Virus Research 265 (2019) 30–33

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Identification of hepatitis E virus subtype 4f in blood donors in Shanghai, China

Xiangxiang Chen[a,1], Ping Gong[b,1], Abram L. Wagner[c], Yixuan Li[a], Guanhong Wang[a], Yihan Lu[a,d,*]

[a] Key Laboratory of Public Health Safety (Ministry of Education), Fudan University School of Public Health, Shanghai, 200032, China
[b] Pudong New Area Blood Center, Shanghai, 200127, China
[c] Department of Epidemiology, University of Michigan, Ann Arbor, MI, 48109, USA
[d] Fudan University Pudong Institute of Preventive Medicine, Shanghai, 200136, China

ARTICLE INFO

Keywords:
Hepatitis E virus
Genotype
Phylogenetics
Blood donor

ABSTRACT

Hepatitis E virus (HEV) has been divided into eight genotypes and approximately thirty subtypes. Past studies of blood donors have revealed a substantial prevalence of HEV infection. We examined anti-HEV antibodies and HEV RNA in Chinese voluntary blood donors (VBDs). Blood specimens were collected during 2010–2011, 2014–2015, and 2018, and tested for anti-HEV IgG and IgM antibodies. HEV RNA was tested using real-time PCR and nested reverse transcription PCR (RT-PCR). Phylogenetic analysis determined the genotype using MEGA 7.0. Among 4044 VBDs, 2774 were men (68.6%). In total, 19.8% and 1.1% of the VBDs were reactive to anti-HEV IgG and IgM, respectively. The seroprevalence of anti-HEV IgG was significantly associated with age and time period ($P < 0.05$), whereas anti-HEV IgM was associated with anti-*Treponema pallidum* and time period ($P < 0.05$). A total of five specimens were positive for HEV RNA with normal ALT levels. Subtype 4f (n = 1; in the specimens reactive to anti-HEV IgM) and 4d (n = 3; 1 in the specimens reactive to anti-HEV IgM and 2 in the anti-HEV negative specimens) were found. The last specimen positive for HEV RNA was not genotyped due to failure in amplifying the partial sequence. In conclusion, our study identified HEV subtype 4f for the first time in China. Additionally, we confirmed the high prevalence of HEV in Chinese VBDs. These findings suggest a substantial risk of transfusion-transmitted HEV. Therefore, screening for HEV among Chinese VBDs might be warranted to prevent further transfusion-mediated spread of HEV.

1. Introduction

Worldwide, hepatitis E contributes to 3.7 million disability-adjusted life years (Murray et al., 2012). In China, hepatitis E incidence has increased since 2005, and overtook hepatitis A as the leading cause of acute hepatitis in 2012 (Zhang et al., 2016). In Shanghai, the largest and most economically developed metropolis in China, the incidence of hepatitis E has continually increased in the last decade, whereas other viral hepatitides (A, B, and C) have declined, with the result that hepatitis E has become the most common cause of acute hepatitis in 2016 with an incidence of 2.72 per 100,000 population (Chen et al., 2018).

Past studies of blood donors have revealed a substantial seroprevalence of anti-HEV IgG antibody, ranging from 3.4% in Japan to 29.2% in China (Takeda et al., 2010; Wang et al., 2016). The first confirmed case of transfusion-transmitted hepatitis E was documented in 2004 (Matsubayashi et al., 2004), with later transfusion-transmitted cases reported in other countries. Screening for HEV RNA in blood donations has been implemented in countries such as Ireland, the UK, and the Netherlands, with Germany and France implementing programs for screening in high-risk populations (Domanović et al., 2017). In recent years, studies have detailed the prevalence of HEV infection in blood donors in China (Wang et al., 2016). However, there is no analysis of hepatitis E among blood donors in Shanghai which can ascertain the possible risk of HEV infection.

