SECOND INTERNATIONAL EPIZOOTIOLOGY SYMPOSIUM

(XIV SERBIAN EPIZOOTIOLOGY DAYS)

PROCEEDINGS

Hotel "Srbija"

April 18-21st, 2012, Belgrade
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8. SEROPREVALENCE OF HEPATITIS E AMONG BLOOD DONORS IN SERBIA

Petrović T., Lupulović D., Vojvodić S., Jiménez de Oya N., Escribano-Romero E., Blázquez A.B., Lazić S., Saiz J.C.*

Abstract

Hepatitis E virus (HEV), an enterically transmitted pathogen, is one of the major causes of acute hepatitis in humans, being responsible for outbreaks and epidemics in regions with sub-optimal sanitary conditions, especially in some parts of Asia, Africa, Mexico and Middle East, in many of which it is endemic. In contrast, hepatitis E is unusually reported in industrialized countries and when reported, it is mostly as sporadic cases in humans who travelled to endemic countries. Recently, some human HEV infection in non-endemic counties could not be explained by the contact of those patients with the virus in the endemic regions. Also, recent studies have revealed quite variable seroprevalence rates among European’s populations and a possible porcine zoonotic transmission has been postulated. Moreover, the human HEV strains described in industrialized countries appears to be closely related to the swine HEV strains found in the same countries. In this regard, and although HEV is highly distributed among Serbian pigs, limited data about HEV seroprevalence in Serbian population are available.

In the present study, the prevalence of anti-HEV IgG antibodies among blood donors in Serbia was investigated by means of an ELISA based on a recombinant open reading frame 2 protein of HEV genotype 3. A total of 200 serum samples from blood donors of different age, sex and occupations, which live in Novi Sad (22/11.06%) and surrounding villages (177/88.94%) in the Vojvodina province, in the northern part of Serbia were examined. We found that 30 (15%) of the 200 analyzed blood donors tested positive in the ELISA test, and that the prevalence increases with age. Anti-HEV IgG positive serum samples had an average P/N of 4.45±1.95 (range 2.5-9.58).

These results indicate that HEV infection is not just occasionally present in human population in Serbia. Further investigation of HEV infection in Serbia is needed for the estimation of the real prevalence and possible health effects on the human population.

Key words: blood donors, HEV serology, ELISA, IgG

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Introduction

Hepatitis E is mostly a waterborne viral disease and was first recognized during an epidemic of hepatitis which occurred in Kashmir valley in 1978. Very soon, hepatitis E was retrospectively traced to an epidemic of acute viral hepatitis which had occurred in Delhi, India in 1955–1956 (Khuroo and Khuroo, 2008). The disease is an important public health problem in many developing countries of Asia and Africa, and is also endemic in many industrialized regions including the United States and several European countries (Meng, 2010). Although the overall mortality of hepatitis E is less than 1% in the general population, it can reach up to 28% in infected pregnant women (Purcell and Emerson, 2001). The etiological agent of disease is hepatitis E virus (HEV) that was recently classified in a new genus Hepeviridae of the family Hepeviridae (Emerson et al., 2004). Hepatitis E has been associated with large waterborne outbreaks of acute hepatitis and sporadic infections due to contamination of water and water supplies, and poor sanitation conditions in developing countries, notably in Asia, Africa and Central America (Seymour and Appleton 2001; Khuroo and Khuroo, 2008; Purcell and Emerson, 2008; Pavio et al., 2010). HEV infection in industrialized countries is sporadic and was often reported in human patients with a history of traveling to endemic countries. However, there are increasing cases of sporadic human HEV infection in non endemic countries without history of traveling. In contrast to regions of endemicity where the water vector has been well characterized, very little is known about the mode of transmission in regions where HEV is nonendemic. Unlike the other hepatitis viruses, HEV has animal reservoirs. After the successful experimental transmission of human HEV to domestic pigs, it was suggested that swine HEV infection may be a zoonosis. The autochthonous origin of the hepatitis E sporadic cases in developed countries is confirmed by several lines of evidence, such as the absence of travel to endemic countries or that HEV strains involved in cases reported from industrialized countries are different than from developing countries (Clemente Casares et al., 2003).

