

STUDIES ON THE EPIDEMIOLOGICAL AND MOLECULAR CHARACTERISTICS OF THE HEPATITIS E VIRUS IN BULGARIA: A COMPREHENSIVE REVIEW

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ABSTRACT:

Hepatitis E virus (HEV) is a RNA virus that belongs to the family *Hepeviridae*. The virus causes self-limited acute hepatitis in immunocompetent individuals, but can become chronic or present with extrahepatic manifestations in immunosuppressed patients. In recent years, due to the increased scientific interest in HEV infection, the number of laboratory-confirmed cases have also increased. The first study of HEV infection in Bulgaria was carried out in mid-90s of the last century by Teoharov et al. Ten years later, more in-depth studies of HEV infection began. The main focus was on the evaluation of HEV seroprevalence among different target populations. Attention was also paid to the zoonotic potential of the infection. The aim of the present review is to summarize studies on HEV conducted by Bulgarian authors in regards to HEV seroprevalence among humans and animals, clinical and epidemiological characteristics of

HEV infection, and molecular-characteristics of HEV.

Key words: *Hepatitis E virus, prevalence, haemodialysis patients, domestic pigs.*

INTRODUCTION

Hepatitis E is a quasi-enveloped single-stranded RNA virus with icosahedral symmetry, measuring 27-30 nm in diameter. The genome has positive polarity (1). Hepatitis E virus (HEV) classification is based on morphology of the infectious particles, mode of transmission and clinical features. The virus has been initially classified in the *Caliciviridae* family. The latest genetic analysis reveals limited genetic similarities with amino acid sequences of the replicative enzymes in rubella virus and alphaviruses of *Togaviridae* family and plants' *Furoviruses* (2). As a result of these discoveries made in 2009, HEV was classified as the only member of the newly formed family *Hepeviridae*, genus *Hepevirus*. In 2019, the family *Hepeviridae* has been divided into two genera: the genus *Orthohepevirus*, which includes viruses isolated from mammals and birds, and the genus *Piscihepevirus*, which consists only of the species *Piscihepevirus A*, isolated from fish. There are four species in *Orthohepevirus* genus, e.g. *Orthohepevirus A*, *Orthohepevirus B*, *Orthohepevirus C*, *Orthohepevirus D*, and each species has different hosts (3). At least, 9 viral genotypes belong to *Orthohepevirus A*. The latter species includes four genotypes of HEV – genotypes 1, 2, 3, and 4, which are important for human pathology (3). Usually HEV infection is asymptomatic. According to the World Health Organization, about 20 million new HEV infections occur in the human population worldwide each year, of which only 3.3 million are symptomatic (4). The most common clinical presentation of HEV infection is acute viral hepatitis, and symptoms may vary from mild to fulminant acute hepatitis in immunocompetent individuals, or progress to chronic hepatitis in immunosuppressed patients. The icteric form of infection can be observed

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in 5% to 30% of the infected individuals. The mortality rate is below 0.5% up to 4% during an outbreak, but can hit up to 25% in pregnant women (5).

HEV was identified in 1983 by Balayan et al (6). From that moment on, HEV studies began worldwide. For the first time, HEV infection in Bulgaria was reported in 1995 by Teoharov and co-authors (7). In 2008, a new case report of HEV infection was published by the latter team (8). From this moment on, a number of studies have been conducted for a better understanding of HEV and the infection caused by it.

The aim of the present review is to summarize studies on HEV conducted by Bulgarian authors and to compile data concerning: 1) HEV seroprevalence among humans (general population, patients with acute viral hepatitis and specific populations) and among animals; 2) clinical and epidemiological characteristics of HEV infection; and 3) molecular characteristics of HEV.

