



# EPIDEMIOLOGY OF HEPATITIS E VIRUS IN CROATIA: A NARRATIVE MINI-REVIEW

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**SUMMARY** – Hepatitis E virus (HEV) infection is an emerging public health problem worldwide, mainly presenting as acute self-limiting hepatitis, but extra-hepatic manifestations, as well as chronic hepatitis in immunocompromised populations, have been more commonly recognized. The seroprevalence ranges from 0.6% to 52.5% in Europe, depending on the geographical region and population group tested. This narrative review focuses on HEV epidemiology in Croatia. The seroprevalence studies show that HEV is widespread in Croatia, with significant differences among population groups; healthcare professionals (2.7%), pregnant women (2.9%), general population (3.4%), injecting drug users (6.1%), animal-related professions (4.0-8.1%), alcohol abusers (8.9%), war-related PTSD patients (8.6%), patients with chronic liver diseases (15.1%), blood donors (21.5%), patients after liver transplantation (24.4%) and patients on hemodialysis (5.2-43.4%). The data show that HEV IgG positivity increases with age and is higher in continental than coastal parts of Croatia and in suburban and rural areas. Phylogenetic analysis show that all detected HEV strains in Croatia were identified as genotype 3. Since the emerging trends of HEV spreading are likely to continue, continuous surveillance is important, especially in immunocompromised persons at risk of chronic hepatitis E.

**Key words:** *hepatitis E virus, Croatia, transplant, hemodialysis, epidemiology*

## Introduction

Hepatitis E virus (HEV) is a non-enveloped RNA virus that belongs to the family *Hepeviridae*, genus *Orthohepevirus*. Genotypes 1 and 2 are restricted to humans only, while 3 to 8 are zoonotic genotypes<sup>1</sup>. HEV genotypes 5 and 6 have been isolated from wild boars in Japan, with no human cases to date. In contrast, HEV genotype 7, isolated from dromedary camel, was found to have significance to humans<sup>1</sup>. HEV infection is an emerging public health problem worldwide, with an estimated 20 million infections and 70,000 deaths annually, representing the most common cause of viral hepatitis<sup>2,3</sup>. In developing countries,

the waterborne transmission is the most common route, mainly restricted to genotypes 1 and 2<sup>3</sup>. HEV has gained considerable attention in resource-rich countries in recent decades, especially in Europe, as zoonotic and foodborne infection of predominantly genotype 3 (and 4)<sup>3</sup>. The consumption of raw and undercooked meat (mainly pork products) is the primary source of infection<sup>3,4</sup>. Occupational exposure in animal-related professions has also been associated with HEV infection<sup>5</sup>. Lately, the waterborne transmission of HEV genotype 3 has been a more commonly recognized route also in resource-rich countries<sup>4</sup>. In the last decade, parenteral transmission via blood products<sup>6</sup> and solid-organ transplants<sup>7</sup> have become important HEV transmission routes. Diagnosis of HEV is confirmed by serology and nucleic acid amplification test, although only HEV RNA testing is recommended for the immunocompromised population<sup>3</sup>.

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In developed countries, hepatitis E usually presents as an acute mild self-limiting illness, mostly affecting older men<sup>8</sup>. However, it may result in severe consequences as patients with pre-existing liver disease are at risk of developing liver failure<sup>9</sup>. Extra-hepatic manifestations have been demonstrated to significantly affect the clinical course involving any of the systems but primary neurological (as Guillain-Barré syndrome or neuralgic amyotrophy) followed by cardiovascular and hematological manifestations (cryoglobulinemia)<sup>10</sup>.

The chronic form of hepatitis E, the entity described in 2008, may develop only in immunocompromised settings, such as solid-organ transplant recipients, HIV-positive patients, and patients with hematological malignancies only caused by genotype 3 and 4<sup>11-14</sup>. The majority of chronic cases are asymptomatic, with mild but persistent liver test abnormalities. After an acute infection, one-third of the transplant recipients will clear the virus after reducing immunosuppression<sup>3</sup>, but more than half of recipients will typically progress to chronic hepatitis and need additional treatments<sup>3,11,14</sup>, to stop the rapid progression of liver fibrosis<sup>11</sup>.

## Methods

We searched PubMed, Web of Science, Medline, Scopus, and ResearchGate with no limitations of the year of publication nor language restriction using a predefined search strategy "hepatitis E virus", human and Croatia. Once a comprehensive list of abstracts has been retrieved and reviewed (by both authors), studies appearing to meet inclusion criteria were reviewed in full. Studies non-relevant for the topic or the ones with data inconsistency were also excluded as assessed by the authors. Books, dissertations, review articles, meta-analyses, non-English articles, and unpublished reports were excluded.

## Hepatitis E virus in Croatia

In Croatia, autochthonous HEV infection was described in 2012<sup>15</sup>. Croatia's first seroprevalence study was conducted between 2011 and 2013 among 504 hepatitis patients negative for acute viral hepatitis A-C and 88 HIV-infected patients. Anti-HEV IgM or IgG antibodies were confirmed in 10.7 % and 1.1% of hepatitis and HIV-infected patients, respectively.

