body fluids for more than 3 months. It is believed that in this group there is a low or undetectable level of anti-HEV antibodies [37].

The phenomenon of HEV reinfection is not well understood. Based on previous observations, it seems to occur, although its scale remains unknown. Cases of HEV reinfection have been described in solid organ transplant recipients. Importantly, in this group, HEV reinfection may lead to chronic infection [20, 38, 39]. The results of an 8-year follow-up of blood donors in the German population showed that, among 495 HEV RNA(+) donors, as many as 78.4% did not have IgM or IgG antibodies against HEV, while 8.5% had only IgG antibodies. Interestingly, only 26.6% of HEV RNA(+) donors showed an increase in ALT activity. These data may indicate ongoing recurrent reinfections in the studied population [40].

Available diagnostic tests and their clinical application for differentiation of phases of infection and limitations, especially in patients with immunodeficiency were presented in Table I. Diagnosis of HEV infection is based on the examination of a molecular (HEV RNA) and serological markers (IgM and IgG antibodies and HEV antigen). The detected markers allow to distinguish between acute, chronic and past infection phase, however their usefulness in immunocompetent patients and with immunodeficiency differs (Table I) [30, 41, 42].

The gold standard for diagnosis of acute and chronic infection in both immunocompetent and immunodeficient individuals is the HEV RNA test. HEV RNA in the acute phase can be detected in both blood and feces from the 3rd to 6th week of infection. HEV RNA present for more than 3 months indicates chronic infection [43, 44]. The HEV RNA test also allows us to monitor the reduction of infection and the effectiveness of antiviral treatment, as well as reinfections [45, 46]. It should be noted

that molecular tests (both qualitative and quantitative) may differ in terms of analytical and clinical sensitivity declared by manufacturer. Analytical sensitivity of tests (the lowest RNAaemia detected by the test) can be reliably determined and compared by analyzing the results of dilution tests of international WHO standards and secondary standards [47, 48, 49], and clinical sensitivity (the efficiency of detecting various polymorphic forms) can be compared by analyzing the results of reference panel tests (IRP) [50]. The most sensitive molecular tests detect even several IU of HEV RNA/ml [20].

Anti-HEV antibody tests remain an important element of HEV diagnostics in immunocompetent individuals. Anti-HEV IgM and IgG antibodies can be detected in up to 98% of this group of patients in the acute phase, provided that tests with high sensitivity and specificity are used [51].

High sensitivity of some tests, e.g. Wantai, results from the use of the μ chain coating strategy, which is used by few manufacturers of anti-HEV tests [52, 53, 54]. The results of some studies indicate that the sensitivity of the so-called rapid anti-HEV IgM tests, e.g. All Diag, can be comparable to ELISA tests [51, 55]. Otherwise, in people with acute hepatitis E and reduced immunity, anti-HEV IgM and IgG antibodies were not detected in even 20% and 85% of infected people, respectively - therefore, in this group of patients, antibody testing should not be the basis for HEV diagnosis [56, 57]. Moreover, when analyzing the result of IgM isotype tests, it should be taken into account that cases of their persistence for more than a year were observed, and in people infected with e.g. EBV, CMV, HCV, there is a risk of a falsely reactive anti-HEV result - therefore, diagnosis and differentiation of the phase of HEV infection, even in immunocompetent people, should not be based solely on the anti-HEV IgM test [58, 59, 60]. The presence of only anti-HEV IgG antibodies indicates a past infection or vaccination. In people with past infection, the sensitivity of anti-IgG tests is estimated at 57.5-75% [51].

HEV ORF2 antigen is detected in blood and urine in both acute and chronic phases of infection, which may be helpful in diagnostics. HEV-Ag tests performed in serum allow for detection of infection with RNAemia at the level of 10^2 - 10^5

copies/ml, and are characterized by high sensitivity (80-94%) and specificity (86-100%) [61, 62, 63]. The results of the studies utilizing new HEV-Ag test in urine indicate, that its effectiveness in diagnosing HEV may be high compared to HEV RNA and anti-IgM tests, because the HEV-Ag level in urine is over 10 times higher than in blood [36]. However, it should be noted, that in immunocompetent individuals a positive HEV-Ag result does not always correlate with presence of HEV RNA. For example, HEV-Ag was detected in patients treated with ribavirin even several dozen months after the elimination of HEV RNA [30]. Studies on HEV cell cultures suggest that the reason for the lack of correlation between the results of HEV antigen and RNA is the detection of glycosylated forms of ORF2 secreted in infected patients at high concentrations by HEV-Ag test, in addition to the less abundant non-glycosylated form of ORF2 present in infectious virions [64]. The use of HEV antigen testing in diagnostics is still not well established, as it has been noted, that this marker may be undetectable during the period of antibody development, and moreover, little is known about its detection in immunocompromised individuals [65].

Epidemiological situation in Poland

Presence of the virus in the ecosystem

Since HEV infections are mainly spread by the oral route, it is particularly important to know the data on the detection of the virus in food. Our knowledge in this area is limited and largely indirect. Studies conducted on liver samples (n=100) and blood (n=146) of pigs obtained from slaughterhouses and retail outlets in central and eastern Poland showed the presence of HEV RNA in 1 and 5 samples, respectively [66]. In addition, viral RNA was detected in the blood of wild boars (25.8%) [67]. The presence of HEV genetic material on the surface of fruits and vegetables was also demonstrated – it was identified on 1 to 2% of leafy vegetables and strawberries, respectively [68, 69].

