SEROPREVALENCE OF EMERGING HEPATITIS E VIRUS IN PATIENTS WITH ACUTE HEPATITIS BETWEEN 2004 AND 2018 IN CSONGRÁD COUNTY, HUNGARY

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SUMMARY

Objectives: Hepatitis E virus (HEV) has recently become endemic in Europe, however, it is often a remnant neglected by clinicians as the causative agent of acute and chronic hepatitis and is often misdiagnosed as a drug-induced liver injury. The infection rate in European pig farms is estimated to be around 15–20%, therefore, the primary source of HEV infections might be poorly prepared pork meat. As HEV infections may occur more often in clinical practice than previously thought, the present paper aims to analyse the seroprevalence of HEV in patients with acute hepatitis over a period of 14 years in Csongrád County, Hungary.

Methods: The sera of 4,270 hepatitis patients collected between 2004–2018 were tested for cumulative anti-HEV IgG/IgM. Furthermore, 170 IgM positive sera were tested for the presence of viral RNA by RT-qPCR.

Results: Between 2012–2018, the cumulative seroprevalence has increased 9.18 times, and between 2013–2018, IgM prevalence has increased 12.49 times. Viral RNA was detectable in 12.35% of IgM positive sera.

Conclusion: The present paper presents data showing that the seroprevalence of hepatitis E virus has increased markedly over the course of the last decade in Hungary and in other European countries as well. The exact reason behind this phenomenon is yet to be determined. To assess the dynamics and the reason for this increase in prevalence, pan-European, multicentre studies should be conducted.

Key words: hepatitis E virus, seroprevalence, molecular detection, serology

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https://doi.org/10.21101/cejph.a7406

INTRODUCTION

Genotype 3 of the hepatitis E virus (HEV3) is a zoonotic foodborne pathogen affecting people of all ages in Europe. This genotype has been known to infect a variety of mammals, including wild boars, deer and swine. Humans are believed to be infected during processing and by the consumption of undercooked meat. In most cases, hepatitis E infection is presented as a mild self-limiting disease, which may often be asymptomatic (1). Even if it causes symptoms, those can be misleading, including fatigue, elevated ALT levels and jaundice; therefore, most clinicians consider it as a non-harmful viral agent. However, in some cases, it can manifest itself as a severe disease when acquired by patients with underlying chronic liver disease, resulting in acute-on-chronic liver failure with a mortality rate in certain risk groups reaching 30% (2). In immunosuppressed patients, HEV infections can progress to a chronic state in 60% of the cases (3), which can lead to liver cirrhosis (4). Approximately 29 million people suffer from chronic liver disease in Europe (5). Therefore, combined with other comorbidities, HEV may pose a threat to a significant part of the population.

Swine is a well-known reservoir of the virus (4). In different European countries, the proportion of infected swine may vary between 13.7% (Slovenia) and 87.5% (Finland) (6, 7).

Based on OECD data, average pork consumption in Europe was 35.5 kg/capita/year in 2018, for Hungary, it is 18.3 kg/capita/year (8, 9).

Contrary to previous expectations, HEV has started to become an endemic disease in Europe over the past decade (10). Since most infections are asymptomatic, the exact reasons and dynamics of this endemicity of HEV are still unknown. There have been several alerting publications recently, regarding the increasing seroprevalence in Europe showing prevalence data as follows: France 52.5%, Poland 49.6%, Italy 49%, Netherlands 31% (11–14).

It is of great clinical importance to identify an acute exacerbation of chronic liver disease caused by HEV, as the infection can be effectively treated with ribavirin therapy and might prevent acute chronic liver failure (15).

The present study aims to gain insight into the real magnitude of HEV infections in Csongrád County, Hungary, by analysing the seroprevalence of HEV in patients with acute hepatitis between 2004 and 2018.

