Low Hepatitis E Virus Prevalence Among Blood Donor in Dali in China

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Research

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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 for HEV and have a relatively low risk of HEV transmission via blood transfusion. Whether to formulate the strategy for HEV screening in blood center needed further researched.

Background

Hepatitis E virus (HEV) is an enterically transmitted Ribonucleic Acid (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]. HEV viruses were classified into subtypes 1-8, with genotypes 1 and 2 being only human pathogens, highly endemic in Asia, Africa, the Middle East, and Mexico[2], causing 20 million new infections and 70,000 deaths from acute liver failure every year[3]. In contrast, HEV-3 and HEV-4 are zoonotic viruses that not only infect humans but also swine, wild boar, deer, rabbits, wild birds and shellfish[4-7]. Genotypes 5 and 6 have been only isolated from wild boar in Japan[8], whereas genotypes 7 and 8 were isolated from camels[9, 10]. Genotype 7 was also isolated from human[11].

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% [12]. 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 [13].

HEV is usually transmitted through drinking water and food which contaminated by faeces of HEV infectors [12]. Since water supplies and sanitary infrastructures have been improved, animals have become a major source of human HEV infection [11]. 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 [14-20]. 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[21], and all blood recipient in Switzerland.[22]

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 [23]. If this kind of people enters the donor team, it will threaten blood safety. This study is to provide an estimation of HEV prevalence among blood donors in Yunnan in China 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 (Fig 1).

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 [24]. 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/H₂O₂ (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 [25]. 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:

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5'- GCAGACTATCGCTGATGGTAAGGCCCATTTTACAGAGACTGTAAACCTGT  
GCTTGATCTTACAAATTCTATCGTACAGCGGATAGAATGAATAACATGTT  
TTGTGCATTGCCCATGGGGTCACCATGTGCCCTAGGGCTGTTCTGTTG  
CTGTTCTTCGTGCTTCTGCCTATGCTGCCCCGCGCCACCGGCCGCCAG  
CCGTCTGGCCGCCGTCTGTTGGGCGGCGCAGCGGCGGTACCGGCGGTGG  
TTTCTGGGGTGACAGGGTTGATTCTCAGCCCTTCGCCCTCCCCTATATT  
CATCCAACCAACCCCTTCGCCGCCGATGTCGTTTCACAATCCGGGGCT  
GGAGCTCGCCCTCGACAGCCGCCCGCCCCCTTGGCTCCGCTTGGCGTGA-3'.
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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. Another principle was also used: results of anti-HEV IgG combined with both IgM and IgA, whatever anti-HEV IgG or IgM or IgA was positive, it was thought as HEV seropositive. 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) or 14.91% (278/1864, IgG or IgM or IgA 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
HEV seroprevalence (IgG combined with both IgM and IgA)	278	14.91

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, HEV seroprevalence and HEV seroprevalence (anti-HEV IgG results combined with IgM and IgA), (Fig 2. A-C). 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 2. D,E). No statistically significant difference in anti-HEV IgG, anti-HEV IgM, anti-HEV IgA, HEV seroprevalence, HEV seroprevalence (anti-HEV IgG results combined with IgM and IgA) was found by age, education, occupation, married status, donation times, and diet history (*Additional file 1*).

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 3). Similar multivariable analysis results were also found in HEV seroprevalence which was based on anti-HEV IgG results combined with both IgM and 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 4.*) (*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. A systematic review and meta-analysis which was published in 2016 showed that the pooled prevalence of HEV infective biomarkers (anti-HEV IgG, anti-HEV IgM, RNA and antigen) among mainland Chinese blood donors was 29.2%, 1.1%, 0.1% and 0.1%, respectively [26]. Ren et al. reported that the prevalence of anti-HEV IgG, anti-HEV IgM and antigen among blood donors in Yunnan province in China was 38.35%, 1.13% and 0.00%, respectively [27]. Dali is a traffic fortress and a famous tourist city with a complex population and epidemic background of infectious diseases in western Yunnan. 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. 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). While Li et al. described the positive rate of anti-HEV IgG, anti-HEV IgM, and HEV RNA was 62.6%, 3.84%, and 12.5%, respectively in Dali among the general population [28], significantly higher than that in this study. It might be caused by blood donors represent lower risk individuals than the general population.