Detection of HEV infection markers in humans

HEV RNA and antigen

The results of tests of markers of ongoing (acute) infection (viral RNA and antigen) in humans and animals in Poland are presented in Table II [66, 67, 70, 72, 73, 74]. The data on incidence based on HEV RNA testing to date come only from blood donor testing. The nationwide analysis conducted in 2015 covered nearly 13 thousand donations from first-time donors, which were tested individually. The genetic material of the virus (confirmed reactive result of the screening test) was found in 6 donors (1/2,109 donors/donations) [70]. From mid-2018 to the end of 2019, the Regional Blood Transfusion Centre (RBTC) in Poznań tested donors, whose plasma was intended for clinical use in England, where HEV RNA is a mandatory qualification test in blood donors. The tests were conducted in mini-pools, initially from 16 donations using the transcription mediated amplification (TMA) method, and then from 24 donations using the real-time PCR method. During screening of almost 40 thousand donations, a total of 10 infected donors were identified (1/approx. 3,900 donations) [data from the Institute of Hematology and Transfusion Medicine in Warsaw (IHTM) and RBTC Poznań, A. Bukowska, PhD]. In Poland according to the current recommendations [71], HEV RNA testing is not obligatory, but is recommended as it increases the safety of transfusions. In April 2024, the RBTC in Warsaw started testing for HEV RNA in IDT - 35 infections were detected in 51,782 donations, (confirmed repeatably reactive (RR) results; frequency 1/1,479 donations) [data from IHTM and RBTC Warsaw, J.Gdowska, MsC].

In several other studies conducted locally, the presence of the virus was also analyzed using direct methods (RNA or antigen detection). Infection markers (Ag by ELISA without verification) were detected in single patients from Podlasie with liver cirrhosis (1.7%) and after transplantation (2.8%) [72], but they were not present in hemodialysis patients from Wielkopolska [73] and in hematological patients, including after allo-HSCT [74].

The number of studies, in which the polymorphic form of the virus occurring in Poland was determined is still limited. As expected, HEV donors were carriers of genotype 3 infections – subtypes 3i and 3c [70]. Subtype 3i was identified in wild boars, while subtypes 3f and 3e were identified in the liver and blood of pigs [66, 75].

Moreover, subtypes 3i and 3c and mixed infections with different/two subtypes were detected in pig feces [76].

IgM anti-HEV isotype

Knowledge about the incidence can be supplemented by the results of tests for anti-HEV IgM antibodies appearing in the early phase of infection and persisting for the first few months – data for populations of blood donors (A), other symptomless person (B) and patients (C) were summarized in Table III [70, 72, 73, 74, 77, 78, 79, 80, 81, 82]. In the only nationwide study conducted so far, the serological marker of acute infection was detected in 0.8% of donations collected in 2015 [70].

In other studies, on healthy individuals from different regions of Poland, a comparable percentage of positive results was obtained - in 0.3% to 0.5% of hunters (samples collected slightly earlier (2010-12) [77, 78] than in the case of the above-mentioned donors) [70] and in 0.8% of soldiers (2016) [79].

More varied results were observed in patients. Anti-HEV IgM ranged from 0% in hemodialysis [73] and hematological patients [74], through about 1% in immunocompromised patients (with HIV and after transplantation) [72] and with autoimmune hepatitis [80], up to 5% in patients with liver cirrhosis [72]. Differences in results between blood donors and patients should be interpreted with caution, because in the second group, unlike donors, the specificity of reactive results in ELISA was not verified in WB. Usually, the percentage of confirmed results is lower as compared to that not verified. Moreover, a large proportion of patients had, to a greater or lesser extent, impaired immunity and the ability to produce antibodies in response to infection. For that reason, we cannot exclude, that a larger proportion than indicated by the test results could have been infected in the months preceding the sample collection.

The frequency of anti-HEV IgM (15.6%) identified in the group of Hindus studying in Białystok clearly differs from the range of the results quoted. During this first population study of specific anti-HEV antibodies in Poland, an appropriate confirmatory test was not yet available. Additionally, the authors note that

seropositivity was most likely a consequence of infections, that occurred during stay in Asia [81].

IgG anti-HEV isotype

When analyzing the results of anti-HEV IgG antibodies studies, several associations from previous, including Polish observations, should be taken into account. The frequency of antibodies increases with age and, as shown by the results of some studies, is higher in men [70]. Therefore, the demographic structure of the studied group may have a significant impact on the obtained results. Moreover, as shown in the section on diagnostics, the percentage of seropositive results depends on the test used. It was noted, that some tests may be characterized by higher sensitivity and therefore give a higher percentage of reactive results in population studies than tests with lower sensitivity.

The summary of screening results of anti-HEV IgG antibodies, indicating a past infection in Poland in animal and human populations (both healthy and patients) are presented in Table IV [67, 70, 72, 73, 74, 77, 78, 79, 80, 82, 83, 84, 85, 86, 87, 88, 89]. The results of tests in humans are discussed in the following section. Especially, we focused on the results obtained using the the most specific and sensitive Wantai test, which has been used in numerous studies worldwide for donors' population assessment.

So far, the results of two nationwide studies on the detection of the anti-HEV IgG isotype in animals have been published. They indicate the commonness of infections in wild boars and pigs/swine. Anti-HEV IgG was detected using tests from different manufacturers in 31% to 44.4% of wild boars and in 44.1% of pigs, however was not detected in other wild animals (deer, roe deer, bison, etc.) [83].

Population studies of IgG anti-HEV in humans

The only nationwide analysis of humans showed anti-HEV IgG seropositivity in an average of 43.5% of donors – from 30% in Podlasie to 60% in Greater Poland [70]. Other studies performed locally in donors in Greater Poland confirmed a high prevalence of antibodies to HEV (from 49.6% to 60.9%) [85-88].

