Disparities in detection of antibodies against hepatitis E virus in US blood donor samples using commercial assays

Mohammad Zafrullah,[1] Xiugen Zhang,[1] Coleen Tran,[1,3] Megan Nguyen,[2,4] Saleem Kamili,[1] Michael A. Purdy (iD)_,[1] and Susan L. Stramer[2]_

BACKGROUND: Reported hepatitis E virus (HEV) antibody assay performance characteristics are variable. Using a subset of surplus US blood donation samples, we compared assays for detecting anti-HEV immunoglobulin M (Ig)M and IgG or total anti-HEV antibodies.

STUDY DESIGN AND METHODS: Samples from 5040 random blood donations, all HEV-RNA negative, collected primarily in the midwestern United States in 2015 were tested for anti-HEV IgM and IgG or total anti-HEV using assays manufactured by Diagnostic Systems, Wantai, and MP Biomedicals.

RESULTS: Overall, the percentage of detection for anti-HEV IgG and total anti-HEV was 11.4%, and for anti-HEV IgM was 1.8%. Nine samples were reactive for anti-HEV IgM by all assays, giving a recent infection rate of 0.18%. Anti-HEV IgG/total anti-HEV detection rates increased with age. Interassay agreement was higher among the IgG anti-HEV/total anti-HEV assays (84%) than the IgM assays (22%). Regression analyses of signal-to-cutoff ratios from IgG/total antibody assay were heteroskedastic, indicating no constant variance among these assays, suggesting they may detect different epitopes or were affected by waning or less avid antibodies in the US donor population.

CONCLUSIONS: Although similar percentages of IgG anti-HEV/total anti-HEV detection were observed across the three commercial assays, each assay detected a unique sample subpopulation and was heteroskedastic when compared pairwise. Discordance was higher among anti-HEV IgM assays, but a recent HEV infection rate of at least 0.18% was estimated based on assay concordance.

Hepatitis E is caused by the hepatitis E virus (HEV).[1] HEV is a member of the _Hepeviridae_ family. Variants that infect humans belong to the species _Orthohepevirus A_.[2,3] This species has eight recognized genotypes, of which five, Genotypes 1, 2, 3, 4, and 7, are known to infect humans.[3,4] Although acute hepatitis E is usually self-limiting with low mortality (about 1%-3%), the mortality rate can reach levels of up to 30% in pregnant women during the second and third

trimester who are infected with Genotypes 1 or 2.[5,6] HEV Genotypes 3 and 4 are frequently associated with chronic infection in immunocompromised individuals, most notably solid-organ transplant recipients, leading to chronic hepatitis, cirrhosis, and liver failure.[7-9] Early epidemiologic studies indicated the virus was restricted to developing countries where it was transmitted by a fecal-oral route through contaminated water, causing large outbreaks. Sporadic cases of hepatitis E seen in developed countries were originally thought to be associated only with travel to endemic areas. However, more recent studies have shown that autochthonous hepatitis E, Genotypes 3 and 4, can be found in developed countries transmitted zoonotically from infected animals through the consumption of raw or undercooked meat and offal.[10-12] Most cases of acute hepatitis E in developed countries tend to be asymptomatic.[13-15] Because asymptomatic HEV infection in blood donors has been documented via HEV RNA detection, and HEV has been documented to be transfusion transmitted, there is increasing concern worldwide about blood safety.[15,16]

Antibody prevalence in blood donors varies depending on geographic location and the assays used. For example, in Europe anti-HEV immunoglobulin (Ig)G seroprevalence in blood donors ranges from 1.3% to 52%,[14] increases with age, and higher IgG positivity is usually seen in males compared to females.[17,18] A study in the Netherlands using a single assay showed that IgG anti-HEV prevalence had decreased from 47% in 1988 to 21% in 2011,[18] and in the United States, the National Health and Nutrition Examination Survey (NHANES) using the same anti-HEV IgG assay found decreases in IgG prevalence from 10.2% during 1988 to 1994 to 6% during 2009 to 2010.[19] An analysis of 1939 US blood donors at the National Institutes of Health (NIH) in 2013 found IgG prevalence of 18.8% and IgM prevalence of 0.4%. IgG prevalence increased with donor age, with prevalence decreasing from 21.8% in 2006 to 16% in 2012. The NIH study used an in-house assay for the first analysis and a commercial assay for the subsequent analysis. None of the NIH donors were HEV-RNA positive.[20] A subsequent study of 4499 HEV-RNA–negative samples at the American Red Cross (ARC), which was a subset of 18,829 donation samples collected in 2013 from which HEV RNA occurred in 1 per 9500 donations (95% confidence interval [CI], 1:2850-1:56,180), showed the antibody detection rates using a different assay were 7.7% for IgG and 0.58% for IgM.[21] Similarly, antibody prevalence increased with age and was highest in the midwestern United States (12.5%; odds ratio of 2.23 vs. other US regions).

