2019

Prevalence and impact of hepatitis E virus infection among persons with chronic hepatitis B living in the US and Canada

David R. McGivern
University of North Carolina at Chapel Hill

Hsing-Hua S. Lin
University of Pittsburgh

Junyao Wang
University of Pittsburgh

Tiffany Benzine
University of North Carolina at Chapel Hill

Henry L.A Janssen
University of Toronto

See next page for additional authors

Open Forum Infectious Diseases

MAJOR ARTICLE

Prevalence and Impact of Hepatitis E Virus Infection Among Persons With Chronic Hepatitis B Living in the US and Canada

David R. McGivern,[1,2] Hsing-Hua S. Lin,[3] Junyao Wang,[3] Tiffany Benzine,[1] Harry L. A. Janssen,[4] Mandana Khalili,[5] Mauricio Lisker-Melman,[6] Robert J. Fontana,[7] Steven H. Belle,[3] and Michael W. Fried[8]

[1]Lineberger Comprehensive Cancer Center and Division of Infectious Diseases, Department of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; [2]Center for Biologics Evaluation and Research, US Food and Drug Administration, Silver Spring, Maryland, [3]Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania; [4]Toronto Centre for Liver Disease, University Health Network, Toronto General Hospital, University of Toronto, Toronto, Ontario, Canada; [5]Department of Medicine, University of California San Francisco, San Francisco, California; [6]Division of Gastroenterology, Washington University School of Medicine, St. Louis, Missouri; [7]Division of Gastroenterology, Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan; [8]UNC Liver Center, Division of Gastroenterology and Hepatology, Department of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

Background. Patients with chronic hepatitis B virus (HBV) may experience spontaneous biochemical flares of liver disease activity. This study aimed to determine (i) the prevalence of prior and possible acute hepatitis E virus (HEV) infection among persons with chronic HBV and (ii) whether HEV infection is associated with liver disease flares among persons with chronic HBV.

Methods. Serum from a random sample of 600 adults in the Hepatitis B Research Network Cohort Study was tested for HEV RNA and anti-HEV IgM and IgG. Logistic regression models were used to estimate crude and adjusted odds ratios of anti-HEV prevalence for participant characteristics.

Results. Anti-HEV IgG and IgM seroprevalence was 28.5% and 1.7%, respectively. No participants had detectable HEV RNA. Of the 10 anti-HEV IgM+ participants, only 1 had elevated serum ALT at seroconversion. The odds of anti-HEV seropositivity (IgG+ or IgM+) were higher in older participants, males, Asians, less educated people, and those born outside the United States and Canada.

Conclusions. Acute HEV infection is a rare cause of serum ALT flares among persons with chronic HBV. The high seroprevalence of anti-HEV IgG among the chronic HBV patients is strongly associated with various demographic factors in this largely Asian American cohort.

Keywords. acute hepatitis; HEV; HBV; alanine aminotransferase; flare.

Hepatitis E virus (HEV) is endemic in many developing countries, where it is transmitted through the fecal–oral route and is an important cause of acute hepatitis, fulminant hepatic failure, and acute-on-chronic liver disease [1]. In contrast, large-scale outbreaks are rare in developed countries due to better infrastructure, water supply, and sanitation. However, clusters of disease have been reported in areas of low endemicity that are not associated with travel to areas of high endemicity but instead are associated with zoonotic transmission [2].

There are at least 5 genotypes of HEV (*Orthohepevirus A*) that infect humans and have different epidemiological features. Genotypes 1 and 2 are restricted to humans and are associated with fecal–oral transmitted epidemics in developing countries. Seroprevalence is estimated to range from 15% to 25% in Africa and parts of Asia [3]. In the United States, infection with genotypes 1 and 2 is typically found only in persons who have recently traveled to countries of high endemicity [4], including Mexico and Central America. Genotypes 3 and 4 are associated with sporadic cases of acute hepatitis in humans and are commonly found in domestic pigs, wild boar, and deer. Genotype 3 is found worldwide, whereas genotype 4 is found mainly in eastern Asia. Zoonotic transmission of genotypes 3 or 4 can occur through consumption of undercooked pig organ meat [5], deer meat [6], or contact with infected swine [7, 8]. Additionally, a recent case report described zoonotic transmission of genotype 7 HEV linked to consumption of camel-derived food products [9].

In Western Europe, genotype 3 HEV infection is increasingly recognized as an emerging infection [10], whose transmission is thought to be primarily foodborne [11]. HEV can also be transmitted by blood transfusion, but this is rare [12]. Although typically an acute, self-limiting infection, HEV can establish persistent infection among immunosuppressed persons such as transplant recipients [13]. Persistent genotype 3 HEV infections have been detected in Europe and the United States in transplant recipients who received blood products from infected but asymptomatic donors [13].

Received 26 February 2019; editorial decision 31 March 2019; accepted 5 April 2019.

Prior presentation. This work was presented in part at the European Association for the Study of the Liver, International Liver Congress 11–15 April 2018; Paris, France.

Correspondence: D.R. McGivern, PhD, Center for Biologics Evaluation and Research, US Food and Drug Administration, 10903 New Hampshire Avenue, Silver Spring, MD 20993 (david.mcgivern@fda.hhs.gov).

Open Forum Infectious Diseases®

DOI: 10.1093/ofid/ofz175

The extent of autochthonous genotype 3 HEV transmission in the United States is unclear, as surveillance efforts have been limited, but it may have been underestimated because, in contrast to genotypes 1 and 2, viremic persons with no comorbidities can be asymptomatic [10, 14, 15]. Furthermore, genotype 3 HEV infections among persons with underlying chronic liver disease may have been missed as clinical features of acute hepatitis E are indistinguishable from acute episodes of chronic hepatotropic virus infections or drug-induced liver injury [16]. In previously asymptomatic persons with chronic hepatitis B virus (HBV) infection, we hypothesize that co-infection with HEV may be responsible for acute exacerbation of liver disease.

