Prevalence of hepatitis E virus antibodies and infection in New Zealand blood donors

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ABSTRACT

AIM: Blood transfusion is one route of transmission of hepatitis E virus (HEV). The aim of this study was to assess both the prevalence of HEV antibodies and HEV infection in New Zealand blood donors.

METHOD: To determine HEV seroprevalence, donor plasma samples (n=1,013) were tested for HEV antibodies using two commercially available ELISA kits, the Wantai HEV IgG ELISA and the MP Diagnostics HEV ELISA 4.0. To assess the prevalence of HEV infection, pooled plasma samples from individual plasma donors (n=5,000) were tested for HEV RNA using RT-qPCR. Samples that tested HEV antibody positive or gave an equivocal result with either ELISA were also tested for HEV RNA.

RESULTS: The HEV seroprevalence in New Zealand blood donors was 9.7% using the Wantai HEV IgG ELISA and 8.1% using the MP Diagnostics HEV ELISA 4.0. The presence of HEV antibodies was significantly and positively correlated with increasing donor age. HEV RNA was not detected in any of the samples tested, indicating no evidence of current infection.

CONCLUSION: This study, the largest to date to assess HEV seroprevalence in New Zealand, provides valuable baseline information on HEV seroprevalence and infection in New Zealand blood donors. The seroprevalence rate in New Zealand is similar to that reported in other developed countries.

Hepatitis E virus (HEV) infection is a common cause of acute hepatitis in developing countries, where faecal-oral transmission via faecally contaminated water is the most common transmission route.[1] Hepatitis E cases identified in developed countries are commonly associated with travel to developing countries where the virus is endemic. However, there are increasing reports of autochthonous (locally acquired) cases of HEV infection in developed countries. These cases are usually associated with HEV genotype 3 (Europe, America) or 4 (Asia).[1,2] The transmission route(s) for HEV in developed countries are not well understood, but there is good evidence to show that zoonotic transmission from pigs and foodborne transmission from undercooked pig and deer meats are important.[3–5] The majority of HEV infections in humans are asymptomatic.[1] One potentially important route of HEV transmission is transfusion of blood components.[6,7] However, the contribution of such transmission to overall HEV disease burden is unclear.[8] There have been several recent HEV seroprevalence studies of blood donors, with a broad range in reported seropositivity. For example, Cleland et al reported an HEV seroprevalence of 4.7% in 1,559 Scottish blood donors in 2012,[9] Slot et al observed a seroprevalence of 26.7% in 5,239 Dutch blood donors in 2011 and 2012,[10] and Lucarelli et al reported a 49% seroprevalence rate in 313 blood donors in central Italy in 2014.[11] Several studies have attempted to determine the presence of HEV viraemia in blood donors, and therefore the more immediate risk to blood and blood product recipients.[12–14] This risk has prompted some countries, including Ireland and the UK, to introduce routine testing of donated blood for HEV RNA to prevent transmission by transfusion. Other countries are considering its implementation either selectivity (eg, intended for high-risk patients) or nationwide.[15]

There are no recent data on HEV seroprevalence among the New Zealand population and no published data on HEV viraemia in New Zealand blood donors. One previous New Zealand study of 265 blood donors published in 2007 observed an HEV IgG seropositivity rate of 4.2%, although limited information on donor characteristics was available.[16] Accordingly, the aims of this study were: (i) to determine the contemporary seroprevalence of HEV in New Zealand blood donors, and (ii) to assess the prevalence of HEV infection (as measured by HEV RNA detection) in New Zealand blood donors.

Methods

Plasma samples collected by the New Zealand Blood Service (NZBS) between 11 November 2014 and 10 March 2015 were used for the seroprevalence study. The sampling strategy was based on the New Zealand population census 2006 distribution with target sample numbers calculated from each of five New Zealand regions (classified as Northern, Auckland, Midland, Central and Southern) covering urban and rural areas and three age groups (<30 years, 30–59 years and >60 years). For each of the donors participating in this study, data on age, sex and region of residence were collected. For statistical analysis, age groups were classified as: 16–30 years, 31–45 years, 46–60 years and >60 years of age.

Previous studies have reported differences in the relative specificity and sensitivity of the ELISA kits used for HEV IgG detection.[17] To allow comparison with other studies that have used various ELISA assays, two different ELISA kits were used to test each donor plasma sample. These were the Wantai HEV IgG ELISA (Beijing Wantai Biological Pharmacy Enterprise Co., Ltd, Beijing, China) and the MP Diagnostics HEV ELISA 4.0 (MP Biomedicals Asia Pacific, Singapore). According to the manufacturer's kit insert, the Wantai HEV IgG ELISA detects HEV IgG only, while the MP Diagnostics HEV ELISA 4.0 detects total (IgG, IgM and IgA) HEV antibody. Testing and calculations (ie, sample to cut-off ratio and determination of equivocal results) were in accordance with the manufacturer's instructions. Samples that tested HEV seropositive by either kit were subsequently tested for the presence of HEV RNA using the method described below.