MATERIAL AND METHODS

To achieve the aim of the present review an electronic keyword literature search in PubMed and the Central Medical Library at the Medical University (MU) in Sofia, was performed. To define the research question of the present review the terms “hepatitis E virus infection”, “acute hepatitis E virus” “hepatitis E virus prevalence”, “hepatitis E virus seroprevalence”, “hepatitis E virus genotype” AND “Bulgaria” were applied during search in PubMed database. Only publications in English were selected. For the databases of the Central Medical Library, the terms applied for the search, were “hepatitis” AND ‘E’. Articles published between 1990 and 2021 year were screened. Reviewing was conducted independently by two of the authors. Initially, 146 articles of potential interest were found (Figure 1): 16 from PubMed and 130 from the database of the Central Medical Library at MU-Sofia. Following screening titles and abstracts and applying the following exclusion criteria: 1)

to be related with methods development, 2) to be a review, and 3) to have restricted free on-line access to full text, finally, 23 online free full-text papers had met exclusion and inclusion criteria and were used for further data analysis.

The review is focused on six different research questions (Table 1), that are important for a better understanding of HEV infection.

STUDIES EVALUATING THE SEROLOGICAL PREVALENCE OF HEPATITIS E AMONG PATIENTS WITH ACUTE VIRAL HEPATITIS (AVH) OR THE GENERAL POPULATION.

In Bulgaria, the first study on HEV prevalence was conducted in 1995 by the National Reference Laboratory “Hepatitis viruses”, National Center of Infectious and Parasitic Diseases (NCIPD), Sofia. The aim was to evaluate the presence of antibodies to HEV (anti-HEV) among 53 patients with non-A non-B AVH (Table 2). The study revealed that 7,55% (4/53) of the patients were anti-HEV positive. Among all positive patients, 5.67% were HEV mono-infected, in four patients anti-HAV IgG was detected, and one patient was hepatitis B virus (HBV) and cytomegalovirus (CMV) co-infected (7). In a later study, conducted in 2010-2012, serum samples from 32 patients with clinical and laboratory data for AVH, were tested for anti-HEV IgM and/or IgG and HEV RNA. Out of the 32 samples tested, 44% (14/32) were anti-HEV positive with detected HEV RNA, of them, 12 were simultaneously positive for both antibody classes – IgM and IgG. Two of the sera were positive for specific IgM (1/14) or IgG antibodies (1/14), respectively (9).

In another study, 806 patients with AVH were retrospectively evaluated. Among them, 16,13% had laboratory confirmed HAV infection, 12,03% – HBV infection, 2,23% – HCV infection, 5,33% – Epstein-Barr virus (EBV) infection, 1,74% – CMV infection, and 60,05% (484/806) were with undifferentiated AVH. Of all AVH, 2,48% patients (20/484) were HEV positive, who comprised 0.29% of all hospitalized patients within the study period (10). HEV seroprevalence among

Table 1. Research questions evaluated within the review.

<i>Research question</i>	<i>Reported results</i>
<i>Seroprevalence of hepatitis E among AVH patients and general population/blood donors</i>	Anti-HEV seroprevalence varies between 2% up to 44 %
<i>Distribution of HEV in different age groups</i>	Age-dependent tendency of anti-HEV IgG prevalence
<i>Molecular/genetic characteristic of HEV</i>	Circulation of HEV genotype 3, subgenotypes e, f and c
<i>Serological prevalence of hepatitis E among swine</i>	Persistence of HEV infection among industrial pigs
<i>Evaluation of clinical and epidemiological characteristics of HEV infection</i>	HEV infection is more severe in co-infected patients with pre-existing liver pathology
<i>Hemodialysis as a risk factor for HEV infection</i>	The established HEV seroprevalence is close to that in general population

Legend: anti-HEV = antibodies against hepatitis E virus; AVH = acute viral hepatitis

Table 2. Characteristics of the studies reporting serological prevalence of hepatitis E among AVH patients and general population / blood donors

Total number of studied samples (N)	Anti-HEV IgM / IgG positive samples (%)	Co-infection/ co-morbidities	Reference
53	7,55 / NS	CMV; ch HBV	(7)
32	44*	NS	(9)
806	2,48 / NS	HBV	(10)
325	13,2 / 20,9	NS	(11).
741	ND / 9,04	NS	(12)
896	11,5 / 16,1	NS	(13)
555	NS / 21,7 to 28,8	NS	(14)

Legend: NS = not studied; CMV = cytomegalovirus; ch HBV = chronic HBV infection; HBV = hepatitis B virus; ND = not detected; * = anti-HEV positive.