The Hepatitis B Research Network (HBRN) is a National Institutes of Health–funded cooperative network that includes 21 clinical sites that recruited adult participants into an observational cohort study from diverse regions and populations in the United States and Canada to better understand the natural history of chronic hepatitis B [17]. This ancillary study aimed to examine (i) the seroprevalence of anti-HEV among adults with chronic HBV and associated demographic and clinical features and (ii) whether acute HEV infection is associated with the occurrence of serum ALT flares among adults with chronic HBV enrolled in the HBRN Adult Cohort Study.

METHODS

Study Design and Sample Selection

Details of the HBRN Cohort Study protocol and characteristics of the adult participants have been described previously [17]. Institutional review board approval was obtained from all clinical sites. All participants provided written informed consent.

Determining Seroprevalence of Anti-HEV in the HBRN Adult Cohort

As of November 21, 2016, 1991 HBsAg+ participants were enrolled in the HBRN Adult Cohort Study (Figure 1). Of these, 1351 had chronic HBV, were not in targeted enrollment groups (eg, acute, pregnant, hepatitis D virus [HDV]+, potentially eligible for 1 of the clinical trials), and had a serum sample at, or within 48 weeks of, study entry. To obtain estimates of the prevalence of HEV viremia and anti-HEV seropositivity, a random sample of 600 participants was selected and tested for anti-HEV IgG and IgM. Most of the participants in the random sample of 600 were noncirrhotic on the sample date: 8 participants had cirrhosis before the sample date, 1 had date of onset of cirrhosis on the sample date, and 1 had date of onset of cirrhosis 4 days after the sample date. The next closest cirrhosis onset was 15 weeks after the sample date.

Association of Anti-HEV Positivity With Unexplained Liver Disease Flares Among Adults With Chronic HBV

At or after enrollment, there were 122 adjudicated ALT flare events, defined as ALT ≥10 times the upper limit of normal (ULN; female ≥200 U/L, male ≥300 U/L) among the 1991 participants (Figure 2). Adjudication included both the date the flare began and its etiology. For the aim (ii) analyses, we

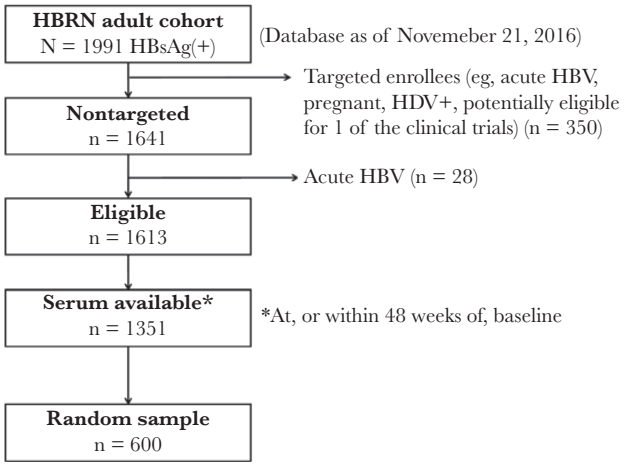

Figure 1. Sample selection flowchart for aim (1) seroprevalence of anti-HEV in the HBRN Adult Cohort. Abbreviations: HBRN, Hepatitis B Research Network; HBV, hepatitis B virus; HDV, hepatitis D virus.

excluded 24 flare events adjudicated as having known etiology (withdrawal of antiviral or immunosuppressive therapy [n = 11], HDV coinfection [n = 2], immune tolerant to immune active transition [n = 3], antiviral or drug-induced liver injury [n = 3], pregnancy or miscarriage [n = 2], and HBV related but cannot determine [n = 3]). The remaining 98 flare events occurred among 88 individuals. Other exclusions were 1 flare that occurred when the participant was receiving peginterferon and 15 for whom no serum was available between 6 weeks before and 12 weeks after the adjudicated flare onset date. In the 10 participants who had more than 1 flare event after these exclusions, only the first was included. Thus, 72 flares occurring among 72 participants are included in these analyses. Of these 72 flares, 5 flare cases had cirrhosis before the sample date (1 of whom was also in the random sample to estimate prevalence), 1 had date of onset of cirrhosis 3 weeks after the sample date, and the 1 furthest from the sample date had date of onset of cirrhosis 7 weeks after the sample date.

To determine whether anti-HEV positivity was associated with flare, the 72 cases with at-flare serum were compared with 288 nonflare controls who were not receiving peginterferon and not pregnant from the aim (i) sample. Matching (1 case to 4 controls) was by propensity of being a flare according to sex, age, place of birth, and HBeAg status.

Postflare serum samples >12 weeks and ≤60 weeks after adjudicated date of flare onset were available for 51/72 (71%) flare cases. Eligible postflare controls were identified by nonflare participants who were not receiving peginterferon and not pregnant from the aim (i) and also had a subsequent serum sample. Similar to at-flare, 51 postflare cases were 1:4 matched to 204 postflare controls. The propensity score included time between samples and sex, age, place of birth, and HBeAg status.