A high percentage of antibodies indicating a past infection was also identified among patients treated in Poznań. Specific IgG were detected in 37.7-50.8% of HIV patients [72, 87], 40.6% of transplant patients [72], 48.3% of patients with liver cirrhosis [72] and in 49.7% of hemodialysis patients [73]. In Mazovia, despite treatment and the underlying disease significantly reducing immunity, anti-HEV IgG was detected in 44.6% of samples collected from hematological patients in 2021-2023 [74].

Other nationwide studies were conducted using tests from other manufacturers (Mikrogen, Euroimmun) than in the case of the above-mentioned studies of donors and patients (Wantai). These analyses showed HEV IgG seropositivity of 22.2-25% of hunters [77, 78] and 6.3% of soldiers [79].

Epidemiological situation in Poland in comparison with data from other regions of Europe and the world

The frequency of anti-HEV IgG (Wantai test) in patients and in blood donors in Poland in comparison with other countries and regions of the world is presented in Figure 3. Taking into account the highest results obtained so far, obtained for donors with the test considered to be among the most sensitive (Wantai), it should be assumed, that Poland is the country with the highest seroprevalence in Europe. Over 40% of donors nationwide have been passed infection, which is the highest percentage among European countries and one of the highest in the world [90, 91, 92, 93]. Polish results are comparable only to Nepal [94], where testing was performed shortly after the catastrophic earthquakes, that with a high degree of probability significantly influenced epidemiological situation. Moreover, it can be assumed, that the dominant genotype transmited in Nepal was genotype 1, while in Poland only genotype 3 infections have been documented so far. Also, the frequencies of anti-HEV IgG antibodies observed locally, in the Greater Poland province (approx. 60%) are among the highest. The seroprevalence in this area exceed the extreme frequencies observed in southern France (39.1%) [95], in two provinces in Italy (40-44%) [96] and are comparable only with Corsica (56.1%) [97] and northern India (60.5%, expected genotype 1) [98] (Figure 3).

The epidemiological data presented in current paper allow for a preliminary estimate of HEV incidence in Poland. For his purpose seroprevalence of specific IgM is helpful, as it is marker of acute infection maintaining in human blood at detectable level for app. 6 months. Extrapolating results of nationwide studies of donors (0,8%) on the whole population of Poland (38 mln.), one of the pioneers of research on genotype 3 HEV in Europe, Dr. John Dalton, estimated the number of new/acute HEV infections per year at approximately 400 thousand [99]. To make more cautious estimates you can also use the lower percentage of IgM positive results obtained in other locally conducted studies (0.3-0.5% - see Table III) and assume, that this isotype of specific antibodies persists on average not for 6, but even for 12 months. Under such assumptions the number of predicted infections would range from about 113 to 189 thousand cases per year (1 year x 37.72 million inhabitants [100] x 0.3-0.5% [percentage of IgM positive infections per year] = 113,160-188,600). Therefore, taking into account more and less cautious estimates, we can expect from about 113 to 400 thousand infections per year in Poland, respectively.

It is worth noting that in Poland, unlike in most European countries [101], HEV is not subject to mandatory epidemiological surveillance. Therefore, in our country we do not have reliable data on the epidemiology of infections, especially symptomatic ones. It is known that acute infections identified in blood donations and at least some infections diagnosed in infectious disease clinics (RNA-positive) are reported to epidemiological surveillance and then presented in the category of "other and unspecified hepatitis" (item 107) in the NIZP-PZH report published every two weeks [102]. Even if we assume, that all cases reported in this way concern HEV (and in 2023 there were 59 reports classified as such) and cautiously estimate, that no more than 1% of infections have a significant clinical course requiring treatment (1% out of 113,160-400,000 estimated infections per year in the whole country = 1,130-4,000 symptomatic cases), it appears, that the number of diagnosed infections is significantly underestimated (from 19 to 68 times).

Currently, no more than several dozen cases of hepatitis E or other symptomatic HEV infections are diagnosed in Poland each year, while in Western

European countries with significantly lower seroprevalence, many times more symptomatic cases are reported. Over the period 2005–2015 a total of 21.018 confirmed hepatitis E cases were reported from 22 countries. The largest numbers of confirmed cases, accounting for 80% of all cases reported, were from 3 countries - Germany, France, and the United Kingdom [5].

Clinical significance

HEV genotype 3 can lead to asymptomatic infections in majority of patients. Less than 5% may develop acute self-limiting hepatitis, sometimes with a jaundice. After an incubation period of 3-8 weeks (on average about 4 weeks), prodromal general symptoms may occur. These include subfebrile state or fever, increasing fatigue, weakness, loss of appetite, nausea, vomiting, muscle and joint pain, which are accompanied by the pain in the right hypochondrium. In some patients, jaundice with pruritus, hepatomegaly, dark urine, discolored stools may appear. Laboratory tests performed during this period show constant increase in transaminase activity. The peak of HEV viremia usually precedes the peak of transaminase activity by about 6 weeks. The course of the disease is significantly influenced by the status of the body's immune response and pre-existing liver diseases. In immunocompetent patients without concomitant liver diseases, acute HEV infection is self-limiting. Symptoms usually subside after 1-2 weeks along with transaminase activity, that usually normalizes after several weeks. Single cases of HEV viremia lasting more than 2 years have been observed in immunocompetent patients, but this was not associated with the progression of liver fibrosis. Symptoms, that may accompany HEV infection were grouped in Table V into the cathegory of different clinical manifestations: hepatic, hematologic, neurological, nephrological and other [23, 103].

A significant clinical problem is the overlap of acute HEV infection with underlying liver disease, especially in the phase of advanced fibrosis or hepatic cirrhosis. This often leads to decompensation of liver function, that sometimes may progress to acute, life-threatening organ failure [22, 23].

In acute infection with HEV genotype 1 during pregnancy a particularly severe hepatitis progressing to fulminant liver failure and death in up to 20-25% of infected women, was noted [41].