AVH in North-Eastern Bulgaria was investigated, too. In still another study, 325 patients (287 hospitalized patients with clinical data of AVH and 38 outpatients with laboratory data of liver dysfunction) were tested for anti-HEV IgG and Ig M. Four of the patients had travelled to HEV endemic areas. The data indicated that 13.2% patients (43/325) were with acute HEV infection. In tested patients, 20.9% were anti-HEV IgG positive (68/325), which is a marker for a past HEV infection (11). In accordance to the general population, overall

anti-HEV IgG prevalence of 9,04% was found by Theoharov et al. (Table 2). Serum samples from 741 individuals were tested for the presence of anti-HEV IgG and IgM. Anti-HEV IgG, as a marker for a past infection, were detected in 9,04% (67/741) of individuals, and in 1,48% (11/741) a concomitant appearance of both classes IgG and IgM antibodies was established. The presence of anti-HEV IgM as a single marker was not detected (12). Another study of the general population revealed anti-HEV IgM and anti-HEV IgG seroprevalence of 11,5% and 16,1%, respectively

(13). Extremely high HEV seroprevalence among Bulgarian blood donors from different districts was established, the percent values varying between 21,7% and 28,8% (23/80) for Shumen district, 23.2% (22/95) for Pleven district, 27.1% (38/140) for Stara Zagora district, 27.5% (44/160) for Plovdiv district, and 21.3% (17/80) for Sofia. The authors evaluated the seroprevalence in connection with hunting activities and detected values of 48,7% to 51,6% (14).

Summarised studies on HEV prevalence suggest spread of the infection among Bulgarian population should not be neglected as HEV is detected from 2% up to 44 % among AVH, in 11,5% of general population and up to 28,8% among blood donors.

STUDIES ON DISTRIBUTION OF HEV IN DIFFERENT AGE GROUPS

Clinical presentation of HEV infection is age dependent (15). The rate of anti-HEV IgG positivity increases with age, which could be explained with the increased risk of exposure to HEV by age (16) The same trend is established in a study of 741 individuals from Plovdiv region, conducted between 2012 and 2013 by Teoharov et al. (12). The age of the population studied varied from 1 year to more than 82 years old. A clear association between the proportion of

anti-HEV-IgG positive individuals and the age was detected – an increase in anti-HEV-IgG positivity from 3,53% in individuals aged 1-9 years to 19,23% in the group over 60 years. The authors established discrepancy to 8,27% for age group 50-59, which is a prerequisite for future studies. No differences were found in the distribution of antibodies against HEV between males and females. Therefore, the age is one of the host factors, which defines the correlation of antibodies against HEV virus.

STUDIES ASSESSING MOLECULAR-GENETIC CHARACTERISTICS OF HEV

Following successful genomic sequencing of isolates from around the world nine HEV genotypes have been established (1 to 9), and their genetic sequences differ from one another by up to 25%, and they infect both humans and animals (17, 18). The genetic characteristics of the virus are relevant to the clinical course of the infection – severity and outcome, and its geographical spread. Several molecular-genetic investigations of HEV strains, isolated from Bulgarian patients, have been conducted. The genotyping of 14 samples by amplification of the 337 nt fragment (4228 nt-4565 nt) of HEV open reading frame 1 (ORF1), revealed the presence of HEV genotype 3 subtypes f and e (12) (Table 3).

Table 3. Characteristics of the studies on HEV genotype distribution.

Total number of studied samples (N)	Amplified genome region	Genotype	Sub-genotype	Reference
14	ORF1	3	e, f	(12)
103	ORF2	3; 1*	e, f, c	(19)