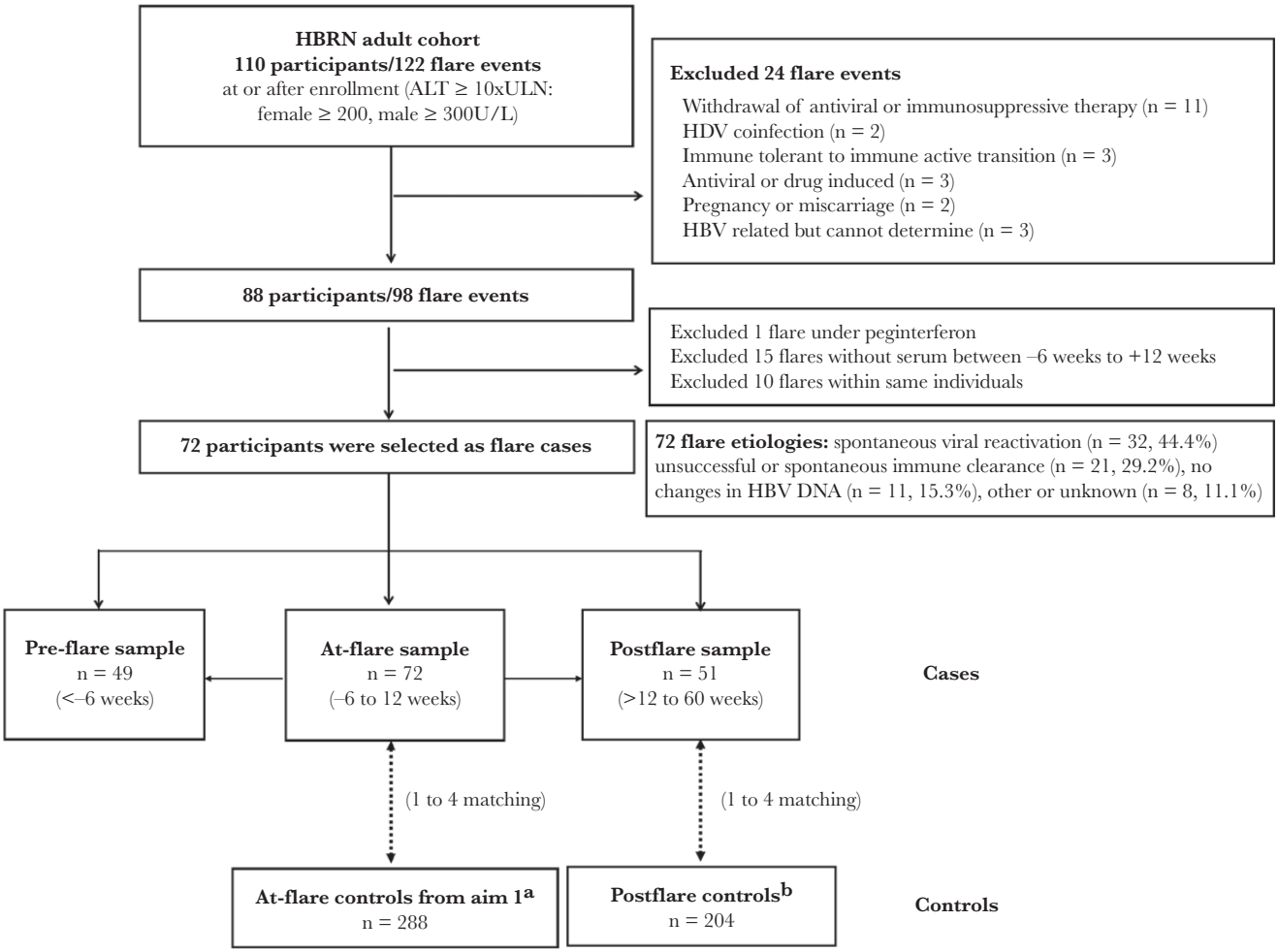

Figure 2. Sample selection flowchart for aim (2): association between HEV infection and liver disease flares among persons with chronic HBV. [a]At-flare samples: cases and controls were matched by propensity scores accounting for sex, age, place of birth, and HBeAg status. [b]Postflare samples: cases and controls were matched by propensity scores accounting for sex, age, place of birth, HBeAg status, and time between samples. [a,b]Exclusion criteria for both cases and controls: samples from participants on peginterferon treatment or from pregnant women. Abbreviations: HBRN, Hepatitis B Research Network; HBV, hepatitis B virus; HDV, hepatitis D virus.

Finally, to test whether anti-HEV positivity differed over time, preflare was compared with at-flare (n = 49) and postflare (n = 35), and at-flare was compared with postflare (n = 51). No participants decompensated in the time between preflare and postflare sample for this study.

Enzyme-Linked Immunosorbent Assays for Anti-HEV IgM and IgG

Serum samples were tested for anti-HEV IgM and IgG by enzyme-linked immunosorbent assay (ELISA). Commercial ELISA kits for the detection of anti-HEV IgM and IgG in human sera were purchased from Wantai (Beijing, China). Each sample was tested in duplicate. Assays were performed according to the manufacturer's instructions, and absorbance at 450 nm was measured using a Synergy Neo2 plate reader (Biotek, Winooski, VT). For those samples that tested positive for anti-HEV IgM, longitudinal analyses were performed to authenticate the initial test result.

RNA Extraction and Quantitative Reverse Transcription Polymerase Chain Reaction Detection of HEV RNA

RNA was extracted from 200-µL serum samples using the ZR-96 Viral RNA Kit (Zymo Research, Irvine, CA). Purified RNA was eluted into 15 µL of RNase-free molecular-grade water. HEV RNA was measured using a previously validated Taqman reverse transcription polymerase chain reaction (RT-PCR) assay with primers and probe targeting a region in the viral ORF3 region that is highly conserved between genotypes [18, 19]. RNA was quantified by comparison with a standard curve based on serial dilutions of genotype 3 HEV Kernow C1 (p6) genomic RNA that were generated by in vitro transcription from a cDNA clone [20]. The standard curve was compared with the World Health Organization international standard for HEV RNA (code number 6329/10) [21] to calculate a limit of detection for the assay of 75 IU.

Statistical Analyses

Determining Seroprevalence of Anti-HEV and Associations With Clinical and Demographic Characteristics

Seroprevalence (%) was calculated by the number of the 600 participants with each type of infection divided by the number tested, multiplied by 100. Ninety-five percent exact confidence intervals about the prevalence estimates were calculated. Demographic and clinical characteristics were summarized by HEV infection status. Categorical data were summarized with frequencies and percentages, and continuous data were summarized as mean (standard deviation) or median (25th and 75th percentiles), depending on the distribution. The Kruskal-Wallis test or Pearson's chi-square test was used to test whether distributions of continuous or categorical characteristics, respectively, differed between anti-HEV+ and anti-HEV- participants. Characteristics included age, sex, race (Asian, non-Asian), time since migration (ie, US/Canada born, migrated >20 years ago, migrated ≤20 years ago), geographical region of enrollment, education level, employment status, ALT, $\log_{10}$ HBV DNA, genotype, and HBeAg. Simple logistic regression models were used to test for differences in the seroprevalence of HEV by participant characteristics.

Multiple logistic regression models were fit stepwise and used to examine the independent (adjusted) associations between seroprevalence and demographic and clinical characteristics. The criterion for both entry and removal from the models was a P value ≤0.05. The results are presented as odds ratios (ORs), 95% confidence intervals (CIs), and P values.