Immunosuppression of various aetiology, particularily after solid organ transplantation, have a significant impact on the immunological control of HEV replication. In 50-60% of immunocompromised patients, infection with HEV genotype 3 or 4 becomes chronic, which is defined as prolonged detectability of HEV RNA in blood for over 6 months. However, later observational studies have shown, that in some solid organ recipients, spontaneous elimination of HEV RNA occurred up to 3 months after infection. The chance of spontaneous elimination of HEV RNA in the period from 3 to 6 months after infection was very low [104]. Hence, the current recommendations of experts from the European Association for the Study of the Liver (EASL) indicate that patients, in whom HEV replication lasts >12 weeks should be considered as chronically infected with HEV [23]. Symptoms of chronic HEV infection are non-specific. Most frequently (in 1/3 of patients), progressive, prolonged fatigue was observed. In majority of patients, a chronic moderate increase in transaminase activity was observed. In some, transaminase activity remained within the norm, serological markers of HEV infection were undetectable, and the only test confirming the infection was the detection of HEV RNA in blood [105]. It is estimated that approximately 10% of patients with chronic hepatitis E will develop liver cirrhosis within 1-2 years, which may result in liver failure and death of the patient or the need for liver transplantation [23].

Hepatological symptoms of acute or chronic hepatitis E may be accompanied by extrahepatic manifestations, which reflect the extrahepatic HEV replication and immunological damage to tissues and organs. Neurological symptoms like bilateral amyotrophic neuralgia (pain, paresthesias and muscle weakness in the shoulder girdle and arm) or Guillain-Barré syndrome more frequently affect immunocompetent individuals infected with genotype 3 HEV. However, these complications have also been observed in immunosuppressed patients with chronic hepatitis E. In patients with immunodeficiencies, extrahepatic nephrological complications

(glomerulonephritis, IgA nephropathy) and hematological complications (monoclonal gammopathy in 25% of individuals with acute HEV infection, thrombocytopenia, cryoglobulinemia, aplastic or hemolytic anemia) are more common. Additionally, cases of pancreatitis, arthritis, myocarditis, or thyroiditis have been reported for genotype 1 HEV infections [21, 23] (Table V).

It is estimated, that 3-13% of patients with suspicion of drug-induced liver injury (DILI) are infected with HEV. Therefore, testing for HEV is higly recommended in this group [103]. In our own material (unpublished data), 25% of patients diagnosed with viral hepatitis E were admitted to hospital with an initial diagnosis of acute drug-induced liver injury.

The disease caused by HEV is self-limiting and treatment is primarily symptomatic, based on the maintaining water and electrolyte balance, vitamin supplementation, UDCA (ursodeoxycholic acid) in patients with the cholestatic hepatitis and pruritus, avoiding paracetamol and other potentially hepatotoxic drugs. Specific therapeutic interventions, such as ribavirin, may be considered only in patients with acute liver failure or chronic hepatitis E in immunocompromised population. Single reports indicate, that in severe acute hepatitis E in patients with underlying liver diseases, ribavirin usage shortened the period of HEV viremia and accelerated the convalescence. Patients with hepatitis E and fulminant liver failure may require liver transplantation [22, 23]. Clinical reports indicate the efficacy of pegylated interferon α in inhibiting HEV replication, however, due to its immunomodulatory effect, most patients with chronic, complicated hepatitis E have contraindications to this therapy. Single reports indicate a potentially beneficial effect of inhibiting HEV replication by sofosbuvir, however emerging resistant variants are of specific concern [22, 23]. Several compounds, that inhibit HEV replication by targeting host or directly virus are currently in the pipeline.

Infection prevention

Infection prevention is particularly important in the case of groups at risk of more significant complications in the course of HEV infection: in patients with liver disease (including cirrhosis, hepatitis B and C, etc.) and patients with reduced

immunity due to the underlying disease (cancer), immunosuppressive treatment (especially in transplant patients) and HIV infection. The significance of HEV infection in pregnant women is discussed - it is known that genotype 1 infections are particularly dangerous, but it is not fully known, what significance genotype 3 identified in developed countries, including Poland, has in this group [22, 41].

The primary prevention of diseases transmitted through the fecal-oral route involves improving sanitary conditions, access to clean drinking water, proper sewage disposal, and educational measures that inhibit virus transmission within the population, such as the habit of washing hands with clean water before meals.

Prevention of zoonotic infections (HEV genotype 3 and 4) includes maintaining good hygiene, washing hands after contact with animals or their feces, and proper cooking of meat. Particular caution is recommended, when it comes to consumption of pork and wild game, that has not been fried or baked. For these meat products, careful and possibly prolonged heat treatment is recommended [22, 41].

Another significant source of infection may be transfusions of blood, its components and blood products. Thus, blood services in many countries, mainly European ones, has introduced HEV RNA testing for donations intended either for all recipients or only those belonging to risk groups. In Poland, there is no such requirement so far, however, some RBTCs (e.g. in Warsaw) have introduced multiplex tests for donor screening, which, in addition to detecting HBV DNA, HCV RNA and HIV RNA, identify HEV RNA. An additional action reducing the risk of HEV transmission through transfusion may be the use of inactivation methods. A significant limitation is the lack of such method for whole blood and red blood cell concentrates and limited effectiveness ensuring a smaller reduction in infectivity/replication capacity (2-3 log), than in the case of enveloped viruses such as HBV, HCV or HIV [21, 23, 25, 92, 103].