MATERIALS AND METHODS

Sample Collection

Sera samples were collected from patients with acute hepatitis (showing signs and symptoms of the disease, including elevated transaminase levels), and anti-HEV IgG/IgM antibody reactivity was tested at the Public Health and Food Chain Safety Service of the Csongrád County Government Office, Hungary. Samples were collected and tested between 2004 and 2018.

Detection of HEV-specific Antibodies (IgM/IgG) and IgM in Sera from Patients with Acute Hepatitis

The detection of specific HEV IgG and IgM antibodies of the patients was performed using the ELISA method (HEV AB ELISA, DiaPro, Diagnostic Bioprobes Srl., Milan, Italy). According to the clinical protocol, in the case of HEV specific total antibody positivity, when it seemed necessary, HEV specific IgM antibody detection was also performed (HEV IgM ELISA, DiaPro, Diagnostic Bioprobes Srl., Milan, Italy).

Molecular Detection of HEV RNA

IgM positive sera of hepatitis patients detected between 2016 and 2018 were examined for the presence of viral RNA.

RNA Extraction and Reverse Transcription Assay

The extraction of HEV RNA from residual IgM positive sera was performed using the MagNA Pure LC Total Nucleic Acid Isolation Kit (La Roche AG, Basel, Switzerland). Complementary DNA was made using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, USA).

Real-time Polymerase Chain Reaction

The real-time polymerase chain reaction (PCR) assay was performed using the SsoFast™ EvaGreen® Supermix with low ROX (Bio-Rad Laboratories, Inc., Hercules, CA, USA). A previously published PCR method was adopted for the real-time detection of HEV3 using the Bio-Rad CFX 96 real-time PCR instrument (Bio-Rad Laboratories, Inc., Hercules, CA, USA) (16). The primers were supplied by Integrated DNA Technologies, BVBA, Leuven, Belgium.

Sequencing Analysis

The cycle sequencing reaction was carried out from purified real-time PCR products using the BigDye Terminator v3.1 cycle sequencing reaction kit (Applied Biosystems, Carlsbad, USA) and sequence data were collected using the ABI PRISM 3130 genetic analyser (both from Applied Biosystems, Carlsbad, CA). Sequence chromatograms were cleaned using BioEdit software. For identification, the HEV sequences were aligned using BLAST searches using the Basic Local Alignment Search Tool.

Table 1. *Characteristics of examined population with acute hepatitis (N = 4,270)*

Age group (years)	Males n (%)	Females n (%)	Total n (%)
Hepatitis patients tested for IgG/IgM			
Below 30	394 (19.45)	374 (16.67)	768 (17.99)
30–35	225 (11.11)	155 (6.91)	380 (8.90)
36–40	184 (9.08)	150 (6.68)	334 (7.82)
41–45	172 (8.49)	150 (6.68)	322 (7.54)
46–50	150 (7.40)	171 (7.62)	321 (7.52)
51–55	202 (9.97)	253 (11.27)	455 (10.66)
Above 55	699 (34.50)	991 (44.16)	1,690 (39.58)
Total	2,026 (100.00)	2,244 (100.00)	4,270 (100.00)
Hepatitis patients tested for IgM			
Below 30	371 (19.65)	350 (16.67)	721 (18.08)
30–35	202 (10.70)	148 (7.05)	350 (8.78)
36–40	176 (9.32)	143 (6.81)	319 (8.00)
41–45	156 (8.26)	142 (6.76)	298 (7.47)
46–50	138 (7.31)	155 (7.38)	293 (7.35)
51–55	183 (9.69)	237 (11.29)	420 (10.53)
Above 55	662 (35.06)	925 (44.05)	1,587 (39.79)
Total	1,888 (100.00)	2,100 (100.00)	3,988 (100.00)

Statistics

The difference between the seven age groups was tested with the χ^2 test for independence, and between the sexes with Fischer's exact test. To test the difference between men and women in every age group, besides χ^2 test and Fisher's exact test, odds ratio was also calculated, and it gave the same results as the χ^2, and Fisher's exact test.

