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ORIGINAL ARTICLE

The Risk of Transfusion-Transmitted Hepatitis E Virus: Evidence from Seroprevalence Screening of Blood Donations

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Abstract Throughout the world, there has been growing concern over the risk of hepatitis E virus (HEV) transmission via blood transfusion. The present study screened blood donor samples for anti-HEV immunoglobulin M (IgM) and immunoglobulin G (IgG). The prevalence of HEV infection was assessed on a total of 1,003 archived serum samples obtained from the National Blood Centre, Malaysia. The samples were collected from healthy blood donor from Klang Valley between 2017 and 2018. All samples were tested for IgM and IgG antibodies to HEV using enzyme-linked immunosorbent assays (ELISA). HEV-specific IgG antibodies were detected in 31/1003 (3.1%; 95% confidence interval [CI] 2.1%–4.4%) and IgM in 9/1003 (0.9%; 95% CI 0.4%–1.7%) samples. In bivariate analysis, there was no significant difference in the prevalence of anti-HEV IgG with respect to gender and district of origin. Although not statistically significant, males had higher odds of having anti-HEV IgG than females (odds

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ratio [OR] = 2.86; 95% CI 0.95–8.64). All anti-HEV IgG positive individuals were people of Chinese descent. Anti-HEV IgG increased significantly with age, from 0.6% (95% CI 0.1%–2.6%) of 18–30-year-old donors to 7.4% (95% CI 2.7%–17.0%) of donors older than 50 years and was highest among non-professional workers (5.3%; 95% CI 2.5%–10.5%). Increasing age and a non-professional occupation remained significant predictors for anti-HEV IgG in the multivariable analysis. Screening of blood donations for HEV in Malaysia is important to safeguard the health of transfusion recipients. The higher rates of HEV infection in blood from older donors and donors who are non-professional workers may provide insights into targeted groups for blood screening.

Keywords Infectious disease · Seroprevalence · HEV · Hepatitis E · Blood donors · Malaysia

Introduction

Hepatitis E infection, caused by the hepatitis E virus (HEV)–one of five hepatitis viruses – is the most common cause of acute viral hepatitis worldwide [1]. A review evaluated studies on HEV seroprevalence across the globe found that the prevalence ranges from 1 to 52% [2]. Hepatitis E is both an epidemic and endemic disease in developing Asian and African countries [3]. In developed countries, HEV has been recognised as an emerging and often undiagnosed disease [4]. HEV transmission usually occurs via the faecal-oral route, principally via contaminated water [5]; however, transfusion of blood and blood products has been acknowledged to play a role in the spread of the HEV [6].

High HEV seroprevalence rates have been found among blood donors in southwest France (52.5%), Denmark (20.6%), the United States (18.3%) and southwest England (16%) [7–10]. The observation of high rates of HEV viraemia in blood donors have prompted many European countries to introduce routine HEV testing or selected screening of blood donated by high-risk individuals to prevent virus transmission by transfusion [11]. In addition, plenty of research-based HEV genomic sequencing studies showing an identical match between blood donors and recipients, confirming the evidence of transfusion-transmitted HEV [12]. Transfusion-transmitted HEV, particularly from asymptomatic individuals, is worrisome to the transfusion medicine community as it could pose a serious threat to public health. Current evidence indicates that HEV infection occurs in nearly half of recipients of HEV-contaminated blood products, and most of the donors of these blood products were asymptomatic at the time of blood donation [13]. The need for screening for HEV screening of blood donations has gained international recognition and there is an increasing trend in the implementation of HEV antibody screening in blood donations worldwide [12]. In Asia, high anti-HEV immunoglobulin G (IgG) seroprevalence rates have been demonstrated among blood donors in China (32.6%) [14], India (17.7%) [15] and Hong Kong (15.5%) [16]. A lower seroprevalence rate has been reported in Japan (3.4%) [17]. The HEV seroprevalence from other Southeast Asian countries is lacking. A study in Cambodia reported anti-HEV IgG in 28.2% of blood donors in 2014 [18]. Nevertheless, in Thailand HEV RNA was detected in 1 out of 1158 blood donations [19]. The lack of data is a worrying fact for recipients of blood in the Southeast Asian countries.