Intensive work is underway on an effective vaccine against hepatitis E. So far, 4 recombinant vaccines have been developed, all of which use ORF-2 antigens administered in 3 doses over 6 months for immunization. Three vaccines were constructed using the genotype 1 antigen, and one using genotype 4. The most

advanced work is on the Chinese Hecoline® vaccine using the genotype 4 antigen. The results of four phases of clinical trials indicate high effectiveness against viruses belonging to genotype 4, it has a weaker effect against genotypes 1 and 2, and the effectiveness of the developed vaccines against genotype 3 remains unknown. Vaccination of risk groups and people in areas of HEV epidemics is being considered. An important observation is that vaccinations do not lead to the development of long-lasting immunity, but prevent progression of HEV infection to symptomatic hepatitis. Furthermore, it has been shown to be safe for pregnant women. [106].

Duscussion

The presented data indicate a high incidence of HEV among people in Poland, with significant underestimation of epidemiological surveillance data. Therefore, it is necessary to expand knowledge of the epidemiology and clinical significance among medical personnel, increase the availability of diagnostics, and implement mandatory separate reporting of hepatitis E. In terms of prevention, primary efforts should be directed toward protecting high-risk groups of patients at high risk of clinical complications from HEV infection – transfusion and transplant recipients (by implementing HEV RNA testing in donors of blood and blood components, as well as organs and tissues). To explain the phenomenon of extremely high seroprevalence in Poland and develop strategies to limit the infection epidemic, research into the sources of HEV infection in Poland – food, water, etc. – is desirable. More accurate forecasting of the infection epidemic in Poland and other countries will be possible after collecting more detailed data confirming or ruling out reinfection and its characteristics. The Polish experience appears to be universal and useful for other countries, especially developed ones.

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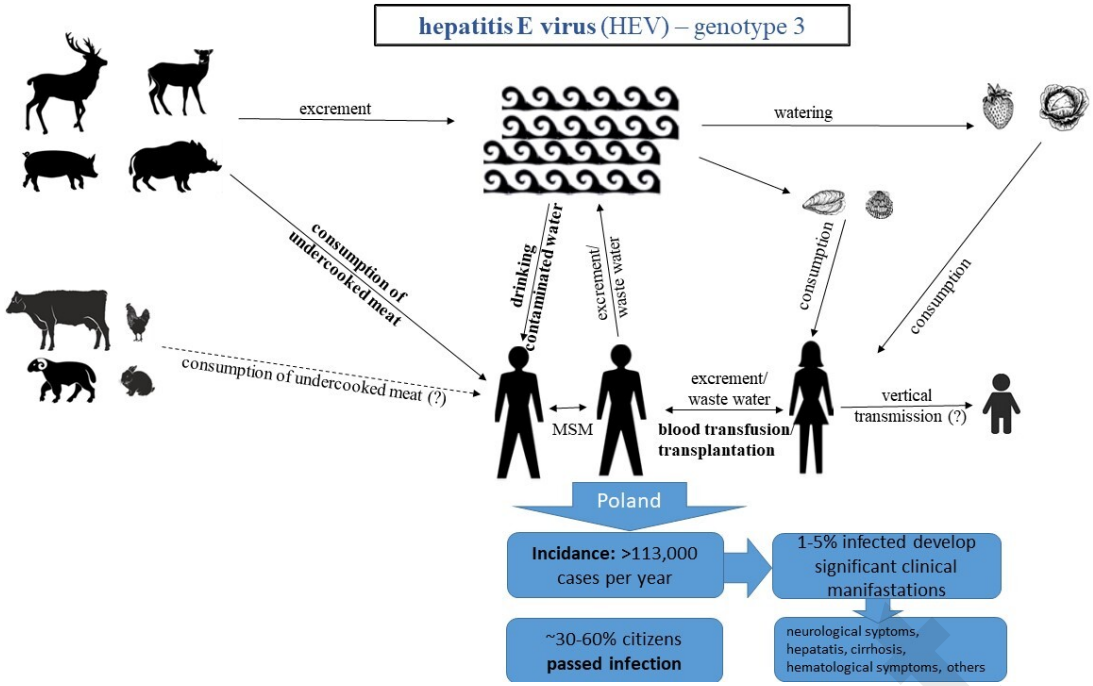

hepatitis E virus (HEV) – genotype 3
excrement
watering
consumption of undercooked meat
drinking contaminated water
excrement/ waste water
consumption
consumption
consumption of undercooked meat (?)
MSM
excrement/ waste water
blood transfusion/ transplantation
vertical transmission (?)
Poland
Incidance: >113,000 cases per year
1-5% infected develop significant clinical manifastations
~30-60% citizens passed infection
neurological syptoms, hepatatis, cirrhosis, hematological symptoms, others

Table I. **Diagnostic tests and their application** [based on 30, 42]

Test (method)	**Phase of infection**	**Aplication in patients**		**Material for testing**
		immunocompetent	**with immunodeficiency**	
IgM anti-HEV (ELISA or rapid test)	acute	diagnostics#	support in phase determination, however application limited (results has to be interpreted with caution)*	serum
IgG anti-HEV (ELISA or rapid test)	acute, persistent, past	diagnostics	support in phase determination, however application limited (results has to be interpreted with caution)*	serum
HEV RNA	acute, persistent	diagnostics	diagnostics	plasma, serum, feces
		monitoring of response for treatment	monitoring of response for treatment	plasma, serum, feces
HEV-Ag (ELISA)	acute, persistent	differential diagnosis - acute (lower level) - persistent (higher level)	diagnostics**, if HEV RNA not available	serum, feces, urine^

should not be based solely on the anti-HEV IgM test, ^Current studies on effectiveness of urine tests mainly concern immunocompetent individuals [42], * there is a significant risk of atypical seroconversion and negative/delayed reactive results despite infection, **significance and interpretation of HEV-Ag in patients with immunodeficiency still under investigation

HEV RNA is also applied for donors (both blood and transplant) screening. All presented methods could be used for population screening