Association of Anti-HEV Positivity With Liver Disease Flares Among Persons With Chronic HBV

The odds of having an ALT flare were compared between participants who were anti-HEV positive and those who were anti-HEV negative using conditional logistic regression to account for the propensity score matching of nonflare controls to flare cases, both at time of flare and postflare. To determine whether flares were associated with seroconversion from anti-HEV negative to positive, McNemar's test was used to test whether seroprevalence of anti-HEV changed pre- vs at-, pre- vs post-, and at- vs postflare.

SAS 9.4 and R 3.4.2 were used for statistical analysis and graphical displays.

RESULTS

Prevalence of HEV Infection in the HBRN Adult Cohort

Among the 600 randomly selected study participants from the HBRN adult cohort, the median age was 42 years, 50.7% were female, 72.5% were Asian, and 19.4% were US or Canada born (Table 1). Seroprevalence of anti-HEV+, defined as either anti-HEV IgM positive or anti-HEV IgG positive, was 29%. There were significant differences in anti-HEV prevalence by age (higher prevalence in older participants), males (34.8%) vs females (23.4%), Asians (31.8%) vs non-Asians (21.2%), immigrants (32.1%) vs those born in the United States or Canada (16.0%), and those with less than a Bachelor's degree (35.3%) vs at least a Bachelor's degree (20.6%). Active viremia (detectable HEV RNA) was not detected in any of the 600 samples. Regarding serological evidence of remote and recent acute infection (Table 2), 71.0% were anti-HEV IgG and IgM negative, 27.3% (n = 164) were anti-HEV IgG (+) only, 0.5% (n = 3) were IgM (+) only, and 1.2% (n = 7) were both IgG (+) and IgM (+). Therefore, the seroprevalence of anti-HEV IgG and IgM was 28.5% (95% CI, 24.9%–32.3%) and 1.7% (95% CI, 0.8%–3.0%), respectively. Median ALT for the 10 anti-HEV IgM+ participants (range) was 27.5 (9–76) U/L.

HEV seroprevalence was examined by geographical region based upon site of enrollment (Supplementary Table 1). HEV seroprevalence among participants from the Canadian site was the highest (42%), followed by sites in the US-West (37%), US-Midwest (26%), US-Southwest (23%), US-Southeast (19%), and US-Northeast (18%). Geographical region was strongly associated with the 2 categories of race (Asian vs non-Asian: chi-square test $P < .0001$): 89% of the participants at the sites in the US-West were Asian, and 85% were Asian at the Canadian site, compared with 56% at sites in the US-Southeast and 58% at sites in the US-Midwest. Thus, in the multivariable model, race (Asian/non-Asian) was used, not region.

In the multivariable (adjusted) logistic regression model, characteristics significantly and independently ($P < .05$) associated with higher seroprevalence of HEV were older age, male, Asian race, less education, and positive HBeAg (Table 3). Although those who migrated to the United States or Canada more than 20 years ago did not have a significantly different seroprevalence of HEV than those born in the United States or Canada, more recent immigrants had significantly higher seroprevalence than those born in the United States or Canada.

The association of education with HEV seroprevalence varies by race (P value for race × education interaction = 0.046). Among participants with less than a Bachelor's degree, Asians had 2.9 times higher odds of HEV seroprevalence than non-Asians, whereas the odds of HEV seroprevalence were similar between Asians and non-Asians among those who had at least a Bachelor's degree (Table 3).

Longitudinal Analyses of Sera From Anti-HEV IgM-Positive Subjects

To further authenticate the 10 anti-HEV IgM-positive subjects, archival sera from before and after the initial positive result were assayed for anti-IgM and IgG (Figure 3). Only 4/10 subjects (Figure 3A–D) were confirmed as recent infections based on anti-HEV IgM and IgG kinetic characteristics of the convalescence phase of acute HEV (anti-HEV IgM positive but declining, anti-HEV IgG increasing). Of these 4 subjects, only 1 (Figure 3 D) had increased ALT around the time of seroconversion. The remaining 6 subjects were unclear or unconfirmed.

Table 1. Participants' Characteristics by Anti-HEV Positivity

Characteristics	Total n = 600	Anti-HEV (-) n = 426 (71%)	Anti-HEV (+) n = 174 (29%)	*P* Value[a]
Age at current study visit, y	n = 600	n = 426	n = 174	<.001
Median (25th %-ile: 75th %-ile)	42.1 (32.8: 53.0)	39.8 (31.4: 50.6)	47.7 (38.1: 58.1)	
Age stratum, No. (%)	n = 600	n = 426	n = 174	<.001
18–<30 y	103 (17.2)	83 (80.6)	20 (19.4)	
30–<40 y	163 (27.2)	135 (82.8)	28 (17.2)	
40–<50 y	143 (23.8)	97 (67.8)	46 (32.2)	
50+ y	191 (31.8)	111 (58.1)	80 (41.9)	
Sex, No. (%)	n = 600	n = 426	n = 174	.002
Female	304 (50.7)	233 (76.6)	71 (23.4)	
Male	296 (49.3)	193 (65.2)	103 (34.8)	
Race, No. (%)	n = 599	n = 426	n = 173	.01
Non-Asian	165 (27.5)	130 (78.8)	35 (21.2)	
Asian	434 (72.5)	296 (68.2)	138 (31.8)	
Time since migration, No. (%)	n = 545	n = 387	n = 158	.001[b]
Born in US/Canada	106 (19.4)	89 (84.0)	17 (16.0)	
Born outside US/Canada	439 (80.6)	298 (67.9)	141 (32.1)	.83[c]
Migrated >20 y ago	187 (42.6)	128 (68.4)	59 (31.6)	
Migrated ≤20 y ago	252 (57.4)	170 (67.5)	82 (32.5)	
Education level, No. (%)	n = 594	n = 426	n = 168	<.001
Bachelor's or higher	282 (47.5)	224 (79.4)	58 (20.6)	
Less than Bachelor's	312 (52.5)	202 (64.7)	110 (35.3)	
Employment status, No. (%)	n = 597	n = 426	n = 171	.28
Employed, full-time or part-time	447 (74.9)	326 (72.9)	121 (27.1)	
Homemaker, not currently working for pay	25 (4.2)	18 (72.0)	7 (28.0)	
Not currently employed	125 (20.9)	82 (65.6)	43 (34.4)	
ALT, U/L	n = 595	n = 425	n = 170	.41
Median (25th %-tile: 75th %-tile)	33 (22: 51)	31 (22: 51)	35 (22: 52)	
HBV DNA, $\log_{10}$ IU/mL	n = 600	n = 426	n = 174	.23
Median (25th %-ile: 75th %-ile)	3.5 (2.3: 5.3)	3.5 (2.3: 5.1)	3.7 (2.6: 5.6)	
Genotype, No. (%)	n = 555	n = 391	n = 164	.79
A	90 (16.2)	65 (72.2)	25 (27.8)	
B	224 (40.4)	154 (68.8)	70 (31.3)	
C	176 (31.7)	123 (69.9)	53 (30.1)	
D	44 (7.9)	32 (72.7)	12 (27.3)	
Other: E, F, or multiple genotypes	21 (3.8)	17 (81.0)	4 (19.0)	
HBeAg, No. (%)	n = 600	n = 426	n = 174	.92
Negative	464 (77.3)	329 (70.9)	135 (29.1)	
Positive	136 (22.7)	97 (71.3)	39 (28.7)	

