Low Hepatitis E virus prevalence among blood donor in Dali in China

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Research

Abstract

BACKGROUND: Hepatitis E virus (HEV) is a nonenveloped RNA virus causing Hepatitis E worldwide. An increasing transfusion transmission cases of HEV infections from asymptomatic blood donors which causing serious illnesses in immunosuppressed recipients have been reported in the past few years. China is one of the highly prevalent regions of HEV, it is important to evaluate the risk of HEV transmission from blood transfusion.

METHODS: A total of 1864 serum samples from blood donors and demographic characteristics were randomly collected from Feb to Mar 2018 in Dali city. Anti-HEV IgG, IgM and IgA antibodies and HEV antigen were examined by enzyme-linked immunosorbent assay (ELISA). HEV RNA was detected by real-time PCR. Multivariable logistic regression modelling was used to examine risk factors associated with HEV prevalence.

RESULTS: Overall, the positive rate of anti-HEV IgG, IgM, and IgA antibodies was 13.36% (249/1864), 1.13%(21/1864), and 1.82%(34/1864), respectively. However, none of the 1864 serum samples was detected as HEV antigen-positive nor HEV RNA positive. The positive rate of anti-HEV IgG antibody is high as 28.57% (2/7) in the donors with isolated elevated alanine aminotransferase (ALT). Females (16.69%) had a significantly higher HEV seroprevalence than males (13.04%) (odds ratio [OR]: 1.34 [95% CI, 1.02-1.75]). Other ethnic minority (24.32%) and Bai (18.85%) donors had a significantly higher HEV seroprevalence when compared to Han (12.21%) blood donors (odds ratio [OR], 2.25 [95% CI, 1.04-4.88] for other ethnic minority, 1.65 [95% CI, 1.24-2.19] for Bai).

Conclusions: Dali, Yunnan province, China is an endemic region f of hepatitis E virus, and women, Bai and other ethnic minorities may be potential risk factors for hepatitis E virus infection.. The risk of transmission of hepatitis E virus through blood transfusion is relatively low, and the specific risk value needs to be further tested by expanding the sample size. Whether to formulate the strategy for HEV screening in blood center needed further researched.

Background

Hepatitis E virus (HEV) is an enterically RNA virus that can cause Hepatitis E all over the world. Like hepatitis A virus infection, only a portion of infections has HEV-related symptoms [1].

In July 2019, the World Health Organization (WHO) reported that about 20 million people infected by HEV each year and 44 000 people died in 2015. China is one of the highly prevalent regions of HEV with a seroprevalence from 0.01% to 48% [2]. A very large outbreak of hepatitis E was reported in the Xinjiang Uighur Autonomous Region during 1986-1988, causing 119,280 cases and more than 700 deaths [3].

HEV is usually transmitted through drinking water and food which contaminated by faeces of HEV infectors[3]. Since water supplies and sanitary infrastructures have been improved, animals have become a major source of human HEV infection [4]. Moreover, an increasing transfusion transmission cases of HEV infections from asymptomatic blood donors which causing serious illnesses in immunosuppressed recipients have been reported in the past few years [5-11]. To protect the patient from transfusion-acquired HEV infection, blood components are implemented by HEV screening before they were provided to at-risk patients in the United Kingdom[12], and all blood recipient in Switzerland.[13]

Dali is a traffic fortress and a famous tourist city with a complex population and epidemic background of infectious diseases in western Yunnan in China. People in Dali eat raw or undercooked food and contact with animals frequently, with the relatively low living standard of resident, causing a higher HEV infection rate [14]. This study is to provide an estimation of HEV prevalence among blood donors in Yunnan in China, to evaluate the risk of HEV transmission by blood donation and to identify the risk factors associated with HEV infection.

Methods

Sample collection

This study was approved by the ethics committees of the Institute of Blood Transfusion, Chinese Academy of Medical Sciences (IBT). A total of 1864 donation blood samples were obtained randomly from Feb to Mar 2018 in Dali, China. All donor samples were tested for Alanine aminotransferase (ALT), HBsAg, anti-HCV, anti-HIV-1/2, and syphilis during routine donor screening. Questionnaires about demographic and donation characteristics were voluntarily filled in by the donors, including gender, age, race/ethnicity, education, occupation, donation times, and history of consumption of raw food such as beef, mutton or milk. Test samples were stored in -80°C freezers at blood center until they were shipped in batches to IBT in dry ice type environments. Among 1864 individual donors, 67.86% were males, 55.79% were Han, 61.53% were middle school and below educated, 34.07% were farmers, 79.61% were married, 50.70% were first donors, 98.39% having no raw milk diet history, and 98.23% having no raw meat diet history (Additional file 1. Fig S1.).