Table II. **Detection of markers of active HEV infection (RNA or Ag) in Poland**

Period of research	**Area** (tested group)	**Available demographic data**	**References**	**Method** (format)	**Number** (frequency) of donations	
					tested	reactive/ confirmed
2012-14	**north-west Poland** wild boars (blood)	not applicable	Dorn-In S. et al. Foodborne Pathog Dis. 2017 [67]	RNA, nested RT-PCR (IDT)	163	**42** (25.8%)
2018-19	**central and eastern Poland** pigs	not applicable	Bigoraj E. et al., Food Environ Virol. 2021 [66]	RNA, rt-PCR (MP)	146	**5** (3.4%)
2015	**Poland** blood donors	random donors, ~60%>40 years old, ~70% males	Grabarczyk P. et al. Transfusion 2018 [70]	RNA, TMA (IDT)	12,664	**6** (0.047%; 1:2,109)
2018-19	**Greater Poland** blood donors	as above	Bukowska A. (RCKiK Poznań)	RNA, TMA (MP16), rt-PCR (MP24)	38,716	**10*** (0.026%; 1:3,872)
V-VIII. 2024	**Mazovia** blood donors	as above	Gdowska J. (RCKiK Warszawa)	RNA, TMA (IDT)	51,782	**35** (0.1%, 1:1,438)
2013-16	**Podlasie** with liver cirrhosis, after transplantation,	Age: mean 50.35; range 21-80 years; 63.56% males	Parfieniuk-Kowerda A. et al. Arch Med Sci. 2021 [72]	ELISA Ag	180	**3** (1.7%)
					180	**5** (2.8%)

	with HIV				90	0 (0%)
2015-18	**Greater Poland** hemodialysed	Age: median 65.6; 57.1% males	Bura M. et al. J Med Virol. 2020 [73]	ELISA Wantai HEV-Ag ELISA Plus	189	0%
2019-23	**Mazovia** hematological	Age: median 62, mean 59, range 8-89 years; 56.8% males	Kalińska A. et al. Acta Haematologica Polonica 2024 [74]	RNA, rt-PCR ELISA Ag	148	0 (0%) 0 (0%)
	after allo-HSCT	Age: median 49, mean 49, range 18-71 years; 52.34% males		RNA, rt-PCR ELISA Ag	166	0 (0%) 0 (0%)

Table III. **Detection of serological markers of the acute HEV infection (anti-HEV IgM) in Poland**

Period of research	**Area** tested group	**Available demographic data**	**References**	**Method of**		**Number** (%) of samples	
				screening	confirmatory testing	tested	**positive**
A.							
2015	**Poland** blood donors	Age: 50 donors from each group 18-27, 28-37, 38-47, 48-57, >57 years; ~70% males	Grabarczyk P. et al. Transfusion 2018 [70]	Wantai HEV-IgM ELISA	recomLine HEV IgM/IgG immunoblot test, Mikrogen	3,079	**39** (0.8%)
before 2018	**Greater Poland** blood donors	Age: mean±SD 44.1±6,5, range 29-58, median 43.5	Bura M. et al. Adv Clin Exp Med. 2018 [82]	Anti-Hepatitis E Virus ELISA [IgM], Euroimmun	no	90	0 (0%)
2020	**Mazovia** blood donors		IHTM data	Rapid Test for IgM Antibody to Hepatitis E (Colloidal Gold Device), Wantai	Wantai HEV-IgM ELISA	650	**3** (0.5%)
B.							
before 2008	**Białystok** Indian students	Age: mean 24.4±0.56, range 18-48; n=45 males	Jaroszewicz J. et al. Przegląd Epidemiologiczny 2008 [81]	DI.PRO, Diagnostics Bioprobes Sri.	no	45	**7** (15.6%)
2010-12	**Poland** hunters	No data	Sadkowska-Todys M. Przegląd Epidmiologiczny 2015 [77]	recomWell HEV IgM, Mikrogen	recomLine HEV IgM/IgG immunoblot test, Mikrogen	1,027	**3** (0.3%)
2010-12	**Poland** hunters	Age: 38-312 person per each age group	Baumann-Popczyk A. et al. Med	recomWell HEV IgM, Mikrogen	recomLine HEV IgM/IgG	1,021	**5** (0.5%)

		(<30, 31-40, 41-50, 51-60, 61-70, >71); 97.7% males	Microbiol Immunol. 2017 [78]		immunoblot test, Mikrogen		
2016	**Poland** soldiers	Age: range 26-57 years; 93.7% males	Korzeniewski K. et al. Int Marit Health. 2018 [79]	Anti-Hepatitis E Virus ELISA [IgM], Euroimmun	recomLine HEV IgM/IgG immunoblot test, Mikrogen	253	**2** (0.8%)
before 2018	**Greater Poland** foresters	Age: mean 45±9.6; median 44.5 years; 70.8% males	Bura M. et al Adv Clin Exp Med. 2018 [82]	Anti-Hepatitis E Virus ELISA [IgM] Euroimmun	MP Diagnostocs ASSURE HEV IgM Rapid Test	48	**1** (2.1%)
C.							
2013-16	**Podlasie** with liver cirrhosis, after transplantation, with HIV	Age: mean 50.35, range 21-80 years; 63.56% males	Parfieniuk-Kowerda A. et al. Arch Med Sci. 2021 [72]	Wantai HEV-IgM ELISA	no	180 180 90	**9** (5%) **2** (1.1%) **1** (1.1%)
2015-18	**Greater Poland** hemodialysed	Age: median 65.6 years; 57.1% males	Bura M. et al. J Med Virol. 2020 [73]	Wantai HEV-IgM ELISA	no	189	0%
2015-19	**Mazovia** with AIH	Age: median 34, range 18-83 years; 68% females	Janik M.K. et al. Pol Arch Intern Med. 2024 [80]	Anti-Hepatitis E Virus ELISA [IgM] Euroimmun	no	379	**5** (1.3%)
2021-23	**Mazovia** hematological	Age: median 62, mean 59, range 8-89 years; 56.8% males	Kalińska A. et al. Acta Haematologica Polonica 2024 [74]	Wantai HEV-IgM ELISA	no	148	0 (0%)