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) [29] (Table 2). Donors with isolated ALT elevation also had a significantly higher anti-HEV IgG positivity than qualified donors (28.57%, 2/7; vs 7.92%, 256/1970), resembling many other articles (33.3% vs 24.9% by Wang Meiyu, 40.02% vs 27.42% by Ren Furong) [27, 30].

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 [27, 31, 32]. Our data showed there were no significant differences in HEV seroprevalence estimates by age, education, occupation, married status, donation times (*Additional file 1*) and HEV seroprevalence was higher in female donors (16.69%) than that in male donors (13.0%). Other ethnic minority and Bai had a significantly higher prevalence ((24.32% and 18.55%, respectively) than Han (12.21%). HEV transmitted from undercooked/raw organs or tissues of HEV-infected animals to human beings have been widely reported [33, 34]. 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 [35-37]. As 70.27% of the Goats (52/74) and 37.14% of the cows were found HEV RNA positive in Dali [38], significantly higher than that in other regions (3.26% in Yaks in the northwest of China, 3.15% in cattle in Shandong) [39, 40], people ate raw milk or meat from HEV infective cows or goats might have a higher risk to be infected with HEV. If this kind of population enters the blood donor troop, it will cause a serious threat to the safety of blood transfusion. 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.

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 3). It was reported that the positive rate of HEV-specific IgG in pigs was 78.9% (490/621) [41] in Yunnan, and the prevalence of HEV RNA was 33.3% in retail pork meat in Yunnan [42]. Phylogenetic and molecular evolutionary analysis revealed that HEV isolates from human and pigs shared 84.2% to 100.0% nucleotide sequence identity with each other, with all belonging to genotype 4 HEV [41]. The reason might be that the Bai nations like to eat uncooked or undercooked meat, especially pork. The limitation of this paper is not to investigate the correlation between the consumption of uncooked pork and HEV prevalence.

As anti-HEV IgM indicates a recently acquired infection [43], the IgA class was firstly described in the early 90s to reflect a recent HEV infection together with IgM. Although the role of the anti-HEV IgA in combination with IgM in the diagnosis of HEV infection is still on the debate, it has been reported that it should increase the specificity of the two single assays and help to minimize false positives and erroneous diagnosis [43-46]. In this study, the prevalence of recent HEV infection among blood donors was 1.13% (21/1864) based on the anti-HEV IgM positivity, while it increased to 2.95% (55/1864) while IgA combination with IgM. The HEV seroprevalence was 14.22% (265/1864) only based on the anti-HEV

IgG results in combination with IgM reactive results while it slightly increased to 14.91% (278/1864) When it was based on the anti-HEV IgG results combined with both IgM and IgA (Table 1).

Conclusions

In general, Dali in Yunnan in China is an endemic region for HEV and have a relatively low risk of HEV transmission via blood transfusion. Whether to formulate the strategy for HEV screening in blood center needed further researched.

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

Declarations

Ethics approval and consent to participate: This study was approved by the ethics committees of the Institute of Blood Transfusion, Chinese Academy of Medical Sciences (IBT).

Consent for publication: Not applicable.

Availability of data and materials: The data analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare no potential conflict of interest.

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Authors' contributions: Ping Fu, Baochai Lin: Conceptualization, methodology, software, investigation, study execution, original draft preparation; Bingting Wu, Ling Ke, Tianfu Yang, Yue'e Du, Lishan Cheng¹, Zhou Li: resources, data curation, samples collection; Tiancheng Li, Yu Liu: supervision, project administration. All authors have read and agreed to the published version of the manuscript. All authors have read and agreed to the published version of the manuscript.

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References

1. Rein DB, Stevens GA, Theaker J, Wittenborn JS, Wiersma ST. The global burden of hepatitis E virus genotypes 1 and 2 in 2005. *Hepatology*. 2012 Apr;55(4):988-97.

