

Prevalence of hepatitis E virus (HEV) antibodies in Serbian blood donors

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Abstract

Introduction: Hepatitis E virus (HEV) infection is rarely reported in industrialized countries, but recent studies have revealed quite variable seroprevalence rates among European populations, including blood donors. In Serbia, very limited data about HEV seroprevalence are available. This study aimed to determine the prevalence of anti-HEV IgG antibodies and HEV RNA in the sera of volunteer blood donors in Serbia.

Methodology: Serum samples from 200 volunteer blood donors were tested for the presence of anti-HEV IgG by enzyme-linked immunosorbent assay (ELISA) using ORF-2 HEV genotype 3 recombinant proteins as antigen, and for the presence of HEV RNA by nested reverse transcriptase polymerase chain reaction (RT-PCR).

Results: In total, 15% of the volunteer blood donors were seropositive. The prevalence increased with age; 21.5%, 14.2%, and 5.4% HEV seroprevalence rates were found in individuals older than 51 years, between 31 and 50 years, and in those younger than 30 years of age, respectively. However, no HEV RNA was detected in any of the individuals analyzed.

Conclusions: The prevalence of anti-HEV IgG among blood donors as representatives of the general population is quite high in Serbia compared to data from many European countries. One of the reasons for this could be the high prevalence of HEV among Serbian pigs and the traditional consumption of piglet meat in the country. The relatively high HEV seroprevalence found among Serbian blood donors indicates the need for further investigation.

Key words: hepatitis E virus; blood donors; serology; ELISA; RT-PCR.

J Infect Dev Ctries 2014; 8(10):1322-1327. doi:10.3855/jidc.4369

(Received 30 October 2013 – Accepted 23 March 2014)

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Introduction

Hepatitis E is an enterically transmitted disease caused by the hepatitis E virus (HEV) that is endemic in regions where sanitary conditions and water supplies are inadequate. Whereas human-to-human transmission seems to be exceptional, mother-to-child and blood transfusion transmission have been described [1-4]. In contrast to the usually waterborne HEV infection in endemic developing countries, in developed countries, sporadic hepatitis E is mainly a zoonotic and foodborne disease [1,5].

HEV are classified into 4 major genotypes, of which genotypes 1 and 2 only infect humans, while

genotypes 3 and 4 are zoonotic [1,2]. The majority of HEV epidemics in Asia and Africa have been caused by genotype 1 strains, while genotype 2 strains have caused epidemics in Mexico and some regions of Africa. In China, epidemics were caused mainly by genotype 1, but genotype 4 has recently become the dominant cause of sporadic hepatitis E in that country. HEV genotype 3 is widely distributed and is the cause of sporadic cases worldwide, as well as of most infections in Europe and the USA, where an increasing frequency of sporadic cases is being described and where HEV is now considered to be an emerging pathogen [2-4,6].

Genotype 3 strains detected in humans and swine from the same geographical areas are genetically closely related [3,7]. This fact, together with the description of cases of acute hepatitis in people who ate uncooked deer meat or liver from pork or wild boar and the recent detection of HEV RNA and infectious virus in commercial pig livers and pork products sold in local grocery stores has raised the hypothesis of a zoonotic potential for HEV [3,8-12]. Several studies have suggested that pig handlers, veterinarians, and other workers with occupational exposure to swine present a higher HEV IgG seropositivity, although other studies did not find such an association [2,13].

Currently, commercially available kits are designed to detect anti-HEV in human sera or plasma, and include short fragments of ORF-2 and ORF-3 of HEV genotypes 1 and 2, but not of HEV genotype 3, the most prevalent in industrialized countries in pigs and humans [7]. Various reports indicate that commercial assays sometimes fail to detect specific antibodies in sera from patients with proven HEV genotype 3 infections, and thus the number of autochthonous HEV infections in industrialized regions may have been underestimated [14-16].

Lately, an increasing number of data describing quite variable seroprevalence rates in different populations from European countries have been reported, and these differences have been attributed, at least partially, to the different methodologies applied [1,4,14,16,17].