Related to the seropositivity data, a second grouping was applied according to the year of collection. Since the data originated between 2004 and 2018, there are 15 age groups to discern. The difference between the age groups was tested with χ^2 test for independence that refers to IgG/IgM and only the seropositivity of IgM.

The calculations were performed using GraphPad InStat V2 (GraphPad Software, V2.05a, USA), $\alpha \leq 0.05$ was chosen for the significance level.

RESULTS

Characteristics of the Study Population

Data were analysed anonymously and retrospectively. Among 4,270 samples tested for total anti-HEV Ig antibodies, 2,026 (47.45%) were males and 2,244 (52.55%) females. Among 3,988 samples tested for IgM alone, 1,888 (47.34%) were males and 2,100 (52.66%) females (Table 1).

Seroprevalence of Hepatitis E in Patients with Acute Hepatitis

Anti-HEV cumulative serological data of IgG/IgM and IgM only originated between 2004 and 2018. Referring to the IgG/IgM data, there is a marked increase in seropositivity between 2012 and 2013. Regarding cumulative IgG/IgM levels, the lowest seroprevalence (4.93%) was detected in 2012, and the highest (45.21%) in 2018. Seroprevalence between 2012 and 2018 has increased more than ninefold (Fig. 1a). There was a significant difference in HEV seroprevalence between different years ($\chi^2 = 513.720$, $p < 0.001$).

The IgM-only data show similar tendency characteristics with a lowercase number. The lowest seroprevalence (1.82%) of anti-HEV IgM was measured in 2013, and the highest (22.71%) was detected in 2018 (Fig. 1b). Between 2013 and 2014, a marked increase was detected, and such a rising pattern continued until 2018, showing a significant difference between the age groups ($\chi^2 = 158.530$, $p < 0.001$).

The seropositivity of HEV IgG/IgM was widely distributed between the age groups. The data were sorted according to the following age groups: under 30, 30–35, 36–40, 41–45, 46–50, 51–55, and above 55. The lowest seroprevalence was detected in the age group under the age of 30 years (Fig. 2). The mean values obtained in these groups were 10.91%, 16.44%, 15.76%, 16.86%, 21.33%, 27.72%, and 30.62% for men; and 7.75%, 11.61%, 16.00%, 20.00%, 25.73%, 18.97%, and 25.93% for women, respectively (Fig. 2). The prevalence increases from younger to older age groups ($\chi^2 = 73.805$, $p < 0.001$ in men and $\chi^2 = 68.709$, $p < 0.001$ in women). Using the Fisher's exact test, a significant difference was found between men and women in age groups 51–55 years ($p = 0.033$) and >55 years ($p = 0.036$).

Molecular Screening of HEV in Sera of Patients with Acute Hepatitis

In total, 170 IgM reactive samples were analysed for the presence of HEV RNA. HEV RNA was detected in 36 (21.17%) samples using RT-qPCR. PCR positive samples were sequenced. Among these, 21 was successful sequencing, which resulted HEV3 genotype in all cases (12.35% of the total of 170 samples).

DISCUSSION

In previous years, high seroprevalence of HEV has been reported in several European countries. The present study observes a marked increase in the prevalence of HEV in patients with acute hepatitis in Hungary and these findings are in correlation