Malaysia is a country in Southeast Asia. The prevalence of anti-HEV antibodies in the general population at large in Malaysia has never been reported. Earlier studies on HEV infections in Malaysia were among two indigenous people communities, locally known as Orang Asli and a small sample of blood donors from an urban setting [20] as well as on human immunodeficiency virus (HIV) type 1-infected subjects [21]. The prevalence of anti-HEV IgG in two Orang Asli communities was reported to be 44% and 50%, respectively, compared with only 2% in the urban blood donor sample [20]. The prevalence of anti-HEV IgG in HIV type 1-infected subjects was reported to be 14.5%. Over two decades since these studies, there have been no further reports on HEV seroprevalence in Malaysia. A 2019 study found an anti-HEV IgG positive rate of 5.9% in a sample of Orang Asli in the state of Negeri Sembilan [22]. With the worrisome upward trend in HEV seroprevalence across the world and the fact that transfusion-transmitted HEV can cause serious clinical consequences, this study screened the previously archived blood donor samples in Malaysia for anti-HEV IgM/ IgG to determine an up-to-date seroprevalence.

Materials and Methods

Population and Sample Collection

Archived plasma samples obtained from the National Blood Centre, Malaysia, were used for the seroprevalence study. The samples were collected between 2017 and 2018 from blood donors living in the region of Klang Valley, Malaysia. All donors had previously completed the national medical questionnaire to verify that they fulfilled the criteria for blood donation. Age, gender, ethnicity, and residential location for each donor were also obtained from the blood bank database.

Anti-HEV Serological Assays

The serum samples were tested with the enzyme-linked immunosorbent assays (ELISAs) for the detection of hepatitis E markers (anti-HEV IgG and anti-HEV IgM) using Wantai's HEV-IgG (catalog no. WE-7196) and HEV-IgM ELISA kits (catalog no. WE-7296) (Beijing Wantai Biological Pharmacy Enterprise Co., Ltd, Beijing, China). Testing and calculations (i.e. sample to cut-off ratio and determination of equivocal results) were in accordance with the manufacturer's instructions. The Wantai HEV IgG assay is known for its superior sensitivity [23, 24] and is also regarded as a "gold standard" for HEV antibody detection [25]. The test is currently one of the most commonly used assays with specificity and sensitivity for the HEV IgG of 97.96% and 99.6%, respectively [26, 27] and has been widely used for HEV screening of blood donations [26].

Statistical Analyses

Statistical analysis was performed using SPSS software version 25.0 (IBM, Armonk, NY, USA). The binomial 95% confidence interval (CI) was determined for seroprevalence rates. Pearson's Chi-square (χ^2) test was used to determine the significance of any observed differences in the seroprevalence rates for different demographic subgroups. P values of less than 0.05 were regarded as statistically significant.

Ethical Approval

Permission to use the archived blood samples was obtained from the National Blood Centre, Malaysia. The study was approved by the Medical Research and Ethics Committee, Ministry of Health Malaysia [NMRR-19–2524-49,403 (IIR)].

Results

The frequency of HEV-specific antibodies detected in 1,003 blood donors was 31 (3.1%; 95% CI 2.1–4.4) for IgG and 9 (0.9%; 95% CI 0.4–1.7) for IgM. In total, 3 donors were positive both for IgM and for IgG against HEV (Table 1). The three donors tested positive for anti-HEV-IgM and IgG were a 45-year-old male chef, 28-year-old female executive, and 18-year-old male student. Demographic details were only available for 711 samples, of which 20 samples (2.8%; 95% CI 1.7–4.3) were IgG positive. Table 2 shows the prevalence of IgG anti-HEV by demographic characteristics. The age range of the donors was from 17 to 60 years, with a mean age of 34.1 ± 10.7 years and a median age of 33 years. The majority were male (n = 419, 58.9%), 18–30 years old (n = 301, 42.3%) and of a professional and managerial occupation (n = 313, 44.0%). The majority were Malay (n = 238, 33.5%) or people of Chinese descent (n = 404, 56.8%). Over one-third of the donors reside in the Federal Territory of Kuala Lumpur (Table 2).

There was a significant association between seropositivity and age. The seroprevalence rates increased gradually across age-groups, with the lowest (0.6%, 95% CI 0.1%–2.6%) and highest (7.4%, 95% CI 2.7%–17.0%) seroprevalence rates in the 18–30-year-old and > 50-year-old age groups, respectively. By occupation category, non-professional workers had a significantly higher seroprevalence rate (5.3%, 95% CI 2.5%–10.5%).

There were no significant differences in the seroprevalence rate between males and females, among ethnic groups or by geographic region. Although not statistically significant, males had higher odds of anti-HEV IgG seroprevalence than females (odds ratio [OR] = 2.86; 95% CI 0.95–8.64). All those positive for anti-HEV IgG were of Chinese descent. Figure 1 shows the geographical distribution of HEV IgG and IgM. Although there is no significant difference in the seroprevalence rate among districts, prevalence rates appear to be concentrated based on geographical location. There appears to be a concentration of HEV seropositivity in the townships located in Ampang, Cheras, and Puchong.