Hepatitis E virus (HEV) has been divided into eight genotypes and approximately thirty subtypes (Smith and Simmonds, 2018). Genotypes 1 and 2 infect only humans, whereas genotypes 3, 4, and 7 infect both humans and animals (Smith and Simmonds, 2018). In China, genotypes 1, 3, 4, and 8 and at least 11 subtypes have been identified (Liu et al., 2012;Smith and Simmonds, 2018). In this study, we examined the seroprevalence of anti-HEV IgG and IgM antibodies, HEV RNA, and determined the genotype of HEV infection in voluntary blood donors

* Corresponding author at: 138 Yi Xue Yuan Road, Shanghai, 200032, China.
E-mail addresses: xx_chen16@fudan.edu.cn (X. Chen), gongping.shen@aliyun.com (P. Gong), awag@umich.edu (A.L. Wagner), yixuanli17@fudan.edu.cn (Y. Li), ghwang15@fudan.edu.cn (G. Wang), luyihan@fudan.edu.cn (Y. Lu).
[1] These authors contributed equally to the manuscript.

https://doi.org/10.1016/j.virusres.2019.03.001
Received 19 October 2018; Received in revised form 1 March 2019; Accepted 1 March 2019
Available online 02 March 2019
0168-1702/

(VBDs) in Shanghai.

2. Materials and methods

We enrolled a total of 4044 VBDs aged 18–55 years from Pudong New Area, Shanghai, China, during 2010–2011 (n = 1266), 2014–2015 (n = 1618), and 2018 (n = 1160). All the VBDs had passed pre-donation screening, including a health history questionnaire and rapid test for hepatitis B surface antigen (HBsAg). Additionally, gender and age were collected at the time of donation.

After blood donation, all the blood specimens were tested for alanine aminotransferase (ALT), hepatitis B virus surface antigen (HBsAg), antibody against hepatitis C virus (anti−HCV), antibody against HIV (anti-HIV), and antibody against *Treponema pallidum* (anti-TP) by ELISA. We further examined anti-HEV IgG and IgM using commercial ELISA kits (Wantai, Biological Pharmacy Co., Beijing, China) per manufacturer's instructions. Statistical analysis was conducted using SPSS 22.0 (SPSS Inc., Chicago, IL, USA). A *P*-value of < 0.05 was considered statistically significant.

Subsequently, we examined HEV RNA in the blood specimens using a commercial real-time PCR kit (Shanghai BioGerm Medical Biotechnology, China) that employed pooled samples (6 individual specimens mixed into 1 specimen), and nested reverse transcription PCR (RT-PCR) with amplification of an HEV ORF1 821-bp partial sequence and an ORF2 348-bp partial sequence, as described elsewhere (Huang et al., 2002; Lu et al., 2013). In the blood specimens that were reactive to anti-HEV IgG or IgM, we conducted both real-time PCR and nested RT-PCR. In the specimens non-reactive to anti-HEV IgG and IgM, we conducted real-time PCR to determine the presence of HEV RNA, in which we further employed nested RT-PCR to amplify the 821-bp and 348-bp partial sequences. The roadmap of the examination was listed in Fig. 1.

PCR products of nested RT-PCR were sequenced on both directions to obtain an accurate nucleotide sequence. Further phylogenetic analysis was performed by using MEGA 7.0. The amplified sequences have been deposited in the GenBank under accession no. MH687873-MH687876, MK397813-MK397816.

3. Results

The study population included 2774 men (68.6%) and 1270 women (31.4%) ($P < 0.001$). The average age was 29.6 ± 7.8 years for men and 30.7 ± 8.3 years for women ($P < 0.001$). In total, 19.8% (n = 799; 95% CI 18.6%–21.0%) of samples were reactive to anti-HEV IgG and 1.1% (n = 43; 95% CI 0.8%–1.4%) reactive to anti-HEV IgM. Of them, 0.8% (n = 33) were reactive to both anti-HEV IgG and IgM. Additionally, the seroprevalence was 0.35% for HBsAg, 0.45% for anti−HCV, 0.20% for anti-HIV, and 0.62% for anti-TP. The seroprevalence of anti-HEV IgG was significantly associated with age ($P_{trend} < 0.001$) and time period ($P_{trend} = 0.004$), whereas anti-HEV IgM was associated with time period ($P_{trend} = 0.031$) and anti-TP ($P = 0.03$) (Table 1).