*total in 4 (8,9%); ** verification of part of the reactive samples in the IgG ELISA test; ^*autoimmune hepatitis;* # after excluding IgM positive patients

Table IV. **Detection of serological markers of past HEV infection (anti-HEV IgG) in Poland**

Period of research	Area (tested group)	Available demographic data	References	Method of		Numer of (%) samples	
				screening	confirmatory	tested	positive
Animals population							
2012-13	**Poland** wild boars deer roe deer bison chamois bear	not applicable	Larska M. et al. Zoonoses Public Health. 2015 [83]	ID Screen Hepatitis E multi species indirect ELISA (ID.vet, France)	no	261 118 38 68 4 1	**116** (44.4%) 0% 0% 0% 0% 0%
2012-14	**north-west Poland** wild boars (blood)	not applicable	Dorn-In S. et al. Foodborne Pathog Dis. 2017 [67]			163	**28** (17.2%)
2014-15	**Poland** wild boards pigs	not applicable	Weiner M. et al. Vet Res 2016 [84]	PrioCHECK HEV Ab porcine (Prionics, Switzerland)	no	290 143	**90** (31%) **63** (44.1%)
Healthy people population							
2015	**Poland** blood donors	Age: 50 donors from each group: 18-27, 29-37, 38-47, 48-57, >57; ~70% males	Grabarczyk P. et al. Transfusion. 2018 [70]	Wantai HEV-IgM ELISA	no	3,079	**1340** (43.5%)
2015	**Greater Poland** blood donors	Age: mean 37.7±7.7, range 18-55 years; 84.4% males	Bura M. et al. Adv Clin Exp Med. 2017 [85]	Anti-Hepatitis E Virus ELISA [Ig] Euroimmun	no	105	**4** (3.8%)

2015	**Greater Poland** blood donors	Age: mean 38.4±7.7, median 40 years; 68.3% males	Bura M. et al. Pol J Microbiol. 2018 [86]	Wantai HEV-IgG ELISA	no	110	**67** (60.9%)
2015-16	**Greater Poland** blood donors	no data on age, 65.5% males	Bura M. et al. Int J Infect Dis. 2017 [87]	Wantai HEV-IgG ELISA	no	246	**122** (49.6%)
before 2018	**Greater Poland** blood donors	Age range: 18-55 years	Bura M. et al. J Med Virol. 2018 [88]	Anti-Hepatitis E Virus ELISA [Ig] Euroimmun - cut of ≥2.2 IU/mL - cut of ≥0.8 IU/mL Wantai HEV-IgG ELISA	no	153	**7** (4.6%) **35** (22.9%) **86** (56.2%)
2010-12	**Poland** hunters	no data	Sadkowska-Todys M. et al. Przegląd Epidemiologiczny 2015 [77]	recomWell HEV IgG (Mikrogen)	recomLine HEV IgM/IgG immunoblot test (Mikrogen)**	1,027	**206** (25%)
2010-12	**Poland** hunters	Age: 38-312 person per each age group (>30, 31-40, 41-50, 51-60, 61-70, >71); 97.7% males	Baumann-Popczyk A. et al. Med Microbiol Immunol. 2017 [78]	recomWell HEV IgG (Mikrogen)	no	1,021	**227** (22.2%)
2014	**Greater Poland** foresters	Age: mean 45±9.6, range 29-65 years, median 4.5	Bura M. et al. Adv Clin Exp Med. 2018 [82]	Anti-Hepatitis E Virus ELISA [Ig] Euroimmun	no	48	0 (0%)
2016	**Poland** soldiers	Age range 26-57; 93.7% males	Korzeniewski K. et al. Int Marit Health. 2018 [79]	Anti-Hepatitis E Virus ELISA [Ig] Euroimmun	recomLine HEV IgM/IgG immunoblot test (Mikrogen)**	253	**16** (6.3%)