Increasing age and occupation as a general worker remained significant predictors for anti-HEV IgG seropositivity in the multivariate analysis. The odds of anti-HEV IgG seropositive was 12.68 times greater in a > 50-year-old donor than one from the 18–30-year-old group. Non-professional workers had 3.52 times higher odds of anti-HEV IgG seropositive than those in the professional and managerial group.

Table 1 Prevalence of HEV IgG and IgM

Classification of immunoglobulin	Sample tested N	Positive n	% positive (95% CI)
IgG	1003	31	3.1 (2.1–4.4)
IgM	1003	9	0.9 (0.4–1.7)
IgG + IgM	1003	3	0.3 (0.1–0.9)

Table 2 HEV IgG by demographic characteristics (N = 711)[†]

	Sample tested n (%)	HEV IgG positive n = 20	HEV IgG% positive (95% CI)	Univariable analysis OR (95% CI)	Multivariable analysis	
					p–value	OR (95% CI)	*p*–value
Age group (years)							
18–30	301	2	0.6(0.1–2.6)	Reference		Reference	
31–40	215	6	2.8(1.1–6.3)	4.29 (0.86–21.47)	0.076	6.14 (1.13–33.5)	0.036
40–50	127	7	5.5(2.4–11.4)	8.72 (1.79–42.58)	0.007	10.29 (1.98–53.38)	0.006
> 50	68	5	7.4(2.7–17.0)	11.87 (2.26–62.54)	0.004	12.68 (2.29–70.25)	0.004
Gender							
Male	419	16	3.8(2.3–6.3)	2.86 (0.95–8.64)	0.063	–	
Female	292	4	1.4(0.4–3.7)	Reference			
Ethnicity							
Malay	238	0	0	–			
Chinese	404	19	4.7 (2.9–7.4)	0.59 (0.07–4.79)	0.623	–	
Indian	49	0	0	–			
Bumiputera Sabah/Sarawak	7	0	0	–			
Non–Malaysian	13	1	7.7(0.4–37.9)	Reference			
Occupation							
Professional and managerial	313	4	1.2(0.4–3.2)	Reference		Reference	
General worker	151	8	5.3(2.5–10.5)	4.32 (1.28–14.59)	0.018	3.52 (1.05–12.27)	0.042
Self–employed	138	4	2.9(0.9–7.7)	2.31 (0.57–9.36)	0.242	4.35 (0.99–19.03)	0.051
Housewife/ Student/ RetireeUnemployed	109	4	3.7(1.2–9.7)	2.94 (0.72–11.98)	0.132	3.34 (0.79–14.04)	0.100
Location (District) [††, §]							
Gombak	57	0	0	–			
Hulu Langat	61	2	3.3(0.6–12.4)	1.04 (0.22–5.04)	0.959	–	–
Klang	27	2	7.4(1.3–25.8)	2.46 (0.50–12.22)	0.271	–	–
Petaling	136	4	2.9(1.0–7.8)	0.92 (0.28–3.15)	0.910	–	–
Wilayah Persekutuan KL	254	8	3.2(1.5–6.3)	Ref			

[†] Number less than total N = 1003 due to missing/demographic characteristics

[††] Number less than 711 due to incomplete/missing address

[§] Number less than 20 IgG positive due to incomplete/missing address

Discussion

In the absence of up-to-date reliable data on HEV seroprevalence in Malaysian blood donors, blood donor samples collected in the Klang Valley region in Malaysia were screened. The Klang Valley is a basin located in the southwestern part of Peninsular Malaysia. Due to its location and status as one of the most developed areas following rapid urbanisation, the Klang Valley is the heartland of Malaysia's industry and commerce. Kuala Lumpur, the federal capital and most populous Malaysian city, is located in the Klang Valley. Malaysia is a multicultural and multiconfessional country, whose official religion is Islam. Malaysia's population can be divided into 69%