Legend: ORF – open reading frame; *HEV genotype 1 was imported for Bulgaria

In another study, 103 anti-HEV IgM positive serum samples from AVH cases (epidemic outbreaks between 2013 and 2015) from all over Bulgaria were analysed for HEV RNA, and viremia was detected in 90 of the samples. Out of these 90 samples, 64 were sequenced by the 355 nt fragment in ORF2 region of the HEV genome. The applied post-sequencing

analysis revealed distribution of HEV genotype 3, subtypes e, f and c. The authors established different circulation of 3e comparing with 3f and 3c, with 3e restricted to South-West Bulgaria while 3f and 3c diffused over the country. During this study an imported HEV genotype 1 was detected (19). Subsequently, after applying a deep Bayesian

phylogenetic analysis, information on the genetic diversity and the spread of HEV genotypes in Bulgaria was obtained. Three different data sets of the HEV viruses were built - for demographic history investigation, and for selective pressure analysis. The evolutionary rate of 351×10^{-3} substitution/site/year for genotype 3e was established. The root of the time to the most recent common ancestor of the Bayesian maximum clade credibility tree of HEV 3e genotype corresponded to year 1965. The demographic history showed a slight growth from 1995 to 2000, followed by a sort of a bottleneck in 2010s, a peak in 2011 and a new growth to 2015. Selection pressure analysis did not reveal positive pressure sites but detected 64 statistically significant sites under negative selection (20). The factors underlying the observed molecular and geographical differences in HEV genotype distribution, remain to be investigated. The application of molecular epidemiological surveillance by Bayesian phylogeny of HEV virus is a suitable scientific approach to public health control.

STUDIES EVALUATING THE SEROLOGICAL PREVALENCE OF HEPATITIS E AMONG SWINE AND WILD BOAR.

HEV genotypes 3 and 4 are zoonotic ones and

have been isolated from a large variety of animals (wild boar, swine, rabbits and deer). These genotypes are transmitted to humans by consumption of undercooked infected meat and by contact with an infected animal and its products, such as offal, and sewage (21). Therefore, veterinarians and animal keepers are in a greater risk to be infected with HEV. The first study on HEV seroprevalence among domestic pigs in Bulgaria was conducted by Pishmisheva and co-authors (22). HEV infection among pigs from five industrial farms was assessed (Table 4). For the purpose of that study, 85 serum samples from healthy pigs (1–6 months) were tested and the result showed that 50% of piglets were positive for anti-HEV antibodies. In the group of fattening pigs, 29,2% were seropositive. The established overall HEV seroprevalence among pigs was 40%. Even higher results were reported in another study that investigated HEV seroprevalence among 171 East Balkan swine (EBS) from North-Eastern and South-Eastern Bulgaria. The established overall HEV seroprevalence was 82,5% (141/171), as for weaner animals it was 77,2% (44/57), for fattening pigs 79% (45/57), and for adult animals 91,2% (52/57) (23).

Table 4. Characteristics of the studies reporting HEV seroprevalence among domestic and wild animals.

Animal species (age in month)	Total number of studied samples	Number of covered pig farms/ slaughterhouses	Percent anti-HEV antibodies positive samples	Reference
Pig (1-6)	85	5	40	(22)
EBS	171	NA	82,5	(23)
Pig,	433	19	60	(24)
Wild boar	32	NA	12,5	

Legend: NA = not applicable; EBS = East Balkan swine.

Another study established 60% anti-HEV IgG seroprevalence in domestic pigs and 12,5% in wild boar. The detected seroprevalence in wild boar was significantly lower in comparison with domestic pigs (24). The established high HEV prevalence among domestic pigs and wild boar suggests that

these animals might be the reason for the increased HEV transmission across Bulgaria. It is important to know the prevalence of HEV infection among domestic and wild animals in order to reduce the risk of human infection with zoonotic strains. Data demonstrate the existence

of HEV infection between industrial pigs and wild boar in the country. These findings are a prerequisite for more in deep studies on the risk of occupational exposure.

STUDIES EVALUATING THE CLINICAL AND EPIDEMIOLOGICAL CHARACTERISTICS OF HEV INFECTION

In the European countries, in most cases HEV infection is an autochthonous one, mild and self-limited. Chronic infection is common for immunocompromised patients, such as transplant patients and hematological patients on chemotherapy, HIV infected patients and those under treatment with corticosteroids and immunosuppressive agents (5). Testing for HEV should be recommended as part of the diagnostic algorithm for all patients diagnosed with hepatitis or with liver biochemical pathology (25). A few years ago data on the clinical presentation of HEV infection were limited due to unawareness and low number of laboratory confirmed cases.