Table 1
Seroprevalence of anti-HEV IgG and IgM antibodies by demographics and prevalence of HBsAg, anti−HCV, anti-HIV, and anti-TP.

	No. tested	IgG antibody against HEV		IgM antibody against HEV	
		No. positive (%)	*P* value	No. positive (%)	*P* value
Age (year)			< 0.001 [b]		0.71 [a]
18-19	191	30 (15.7)		1 (0.52)	
20-29	2070	286 (13.8)		23 (1.1)	
30-39	1211	270 (22.3)		11 (0.91)	
40-49	505	178 (35.3)		8 (1.6)	
50-	67	35 (52.2)		0 (0.0)	
Gender			0.09		0.62
men	2774	568 (20.5)		31 (1.1)	
women	1270	231 (18.2)		12 (0.94)	
Time period			0.004 [b]		0.031 [b]
2010-2011	1266	273 (21.6)		22 (1.7)	
2014-2015	1618	331 (20.5)		11 (0.68)	
2018	1160	195 (16.8)		10 (0.86)	
ALT (U/L)			0.58		1.00 [a]
≤40	3837	755 (19.7)		41 (1.1)	
> 40	207	44 (21.3)		2 (0.97)	
HBsAg			0.50 [a]		1.00 [a]
+	14	4 (28.6)		0 (0.0)	
-	4030	795 (19.7)		43 (1.1)	
Anti-HCV			0.55 [a]		1.00 [a]
+	18	2 (11.1)		0 (0.0)	
-	4026	797 (19.8)		43 (1.1)	
Anti-HIV			1.00 [a]		1.00 [a]
+	8	1 (12.5)		0 (0.0)	
-	4036	798 (19.8)		43 (1.1)	
Anti-TP			1.00 [a]		0.03 [a]
+	25	5 (20.0)		2 (8.0)	
-	4019	794 (19.8)		41 (1.0)	

[a] *Fisher* exact probability method was employed.
[b] Chi-square test for trend was employed.

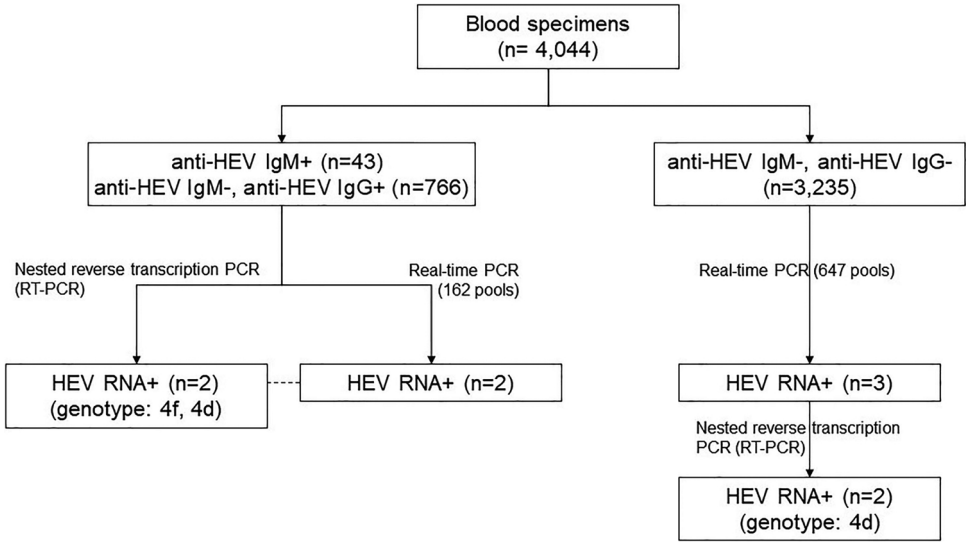

Fig. 1. The roadmap of anti-HEV antibodies and HEV RNA examination in the study.