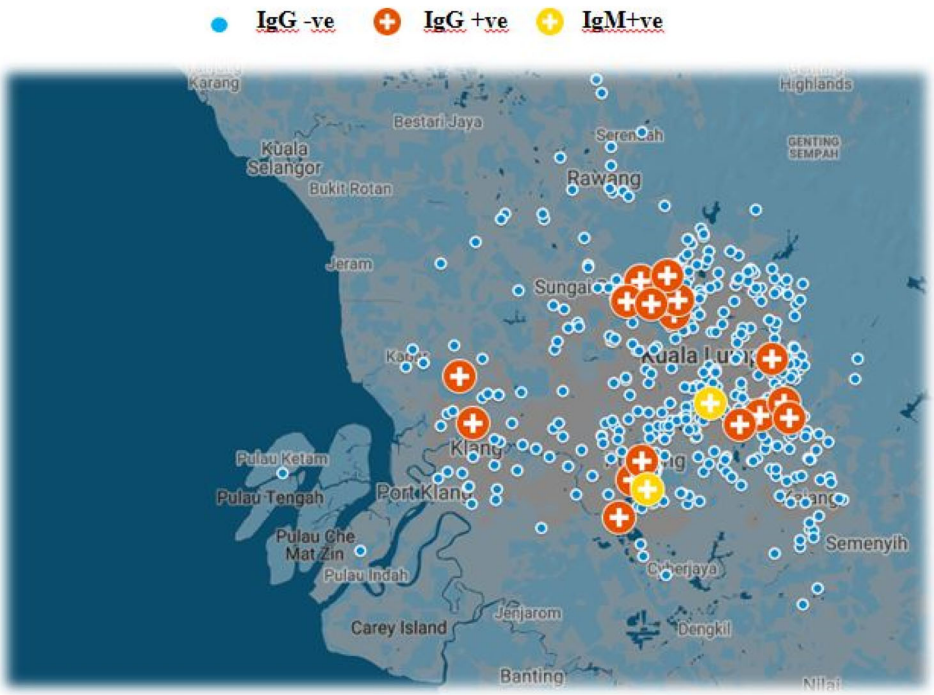

Fig. 1 Geographical distribution of HEV IgG and IgM

Malay, 23% Chinese, 7% Indian, and 1% mostly indigenous people of Borneo [28]

In the present study, the HEV-specific IgG antibody seroprevalence detected with the Wantai HEV IgG assay was at 3.1%. This seroprevalence rate is considerably lower than that reported in blood donors in the United States, the United Kingdom and Western European countries [2, 29]. However, our rate was similar to that reported in Japan (3.4%) in 2005 [17]. Compared with other Southeast Asian countries, the HEV IgG seroprevalence rate in our study is lower than that detected -among the Cambodian blood donors [18]. Furthermore, the seroprevalence in this study is slightly higher compared with the seroprevalence of blood donors in Malaysia conducted nearly two decades ago on a relatively small sample of 100 donors (2%) [20].

An important finding of this study is that 0.9% (9 out of 1,003) had an acute or recent infection at the time of donation, implying that these donors may silently transmit the virus to prospective blood recipients. In addition, 3 of them (0.3%) were also positive for IgG, a finding that implies these donors had previously been exposed to HEV infection. Since HEV is not routinely screened during blood donation in Malaysia, recipients of blood products that may have possibly been exposed to HEV is not known. Although most recipients remain asymptomatic after accepting infected blood products, HEV infection can induce chronic infection in immunocompromised individuals or patients with underlying liver diseases [29]. This finding suggests that it is necessary to strengthen the safety monitoring of donated blood to reduce the risk of transmission of HEV through blood transfusion. Further, HEV infection should also be considered when investigating post-donation illnesses. To date, there is no specific treatment for HEV infection. Therefore, prophylactic vaccination could potentially be an effective method to protect people from HEV infection. Several vaccine candidates based on virus-like particles (VLPs) have progressed into the clinical development stage, and one of them currently approved in China, with evidence on safety and efficacy including in pregnant women [30].

The finding of an increasing trend in HEV IgG antibody prevalence with age in this study is consistent with a meta-analysis on HEV seroprevalence in Europe [31] as well as in donors from Middle Eastern countries [32–34] and India [15]. The increase of anti-HEV IgG seroprevalence with increasing age possibly suggests a cumulative lifetime exposure to HEV, as evident in a previous study in European countries [35]. As seroprevalence was higher in older donors, it is particularly important to carry out HEV screening of older donors.

Our findings also showed that non-professional workers have an increased likelihood of HEV seropositivity than those in the professional and managerial occupational category. The non-professional jobs of the donors include craftsmen, plumbers, electricians, installers, and food service workers or, in general, positions that primarily entail manual labor duties. In other studies, HEV seropositivity has been significantly associated with occupational exposure to animals, particularly pigs [36]. Studies also showed

that professional occupations with animal reservoirs such as veterinarians, slaughterhouse and forestry workers and hunters had a higher seroprevalence than the related general population [37–39]. Our finding potentially provides new insights into the risk of occupational exposure to HEV in Malaysia. Given that being a general worker constitutes a risk factor for contracting HEV, when possible, donors in this occupation group should be questioned about possible HEV exposure risk.