Detection of Anti HEV-IgG, Anti HEV-IgM and HEV IgA antibodies

ELISAs for detection of anti-HEV antibodies were established by using HEV-like-particles (HEV-LPs) as the antigen, which was produced by recombinant baculoviruses [15]. Microplates (96-well) were coated with 200 ng/well HEV-LPs with 0.05 M carbonate-bicarbonate buffer (pH 9.6) at 4°C overnight and blocked with 100 μl 10% Non-fat milk (Sigma, China) at 37°C for 2 h. After washing with PBS-T three times, 100 μL of 1:200 diluted plasma samples were added and incubated at 37°C for 1 h. After five times washing, each well was supplemented with 100 μl of horseradish peroxidase (HRP)-conjugated goat anti-human IgG (1:20000 diluted) (Cappel, Durham, NC) or IgM (1:10000 diluted) (Bethyl, USA) or IgA (1:10000 diluted) (Bethyl, USA) antibody and incubated at 37°C for 1 h. Then the plates were washed four times with PBS-T, added 100 μl of TMB/H_2O_2 (Beyotime, Shanghai, China), and incubated at darkroom for 15 min at room temperature. The enzymatic reaction was stopped with 50 μl 0.3 M sulphuric acid and the optical density (OD) values were measured at 450 nm. A cut-off value was determined on the mean OD_{450nm} value of the negative control (NC) by the formula: Cut-off = 2.1 * NC_{mean}. Values of OD_{450nm} < Cut-off

indicated a negative sample and ≥ Cut-off indicated a positive sample. Ten plasma samples collecting from donors without a history of HEV infection were used as a negative control.

Detection of capsid antigen of HEV

HEV antigen was detected by a two-step incubation antibody-based sandwich ELISA kit (Wantai, Beijing, China). The procedure was carried out according to the manufacturer's instruction. The cutoff values of the assay were statistically established as the mean optical density value of negative controls at 450-nm optical wavelength plus 0.12.

HEV-RNA detection

Nucleic acids were extracted from 200 μL of each sample using the Magen virus RNA kit (Shanghai, China), according to the instructions of the manufacturer. HEV RNA detection was accomplished by TaqMan® real-time fluorescence reverse transcription-polymerase chain reaction (RT-PCR). After extraction of viral RNA from 200 μl of serum by a viral DNA/RNA mini kit (Magen, Shanghai, China), 30 μl of diethylpyrocarbonate (DEPC)-treated water was added. For TaqMan® RT-PCR, the 20 μl reaction contained 4 μl of 5× QuantiTect Probe RT-PCR kit Master Mix (Magen, Shanghai, China), 0.2 μl of enzyme, 10 μl of RNA, and primers and probe at concentrations of 250 and 100 nM, respectively. The primers and probe were widely used in many reports for HEV four major genotypes based on the multiple sequence alignments of 27 sequences of the ORF3 region [16]. PCR was performed on a sequence detection system platform (ABI Prism 7500, Applied Biosystems) as follows: Reverse transcription was carried out at 50°C for 5 min, followed by denaturation at 95°C, then 40 cycles of denaturation at 95°C for 10 seconds and annealing and extension at 60°C for 30 seconds. The TaqMan® assay detected as few as 5 genome equivalent (GE) copies of HEV plasmid DNA. The sequence of the plasmid is:

5'-GCAGACTATCGCTGATGGTAAGGCCCATTTTACAGAGACTGTTAAACCTGTGCTTGATCTTACAAATTCTATCGTACAGCGGATAGAATGAATAACATGTTTTGTGCATTG 3'.

Statistical analysis

Chi-square test was used to assess the anti-HEV IgG, IgM, IgA positive rate by donor's demographic, donation characteristics, and history of consumption of raw food.   The multivariable logistic regression model was then fit to examine factors associated with anti-HEV positivity. The traditional principle was used to define HEV seroprevalence: results of anti-HEV IgG in combination with IgM, whatever anti-HEV IgG or IgM was positive, it was thought as HEV seropositive which was used in this study. The multivariable logistic regression model was fit to examine factors associated with HEV seroprevalence. All statistical analyses were performed using the statistical software package SPSS17.0 (SPSS Inc., Chicago, IL). A p-value of 0.05 or less was considered significant.

Results

HEV seroprevalence

Among 1864 blood samples collected from donors in Dali blood center, the positive rate for anti-HEV IgG, IgM, and IgA among Dali donors was 13.35% (249/1864), 1.12% (21/1864), and 1.82% (34/1864), respectively (Table 1). When donors with any reactive results of anti-HEV IgG, IgM, or IgA, they were defined as HEV seropositive. The HEV seroprevalence was 14.22% (265/1864, IgG or IgM reactive) (as listed in Table 1).