Even though we found in our study from 2008 that 30%–45% of the tested stool and tissue samples from commercial farm pigs in Serbia were positive for HEV RNA by reverse transcriptase polymerase chain reaction (RT-PCR) (unpublished data), and that more than 34% of the backyard pigs' sera tested were anti-HEV IgG positive [18], indicating that HEV infection is widespread in Serbian pigs, very limited data about HEV seroprevalence in Serbian human populations or in surrounding countries of the former Republic of Yugoslavia are available. Even more, according to our knowledge, no human HEV strain has been detected so far in Serbia. In order to provide more data about the presence and prevalence of HEV infection in human population in Serbia, we performed a small pilot study to determine the prevalence of anti-HEV IgG antibodies and HEV RNA in the sera of blood donors as representatives of the general population.

Methodology

Population and sample collection

During the spring of 2010, serum samples from volunteer blood donors ($n = 200$) were anonymously collected by medical staff of the Institute for Blood Transfusion of Novi Sad. All blood donors provided written consent for the use of their blood samples in medical research and fulfilled the Serbian criteria for blood donations. Blood donors' (average age 39.3, range 19–65) samples were routinely tested by ELISA for HBV-Ag, anti-HCV, HIV Ag/Ab, and anti-TP (*Treponema pallidum pallidum*) at the Institute for Blood Transfusion. A questionnaire including personal data, disease history, travelling data, previous transfusion records, previous hepatitis infection, and frequent contact with pets, domestic and farm animals (pigs, cattle, horses, cats, dogs, sheep, goats, rabbits and hamsters) was completed. Age and gender were recorded. The study was approved by the Ethical Committee of the Medical Faculty, University of Novi Sad.

HEV infection assessment

Anti-HEV IgG was detected in blood sera by a previously validated ELISA based on the use of purified truncated ORF-2 HEV genotype 3 recombinant protein expressed in insect larvae [15,19]. Absorbance values were expressed as P/N (absorbance value of the test sample/absorbance value of the negative control). Samples above the cut-off value ($P/N \geq 2.5$) were considered positive. In cases where P/N values were closed to the cut-off value, samples were tested by western-blot as previously described [19].

HEV RNA detection was conducted in all collected blood samples by nested RT-PCR with previously described primers sets [20] and using the commercial kits QIAGEN OneStep RT-PCR Kit and HotStar Taq Master Mix (Qiagen, Hilden, Germany) following the manufacturer's instructions. As positive controls, previously characterized (NCBI GeneBank accession numbers: HM483380–HM483385) Serbian swine HEV genotype 3 isolates were used.

Statistical analyses

Chi square test and Chi square test for trend were performed using GraphPad PRISM (GraphPad Software Inc, La Jolla, USA). Analysis of the variance (ANOVA) was performed with SPSS version 15.0 for Windows (SPSS Inc., Chicago, USA).

Results

General characteristics of blood donors tested are summarized in Tables 1 and 3. In total, 15% (30/200) of the blood donors tested positive for anti-HEV IgG with an average P/N of 4.45 ± 1.95 (range 2.5–9.58). In seven cases in which the P/N values were around the cut-off value, ELISA results were confirmed by western blot (data not shown).

No significant differences in terms of anti-HEV IgG seropositivity were found between men and women (14.6% and 16.7% were positive, respectively). HEV seroprevalence increased with age,

as higher rates were recorded in individuals older than 51 years of age (21.5%) than in those between 31 and 50 years of age, or than in those younger than 30 years of age (14.2% and 5.4%, respectively, $p < 0.027$) (Table 1). Although not available in all cases, median alanine aminotransferase (ALT) values were 25 IU/mL (range 6–148), presenting values higher than 41 IU/mL in 36.8% and 25% of those positive and negative for anti-HEV IgG, respectively. None of the eight blood donors in whom a history of past hepatitis A virus (HAV) was recorded presented positive HEV serology, and none of the 200 blood donors tested

Table 1. Blood donor data and results of HEV serology investigation based on gender and age

Blood donors (n = 200)		% HEV IgG (+)	HEV IgG (-)
GENDER	Male	23/158 (14.6%)	135/158 (85.4%)
	Female	7/42 (16.7%)	35/42 (83.3%)
AGE	18–30	2/37 (5.4%)	35/37 (94.6%)
	31–50	14/98 (14.2%)	84/98 (85.8%)
	51–>70	14/65 (21.5%)*	51/65 (78.5%)