2. Kamar N, Izopet J, Pavio N, Aggarwal R, Labrique A, Wedemeyer H, Dalton HR. Hepatitis E virus infection. *Nat Rev Dis Primers* 2017, 3:17086.
3. Rein DB, Stevens GA, Theaker J, Wittenborn JS, Wiersma ST. The global burden of hepatitis E virus genotypes 1 and 2 in 2005. *Hepatology* 2012, 55:988-997.
4. Colson P, Borentain P, Queyriaux B, Kaba M, Moal V, Gallian P, Heyries L, Raoult D, Gerolami R. Pig liver sausage as a source of hepatitis E virus transmission to humans. *J Infect Dis* 2010, 202:825-834.
5. Dremsek P, Wenzel JJ, Johne R, Ziller M, Hofmann J, Groschup MH, Werdermann S, Mohn U, Dorn S, Motz M, et al. Seroprevalence study in forestry workers from eastern Germany using novel genotype 3- and rat hepatitis E virus-specific immunoglobulin G ELISAs. *Med Microbiol Immunol* 2012, 201:189-200.
6. Chaussade H, Rigaud E, Allix A, Carpentier A, Touzé A, Delzescaux D, Choutet P, Garcia-Bonnet N, Coursaget P. Hepatitis E virus seroprevalence and risk factors for individuals in working contact with animals. *J Clin Virol* 2013, 58:504-508.
7. Zhang X, Bilic I, Troxler S, Hess M. Evidence of genotypes 1 and 3 of avian hepatitis E virus in wild birds. *Virus Res* 2017, 228:75-78.
8. Takahashi M, Nishizawa T, Sato H, Sato Y, Jirintai, Nagashima S, Okamoto H. Analysis of the full-length genome of a hepatitis E virus isolate obtained from a wild boar in Japan that is classifiable into a novel genotype. *J Gen Virol* 2011, 92:902-908.
9. Lee GH, Tan BH, Teo EC, Lim SG, Dan YY, Wee A, Aw PP, Zhu Y, Hibberd ML, Tan CK, et al. Chronic Infection With Camelid Hepatitis E Virus in a Liver Transplant Recipient Who Regularly Consumes Camel Meat and Milk. *Gastroenterology* 2016, 150:355-357.e353.
10. Woo PC, Lau SK, Teng JL, Cao KY, Wernery U, Schountz T, Chiu TH, Tsang AK, Wong PC, Wong EY, Yuen KY. New Hepatitis E Virus Genotype in Bactrian Camels, Xinjiang, China, 2013. *Emerg Infect Dis* 2016, 22:2219-2221.
11. Woo PC, Lau SK, Teng JL, Tsang AK, Joseph M, Wong EY, Tang Y, Sivakumar S, Xie J, Bai R, et al. New hepatitis E virus genotype in camels, the Middle East. *Emerg Infect Dis* 2014, 20:1044-1048.
12. Hepatitis E. <https://www.who.int/en/news-room/fact-sheets/detail/hepatitis-e>. Accessed 27 July 2020.
13. Zhuang H, Cao XY, Liu CB, Wang GM. Epidemiology of hepatitis E in China. *Gastroenterol Jpn* 1991, 26 Suppl 3:135-138.
14. Hewitt PE, Ijaz S, Brailsford SR, Brett R, Dicks S, Haywood B, Kennedy IT, Kitchen A, Patel P, Poh J, et al. Hepatitis E virus in blood components: a prevalence and transmission study in southeast England. *Lancet* 2014, 384:1766-1773.
15. Baylis SA, Gärtner T, Nick S, Ovemyr J, Blümel J. Occurrence of hepatitis E virus RNA in plasma donations from Sweden, Germany and the United States. *Vox Sang* 2012, 103:89-90.
16. Sauleda S, Ong E, Bes M, Janssen A, Cory R, Babizki M, Shin T, Lindquist A, Hoang A, Vang L, et al. Seroprevalence of hepatitis E virus (HEV) and detection of HEV RNA with a transcription-mediated