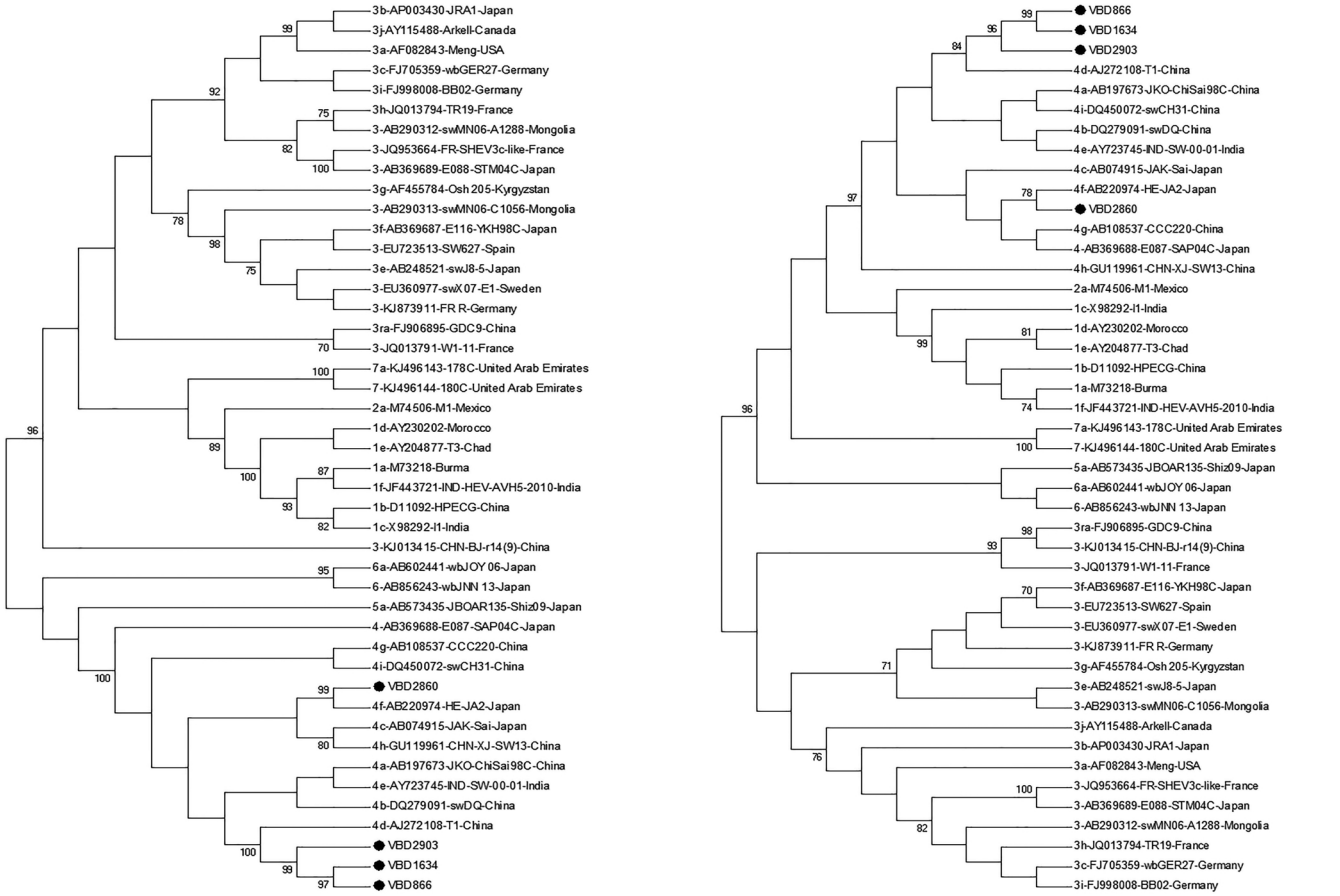

Fig. 2. Phylogenetic tree reconstructed using maximum likelihood method based on hepatitis E virus (HEV) ORF1 821-bp partial sequence (left) and ORF2 348-bp partial sequence (right), with bootstrap tests of 1000 replications (values of > 70% were displayed at the nodes). A total of 41 HEV reference strains with known genotype or subtype were included (Smith et al., 2016). ● indicates the partial sequences isolated from four voluntary blood donors (VBDs) in the study.

Of the 43 anti-HEV IgM positive blood specimens, two specimens (VBD1634 in 2014 and VBD2860 in 2011) were RNA positive by both real-time PCR and nested RT-PCR. Compared with the reference strains (Smith et al., 2016), VBD1634 shared 88.2%–93.0% identities (ORF1 821-bp) and 86.9%–93.5% identities (ORF2 348-bp) with Chinese strain T1 (GenBank asscession no. AJ272108) that is 4d reference strain, whereas VBD2860 shared 90.2%–94.6% identities (ORF1 821-bp) and 87.9%–94.6% identities (ORF2 348-bp) with Japanese strain HE-JA2 (GenBank asscession no. AB220974) that is 4f reference strain. These two isolates were classified as subtype 4d and 4f, respectively (Fig. 2). However, of the blood specimens that were both anti-HEV IgG positive and IgM negative (n = 766), no HEV RNA was detected by real-time PCR or nested RT-PCR.

Of the anti-HEV negative specimens (n = 3235), three specimens were positive by real-time PCR. The 821-bp and 348-bp were successfully amplified in two specimens. Both specimens (VBD866 in 2014 and VBD2903 in 2018) belonged to subtype 4d, sharing 86.9%–93.5% and 88.2%–94.4% (ORF2 348-bp), 87.7%–92.5% and 89.0%–93.4% (ORF1 821-bp) identities with Chinese strain T1, respectively. Additionally, all five specimens positive for HEV RNA had normal ALT levels.

4. Discussion