Molecular analysis was carried out in 14 anti-HEV IgM positive patients, and HEV RNA tested positive in five patients<sup>16</sup>.

In 2014-2015, a pilot study evaluating HEV seroprevalence in different population groups was conducted. The study showed the overall HEV IgG seropositivity of 5.6% with 1.9% IgM positive samples but no HEV RNA detection. HEV IgG antibodies were the most prevalent in alcohol abusers (8.9%) and war veterans (8.6%), followed by 6.1% among persons who inject drugs and 2.7% in healthcare professionals<sup>17</sup>. No individual with high-risk sexual behavior was anti-HEV seropositive. The study confirmed that HEV IgG positivity increases with age from 1.8%-2.3% (<40 years) to 11.3% (>50 years) and with the members within the household (12.1%; 4 members *vs.* 1.8%; 2 members). Additionally, the seroprevalence rates were higher in residents of suburban/rural areas compared to the residents of urban areas (14.5% *vs.* 2.5%), and the area of residence has been identified as the main risk factor for HEV seropositivity in our country<sup>17</sup>.

Data on the HEV seroprevalence among 1036 Croatian voluntary blood donors from six north-eastern counties (2014) showed a significantly higher overall IgG seropositivity rate of 21.5%. HEV RNA was not detected in any of 4.4% HEV IgM reactive samples. There was a significant association between age and seroprevalence, with a higher HEV seropositivity in blood donors  $\geq 40$  years. The difference between males and females was not significant. However, significant differences in the seropositivity were found among counties ranging from 7.5% (Zagreb) to 50.3% (Bjelovar-Bilogora County)<sup>18</sup>.

From 2014 to 2017, 68 pregnant women serum samples were tested for HEV showing an IgG seroprevalence rate of 2.9%<sup>19</sup>. A pilot study conducted in 2016-2017 among professionally exposed persons from continental Croatia showed higher HEV IgG seropositivity among forest workers (8.1%) and hunters (4.0%) compared with the general population (3.4%)<sup>20</sup>.

In 2018, a seroprevalence study was conducted among 394 hemodialysis patients from six medical facilities in five cities (three sites in the continental and three sites in the Croatian coastal region). The overall IgG seroprevalence rate was high (27.9%), with significant variations between dialysis centers: Dubrovnik

Table 1. Epidemiology of hepatitis E in different population groups in Croatia

Year	Population	N tested	Seroprevalence/ HEV RNA prevalence	Method	Reference
2011-2013	Hepatitis patients negative for acute viral hepatitis A-C	504	IgM/IgG 10.7% HEV RNA 5/14 anti-IgM positive	Serology, RT-PCR	16
	HIV-infected patients	88	IgM/IgG 1.1%		
2014	Voluntary blood donors	1036	IgG 21.5%; IgM 4.4% HEV RNA 0%	Serology, RT-PCR	18
2014-2015	Alcohol abusers	56	IgG 8.9%	Serology, RT-PCR	17
	War-related PTSD patients	35	IgG 8.6%		
	Injecting drug users	49	IgG 6.1%		
	Healthcare professionals	37	IgG 2.7%,		
	Persons with high-risk sexual behavior	37	IgG 0%		
	OVERALL	214	IgG 5.6%; IgM 1.9% HEV RNA 0%		
2014-2017	Pregnant women	68	IgG 2.9%	Serology, RT-PCR	19
2016-2017	Forest workers	62	IgG 6.5%	Serology	20
	Hunters	25	IgG 4.0%		
	General population	87	IgG 3.4%		
2017	Liver transplant recipients	242	IgG 24.4%; IgM 0.8% HEV RNA 0%	Serology, RT-PCR	23
2016-2018	Different CLD patients	438	IgG 15.1%; IgM 4.5% HEV RNA 0%	Serology, RT-PCR	22
2018	HD patients (Dubrovnik)	39	IgG 5.2%	Serology, RT-PCR	21
	HD patients (Pula)	45	IgG 17.8%		
	HD patients (Šibenik)	59	IgG 23.7%		
	HD patients (Zagreb)	145	IgG 27.6%		
	HD patients (Osijek)	106	IgG 43.4%		
	OVERALL	394	IgG 27.9%; IgM 0.04% HEV RNA 0%		

5.2%, Pula 17.8% Šibenik, Zagreb 27.6%, and Osijek 43.4%. Factors associated with IgG seropositivity were age > 60 years (OR=8.17; 95%CI=1.08-62.14), living in the continental regions (OR=2.58; 95% CI=1.55-4.30), and transfusion of blood products (OR=1.66; 95% CI=1.01-2.73). Seroprevalence increased significantly with age, from 5.3% in patients under 40 to 31.2% in patients over 60. No significant difference was found in HEV IgG seropositivity according to gender, level of education, duration of hemodialysis, rural or urban area of residence, eating habits, profession, travelling habits, source of drinking water, or the type of sewage systems<sup>21</sup>.