- amplification assay in blood donors from Catalonia (Spain). *Transfusion* 2015, 55:972-979.
17. Hogema BM, Molier M, Sjerps M, de Waal M, van Swieten P, van de Laar T, Molenaar-de Backer M, Zaaier HL. Incidence and duration of hepatitis E virus infection in Dutch blood donors. *Transfusion* 2016, 56:722-728.
 18. Juhl D, Baylis SA, Blümel J, Görg S, Hennig H. Seroprevalence and incidence of hepatitis E virus infection in German blood donors. *Transfusion* 2014, 54:49-56.
 19. Westhölter D, Hiller J, Denzer U, Polywka S, Ayuk F, Rybczynski M, Horvatits T, Gundlach S, Blöcker J, Schulze Zur Wiesch J, et al. HEV-positive blood donations represent a relevant infection risk for immunosuppressed recipients. *J Hepatol* 2018, 69:36-42.
 20. Beale MA, Tettmar K, Szypulska R, Tedder RS, Ijaz S. Is there evidence of recent hepatitis E virus infection in English and North Welsh blood donors? *Vox Sang* 2011, 100:340-342.
 21. Tedder RS, Ijaz S, Kitchen A, Ushiro-Lumb I, Tettmar KI, Hewitt P, Andrews N. Hepatitis E risks: pigs or blood-that is the question. *Transfusion* 2017, 57:267-272.
 22. Niederhauser C, Widmer N, Hotz M, Tinguely C, Fontana S, Allemann G, Borri M, Infanti L, Sarraj A, Sigle J, et al. Current hepatitis E virus seroprevalence in Swiss blood donors and apparent decline from 1997 to 2016. *Euro Surveill* 2018, 23.
 23. Li LJ, Shen YY, Ai ZQ, Guo L, Geng CR. Seroepidemiology and genetic characterization of hepatitis E virus in western Yunnan Province. *Asian Pac J Trop Med*. 2014 Nov;7(11):909-12.
 24. Li TC, Takeda N, Miyamura T, Matsuura Y, Wang JC, Engvall H, Hammar L, Xing L, Cheng RH. Essential elements of the capsid protein for self-assembly into empty virus-like particles of hepatitis E virus. *J Virol* 2005, 79:12999-13006.
 25. Jothikumar N, Cromeans TL, Robertson BH, Meng XJ, Hill VR. A broadly reactive one-step real-time RT-PCR assay for rapid and sensitive detection of hepatitis E virus. *J Virol Methods* 2006, 131:65-71.
 26. Wang M, Fu P, Yin Y, He M, Liu Y. Acute, Recent and Past HEV Infection among Voluntary Blood Donors in China: A Systematic Review and Meta-Analysis. *PLoS One*. 2016 Sep 6;11(9):e0161089.
 27. Ren F, Zhao C, Wang L, Wang Z, Gong X, Song M, Zhuang H, Huang Y, Shan H, Wang J, Liu Q, Ness P, Nelson KE, Wang Y. Hepatitis E virus seroprevalence and molecular study among blood donors in China. *Transfusion*. 2014 Mar;54(3 Pt 2):910-7.
 28. Li Yi WJ, Hao Xianhui, Situ Jianwen, Zhao Yongqin, Huang Fen, Hua Xiuguo. [Molecular epidemiological analysis of hepatitis E in Dali region of Yunnan province.] *J Shanghai Jiaotong University (Agricultural Science)*. 2018;36(05):46-50. <https://kns.cnki.net/kcms/detail/detail.aspx?FileName=SHNX201805007&DbName=CJFQ2018> . (In Chinese)
 29. Zhou S, Ren L, Xia X, Miao Z, Huang F, Li Y, Zhu M, Xie Z, Xu Y, Qian Y, Pan Q, Wang K. Hepatitis E virus infection in HIV-infected patients: A large cohort study in Yunnan province, China. *J Med Virol*. 2018 Jun;90(6):1121-1127.
 30. Wang M, He M, Wu B, Ke L, Han T, Wang J, Shan H, Ness P, Guo N, Liu Y, Nelson KE. The association of elevated alanine aminotransferase levels with hepatitis E virus infections among blood donors in China. *Transfusion*. 2017 Feb;57(2):273-279.