Findings of gender differences in HEV seroprevalence have been inconclusive. In the present study, the overall seroprevalence in male donors was over 3 times greater than in female donors, but this difference was not significant, perhaps due to the small sample size. Our findings, however, were consistent with that of previous studies with larger samples conducted in China where HEV IgG seropositivity was higher in males than in females [14]. In many of the surveyed European countries, there were no significant differences in the anti-HEV IgG seroprevalence by gender [31, 38]. It has been suggested that exposure to HEV is not directly related to gender but rather to individual behaviour [37].

Of note, this study found that of the three major ethnic groups in Malaysia, all HEV IgG seropositivity occurred among people of Chinese descent, and none among the Malays. Contact with pigs or consumption of under-cooked pork has been reported as risk factors for HEV transmission [40, 41]. The absence of HEV seropositivity among the Malays could be due to the non consumption of pork and also the absence of activities involving contact with - pigs and pork products. In support of this assertion is a study in Thailand which reported that the Muslim communities where most abstain from pork and residing in an environment with fewer swine farms are associated with a quite low anti-HEV IgG seroprevalence [42]. HEV strains of genotypes 3 and 4 have been detected in pigs in many studies [43, 44] and ample evidence have also documented that zoonotic infection of genotypes 3 and 4 HEV was associated with food-borne infections linked to pigs, wild boar and deer meat [45, 46]. Therefore, it is possible that genotypes 3 and 4 HEV are currently circulating in the Malaysian community and may be related to zoonotic transmission and food-borne infection. Nonetheless, future larger-scale studies are warranted to provide conclusive empirical evidence of the ethnic disparities in HEV IgG seropositivity among blood donors in Malaysia and the presence of HEV genotypes 3 and 4.

Although there was no significant difference in the seroprevalence rate among districts, it is interesting that HEV seroprevalence appears concentrated based on the geographical distribution map (Fig. 1). This finding suggests a potentially high infection risk in areas of concentrated infections in Ampang, Cheras, and Puchong, where the majority of residents are of Chinese descent. Most recently, the presence of the HEV genomes was found in commercial pork livers and pork meat products in Germany, with prevalence ranging from 5 to 15% [47]. The study adds to the evidence of the risks of pork-to-human transmission through handling and consumption of infected products, which have been evident over the last decade [48]. Of important highlight, in our study, a donor whose occupation is chef was both anti-HEV IgM and IgG positive, probably the consequences of occupational exposure to infected products.

This study has several limitations. First, we investigated the seroprevalence of anti-HEV antibodies from archived samples and almost 30% of the samples were missing the donor's demographic characteristics. Second, the blood donor samples are from Klang Valley and may not be a nationally representative samples. Therefore, there is a need to initiate larger-scale screening for HEV antibodies on blood donor samples that also include donors from other states to provide a more accurate view of the prevalence of HEV in Malaysia. It is important to note that, HEV serology should be interpreted with caution. The detection by antibody screening may not exclude HEV in blood products. It is also equally important to acknowledge the potential for false positives in serological testing. Diagnosis of acute hepatitis E relies upon the detection of HEV RNA by nucleic acid amplification techniques (NAT or NAAT), nonetheless requires specific equipment that is not necessarily available in all laboratories. Despite these limitations, this is the first large-scale HEV screening of blood donations in the country. The findings provide insights into the importance of investigation of post-transfusion hepatitis in future studies.

Conclusion

Although the HEV seroprevalence rate in this study is low, it may still pose a threat to potential blood recipients. The findings suggest that screening donated blood for HEV in Malaysia is important to safeguard the health of blood transfusion recipients. Our findings resemble many other HEV seroprevalence studies in blood donors that have demonstrated increased anti-HEV seroprevalence with increasing age. The higher rates of HEV infection in older blood donors and occupational factors that predict anti-HEV IgG seroprevalence could provide insights into specific groups that should be screened for HEV upon blood donation.

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Authors' Contributions LPW, SAB, ZZ, TW, QZ and NX designed and conceived the study. HYL, CSK, JAJ, NAA, MMR, NAR and HNM prepared and analyzed the blood sample. LPW and HA performed the statistical analysis. LPW wrote the manuscript. All authors have read and approved the final version of this manuscript

Declaration

Conflict of interest The analyses of HEV were performed using Wantai HEV IgG ELISA, of which all the HEV ELISA kits were sponsored by Beijing Wantai Biological Pharmacy Enterprise Co., Ltd, Beijing, China).

Ethics Statement The study was approved by the Medical Research and Ethics Committee, Ministry of Health Malaysia [NMRR-19–2524-49,403 (IIR)].

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