More in depth study of the clinically acquired HEV infection in Bulgaria began after 2008 (26). Laboratory confirmation of suspected HEV infections among patients with AVH, was done by enzyme immunoassay detection of anti-HEV IgM and/or IgG antibodies. The main clinical symptoms associated with acute HEV infection were nausea, loss of appetite, dark urine and jaundice. In both reports the patients in admission presented with extremely elevated liver enzymes: alanine aminotransferase (ALAT) up to 4264, aspartate aminotransferase (ASAT) – to 3752 and total bilirubin – up to 344,5 (Table 5). Co-infection with HBV and HCV, as well as past HBV infection were detected (27; 10). In another study, 78 patients with AVH were retrospectively evaluated. In addition to liver pathology, prolonged anti-HEV IgM seropositivity up to two years was established in 12% (5/78) of the patients after resolving HEV infection. In 28% of the anti-HEV positive patients concomitant liver disorders were reported (28).

Table 5. Characteristics of the studies evaluating the clinical characteristics of HEV infection.

Clinical diagnosis	Number studied cases (N)	Median age	Liver enzymes maximal value			Co-infection/ co-morbidities	Reference
			ALAT [IU/L]	ASAT [IU/L]	T o t a l bilirubin [mmol/L]		
AVH	2	43,5	4264	3752	344,5	HBV	(27)
AVH	20	51	1851,8	1324,9	123,6	HBV HCV	(10)
AVH	78	57	4000	3000	NS	LD	(28)
ALF	1	42	2940	4844	1088	RhA; SpA	(29)

Legend: AVH – acute viral hepatitis; ALF-acute liver failure; NS-not studied; LD = liver disease; RhA = rheumatoid arthritis; SpA= spondylo-arthritis

Acute HEV infection is reported with clinical presentation of acute liver failure (29), thrombocytopenia (30), holestatic (31) and hemophagocytic syndrome (32). Thereby, the differentiation of acute HEV infection is a complex process that requires combining epidemiological, clinical and serological data. In accordance with Ordinance No. 21 on the Procedure for

Registration, Reporting and Control of Infectious Diseases, since 2019 the acute form of viral hepatitis E is a subject to mandatory registration and reporting in Bulgaria.

HEMODIALYSIS AS A RISK FACTOR FOR HEPATITIS E VIRUS INFECTION

In addition to the main route, transmission

through contaminated blood products has also been described for HEV infection (33). As a result, hemodialysis patients are in a greater risk to be infected with HEV. In a study from 2020, Pishmisheva and co-authors investigated the distribution of HEV infection in hemodialysis patients from Pazardzhik region. Among 102 patients on dialysis treatment 14,7% (15/102) were positive for antibodies against HEV (34). In another study, among 80 haemodialysis patients from Plovdiv, 8,75% of the tested samples were anti-HEV IgM and/or IgG positive. All serologically positive samples were negative for HEV RNA (35). In both studies the authors concluded that the established HEV seroprevalence is close to that in the general population. However, because of the possible transmission of HEV by blood products, a periodic serological testing for Hepatitis E in patients on hemodialysis is needed.

CONCLUSION

Overall, this review comprehensively addresses the current knowledge on HEV in Bulgaria. The first studies on HEV infection in Bulgaria began in the mid-90s of the last century and they were conducted by the team of the National Reference Laboratory (NRL) for Hepatitis Viruses. Studies have been focused on the prevalence of serological markers among patients with acute viral hepatitis and among the general population. Worldwide, increased scientific interest in HEV infection is observed after the establishment of locally circulating viral strains in Europe and the zoonotic nature of the infection. The team of NRL for Hepatitis Viruses carries out studies on HEV viral strains circulating in Bulgaria. Research on the spread of HEV among animals has also begun. After the inclusion in 2019 of HEV infection in the ordinance on reports of AVH, the medical and scientific interest in the etiological cause of the infection in Bulgaria has increased. Identification and isolation of HEV from patients with different extrahepatic manifestation suggests expanded study of different immunosuppressed patients' populations. Seroepidemiological surveys will

help to identify changes in epidemiology of HEV infection in Bulgaria. In addition, more in deep studies are needed to understand HEV mechanisms for crossing the inter-species barrier, and to fill in the missing data on Bulgarian HEV phylogenetics.

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