A recently published study conducted on a large number of adult patients (n=438) with different liver diseases (2016-2018) demonstrated that the HEV burden in Croatia is high. The previous exposure was detected in 15.1% of patients, with 4.5 % being IgM positive, but not a single one tested HEV RNA positive. The seroprevalence increased with age, from 9.7% (<45 years) to 17.4% (>60 years, p = 0.368). No differences in HEV-IgG seropositivity related to gender, level of education, geographic region, area of residence, liver disease, or hepatocellular carcinoma presence were detected<sup>22</sup>.

To date, only one study addressed the HEV prevalence in the transplant population. In 2017, a large

cross-sectional study was conducted in a cohort of 242 adult liver recipients, transplanted from 1994 to 2013 in a single high volume liver transplant center, and showed an HEV IgG seroprevalence rate of 24.4%. HEV IgM antibodies were found in only one recipient, and one patient showed an equivocal result, but HEV RNA was not detected<sup>23</sup>. The study identified several independent risk factors for HEV seropositivity, such as female gender, older age, and sewage system connected to a septic tank, whereas the highest level of education was identified as a protective factor<sup>23</sup>.

Phylogenetic analysis was performed on anti-HEV positive human serum samples (174 in total) collected from 2012–2017 from different Croatian regions. The panel also included 12 archive samples of a patient with hepatic lesions of unknown origin. HEV RNA was detected in 26/14.9% samples, including an archive sample from 2010. Detected HEV strains were identified as members of species *Orthohepevirus A*, genotype 3, and clustered within four subtypes (subtypes 3a, 3c, 3e, and 3f). The HEV strains mostly cluster in regards to the year of detection, with the majority belong to subtypes 3a and 3c. The largest genetic diversity was found in Zagreb County<sup>24</sup>.

## Conclusion

The seroprevalence studies results showed that HEV is widespread in Croatia, with significant differences among population groups. The preliminary results indicate that the seroprevalence of hepatitis E among occupationally exposed persons is higher compared to the general population. The reported seropositivity rates detected in the Croatian population are within the range of the seroprevalence reported in other European countries (0.6%–52.5%)<sup>25</sup>.

It is well established that HEV-IgG seroprevalence increases with age, reflecting a cumulative lifetime exposure to the virus. Additionally, there are significant differences in the seropositivity among counties as well as rural/urban area of residence. However, it is important to note that differences in the seropositivity may, at least partially, reflect the differences in the sensitivity and specificity of immunoassays used for detection of HEV antibodies. In addition, some tested groups include a small number of participants; therefore, the seroprevalence results should be interpreted with caution. Further large-scale studies are needed to deter-

mine the true prevalence of this emerging zoonosis in the Croatian population.

Studies on HEV RNA detection and phylogenetic characterization showed that human HEV strains from the European countries mainly belong to the genotype 3<sup>3</sup>. Similarly, detected HEV strains in Croatia showed to be genetically highly related to members of the *Orthohepevirus A* genotype 3.

Since the emerging trends of HEV spreading are likely to continue, continuous surveillance is essential, especially in immunocompromised persons who are at risk of chronic hepatitis E.

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#### Sažetak

### EPIDEMIOLOGIJA HEPATITIS E VIRUSNE INFEKCIJE U HRVATSKOJ: KRATKI PREGLED

*A. Mrzljak i T. Vilibić-Čavlek*

HEV (hepatitis E virus) infekcija je rastući javno zdravstveni problem u svijetu. HEV se većinom očituje kao samolimitirajući akutni hepatitis, međutim ekstrahepatalne manifestacije kao i kronični hepatitis u imunokompromitiranih osoba ve su češći. U Europi seroprevalencija varira od 0,6 % do 52,5 %, ovisno o zemljopisnoj regiji i testiranoj populaciji. Ovaj pregledni rad prikazuje epidemiološke značajke HEV-a u Hrvatskoj. Istraživanja seroprevalencije pokazuju da je HEV široko rasprostranjen u Hrvatskoj, sa značajnim razlikama između pojedinih populacijskih skupina; zdravstveni djelatnici (2,7 %), trudnice (2,9 %), opća populacija (3,4 %), intravenski korisnici droga (6,1 %), zanimanja vezane uz životinje (4,0-8,1 %), alkoholičari (8,9 %), pacijenti s PTSP-om (8,6 %), pacijenti s kroničnim bolestima jetre (15,1 %), dobrovoljni darivatelji krvi (21,5 %), pacijenti nakon transplantacije jetre (24,4 %) i pacijenti na hemodijalizi (5,2-43,4 %). Podaci pokazuju da HEV IgG pozitivitet raste s dobi, te da je viši u kontinentalnim krajevima Hrvatske odnosu na priobalno područje kao i u prigradskim i ruralnim sredinama. Filogenetskom analizom detektiranih sojeva u Hrvatskoj dokazan je genotip 3. S obzirom na rastući trend širenja HEV-a, nepochodno je praćenje infekcije, osobito u imunokompromitiranih osoba koje su pod rizikom od razvitka kroničnog hepatitisa.

**Ključne riječi:** *hepatitis E virus, Hrvatska, transplantacija, hemodijaliza, epidemiologija*