In our study, the overall seroprevalence of anti-HEV IgG in the VBDs in Shanghai was determined to be 19.8%, with a declining trend across time that might be attributable to improvement in people's living habits, which is associated with exposure to HEV infection (Mansuy et al., 2016). The findings were very similar to results in Shanghai's general population, where seroprevalence was 20.6% during 2000–2012 (Ren et al., 2013) and 17.7% in 2016 (H. Ren, pers. comm.). The pooled seroprevalence estimated from Chinese VBDs was 29.2% at a nationwide level and ranged between 17.9% and 40.7% within specific studies (Wang et al., 2016), suggesting that HEV incidence varies by area. Because seroprevalence differs within China and between countries (Takeda et al., 2010; Mansuy et al., 2016; Wang et al., 2016), examining the local context of VBDs can be crucial for determining risk of HEV transmission. Otherwise, our findings were consistent with previous studies. The seroprevalence of anti-HEV IgG increased by age, as would be expected with cumulative exposure to HEV across the lifespan (Fearon et al., 2017). Additionally, there was no association by gender (Shrestha et al., 2016) or by co-infection with HBV, HCV, or HIV (Zhuang et al., 2014; Bura et al., 2017). The difference in seroprevalence might be attributable to variation in the VBD population and other contexts. The overall seroprevalence of anti-HEV IgM was 1.1% in our study, and it was significantly associated with time period and anti-TP. However, we may be limited in detecting statistical differences by anti-IgM status; the low number of individuals who were anti-IgM positive led to limited statistical power. Compared to anti-HEV IgG that symbolizes past infection, anti-HEV IgM is much more characteristic of current infection. Overall, considering the seroprevalence of anti-HEV, HBsAg, anti－HCV, anti-HIV, and anti-TP in our study, VBDs could be susceptible to a variety of infections including HEV in the absence of adequate screening.