31. Meng XJ, Wiseman B, Elvinger F, Guenette DK, Toth TE, Engle RE, Emerson SU, Purcell RH. Prevalence of antibodies to hepatitis E virus in veterinarians working with swine and in normal blood donors in the United States and other countries. *J Clin Microbiol*. 2002 Jan;40(1):117-22.
32. Tsoi WC, Zhu X, To AP, Holmberg J. Hepatitis E virus infection in Hong Kong blood donors. *Vox Sang* 2020, 115:11-17.
33. Chan MCW, Kwok K, Hung TN, Chan PKS. Molecular Epidemiology and Strain Comparison between Hepatitis E Viruses in Human Sera and Pig Livers during 2014 to 2016 in Hong Kong. *J Clin Microbiol*. 2017 May;55(5):1408-1415.
34. Masuda J, Yano K, Tamada Y, Takii Y, Ito M, Omagari K, Kohno S. Acute hepatitis E of a man who consumed wild boar meat prior to the onset of illness in Nagasaki, Japan. *Hepatol Res*. 2005 Mar;31(3):178-83.
35. Huang F, Li Y, Yu W, Jing S, Wang J, Long F, He Z, Yang C, Bi Y, Cao W, Liu C, Hua X, Pan Q. Excretion of infectious hepatitis E virus into milk in cows imposes high risks of zoonosis. *Hepatology*. 2016 Aug;64(2):350-9.
36. Hoofnagle JH, Nelson KE, Purcell RH. Hepatitis E. *N Engl J Med* 2012, 367:1237-1244.
37. Lee GH, Tan BH, Teo EC, Lim SG, Dan YY, Wee A, Aw PP, Zhu Y, Hibberd ML, Tan CK, Purdy MA, Teo CG. Chronic Infection With Camelid Hepatitis E Virus in a Liver Transplant Recipient Who Regularly Consumes Camel Meat and Milk. *Gastroenterology*. 2016 Feb;150(2):355-7.e3.
38. Long F, Yu W, Yang C, Wang J, Li Y, Li Y, Huang F. High prevalence of hepatitis E virus infection in goats. *J Med Virol*. 2017 Nov;89(11):1981-1987.
39. Hu GD, Ma X. [Detection and sequences analysis of bovine hepatitis E virus RNA in Xinjiang Autonomous Region]. *Bing Du Xue Bao*. 2010 Jan;26(1):27-32. Chinese. PMID: 20329555. <https://kns.cnki.net/kcms/detail/detail.aspx?FileName=BDXB201001007&DbName=CJFQ2010>. (In Chinese)
40. Cao Wenbing MX, Li Yan, Hu Guangdong. [The Seroepidemiological Survey of HEV of Sheep and Cattle in Xinjiang Province.] *China Animal Husbandry & Veterinary Medicine* 2009,36(08):157-159. <https://kns.cnki.net/kcms/detail/detail.aspx?FileName=GWXK200908046&DbName=CJFQ2009>. (In Chinese)
41. Li W, Shu X, Pu Y, Bi J, Yang G, Yin G. Seroprevalence and molecular detection of hepatitis E virus in Yunnan Province, China. *Arch Virol*. 2011 Nov;156(11):1989-95.
42. Hao X, Cao W, Situ J, Zhao Y, Huang F, Yu W. Hepatitis E Virus Detected in Pork Products. *Food Environ Virol*. 2018 Dec;10(4):391-393.
43. Ricco G, Bonino F, Lanza M, Scatena F, Alfieri CM, Messa P, Marchisio E, Mascolo G, Romano L, Galli C, et al. New immunoassays for total, IgA and IgM antibodies against hepatitis E virus: Prevalence in Italian blood donors and patients with chronic liver or kidney diseases. *Dig Liver Dis* 2016, 48:536-541.
44. Kamar N, Garrouste C, Haagsma EB, Garrigue V, Pischke S, Chauvet C, Dumortier J, Cannesson A, Cassuto-Viguier E, Thervet E, Conti F, Lebray P, Dalton HR, Santella R, Kanaan N, Essig M, Mousson C,

- Radenne S, Roque-Afonso AM, Izopet J, Rostaing L. Factors associated with chronic hepatitis in patients with hepatitis E virus infection who have received solid organ transplants. *Gastroenterology*. 2011 May;140(5):1481-9.
45. Chau KH, Dawson GJ, Bile KM, Magnus LO, Sjogren MH, Mushahwar IK. Detection of IgA class antibody to hepatitis E virus in serum samples from patients with hepatitis E virus infection. *J Med Virol*. 1993 Aug;40(4):334-8.
46. Zhang S, Tian D, Zhang Z, Xiong J, Yuan Q, Ge S, Zhang J, Xia N. Clinical significance of anti-HEV IgA in diagnosis of acute genotype 4 hepatitis E virus infection negative for anti-HEV IgM. *Dig Dis Sci*. 2009 Nov;54(11):2512-8.

Figures

Figure 1

Demographic characteristics of 1864 donors who completed the questionnaires.

Figure 2

Comparison of the difference of HEV biomarker positive rate among different nationalities and genders. A: anti-HEV IgG positive rate in different races. B: HEV seropositive rate in different races. C: HEV seropositive rate (anti-HEV IgG results combined with IgM and IgA) in different races. D: anti-HEV IgM positive rate in different genders. E: HEV seropositive rate in different genders.

Figure 3

Figure 4

Multivariable logistic regression analysis of HEV seroprevalence (based on results of anti-HEV IgG combined with both IgM and IgA) in different ethnic minorities.

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