Abbreviations: HBV, hepatitis B virus; HEV, hepatitis E virus.

[a] *P* values were obtained using the Kruskal Wallis test for continuous variables and Pearson's chi-square test for categorical variables.

[b] *P* value was obtained using a Pearson's chi-square test between those born in the US/Canada and born outside the US/Canada.

[c] *P* value was obtained using a Pearson's chi-square test between those who migrated >20 years ago and those who migrated ≤20 years ago.

Table 2. Anti-HEV IgG and Anti-HEV IgM Distributions Among 600 Participants

Anti-HEV Status	HEV Infection Status	Anti-HEV IgM Status	Anti-HEV IgG Status	No.	% (95% CI[a])
Negative	No infection	Negative	Negative	426	71.0 (67.2–74.6)
Positive	Remote	Negative	Positive	164	27.3 (23.8–31.1)
	Recent	Positive	Negative	3	0.5 (0.1–1.5)
		Positive	Positive	7	1.2 (0.5–2.4)

Abbreviations: CI, confidence interval; HEV, hepatitis E virus.

[a] Exact binomial 95% confidence intervals.

Table 3. Adjusted Association Between Anti-HEV Positivity and Participants' Characteristics

Characteristics	*P* Value[a] Adjusted Odds Ratio (95% Wald CI) (n = 540)
Age at current study visit, y	*P* < .001
Per-year increase	1.05 (1.03–1.07)
Sex	*P* < .001
Female	1.00 (reference)
Male	2.10 (1.39–3.19)
Race and education level	*P* = .046
Bachelor's or higher: Asian vs non-Asian	1.08 (0.50–2.33)
Less than Bachelor's: Asian vs non-Asian	2.92 (1.42–6.01)
Time since migration	*P* = .045
Born in US/Canada	1.00 (reference)
Migrated >20 y	1.30 (0.62–2.74)
Migrated ≤20 y	2.09 (1.03–4.25)
HBeAg	*P* = .036
Negative	1.00 (reference)
Positive	1.74 (1.04–2.93)

Abbreviations: CI, confidence interval; HEV, hepatitis E virus.

[a]Type III *P* values were obtained using a multivariable logistic regression model.

Subjects E–H were anti-HEV IgM positive at week 0 with IgM declining over time but no increase in anti-HEV IgG. Subjects E–H may have been exposed to HEV but failed to develop long-lived IgG responses. Alternatively, the initial anti-HEV IgM may have been a false positive. Subjects I and J only had sera available from 1 additional time point; thus the longitudinal analyses are difficult to interpret. Subject I was initially anti-HEV IgG positive and IgM negative and 28 weeks later remained positive for IgG (albeit at lower levels) but became anti-HEV IgM positive, possibly indicating re-infection. Subject J was anti-HEV IgM positive and IgG negative at week 0. The only additional sample for subject J was from 184 weeks later, which also tested positive for anti-HEV IgM but was negative for IgG. This case could either represent anti-HEV IgM false positives or mild, acute infections that were not accompanied by development of HEV-specific IgG.

In summary, using highly stringent criteria for recent infection based on longitudinal analyses (anti-HEV IgM positive but declining with anti-HEV IgG increasing over time), the prevalence of recent HEV infection was 0.7% (4/600 samples; 95% CI, 0.2%–1.7%).

Testing Whether Anti-HEV Positivity Is Associated With Liver Disease Flares Among Persons With Chronic HBV

The etiologies of the 72 flare cases included spontaneous viral reactivation (n = 32, 44.4%), unsuccessful or spontaneous immune clearance (n = 21, 29.2%), no changes in HBV DNA (n = 11, 15.3%), and other or unknown (n = 8, 11.1%) (Figure 2). The seroprevalence of anti-HEV (IgM+ and/or IgG+) was similar among at-flare samples in cases (36%; median ALT, 365 U/L) and matched controls (35%; median ALT, 29 U/L) (Supplementary Figure 1A). The seroprevalence rates of anti-HEV were also similar among flare cases' postflare samples (31%; median ALT, 68 U/L) compared with matched postflare controls (29%; median ALT, 32 U/L) (Supplementary Figure 1B). The odds of flaring were not significantly different between anti-HEV positive (IgG+ or IgM+) and anti-HEV negative (IgG- and IgM-) participants (OR, 1.3; 95% CI, 0.7–2.5) for the at-flare and matched samples (Supplementary Table 2). Similar results were found using postflare matched samples (OR, 1.2; 95% CI, 0.6–2.6).

Among 72 flare cases, seroprevalence of anti-HEV was similar pre- (29%), at- (36%), and postflare (31%) (Supplementary Figure 1C), and seroprevalence of anti-HEV did not differ significantly between pre- vs at- (*P* = .45), pre- vs post- (*P* = .51), and at- vs postflare (*P* = .55) (Supplementary Table 3).