The human HEV discovered in industrialized non endemic countries appears to be closely related to the swine HEV found in the same areas. The discovery in pigs of hepatitis E virus (HEV) strains related to human strains was potentially significant in regard to the possibility of interspecies transfer and zoonotic infection (Lu et al., 2006; Pintó and Saiz, 2007). Accumulating evidences indicate that hepatitis E is a zoonotic disease, and domestic pigs, wild boars and maybe other animal species are reservoirs for HEV. The ubiquitous nature of the virus (genotypes 3 and 4) in domestic pigs and wild boars as well as in other animal species raises public health concern for zoonosis and food safety (Meng, 2010; Kaufmann et al., 2011). Recent studies have indicated that consumption of contaminated and undercooked meat from infected pig species may be a risk factor for acquisition of this disease. Food-borne transmission of HEV was confirmed in people that ate dear, pork, or wild boar uncooked meat and liver (Tei et al., 2003; Yazaki et al., 2003; Matsuda, 2003), and HEV-RNA has been detected in livers sold in local markets (Bouwknecht et al., 2007; Feagins et al., 2007, Yazaki et al., 2003).

Seroprevalence of anti-HEV IgG in the adult population in endemic regions with epidemic and endemic disease varies from 4 to 16% (Assarehazdegan et al., 2008; Khuroo and Khuroo, 2008) or even up to 32.6% in blood donors (Guo et al., 2010). In the industrialized countries of the United States, Europe, and Japan, HEV is responsible
for sporadic cases, and genotyping combined with phylogenetic analysis has shown that
the origin of these cases is mostly autochthonous (mainly genotype 3), thus suggesting
that the virus is present locally (Banks et al., 2004). The seroprevalence of HEV in those
countries varied from 1.1 to 3.9% or even up to 18-21% in certain geographical regions
of the United States (Khuroo and Khuroo, 2008). In Europe (The Netherlands, the
United Kingdom, Spain, Italy, Sweden and Netherlands), the prevalence of anti-HEV
antibodies ranges from 2.2 to 9.3% (Clemente-Casares et al., 2003; Meng et al., 2002;
Olsen et al., 2006; Widdowson et al., 2003; Boutrouille et al., 2007). More recently, a
6% anti HEV IgM positivity was found among Montenegro patients with acute hepatitis
(Terzic et al, 2009).

For reasons that are incompletely understood, high HEV seroprevalence rates
have been found among blood donors in southwest France (16.6%), southwest England
(16%), Denmark (20.6%) and the USA (18.3%), with a large proportion of infections
acquired locally (Christensen et al., 2008; Dalton et al., 2008; Mansuy et al., 2008;
Meng et al., 2002). Zoonotic infections were considered as one of the possible reasons
for this high prevalence (Kaufmann et al., 2011).

In Serbia, the prevalence of anti-HEV antibodies in the general population has
never been studied. Some, very limited, data about HEV seroprevalence in Serbian
human populations or in surrounding countries of the former Yugoslavia are available.
To determine whether hepatitis E is a frequent disease in Serbia and to establish an
additional starting point for further studies on HEV epidemiology, HEV prevalence was
estimated by the presence of anti HEV antibodies in sera from blood donors from Novi
Sad and surrounding area.

Material and Methods
During 2010, serum samples from no remunerated blood donors (n=200) from
Novi Sad and surrounding towns and villages were anonymously collected by the
Institute for Blood Transfusion in Novi Sad. All participants provided written consent
for the use of their blood samples in medical research. Blood donors fulfilled the
Serbian criteria for blood donations and completed a medical questionnaire, including
possible contact with farm or domestic animals.

Blood donor (average age 39.3, range 19-65) samples were routinely tested by
ELISA for HBsAg, anti-HCV, HIV Ag/Ab, and anti-TP (Trepanoma Pallidum Pallidum)
at the Institute for Blood Transfusion. A questionnaire including previous
transfusion records, previous HAV infection, and frequent contact with domestic and
farm animals (pigs, cattle, horses, cats, dogs, sheep, goats, rabbits and hamsters) was
fulfilled. Age and gender were recorded.

Serum anti-HEV IgG was detected by a previously validated ELISA based on
the use of purified truncated ORF-2 HEV genotype 3 recombinant protein expressed in
insect larvae (Jiménez de Oya et al., 2009a; Jiménez de Oya et al., 2009b). 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 ≥ 2.5) were considered
positive. In cases where P/N values were closed the cut-off value, samples were tested
by Western-blot as described (Jiménez de Oya et al., 2009b).