Table 1. HEV serologic test results among 1864 blood samples in Dali

HEV biomarkers	Reactive Number	Reactive rate (%)
Anti-HEV IgG	249	13.36
Anti-HEV IgM	21	1.13
Anti-HEV IgA	34	1.82
Anti-HEV IgG+IgM	5	0.27
Anti-HEV IgG+IgA	21	1.13
HEV seroprevalence	265	14.22
Anti-HEV IgG/IgM/IgA	278	14.91

Anti-HEV IgG+IgM: has reactive results of both anti-HEV IgG and anti-HEV IgM; HEV seroprevalence: has any reactive result of anti-HEV IgG and anti-HEV IgM; HEV seroprevalence: has any reactive result of anti-HEV IgG, anti-HEV IgM and anti-HEV IgA)

HEV antigen and HEV RNA screening results

Among 1864 donation samples, none of them was detected as HEV antigen-positive nor HEV RNA positive.

Thirteen samples were identified as unqualified, of which 7 samples were with ALT level higher than 50U/L which is the blood screening limit in China, and 6 samples were HBsAg/anti-HEV reactive or in gray zone. 38.46% (5/13) were detected as anti-HEV IgG reactive. The positive rate of anti-HEV IgG antibody is high as 28.57% (2/7) in the donors with isolated elevated alanine aminotransferase (ALT). (Table 2).

Table 2. HEV results of routine screening unqualified or gray zone samples

	S/CO*	anti-HEV IgG	anti-HEV IgM	anti-HEV IgA	HEV Antigen	HEV RNA	
HBsAg	0.66	+	-	-	-	-	-
	1.65	+	-	-	-	-	-
	0.97	-	-	-	-	-	-
	0.67	-	-	-	-	-	-
anti-HIV	1.64	-	-	-	-	-	-
	0.61	+	-	-	-	-	-
Value		anti-HEV IgG	anti-HEV IgM	anti-HEV IgA	HEV Antigen	HEV RNA	
ALT	52U/L	-	-	-	-	-	-
	55U/L	+	-	-	-	-	-
	69U/L	-	-	-	-	-	-
	51U/L	-	-	-	-	-	-
	51U/L	-	-	-	-	-	-
	51U/L	+	-	-	-	-	-
	52U/L	-	-	-	-	-	-

*: enzyme-linked immunosorbent assay (ELISA) signal-to-cutoff (S/CO) ratios: S/CO ≥ 1.0 is considered reactive; 0.5<S/CO<1 is considered gray zone.

+: reactive; -: Nonreactive.

Risk factors

The results of chi-square test indicated that Han population had a significantly lower prevalence than Bai ($P<0.001$) and other ethnic minority ($P<0.05$) in anti-HEV IgG and HEV seroprevalence, (Fig 1. A-B). Females have a significantly higher prevalence than males in anti-HEV IgM (1.84% vs. 0.79%) and HEV seroprevalence (16.69% vs. 13.04%) ($P<0.05$) (Fig 1. C-D). In the multivariable logistic regression analysis of HEV seroprevalence, females (16.69%) had a significantly higher seroprevalence than males (13.04%) (odds ratio [OR]: 1.34 [95% CI, 1.02-1.75]). Other ethnic minority (24.32%) and Bai (18.85%) donors had a significantly higher seroprevalence when compared to Han (12.21%) blood donors (odds ratio [OR], 2.25 [95% CI, 1.04-4.88] for other ethnic minority, 1.65 [95% CI, 1.24-2.19] for Bai) (Fig 1.E). (Additional file 2,3)

Similar results of chi-square test and multivariable analysis were also found in anti-HEV IgG/IgM/IgA when compared to Han blood donors (OR, 2.25 [95% CI, 1.04-4.88] for other ethnic minority, 1.73 [95% CI, 1.31-2.30] for Bai) (*Fig 2.*) (*Additional file 2,3*) No statistically significant difference in anti-HEV IgG, anti-HEV IgM, anti-HEV IgA, HEV seroprevalence, anti-HEV IgG/IgM/IgA was found by age, education, occupation, married status, donation times, and diet history (Additional file 2)

Discussion

Yunnan, a HEV high endemic region reported by the data-center of China public health science (http://www.phsciencedata.cn), showed a rapidly growing trend of hepatitis E incidence, ranging from 1.83% to 3.01% between 2014-2016 (1.83 in 2014, 2.60 in 2015, 3.01 in 2016, Mean: 2.48 ± 0.60 per 100,000 person-years). Although Chinese blood centers do not routinely perform screening testing for HEV yet, anti-HEV IgG and anti-IgM prevalence among blood donors in various Chinese regions have been reported by many articles [17]. This study showed a significantly lower prevalence of anti-IgG (13.35% vs 38.35%), an equal prevalence of antigen (0.00% vs 0.00%), HEV RNA (0.00% vs 0.00%) and anti-IgM (1.12% vs 1.13%) among blood donors in Dali in western of Yunnan in China compared with Ren's study[18]. The differences were in part due to performance characteristics of the anti-HEV IgG assay, and different sources of the subject population (different cities in Yunnan).