DISCUSSION

This study showed that acute HEV infection is a rare cause of spontaneous flares in persons with chronic hepatitis B in the United States and Canada. Initial screening of 600 serum samples randomly selected from eligible HBRN adult participants identified 10 subjects who were positive for anti-HEV IgM. Previous studies have noted variation in the performance of commercial assays for the detection of HEV-specific IgM [22]. There is no confirmatory assay for anti-HEV IgM antibodies, but false-positive rates are very low [22]. To further authenticate the 10 IgM-positive results, longitudinal analyses were performed to determine whether these anti-HEV IgM-positive measurements were biologically plausible. Of the 10 subjects, only 4 showed anti-HEV IgM declining and IgG increasing over time, providing a more stringent estimate of HEV IgM seroprevalence at 4/600 or 0.7%. This leaves 6 out of the 10 cases that were originally identified as anti-HEV IgM positive as unconfirmed. However, this may be overly stringent, as recent studies suggest that immunocompetent people infected with HEV in industrialized countries do not always develop a persistent humoral response to the virus and a sustained specific IgG response may be associated with repeated exposures [23]. Of the 10 anti-HEV IgM-positive subjects, only 1 showed elevated ALT just before seroconversion. This flare was transient and without jaundice. ALT elevation may have been missed in the other IgM-positive subjects if it was transient and normalized by the time of the subject's clinic visit. Asymptomatic infection with genotype 3 HEV is not uncommon, and studies of blood donors have detected HEV infection in the context of normal ALT levels [24].

For 2 of the anti-HEV IgM-positive subjects, there was evidence for remote infection followed by reinfection. There is evidence in the literature of sero-reversion and reinfection

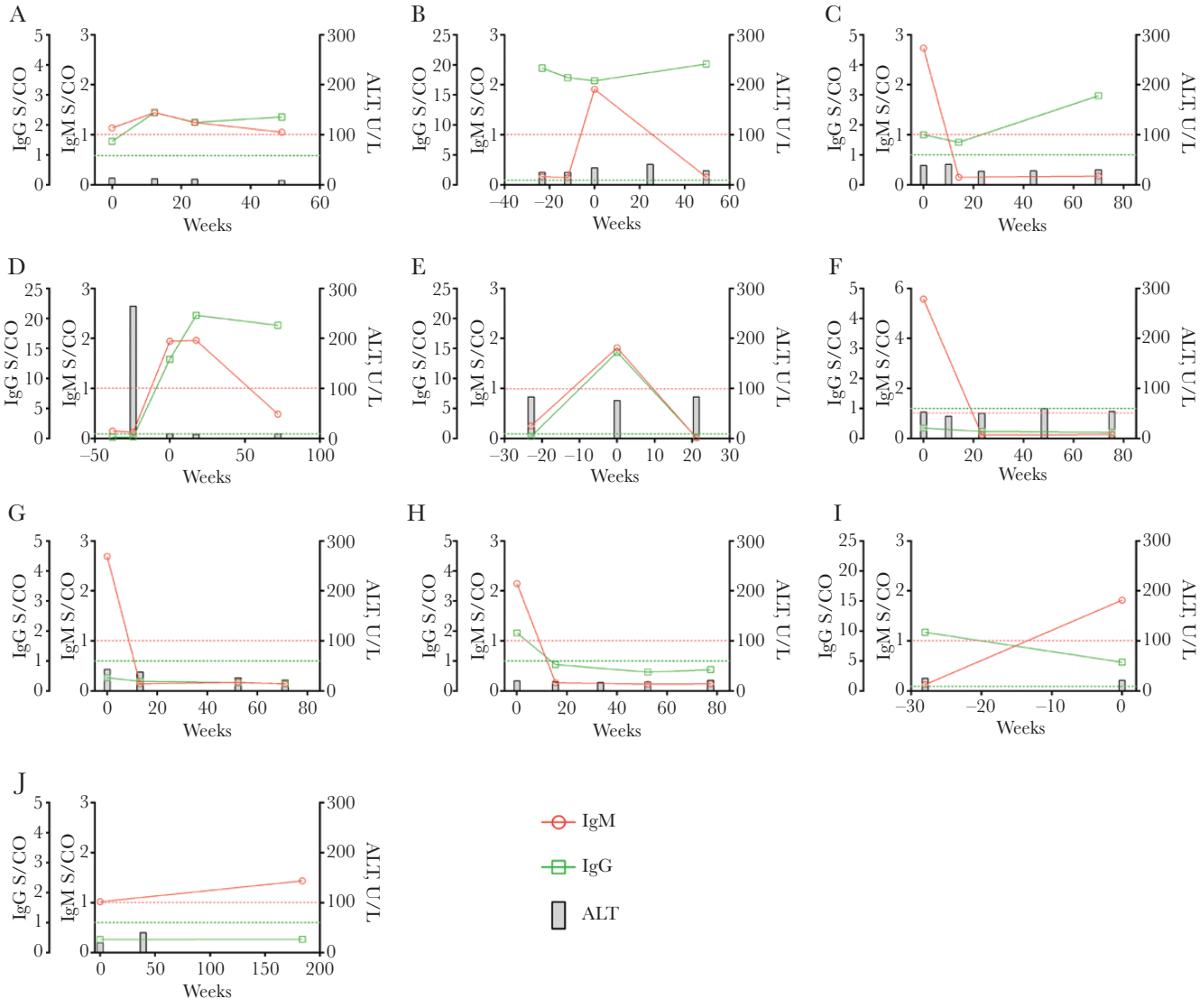

Figure 3. Follow-up longitudinal analysis of antibody responses to HEV among 10 subjects who tested positive for anti-HEV IgM out of a random sample of 600 subjects from the adult HBRN cohort. To authenticate anti-HEV IgM positive samples, further samples were tested from the same subjects, both before and after the initial IgM positive result. For each subject, antibody responses to HEV are expressed as signal/cut-off (S/CO) and plotted against time (weeks) where the initial anti HEV IgM positive test is shown as week 0. Anti-HEV IgM is plotted on the left y-axis (red open circles) and IgG is plotted on the far-left y-axis (green open squares). Serum ALT is plotted on the right y-axis (gray bars). Dashed red and green lines indicate the cutoff for the assay (S/CO=1).

from longitudinal studies of immunocompetent patients with hemoglobinopathies who received multiple transfusions [23].