* $p < 0.027$

Table 2. Anti-HEV IgG positive blood donors and contact with animals

Animals in backyards	No. of donor backyards with animals/total (%):	No. of HEV seropositive owners/total owners (%)	No. of seropositive owners/total HEV seropositive (%)
Pigs	84/200 (42%)	11/84 (13.1%)	11/30 (36.6%)
Cattle	13/200 (6.5%)	0/13	0/30
Horses	6/200 (3%)	1/6 (16.7%)	1/30 (3.3%)
Poultry	88/200 (44%)	13/88 (14.8%)	13/30 (43.3%)
Sheep/goats	28/200 (14%)	1/28 (3.5%)	1/30 (3.3%)
Dogs	118/200 (59%)	19/118 (16.1%)	19/30 (63.3%)
Cats	73/200 (36.5%)	10/73 (13.7%)	10/30 (33.3%)
Rabbits	17/200 (8.5%)	1/17 (5.8%)	1/30 (3.3%)
No animals	33/200 (16.5%)	3/33 (9%)	3/30 (10%)

Table 3. Blood donor data and results of HEV serology investigation based on occupation

Occupation	No. of blood donors	% of blood donors	No. (%) HEV IgG (+)
Worker	90	45.0	16 (17.7%)
Farmer	20	10.0	3 (15.0%)
Pensioner	14	7.0	3 (21.4%)
Merchant	9	4.5	2 (22.2%)
Unemployed	10	5.0	3 (30%)
Engineer	7	3.5	0
Clerk	6	3.0	0
Nurse	4	2.0	0
Policeman	4	2.0	1 (25%)
Pupil	4	2.0	0
Postman	2	1.0	0
Confectioner	2	1.0	0
Doctor	2	1.0	0
Cook	2	1.0	0
Student	3	1.5	0
Other	11	5.5	2 (18.2%)
TOTAL	200	100	30 (100%)

positive for HIV, HBV, or HCV markers. Also, none of 200 blood sera samples examined for anti-HEV antibodies, including the anti-HEV IgG positive sera, gave positive results on the presence of HEV RNA in a nested RT-PCR test.

Most of the blood donors (89%, 178/200) lived in villages and rural areas, and most of them had two or more different animal breeds in their backyard (Table 2), but no relationship between contact with a specific species and a higher seropositivity was found. Likewise, no differences were observed between those with a job with a potential high risk for HEV infection and those with a less risky profession (Table 3). For instance, the seroprevalence found was similar in manual workers (17.7%, 16/90) and farmers (15%, 3/20).

Discussion

Lately, a quite high prevalence of HEV antibodies has been documented in industrialized countries [1,4,16,17], but until now, very limited information from Serbia and the surrounding countries of the former Republic of Yugoslavia was available. For instance, a possible endemicity of HEV infection was suggested [21] after an average of 14.4% of anti-HEV IgG positive samples among different Serbian populations, including blood donors (16.9%), was found. More recently, a 6% and 9.6% anti-HEV IgM positivity was found among patients with acute hepatitis in neighboring countries Montenegro and Hungary [22,23]. In the present report, HEV seroprevalence in Serbia was addressed by testing a Serbian blood donor population for the presence of anti-HEV IgG.

The prevalence (15%) of anti-HEV IgG-positive individuals found among Serbian blood donors in the present study is similar to that found previously in an earlier pilot study (16.9%) conducted in the country [21], as well as in some other European countries and in the United States [6,24-26]. However, lower prevalence rates (1% to 5%) have also been described in northern France, Italy and Switzerland [17,27,28], and even an outstanding rate of 52.2% has been recently reported in blood donors from south-western France [29].

Apart from demographic and behavioral factors of the populations studied, it has been suggested that the differences found in the different reports could be due to the various methodologies used, which included commercial kits that, in some instances, present remarkable differences in sensitivity and/or specificity [1,4,14,16]. Although HEV RNA was not amplified in

any of the samples tested, HEV infecting pigs in the same region belonged to genotype 3 (unpublished data found in our study from 2010), and thus it could be expected that most human infections correspond to the same genotype 3 from which the ELISA antigen, a recombinant truncated ORF-2 protein that we used as an antigen in our ELISA test, was derived [19]. The assay used has been previously validated by western blot and compared with a widely used commercial kit [19], showing a quite good specificity (96.4%) and sensitivity (100%), and has been further applied to test for anti-HEV IgG antibodies in pigs from different countries [18,30].