Theoretically, the presence of HEV antigen, HEV RNA, and anti-HEV IgM provides evidence of recent HEV infection[19, 20].As anti-HEV IgM indicates a recently acquired infection [21], the IgA class was firstly described in the early 90s to reflect a recent HEV infection together with IgM. It has been reported that it should increase the specificity of the two single assays and help to minimize false positives and erroneous diagnosis [21-24]. A study was recently reported from the Netherlands in which 5239 donors were tested for anti-HEV IgM and those who were positive were tested for HEV RNA. Overall, 17 HEV RNA positive samples were detected.[25] Although no HEV antigen or HEV RNA positive samples were detected in the 1864 samples included in this study, which is similar to the results of Ren F[26], our study detected 21(1.13%) anti-HEV IgM positive samples that also represented recent infection. When combined with anti-HEV IgA, the recent infection rate reached 2.95%.

Existing studies have shown that the prevalence of HEV RNA among blood donors is 0.045% in France[27], 0.031% in Netherlands[25] and 0.03% in Spain[7]. Although none of the HEV RNA or HEV antigen positive samples were detected in this study, this may be caused by the limited sample size, only 1864 cases. Therefore, this does not mean that transfusion will not be infected HEV. It only means that the risk of HEV transmission by blood donation may not be as high

as we thought, but the risk still exists, as 1.31% (21/1864) of blood donors have tested positive for anti-HEV IgM, which represents recent HEV infection. It is suggested that scholars who study the risk of HEV transmission by blood donation should increase the sample size.

Zhou Shiyi et. al reported a 44.42% anti-HEV IgG prevalence and a 0.78% anti-HEV IgM prevalence in HIV-infected patients, which was in consist with our results (a 50% anti-HEV IgG prevalence in anti-HIV reactive donors) [28] (Table 2).

Some earlier population-based studies reported that the HEV antibody prevalence varied significantly by age among donors and it was significantly higher among male donors than female donors [18, 29, 30]. Our data also showed that anti-HEV IgG/IgM was higher in female donors (16.69%) than that in male donors (13.0%). What's more, other ethnic minority and Bai had a significantly higher anti-HEV IgG/IgM prevalence ((24.32% and 18.55%, respectively) than Han (12.21%).

In the multivariable logistic regression analysis by gender and race/ethnicity in this study, females, Bai and other ethnic minority might be the potential risk factors for HEV infection (OR, 1.34 [95% CI, 1.02-1.75] for females compared to males, 2.25 [95% CI, 1.04-4.88] for other ethnic minority and 1.65 [95% CI, 1.24-2.19] for Bai compared to Han) (Fig 1). We speculate that it may be caused by the poor living conditions, polluted water sources and the custom of eating raw meat in Bai and other ethnic minorities. It is believed that consumption of uncooked or undercooked infectious HEV contaminated meat or milk shall be a new zoonotic source that bears a high risk of transmitting to human [31-33]. As 70.27% of the Goats (52/74) and 37.14% of the cows were found HEV RNA positive in Dali [34], significantly higher than that in other regions (3.26% in Yaks in the northwest of China, 3.15% in cattle in Shandong) [35, 36]. We wonder whether the HEV prevalence is related to the consumption of uncooked meat or milk. In this study, no significant differences in HEV seroprevalence estimates were found by the consumption of uncooked milk or meat (beef or mutton). The reason might be that in this study, our sample size is relatively small, and only a small percentage of donors admitted to having a history of raw food. The limitation of this paper is not to investigate the correlation between the consumption of uncooked pork and HEV prevalence.

Conclusions

In general, Dali in Yunnan in China is an endemic region of hepatitis E virus, and women, Bai and other ethnic minorities may be potential risk factors for hepatitis E virus infection. The risk of transmission of hepatitis E virus through blood transfusion is relatively low, and the specific risk value needs to be further tested by expanding the sample size. Whether to formulate the strategy for HEV screening in blood center needed further researched.

Declarations

Abbreviations

HEV⬚ Hepatitis E virus⬚RNA⬚Ribonucleic Acid⬚DNA⬚deoxyribonucleic acid⬚WHO⬚World Health Organization⬚IBT⬚Institute of Blood Transfusion⬚ALT⬚Alanine aminotransferase⬚HEV-LPs⬚HEV-like-particles

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