The seroprevalence of 28.5% (95% CI, 24.9%–32.3%) for anti-HEV IgG among persons with chronic HBV is higher compared with the general population in the United States, where seroprevalence was 21.0% (95% CI, 19.0%–22.9%) based on testing of participants in the Third National Health and Nutrition Examination Survey (NHANES III) from 1988 to 1994 [25]. More recently, anti-HEV IgG seroprevalence was found to be 6.0% (95% CI, 5.1%–6.9%) based on data from the NHANES 2009–2010 survey [26]. Differences in assay sensitivity between the 2 studies may partly account for the decline in HEV IgG seroprevalence, but analyses of samples from the different time periods using the same assay showed that anti-HEV prevalence was decreasing in the United States [27]. However, neither of the studies of NHANES participants used the Wantai commercial assay that was used in this study. Of commercial assays, several studies have found the Wantai anti-HEV IgG assay to have high sensitivity (>99%) and specificity (>96%) [28–33]. A study that tested US blood donors for anti-HEV IgG using the Wantai ELISA found a seroprevalence of 18.8% (95% CI 17.0%–20.5%) in samples donated in 2006 and 2012 [34]. Compared with US blood donors, anti-HEV IgG prevalence among persons with chronic HBV in the HBRN Adult Cohort Study is significantly

higher (28.5%; 95% CI, 24.9%–32.3%; $P < .001$). This difference could be due to HEV exposure in endemic regions of the world, because in our sampling of adult HBRN participants, 80.6% were born outside the United States or Canada. We found higher seroprevalence among persons born outside the United States and Canada, likely reflecting acquisition of acute HEV infection in areas of high endemicity and virus clearance before immigration. Additionally, higher seroprevalence was associated with lower education levels (less than a Bachelor's degree) among persons of Asian race. This may reflect higher risk of remote exposure to HEV (ie, before migration) among persons of lower socioeconomic status who immigrated from endemic regions.

HEV RNA was not detected in any of the participants, consistent with the short duration of viremia during acute HEV infection and previous reports of low prevalence of HEV viremia in the United States—1:42 000 or 1:9500 based on screens of blood donors [35] or plasma donors [36], respectively. HEV RNA was not measured in liver tissue or stool, neither of which was collected as part of the current observational study.

We did not find an association between ALT flares and previously undiagnosed HEV infection (as determined by seroconversion to anti-HEV IgG positive after the flare) among 72 flare cases. The lack of association between ALT flares and HEV infection in this study may be due to the small sample sizes that were available longitudinally among flare cases (Supplementary Table 2) or the low prevalence of acute HEV infection in our study sample (ie, only 1 subject who experienced an ALT flare tested IgM+). A recent study showed that among patients with acute HEV infection at public hospitals and clinics in Hong Kong, those with underlying chronic hepatitis B had significantly worse disease outcomes and higher mortality [37].

As discussed above, the seroprevalence of HEV-specific IgG was higher among persons of Asian race and persons born outside the United States or Canada. No information is available concerning whether these participants immigrated from regions of high HEV prevalence. It is interesting to speculate that they may have been exposed to acute (genotype 1 or 2) HEV infection and developed protective immunity before migration and thus be less likely to develop HEV infection following subsequent exposure to genotype 3 HEV in North America.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Acknowledgments

The Hepatitis B Research Network is funded by the National Institute of Diabetes and Digestive and Kidney Diseases. The authors acknowledge the use of HBRN data and samples as the sole contribution of the HBRN. The investigators recognize and appreciate the contributions of the HBRN participants, without whom this research would not be possible.

Financial support. This work was supported by the National Institute of Diabetes and Digestive and Kidney Disease grant U01 DK082867 to Dr Michael W. Fried and U01 DK082864 to Dr Steven H. Belle as an ancillary study of the Hepatitis B Research Network.

Disclaimer. This article reflects the views of the authors and should not be construed as representing the Food and Drug Administration's views or policies.

Potential conflicts of interest. D.R.M. has received research support from Gilead Sciences and AbbVie. M.K. has received research support from Gilead Sciences, Intercept Pharmaceuticals, and AbbVie, and she has served on a scientific advisory panel for Gilead Sciences. M.L.M. is on the speakers' bureau for Gilead, Simply Speaking, and AbbVie. R.J.F. has received research support from AbbVie, Gilead Sciences, and Bristol Myers Squibb and has served as a consultant for Alnylam Pharmaceuticals. M.W.F. is a consultant for and has received grant support from AbbVie, Bristol-Myers Squibb, Gilead, and Merck; he is a consultant for and owns stock in TARGET PharmaSolutions. H.S.L., J.W., T.B., H.L.A.J., and S.H.B. have no conflicts of interest to report. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Aggarwal R, Aggarwal RA. Hepatitis E: clinical presentation in disease-endemic areas and diagnosis. Semin Liver Dis **2013**; 33:30–40.
2. Meng XJ. Zoonotic and foodborne transmission of hepatitis E virus. Semin Liver Dis **2013**; 33:41–9.
3. Rein DB, Stevens GA, Theaker J, et al. The global burden of hepatitis E virus genotypes 1 and 2 in 2005. Hepatology **2012**; 55:988–97.
4. Barbre KA, Jentes ES, Drobeniuc J, et al. Seroprevalence of hepatitis E among Boston area travelers, 2009–2010. Am J Trop Med Hyg **2017**; 96:929–34.
5. Feagins AR, Opriessnig T, Guenette DK, et al. Detection and characterization of infectious hepatitis E virus from commercial pig livers sold in local grocery stores in the USA. J Gen Virol **2007**; 88:912–7.
6. Tei S, Kitajima N, Ohara S, et al. Consumption of uncooked deer meat as a risk factor for hepatitis E virus infection: an age- and sex-matched case-control study. J Med Virol **2004**; 74:67–70.
7. Drobeniuc J, Favorov MO, Shapiro CN, et al. Hepatitis E virus antibody prevalence among persons who work with swine. J Infect Dis **2001**; 184:1594–7.
8. Withers MR, Correa MT, Morrow M, et al. Antibody levels to hepatitis E virus in North Carolina swine workers, non-swine workers, swine, and murids. Am J Trop Med Hyg **2002**; 66:384–8.
9. Lee GH, Tan BH, Teo EC, et al. Chronic infection with camelid hepatitis E virus in a liver transplant recipient who regularly consumes camel meat and milk. Gastroenterology **2016**; 150:355–7 e3.
10. Dalton HR, Bendall R, Ijaz S, Banks M. Hepatitis E: an emerging infection in developed countries. Lancet Infect Dis **2008**; 8:698–709.
11. Drobeniuc J, Greene-Montfort T, Le NT, et al. Laboratory-based surveillance for hepatitis E virus infection, United States, 2005–2012. Emerg Infect Dis **2013**; 19:218–22; quiz 353.
12. Hewitt PE, Ijaz S, Brailsford SR, et al. Hepatitis E virus in blood components: a prevalence and transmission study in Southeast England. Lancet **2014**; 384:1766–73.
13. Kamar N, Selves J, Mansuy JM, et al. Hepatitis E virus and chronic hepatitis in organ-transplant recipients. N Engl J Med **2008**; 358:811–7.
14. Kamar N, Garrouste C, Haagsma EB, et al. Factors associated with chronic hepatitis in patients with hepatitis E virus infection who have received solid organ transplants. Gastroenterology **2011**; 140:1481–9.
15. Slot E, Hogema BM, Riezebos-Brilman A, et al. Silent hepatitis E virus infection in Dutch blood donors, 2011 to 2012. Euro Surveill **2013**; 18(31).
16. Davern TJ, Chalasani N, Fontana RJ, et al. Acute hepatitis E infection accounts for some cases of suspected drug-induced liver injury. Gastroenterology **2011**; 141:1665–72 e1-9.
17. Ghany MG, Perrillo R, Li R, et al; Hepatitis B Research Network; Hepatitis B Research Network. Characteristics of adults in the hepatitis B research network in North America reflect their country of origin and hepatitis B virus genotype. Clin Gastroenterol Hepatol **2015**; 13:183–92.
18. Jothikumar N, Cromeans TL, Robertson BH, et al. 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.
19. Kodani M, Mixson-Hayden T, Drobeniuc J, Kamili S. Rapid and sensitive approach to simultaneous detection of genomes of hepatitis A, B, C, D and E viruses. J Clin Virol **2014**; 61:260–4.