To the best of our knowledge, this study represents the first identification of HEV subtype 4f in China, as we searched GenBank according to the new classification criteria that were published in 2016 (Smith et al., 2016). Past studies have reported the isolation of subtype 4f from humans and swine in China in 2008 and 2009, respectively (De

et al., 2008; Ma et al., 2010); however, there was no nucleotide sequence available for further confirmation. Additionally, we identified two partial sequences (EU107427 and GU117764) of 98-bp in length that was clustered with the 4f reference strain with a low bootstrap value (< 30%); considering short length, they might not be appropriate for genotyping. In our study, the donor infected with subtype 4f was a male worker aged 31 years old, who reported no history of travelling abroad. Subtype 4f was firstly identified in a Japanese patient with acute self-limited hepatitis E in the early 2000s (Inoue et al., 2006); however, there has been no report of this subtype in the following years. China and Japan are both endemic for genotype 4 HEV, sharing many subtypes such as 4a, 4b, 4c, 4 g, and 4i that are prevalent among human and/or swine (Smith et al., 2016). Our isolation of subtype 4f indicated that transmission remains available between these two countries, such as through personal communication and bilateral trade. Additionally, we isolated three strains classified as subtype 4d that is prevalent in eastern China (Lu et al., 2013).

The major limitation to our study is that we could not trace the VBDs who were tested positive for HEV RNA to observe if recipients would develop hepatitis E. Currently, HEV infection is not routinely screened in blood donation in China, so we are not allowed to access the records of their blood products. Another limitation is the validity of the real-time PCR in our study. In the anti-HEV positive specimens, we identified the same specimens positive for HEV RNA by real-time PCR and nested RT-PCR; however, the number of anti-HEV negative specimens (n = 3235) was larger than the number of anti-HEV positive specimens (n = 809), and so we utilized real-time PCR followed by nested RT-PCR, which might underestimate the prevalence of HEV RNA.

In conclusion, our study identified HEV subtype 4f for the first time in China. Additionally, we confirmed the presence of HEV in Chinese VBDs, suggesting risk of further transmission through transfusion to blood recipients. Therefore, screening for HEV in VBDs might be warranted in China.

Funding

This work was supported by the China National Natural Science Funds (grant number 81370081), Shanghai Municipal Blood Center (grant number 05J1702-07), and Shanghai Municipal Health and Family Planning Commission (grant number GWTD2015S05). ALW received funding through a Fulbright Scholarship by J. William Fulbright Foreign Scholarship Board.

Ethical approval

The study was approved by the Institutional Review Board (IRB) of the Fudan University School of Public Health (IRB 00,002,408 and FWA 00,002,399) under IRB #2013-03-0415 and by the Ethics Committee of the Shanghai Pudong New Area Blood Center.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

PG and YL conceived and designed the study. XC, YL, and GW collected the blood samples and performed the laboratory examination. XC and ALW conducted the analysis and prepared the manuscript. ALW and YL critically revised the manuscript.

Acknowledgements

We appreciate the work of the phlebotomists and other health care workers who contributed to this project.

References

Chen, X.X., Shi, Y., Lu, Y.H., Chen, Y.H., Chen, K.Y., Ren, H., 2018. Spatial-temporal characteristics of hepatitis e in Shanghai, 2006-2016 in Chinese. Chin. J. Epidemiol. 39 (7), 954–958.

De DN, Casas M., Peralta, B., Nofrarías, M., Pina, S., Martín, M., et al., 2008. Hepatitis E virus infection dynamics and organic distribution in naturally infected pigs in a farrow-to-finish farm. Vet. Microbiol. 132 (1), 19–28.

Domanović, D., Tedder, R., Blümel, J., Zaaijer, H., Gallian, P., Niederhauser, C., et al., 2017. Hepatitis E and blood donation safety in selected European countries: a shift to screening? Euro Surveill. 22 (16), 30514.