Patients population

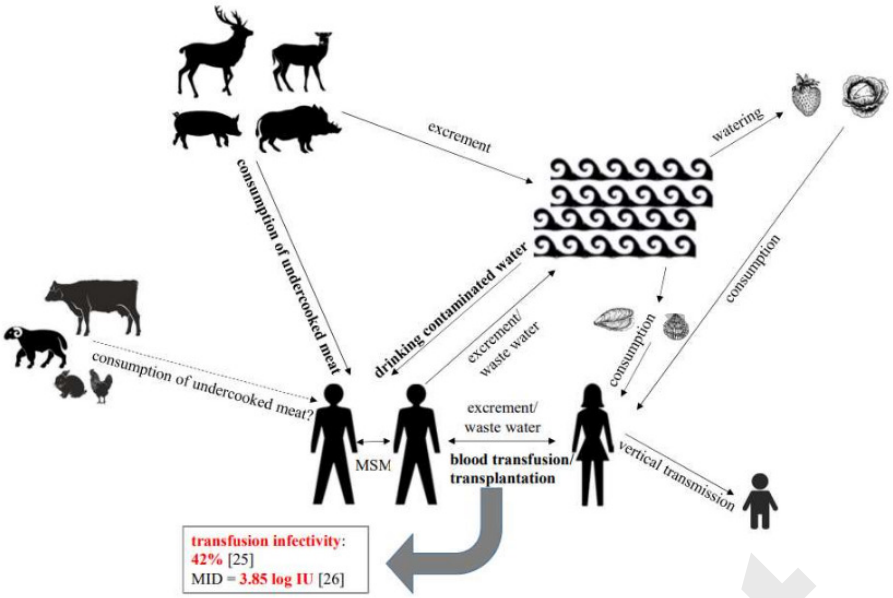
2013	**Greater Poland** from the Infectious Diseases Clinic	Age: mean 47.2±14.2, range 19-85 years, 55.5% males	Bura M. et al. Postepy Hig Med Dosw 2015 [89]	EIA-gen HEV IgG kit (Adaltis)	no	178	**29** (16.3%)
2013-16	**Podlasie** with liver cirrhosis, after transplantation, with HIV	Age: mean 50.35; range 21-80; 63.56% males	Parfieniuk-Kowerda A. et al. Arch Med Sci. 2021 [72]	Wantai HEV-IgG ELISA	no	180 180 90	**87** (48.3%) **73** (40.6%) **34** (37.7%)
2015	**Greater Poland** with HIV	Age: mean 37.7±7.7; range 18-55 years; 84,8% males	Bura M. et al. Adv Clin Exp Med. 2017 [85]	Anti-Hepatitis E Virus ELISA [Ig] Euroimmun	no	105	**1** (0.95%)
2015-16	**Greater Poland** with HIV	Age: range 18-55; 84% males	Bura M. et al. Int J Infect Dis. 2017 [87]	Wantai HEV-IgG ELISA	no	244	**124** (50.8%)
2015-18	**Greater Poland** hemodialysed	Age: median (Q1-Q3) 65.6 (55.6-74.2); 57.1% males	Bura M. et al. J Med Virol. 2020 [73]	Wantai HEV-IgG ELISA	no	189	**94** (49.7%)
2015-19	**Mazovia** with AIH^	Age: median (IQR): 34 (18-83)	Janik M.K. et al. Pol Arch Intern Med. 2024 [80]	Anti-Hepatitis E Virus ELISA [IgM] Euroimmun	no	374#	**55** (14.7%)
2021-23	**Mazovia** hematological	Age: median 62, mean 59; 56.8% males	Kalińska A., et al. Acta Haematologica Polonica 2024 [74]	Wantai HEV-IgM ELISA	no	148	**66** (44.6%)

*total in 4 (8,9%)
** verification of part of the reactive samples in the IgG ELISA test
^*autoimmune hepatitis*
after excluding IgM positive patients

Table V. **Clinical symptoms of HEV infection** [based on: 21, 23, 103]

Cathegory of clinical manifestation	Clinical symptoms
hepatic	• increased ALT activity (including suspected DILI) • hepatitis
hematologic	• severe thrombocytopenia • autoimmune hemolytic anemia • nonimmune hemolytic anemia in G6PD deficiency • pure red cell aplasia • secondary hemophagocytic syndrome • agranulocytosis • primary cutaneous T-cell lymphoproliferative disease CD30(+) • monoclonal gammopathy of uncertain significance
neurologic	• Guillain-Barr syndrome • acute transverse myelitis • neural amyotrophy • cranial nerve palsies • meningitis and encephalitis
nephrological	• glomerulonephritis + cryoglobulinemia
other	• acute pancreatitis • mixed cryoglobulinemia

Figure 1. **Routes of HEV transmission** [based on 11, 17, 19, 20, 21, 23, 24, 27, 28, 29].



Legend: MSM - Men who have sex with men, MID – minimal infection doze, IU – International Units

Figure 2. **HEV – typical infection profile** (antibodies appearance in immunocompetent person) (based on [23, 41])

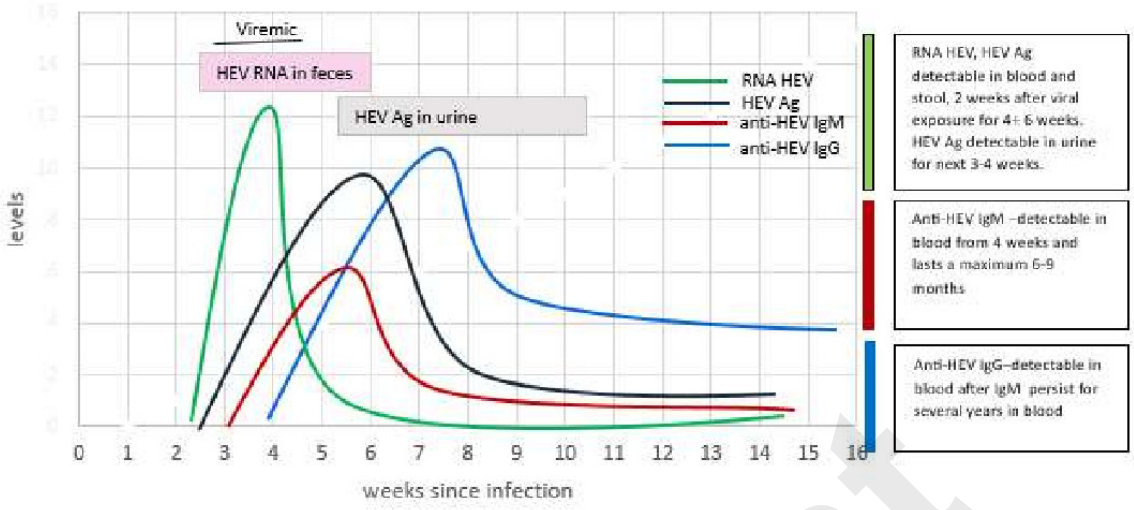


Figure 3. **Frequency of anti-HEV IgG (Wantai test) in patients and blood donors in Poland and in selected countries and regions of the world** (country/region,year of study, references)