20. Shukla P, Nguyen HT, Torian U, et al. Cross-species infections of cultured cells by hepatitis E virus and discovery of an infectious virus-host recombinant. Proc Natl Acad Sci U S A **2011**; 108:2438–43.
21. Baylis SA, Blümel J, Mizusawa S, et al; HEV Collaborative Study Group. World Health Organization International Standard to harmonize assays for detection of hepatitis E virus RNA. Emerg Infect Dis **2013**; 19:729–35.
22. Zafrullah M, Zhang X, Tran C, et al. Disparities in detection of antibodies against hepatitis E virus in US blood donor samples using commercial assays. Transfusion **2018**; 58:1254–63.
23. Servant-Delmas A, Abravanel F, Lefrère JJ, et al. New insights into the natural history of hepatitis E virus infection through a longitudinal study of multitransfused immunocompetent patients in France. J Viral Hepat **2016**; 23:569–75.
24. Kamar N, Bendall R, Legrand-Abravanel F, et al. Hepatitis E. Lancet **2012**; 379:2477–88.
25. Kuniholm MH, Purcell RH, McQuillan GM, et al. Epidemiology of hepatitis E virus in the United States: results from the Third National Health and Nutrition Examination Survey, 1988–1994. J Infect Dis **2009**; 200:48–56.
26. Ditah I, Ditah F, Devaki P, et al. Current epidemiology of hepatitis E virus infection in the United States: low seroprevalence in the National Health and Nutrition Evaluation Survey. Hepatology **2014**; 60:815–22.
27. Teshale EH, Denniston MM, Drobeniuc J, et al. Decline in hepatitis E virus antibody prevalence in the United States from 1988–1994 to 2009–2010. J Infect Dis **2015**; 211:366–73.
28. Al-Absi ES, Al-Sadeq DW, Younis MH, et al. Performance evaluation of five commercial assays in assessing seroprevalence of HEV antibodies among blood donors. J Med Microbiol **2018**; 67:1302–9.
29. Pas SD, Streefkerk RH, Pronk M, et al. Diagnostic performance of selected commercial HEV IgM and IgG ELISAs for immunocompromised and immunocompetent patients. J Clin Virol **2013**; 58:629–34.
30. Bendall R, Ellis V, Ijaz S, et al. A comparison of two commercially available anti-HEV IgG kits and a re-evaluation of anti-HEV IgG seroprevalence data in developed countries. J Med Virol **2010**; 82:799–805.
31. Mansuy JM, Bendall R, Legrand-Abravanel F, et al. Hepatitis E virus antibodies in blood donors, France. Emerg Infect Dis **2011**; 17:2309–12.
32. Mansuy JM, Legrand-Abravanel F, Calot JP, et al. High prevalence of anti-hepatitis E virus antibodies in blood donors from South West France. J Med Virol **2008**; 80:289–93.
33. Kmush BL, Labrique AB, Dalton HR, et al. Two generations of "gold standards": the impact of a decade in hepatitis E virus testing innovation on population seroprevalence. Am J Trop Med Hyg **2015**; 93:714–7.
34. Xu C, Wang RY, Schechterly CA, et al. An assessment of hepatitis E virus (HEV) in US blood donors and recipients: no detectable HEV RNA in 1939 donors tested and no evidence for HEV transmission to 362 prospectively followed recipients. Transfusion **2013**; 53:2505–11.
35. Stramer SL, Moritz ED, Foster GA, et al. Hepatitis E virus: seroprevalence and frequency of viral RNA detection among US blood donors. Transfusion **2016**; 56:481–8.
36. Roth NJ, Schäfer W, Alexander R, et al. Low hepatitis E virus RNA prevalence in a large-scale survey of United States source plasma donors. Transfusion **2017**; 57:2958–64.
37. Lai JC, Wong GL, Yip TC, et al. Chronic hepatitis B increases liver-related mortality of patients with acute hepatitis E: a territorywide cohort study from 2000 to 2016. Clin Infect Dis **2018**; 67:1278–84.