HEV-RNA detection was conducted by a nested RT-PCR as previously
described (Schlauder et al., 1999; Wang et al., 1999; van der Poel et al., 2001).
Chi square test and Chi square test for trend were performed using GraphPad PRISM® (GraphPad Software Inc, La Jolla, CA). Analysis of the variance (ANOVA) was performed with SPSS 15.0 for Windows (SPSS Inc., Chicago, IL).

Results and Discussion

General characteristics of blood donors tested are summarized in Tables 1, 2 and 3. In total, 15% (30/200) of the blood donor resulted positive for anti HEV IgG with an average P/N of 4.45±1.95 (range 2.5-9.58). In 7 cases in which the P/N values were around the cut-off value, ELISA results were confirmed by Western blot.

No significant differences in terms of anti-HEV IgG seropositivity were found between men and women (14.6% and 16.7% of them were positive, respectively; Table 2.). HEV seroprevalence increased with age, as a higher one was recorded in individuals older than 51 years (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.).

**Table 1.** Blood donors data and results of HEV serology investigation (depending on the age)

<table>
<thead>
<tr>
<th>Age</th>
<th>No. of persons</th>
<th>% of persons</th>
<th>% HEV IgG (+)</th>
<th>HEV IgG (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-30</td>
<td>37</td>
<td>18.5</td>
<td>2 (5.4%)</td>
<td>35 (94.6%)</td>
</tr>
<tr>
<td>31-50</td>
<td>98</td>
<td>49.0</td>
<td>14 (14.2%)</td>
<td>84 (85.8%)</td>
</tr>
<tr>
<td>51- &gt;70</td>
<td>65</td>
<td>32.5</td>
<td>14 (21.5%)*</td>
<td>51 (78.5%)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>200</td>
<td>100</td>
<td>30 (15.0%)</td>
<td>170 (85.0%)</td>
</tr>
</tbody>
</table>

*p<0.027

**Table 2.** Blood donors data and results of HEV serology investigation (depending on the gender)

<table>
<thead>
<tr>
<th>Gender</th>
<th>No. of persons</th>
<th>% of persons</th>
<th>% HEV IgG (+)</th>
<th>HEV IgG (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>158</td>
<td>79.0</td>
<td>23 (14.6%)</td>
<td>135 (85.4%)</td>
</tr>
<tr>
<td>Female</td>
<td>42</td>
<td>21.0</td>
<td>7 (16.7%)</td>
<td>35 (83.3%)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>200</td>
<td>100</td>
<td>30 (15.0%)</td>
<td>170 (85.0%)</td>
</tr>
</tbody>
</table>

No relationship between seroprevalence and blood donors occupation was found (Table 3.). For instances, the seroprevalence found was similar in manual workers (17.7%, 16/90) than in farmers (15%, 3/20). None of the 8 blood donors in which a history of passed HAV was recorded presented positive HEV serology and none of the 200 blood donors tested positive for HIV, HVB or HCV markers. Also, none of the anti HEV IgG positive sera give positive results on the presence of HEV RNA in nested RT-PCR test.

The prevalence (15%) of anti-HEV IgG positive individuals found among Serbian blood donors is similar to that found previously in an early pilot study (16.9%) conducted in the country (Delić et al, 2003), but also found (11-20.6%) in other European countries and in the USA (Dremsek et al., 2011; Christensen et al., 2008; Dalton et al., 2008b; Mansuy et al., 2008 and Meng et al., 2002). In contrary, lower
prevalence (1% to 5%) have also being described in Northern France, Italy and Switzerland (Boutrouille et al., 2007; Zanetti and Dawson, 1994; Kaufmann et al., 2011).

Table 3. Blood donors data and results of HEV serology investigation (depending on the occupation)

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

Apart from demographic and behavior 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, including commercial kits, that, in some instances, present remarkable differences in sensitivity and/or specificity (Dalton et al., 2008; Purcell and Emerson, 2010). In our study, an in house ELISA based on the use of purified truncated ORF-2 HEV genotype 3 recombinant protein was used. Although HEV-RNA was not amplified in any of the samples tested, HEV found in pigs in the same region belongs to genotype 3 (Petrović et al., 2010). So, it could be expected that most human infections correspond to the same HEV genotype 3 from which the ELISA antigen, a recombinant truncated ORF-2 protein, was derived (Jiménez de Oya et al, 2009a). The assay used have been previously validated by Western blot and compared with a widely used commercial kit (Jiménez de Oya et al., 2009a; 2009b), showing a quite good specificity (96.4%) and sensitivity (100%). Also, the ELISA test based on the same antigen was previously successfully used for testing of anti-HEV IgG antibodies in pigs from different countries (Lupulović et al., 2010, Jiménez de Oya et al., 2011).