To assess the prevalence of HEV infection, pooled plasma samples were prepared from individual plasma donations collected by the NZBS between 26 June and 8 October 2015. Between eight and 12 plasma aliquots from individual donations were pooled. Pooled samples were tested for HEV RNA using a commercially available real-time reverse transcription (RT)-qPCR assay (RealStar® HEV RT-PCR Kit 1.0, Altona Diagnostics, Hamburg, Germany) according to the manufacturer's instructions. The analytical sensitivity of RealStar® HEV RT-PCR Kit is 0.31 International Units (IU)/µl as reported by Altona Diagnostics.

Statistical analysis was performed using Graphpad Prism (GraphPad Software Inc., San Diego, CA) and R.[18] The binomial 95% confidence interval (CI) was determined for seroprevalence rates. The Pearson's Chi-squared test (χ^2) was used to determine the significance of any observed differences in the seroprevalence rates for different demographic subgroups. A P value of ≤0.05 was considered significant.

Results

In total, 1,013 plasma samples were tested for HEV antibodies. The Wantai HEV IgG ELISA gave a positive result in 98/1,013 samples (9.7%, 95% CI 7.9–11.7%). The MP Diagnostics HEV ELISA 4.0 gave a positive result in 82/1,013 samples (8.1%, 95% CI 6.5-10.0%) (Table 1). The difference between the kits was not statistically significant (p<0.05). A total of 79 (7.8%, 95% CI 6.2–9.6%) samples tested positive using both ELISA assays.

Table 1: Comparison of HEV antibody test results using Wantai and MP Diagnostics HEV ELISA kits.

		MP Diagnostics (HEV IgG, IgM, IgA)			
		Positive	Equivocal	Negative	Total
Wantai (HEV IgG)	Positive	79 (7.8%)	0 (0%)	19 (1.9%)	**98 (9.7%)**
	Equivocal	0 (0%)	0 (0%)	2 (0.2%)	2 (0.2%)
	Negative	3 (0.3%)	0 (0%)	910 (89.8%)	913 (90.1%)
	Total	**82 (8.1%)**	0 (0%)	931 (91.9%)	1,013

Of the 1,013 samples tested, 103 gave a positive or an equivocal result for HEV antibodies with either ELISA kit. Discordant results were obtained for 24/1,013 (2.4%) samples. Of the 98 Wantai HEV IgG positive samples, 19 were negative using the MP Diagnostics ELISA kit (that detects HEV IgG, IgM and IgA), whereas, of the 82 MP Diagnostics HEV antibody-positive samples, three were negative using the Wantai HEV IgG ELISA. Two samples gave an equivocal result using the Wantai HEV IgG ELISA but were negative using the MP Diagnostics HEV ELISA.

No significant difference in the seroprevalence rate between males and females, or by geographic region was observed. However, there was a significant association between seropositivity and age, with the lowest (3%, 95% CI 1.5–5.5%) and highest (18.0%, 95% CI 12.6–24.6%) seroprevalence rates in the 16–30 year and over 60 year age groups respectively using the Wantai HEV IgG ELISA (Table 2).

A total of 625 pooled plasma samples prepared from 5,000 individual donors were tested for HEV RNA. HEV RNA was not detected in any of the pooled samples. HEV RNA was not detected in any of the 103 individual samples that tested HEV antibody positive or that gave an equivocal result with either ELISA kit.

Table 2: HEV seroprevalence by sex, age group and New Zealand region using Wantai and MP Diagnostics ELISA kits.

	Samples tested	Wantai			MP Diagnostics		
		Positive	% positive (95% CI)	P value	Positive	% positive (95% CI)	P value
Overall	1,013	98	9.7 (7.9–11.7)		82	8.1 (6.5–10.0)	
Sex				0.428[a]			0.475[b]
Male	483	43	8.9 (6.5–11.8)		36	7.5 (5.3–10.2)	
Female	530	55	10.4 (7.9–13.3)		46	8.7 (6.4–11.4)	
Age group				<0.01[c]			<0.01[d]
16–30	331	10	3.0 (1.5–5.5)		9	2.7 (1.3–5.1)	
31–45	206	12	5.8 (3.0–10.0)		13	6.3 (3.4–10.6)	
46–60	304	45	14.8 (11.1–19.3)		36	11.8 (8.4–16.2)	
61+	172	31	18.0 (12.6–24.6)		24	14 (9.2–20.1)	
Region				0.073[e]			0.129[f]
Northern[g]	152	20	13.2 (8.2–19.6)		15	9.9 (5.6–15.8)	
Auckland[h]	215	17	7.9 (4.7–12.4)		16	7.4 (4.3–11.8)	
Midland[i]	233	29	12.5 (8.5–17.4)		24	10.3 (6.7–14.9)	
Central[j]	167	17	10.2 (6.0–15.8)		16	9.6 (5.6–15.1)	
Southern[k]	246	15	6.1 (3.5–9.9)		11	4.5 (2.2–7.9)	

[a]$\chi^2 = 0.629$, degrees of freedom (d.f.)=1; [b]$\chi^2 = 0.510$, d.f.=1; [c]$\chi^2 = 43.13$, d.f.=3; [d]$\chi^2 = 27.413$, d.f.=3; [e]$\chi^2 = 8.579$, d.f.=4; [f]$\chi^2 = 7.126$, d.f.=4.
[g]Northland and Waitemata District Health Boards (DHBs).
[h]Auckland and Counties Manukau DHBs.
[i]Waikato, Lakes, Bay of Plenty, Tairawhiti, Taranaki and Hawke's Bay DHBs.
[j]Whanganui, MidCentral, Hutt Valley, Capital & Coast and Wairarapa DHBs.
[k]All South Island (Nelson Marlborough, West Coast, Canterbury, South Canterbury and Southern DHBs).