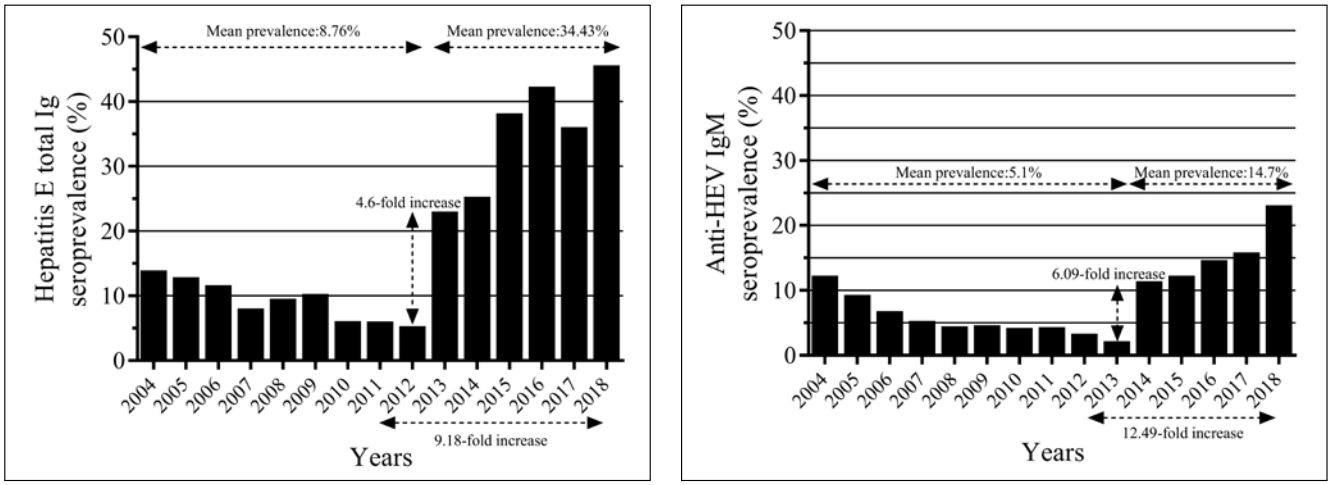

Fig. 1a, b. *Seroprevalence of hepatitis E in patients with acute hepatitis.*

1a: Total seroprevalence of HEV IgG and IgM seroprevalence in patients with acute hepatitis.

Between 2012 and 2013, there was a marked 4.6-fold increase in the prevalence of anti-HEV antibodies in the sera of patients with acute hepatitis. Between 2012–2018, the presence of anti-HEV antibodies increased 9.18 times.

1b: HEV IgM seroprevalence in patients with acute hepatitis.

Between 2013 and 2014, there was a significant 6.09-fold increase in the prevalence of anti-HEV IgM, while between 2012 and 2018, a 12.49-fold increase was observed.

with data from other European countries (10). In the examined period, in the first few years, between 2004 and 2012, the seroprevalence of HEV stagnated. The sharp increase occurred 1 year later in the case of IgM. The marked rise in HEV seropositivity without need of any medical attention highlights the fact that most HEV infections are mild and self-limiting, thus, these data are often absent from medical statistics. The findings of the present study that describe a 12.49-fold increase in the seroprevalence of HEV IgM in the past 5 years are alarming. With the increase in the number of cases, the number of fulminant, acute-on-chronic liver failure due to HEV infection is also expected to rise. Therefore, based on this increasing prevalence trend, HEV should be considered as an underlying cause when it comes to the differential diagnosis of acute hepatitis. HEV should be considered a potential aetiological agent, especially when symptoms arise in the form of drug-induced cholestasis, as clinicians often misdiagnose HEV infections with drug-induced liver injury (17), or there is an underlying hepatitis B infection in the patient's history (18).

In our set of samples, 170 IgM reactive sera were tested for viremia by detection of HEV3 RNA with qPCR, of which 21 (12.35% of the samples) were aligned as HEV3 RNA after sequencing, thus confirming virus replication. There is reason to believe that the findings of recent articles that report the increasing prevalence indicate an alarming sign of the silent emergence of HEV-induced acute-on-chronic hepatitis, which should raise the awareness of physicians. It should be noted that the absence of viral RNA in sera samples does not necessarily rule out HEV infections, therefore, the number of infections may be much higher (19).

The present paper reports a significant rise in HEV seroprevalence in Hungary between 2012 and 2013, and similar observations were made by Suin et al. in Belgium between 2014 and 2015 (20). It is estimated that there are 2 million new HEV infections in Europe every year (21), placing a substantial burden on the healthcare systems.