Our data also showed a significant higher seroprevalence among blood donors over 51 years of age, confirming previous data indicating that in older population higher HEV seropositivity could be found (Christensen et al., 2008, Meng et al., 2002;
Boutroille et al., 2007, Dalton et al., 2008). The reasons for this are not known, but it could be due to the higher HEV activity in the past period or a bigger probability to acquire HEV infection during the longer lifetime.

Possibility of zoonotic and food borne transmission of HEV were already reported (Tei et al., 2003; Yazaki et al., 2003; Matsuda, 2003; Lu et al., 2006; Pinto and Saiz, 2007; Meng, 2010). Connected to that, it has been proposed that close contact with pigs, or other potentially HEV animal reservoir, may represent a risk for HEV infection. Also, an association between occupational exposure to swine and a higher HEV IgG seropositivity have been reported (Wilhelm et al., 2011). In our study no association were found between HEV positive serology and people working in possible risk professions, such as farmers, veterinarians, or medical staff, than among those with less potentially risky jobs, such as manual workers, students, etc.

Conclusion

The HEV transmission through blood transfusion is known especially in HEV endemic regions (Khuroo et al., 2004). Even we have found a relatively high seroprevalence (15%) of anti-HEV IgG among Serbian blood donors; none of them presented HEV-RNA in their blood, so we cannot conclude whether this relatively high anti-HEV seroprevalence actually represents a risk for blood transfusion. Concerning to this and regarding the data from our analysis further investigations is needed to determine the real risk of HEV infection through blood transfusion in Serbia.

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СЕРОПРЕВАЛЕНЦА ХЕПАТИТИСА Е КОД ДОБРОВОЉНИХ ДАВАЛАЦА КРВИ У СРБИЈИ

Петровић Т., Лупуловић Д., Војводић С., Jiménez de Oya N., Escribano-Romero E., Blázquez A.B., Лазић, С., Saiz J.C.

Кратак садржај

Хепатитис Е вирус (HEV), патоген који се преноси феко-оралним путем, један је од главних узрока акутног хепатитиса код људи, нарочито у регионима са субоптималним санитарним условима, у којима се је одговоран за избијање епидемија. Побесно се то односи на неке депове Азије, Африке, Мексика и Блиског Истока, у којима је најчешће и ендемски присутан. Насупрот томе, хепатитис Е се ретко јавља у индустријализованим земљама, углавном као спорадични случајеви код људи који су путовали у ендемске земље. Недавно, HEV инфекција код неких пацијената у неким ендемским земљама се није могла објаснити контактом са болесником код ендемским подручјима. Такође, новија испитивања су утврдила веома различити ниво сропреваленца међу европским становништвом, а постављена је и могућност зооотског преноса вируса са свиња. Штавише, HEV утврђен код људи у индустријализованим земљама је био веома сличан са HEV који су нађени код свиња у истим тим земљама. У том смислу и с обзиром да је већ предходно утврђено да је ХЕВ је високо превалентан у популацији свиња у Србији, постоје изузетно ограничени подаци о сропреваленци HEV у хуманој популацији у Србији.

У овом раду, испитивана је преваленцата антитела против вируса HEV код добровољних давалаца крви у Србији и то помоћу ЕЛИСА теста заснованог на рекомбинантног антитен на кодираног од стране ORF2 дела генома генотипа 3 HEV. Испитано је укупно 200 узораца серума добровољних давалаца крви различитог узраста, пола и занимања, који живе у Новом Саду (22/11.06%) и околним селима (177/88.94%) у Војводини, у северном делу Србије. Установили смо да је 30 (15%) од 200 анализираних давалаца крви реаговало позитивно на ЕЛИСА тесту, и да се сропреваленца повећавала са годинама старости добровољних давалаца. Узорци крвних серума позитивни на IgG антитела против HEV су имали просечан P/N однос у ЕЛИСА тесту од 4,45 ± 1,95 (у опсегу 2,5-9,58).

Добијени резултати испитивања указују на то да HEV инфекција није само спорадично присутна у хуманој популацији у Србији. Даља истраживања HEV инфекције у Србији су неопходна за процену њене реалне учењенности и могућих здравствених ефеката на хуману популацију.

Кључне речи: добровољни даваоци крви, HEV серологија, ЕЛИСА, IgG