Discussion

This study reports on the HEV seroprevalence rate and presence of HEV RNA in New Zealand blood donors, 2014–2015. The seroprevalence rates of 9.7% and 8.1%, as determined by the Wantai and MP Diagnostics ELISA kits respectively, are similar to results from some developed countries where the HEV seroprevalence rates were determined in blood donors using either of these ELISA kits. These include England/northern Wales (10%),[19] Ireland (5.3%),[20] Australia (6%)[21] and US (7.7%).[12] This compares to countries with HEV seroprevalence of >10% to ≤20% among blood donors, such as Austria (13.6%),[22] Denmark (10.7%)[23] and Norway (14%).[24] Higher seroprevalence rates (>20%) reported for blood donors include The Netherlands (26.7%),[10] France (22.4%)[25] and China (21.1%).[26]

Differences in specificities and sensitivities of HEV ELISAs have previously been reported.[17,27] Overall, higher seroprevalence rates are reported for studies that use the Wantai HEV IgG ELISA. In an Australian study, 194 plasma samples that tested positive and 200 samples that tested negative using the Wantai HEV IgG ELISA were subsequently tested using three MP Diagnostics HEV ELISA kits (ie, IgG only, IgM only and total [IgM, IgG, IgA]).[28] That study demonstrated poor agreement between the assays but found a higher concordance between the Wantai and MP Diagnostics HEV total antibody kits (both used in our study).[28] A meta-analysis of 73 published HEV IgG seroprevalance studies from Europe showed significantly higher seroprevalence rates across all cohorts in studies using the Wantai IgG ELISA assay.[29] The reason for this is unclear, and while it is possible that the Wantai assay may be overly sensitive (less specific), this ELISA is widely used (hence useful for comparisons) and considered one of the best performing HEV IgG ELISA kits available.[29–31]

Our study measured a higher seroprevalence than detected in an earlier New Zealand study of blood donors (11/265, 4.2%) which also used the HEV IgG Wantai ELISA.[16] It cannot be confirmed whether this is a true increase in seroprevalence among blood donors. The difference may be a result of differences between the study populations (age, sex and geographical distributions, eg, the earlier study only obtained samples from Auckland) and the sample numbers (265 vs 1,013).

As reported in other studies, age was shown to be a significant risk factor for previous HEV exposure, with seropositivity increasing significantly with age.[24–26] A higher seroprevalence in older persons is indicative of an age-related cohort effect due not only from a cumulative exposure throughout life but increased infection pressure in the past.[32,33]

In our study, we found no significant difference between seropositivity among males and females. This is in agreement with Hartl el al (2016) who, from a meta-analysis of 45 European studies on HEV seroprevalence, showed no significant difference in prevalence between genders.[29] However, some studies have observed a significantly higher HEV IgG prevalence in males than in females. For example, Zhuang et al demonstrated a significantly higher HEV IgG seropositivity in male (25.3%) than female (17.7%) blood donors in China.[34] The reason for this observation is unclear but may reflect different exposure risks between genders in different populations (eg, occupational or food preparation/consumption practices).

The lack of detection of HEV RNA in our study was not unexpected considering the low prevalence rates of HEV RNA in blood donors from other HEV-sporadic countries and the number of specimens tested (n=5,000). Examples of reported rates include 0.007% (1:15,000, Australia and Scotland), between 0.04–0.01% (1:3,000–1:10,000, England, Ireland, Austria and US) and 0.08% (1:1,300, Germany and The Netherlands).[9,12–14,20,22]

Information on potential risk factors for HEV exposure such as overseas travel (eg, travel to HEV endemic areas), occupation (eg, abattoir work, animal handling) and food consumption habits (eg, consumption of undercooked meats or pork) were not available for donors in our study. Several overseas studies have demonstrated the presence of HEV RNA in the food chain, most notably in pork products and an association between HEV seropositivity and exposure to pigs.[35,36] HEV IgG has been

detected in 20/22 (91%) of New Zealand pig herds.[37] It is plausible that for a proportion of New Zealand blood donors, HEV acquisition is autochthonous rather than overseas-acquired. As most cases of HEV infection are asymptomatic, with risk factors unclear, identification of HEV viraemic blood donors via additional questions on the current New Zealand Blood Service Donor Health Questionnaire is not feasible.

Any future studies (eg, a case-control study of HEV exposure risk) should attempt to identify specific risk factors for HEV exposure, particularly those related to transfusion and food consumption. A larger sample size would provide a better test of any regional differences in seroprevalence and a more accurate measure of HEV infection in the blood donor population.

Competing interests:
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