There are several hypotheses regarding the increasing trend, including methodical and analytical differences in serologic tests, the appearance of a new regional virus subtype, or changes in dietary habits (22). There are reports of the emergence of new HEV strains in Europe, however, their exact role in the observed increase in HEV seroprevalence has not been formally demonstrated (23).

The emergence of swine flu in Europe was thought to decrease pork consumption, however, according to data, pork consumption has been stagnating over the past few years (8). Although the carriage of the virus by pigs would not cause a public health problem alone, as meat processing protocols have changed in the last decades, inadequately processed pork may be a viable source of infection (24). Instead of traditional long smoking, accelerated procedures are often used nowadays; concomitantly, studies examining meat products on the Dutch market found that 68.9% of pate and 70.7% of liverwurst were contaminated with HEV3 (25).

In developed countries, the main risk of infection is eating poorly prepared pork (26), and it is also found that dietary habits may affect HEV prevalence (27). The virus inactivation may be inadequate or completely absent in some raw or uncooked semi-processed pork products (e.g., hamburger patties, smoked sausages, or ham and pate). The classic smoking process is a lengthy procedure, with microbe inactivation achieved via protein methylation by formaldehyde, an active smoke compound (28). On the contrary, traditional smoking is often replaced with accelerated smoking, such as the aroma of 'smoke flavour' and pickling liquid with nitrite salts. Nitrite salts have bacteriostatic effects, with questionable antiviral potential (29).

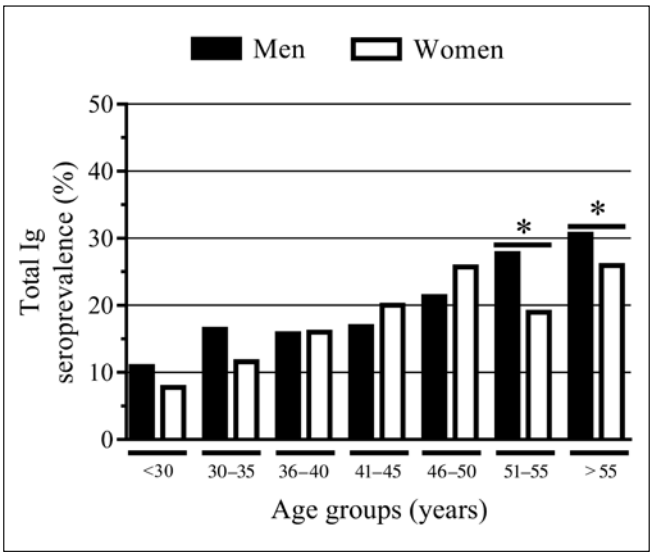

Fig. 2. *Total Ig seroprevalence in patients with acute hepatitis, by sex and age groups.*

Faecal-oral transmission has also been shown to play a role in viral transmission. Although direct person-to-person transmission is rare (30), transmission through sewage-contaminated vegetables has been demonstrated.

CONCLUSIONS

The purpose of this article has been to examine the seroprevalence of HEV among patients with acute hepatitis in Hungary. We have found an alarmingly high rate of IgM seropositivity combined with viremia in IgM positive sera. In 2012 (IgG/IgM) and 2013 (IgM), a significant change occurred in the epidemiology of HEV, driven by an unknown factor. Based on economic statistics and previous veterinary studies performed in European countries, the main source of HEV infection is believed to be inadequately prepared pork (27). According to other European reports, our viral detection and seroprevalence data highlight that HEV3 has become an emerging pathogen behind some serious and acute diseases in the general population. Our data also suggest that large, pan-European multicentre studies should be conducted to evaluate the presence of infective HEV particles in the food chain.

Acknowledgements

We thank Judit Jakus and János Kenyeres who moderated this article and, in that line, improved the manuscript significantly. Additionally, we are grateful to Fanni Rapcsák for her excellent technical assistance.

Conflicts of Interest

None declared

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Received March 25, 2022
Accepted in revised form September 3, 2023