Our data also showed a significantly higher seroprevalence among blood donors over 51 years of age, confirming previous data indicating that older age seems to be a risk factor for anti-HEV positivity [1,24,25,27,31]. In contrast to the data of HEV infection mostly among younger persons in the developing world, in developed countries, most autochthonous HEV infections are reported in middle-aged and elderly populations [1,6]. This phenomenon is still unexplained, but could include host or viral factors. Of these, host factors, such as immune response, age, and pre-existing liver disease, may also play a role in infection [32]. Autochthonous hepatitis E in developed regions is frequently misdiagnosed as drug-induced liver injury, a common problem that occurs with increased frequency in elderly people [1]. It could be assumed that drug-induced liver injury, even of very low intensity or the other influences on liver physiology that usually arise later in human life (in adults and the elderly) are the predisposition factors for HEV infection with virus genotypes 3 and 4 that are mostly present in developed countries. Unlike HEV genotypes 1 and 2 that are found only in humans, genotypes 3 and 4 are found also in animals, including pigs, as main virus reservoirs in nature [1,2], so these viruses maybe have a different affinity for humans, thus resulting in infection of older humans in contrast to the usual HEV genotype 1 and 2 infection in younger humans in endemic developing countries.

ALT values were similar among those blood donors presenting anti-HEV IgG and among those that did not, confirming that these parameters are of low accuracy for HEV screening [17], as the ALT elevation after HEV infection is short in time and occurs once HEV RNA has already being cleared from the blood. Contrary to other studies [31], no relationship between a history of previous HAV infection and anti-HEV IgG positivity was found in our study cohort.

Because of the close genomic similarity between HEV genotype 3 strains infecting humans and pigs [7] and the evidence that infectious virus can be recovered from pork products [3,10], it has been proposed that close contact with pigs or other potential HEV animal reservoirs may represent a risk for HEV infection. Also, several studies have reported an association between occupational exposure to swine and a higher HEV IgG seropositivity [13]. In our study of the blood donor population, no association between HEV positive serology and direct contact with domestic or farm animals, including pigs, was found. Likewise, no higher seroprevalence was found among people working in potentially risky professions, such as farmers, veterinarians, or medical staff, than among those with less potentially risky jobs, such as manual workers and students.

However, since foodborne transmission of HEV had already been reported [2,8,9], as well as the presence of HEV in pork products from markets in many countries [10-12], including Serbia, where HEV has been recently detected in livers (16%) and meat (10%) of two- to three-month-old piglets on the line of slaughterhouse (unpublished data), the obtained high HEV seroprevalence among Serbian blood donors as representatives of the general population may be connected to the traditional food consumption custom of often eating piglet meat in Serbia.

Although an earlier report [21] suggested that a poor socioeconomic situation, unregulated supply of drinking water, and low level of personal and collective hygiene could be related to the high HEV seroprevalence found, this does not seem to be the case in our study, as all the tested blood donors originated from highly developed region in Serbia.

Even though the relatively high prevalence of anti-HEV antibody positive blood donors detected, no HEV RNA positive samples were found among all 200 tested blood sera samples, in line with the very low prevalence of HEV RNA positive blood donors reported in the literature. For example, in a recent study from the Netherlands, only 17 (0.04%) HEV RNA positive samples were detected among 45,415 blood donors [33], and a similar prevalence (0.07%) was also found among 44,816 Chinese blood donors [34].

Conclusions

In summary, the obtained prevalence of anti-HEV IgG among blood donors, which could represent the general population, is quite high in Serbia compared to the available literature data from some other European

countries. According to the zoonotic potential of HEV, one of the reasons for this high prevalence could be the high prevalence of HEV among Serbian pigs and pork products, especially from piglets, and the traditional food consumption custom of often eating barbeques piglet meat in Serbia. Although we found a relatively high seroprevalence (15%) of anti-HEV IgG among Serbian blood donors, none of them presented HEV RNA in their blood; therefore, we cannot conclude whether this actually represents a risk for blood transfusion. Thus, this point surely needs further investigation to determine the real need for HEV screening of blood products.

Acknowledgements

This work was supported by grants CSD2006-0007 and AIB2010SE-00316 from the Spanish Ministerio de Ciencia e Innovación (MICINN), and by grants TR 31084 funded by the Serbian Ministry of Education and Science, and by the bilateral project “Assessment of zoonotic viral activity in Serbia” funded by both Ministries.

Authors' contributions

All the authors of this manuscript have seen and approved the content and have contributed significantly to the work and were actively involved in obtaining the results.

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Conflict of interests: No conflict of interests is declared.