Huang, F.F., Haqshenas, G., Guenette, D.K., Halbur, P.G., Schommer, S.K., Pierson, F.W., et al., 2002. Detection by reverse Transcription-PCR and genetic characterization of field isolates of swine hepatitis e virus from pigs in different geographic regions of the United States. J. Clin. Microbiol. 40 (4), 1326–1332.

Inoue, J., Nishizawa, T., Takahashi, M., Aikawa, T., Mizuo, H., Suzuki, K., et al., 2006. Analysis of the full-length genome of genotype 4 hepatitis E virus isolates from patients with fulminant or acute self-limited hepatitis E. J. Med. Virol. 78 (4), 476–484.

Liu, P., Li, L., Wang, L., Bu, Q., Fu, H., Han, J., et al., 2012. Phylogenetic analysis of 626 hepatitis E virus (HEV) isolates from humans and animals in China (1986-2011) showing genotype diversity and zoonotic transmission. Infect. Genet. Evol. 12 (2), 428–434.

Lu, Y.H., Qian, H.Z., Hu, A.Q., Qin, X., Jiang, Q.W., Zheng, Y.J., 2013. Seasonal pattern of hepatitis E virus prevalence in swine in two different geographical areas of China. Epidemiol. Infect. 141 (11), 2403–2409.

Ma, Z., Feng, R., Zhao, C., Harrison, T.J., Li, M., Qiao, Z., et al., 2010. Seroprevalence and distribution of hepatitis E virus in various ethnic groups in Gansu province. China. Infect. Genet. Evol. 10 (5), 614–619.

Mansuy, J.M., Gallian, P., Dimeglio, C., Saune, K., Arnaud, C., Pelletier, B., et al., 2016. A nationwide survey of hepatitis E viral infection in French blood donors. Hepatology 63 (4), 1145–1154.

Matsubayashi, K., Nagaoka, Y., Sakata, H., Sato, S., Fukai, K., Kato, T., et al., 2004. Transfusion-transmitted hepatitis E caused by apparently indigenous hepatitis E virus strain in Hokkaido, Japan. Transfusion 44 (6), 934–940.

Murray, C.J.L., Vos, T., Lozano, R., Naghavi, M., Flaxman, A.D., Michaud, C., et al., 2012. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380 (9859), 2197–2223.

Ren, H., Li, Y.T., Zhou, X., Wang, Y., Zheng, Y.J., Zhu, Y.Y., et al., 2013. Epidemiologic and genetic characteristics of hepatitis E virus in Shanghai, 1997-2012 in Chinese. Chin. J. Epidemiol. 34 (5), 419–423.

Smith, D.B., Simmonds, P., 2018. Classification and genomic diversity of enterically transmitted hepatitis viruses[J]. Cold Spring Harb. Perspect. Med. 8, a031880.

Smith, D.B., Simmonds, P., Izopet, J., Oliveira-Filho, E.F., Ulrich, R.G., Johne, R., et al., 2016. Proposed reference sequences for Hepatitis E virus subtypes. J. Gen. Virol. 97 (3), 537–542.

Takeda, H., Matsubayashi, K., Sakata, H., Sato, S., Kato, T., Hino, S., et al., 2010. A nationwide survey for prevalence of hepatitis E virus antibody in qualified blood donors in Japan. Vox Sang. 99 (4), 307–313.

Wang, M., Fu, P., Yin, Y., He, M., Acute, Liu Y., 2016. Recent and past HEV infection among voluntary blood donors in China: a systematic review and meta-analysis. PLoS One 11 (9), e0161089.

Zhang, X., Hou, F., Li, X., Zhou, L., Liu, Y., Gilbert, K., et al., 2016. Study of surveillance data for class B notifiable disease in China: from 2005 to 2014. Int. J. Infect. Dis. 48, 7–13.