In spite of documented declines in HEV seroprevalence, an increase in prevalence among 18- to 21-year-old Dutch blood donors was observed.[18] There has also been an increase in the number of reported hepatitis E cases in several countries where it is a reportable disease.[22-24]

However, it is not known whether this increase is due to increased HEV incidence or to increased reporting. Part of the problem is that clinical assays are validated to determine the status of an analyte in a symptomatic individual and not specifically for epidemiologic studies of healthy populations. This is particularly true of HEV serological assays because the lack of concordance has led to variable findings in asymptomatic populations when commercial assays have been compared.[25-27] Currently, there are no Food and Drug Administration–approved anti-HEV or HEV-RNA assays.

This study examined samples from 5040 US blood donations using three different commercial IgG and IgM anti-HEV assays to determine the anti-HEV prevalence rates and to examine the concordance among the immunoassays used.

MATERIALS AND METHODS

Sample selection and preparation

Residual samples from blood donations made to the ARC from March 22 to April 3, 2015, were obtained. Samples from donations positive for routine disease markers (e.g., hepatitis B virus, hepatitis C virus, and human immunodeficiency virus) were excluded. All samples used in this study were selected from approximately 50,000 samples screened by research-use-only HEV-RNA assays to exclude RNA positives (the results of HEV-RNA screening are not considered in the current study). A total of 5040 random samples were enrolled. Blood was collected in plasma preparation tubes; the plasma from these tubes was stored at −70°C until tested.[21] Samples were tested under code, with donor identities not available to investigators. Where appropriate, basic demographics were obtained from an ARC research database associated with this study. The study was approved by the ARC Institutional Review Board. As part of the donation consent, all donors were provided with an information sheet describing future potential uses of their surplus samples for studies on transfusion-transmissible infections. The samples were anonymized and sent to the Centers for Disease Control and Prevention (CDC) for testing.

Antibody testing

This study used six enzyme immunoassays from three commercial companies: Diagnostic Systems Incorporated (DSI S.r.l. [hereafter, DSI]); MP Biomedicals Asia Pacific Pte. Ltd. (MP); and Beijing Wantai Biological Pharmacy Enterprise Co., Ltd. (Wantai). The detection of HEV IgM antibodies used three assays: DS-EIA-ANTI-HEV-M (batch: E-152, DSI), HEV IgM ELISA 3.0 (23162-096, MP), and HEV-IgM ELISA (WE-7192, Wantai). The detection of HEV IgG antibodies used two assays: DS-EIA-ANTI-HEV-G (E-151, DSI) and HEV-IgG ELISA (WE-7292, Wantai). The

TABLE 1. Anti-HEV results by assay for 5040 blood donor samples*					
	Negative		Gray zone		Positive	
	IgG	IgM	IgG	IgM	IgG	IgM
DSI	4471 (88.71)	4894 (97.10)	0 (0.00)	0 (0.00)	569 (11.29)	146 (2.90)§
MP	4503 (89.35)†	4947 (98.15)	0 (0.00)†	0 (0.00)	537 (10.65)†‡	93 (1.85)§
Wantai	4415 (87.60)	5006 (99.33)	6 (0.1)	0 (0.00)	619 (12.28)‡	34 (0.67)§
Mean	88.55%	98.19%	0.04%	0.00%	11.41%	1.81%

* Results are listed as numbers per category (%).
† Assay detects total antibody rather than IgG alone.
‡ The MP Biomedicals (MP) and Wantai IgG assays show a significant difference at $p < 0.025$.
§ All three IgM assays show significant differences between each other at $p < 0.00075$.
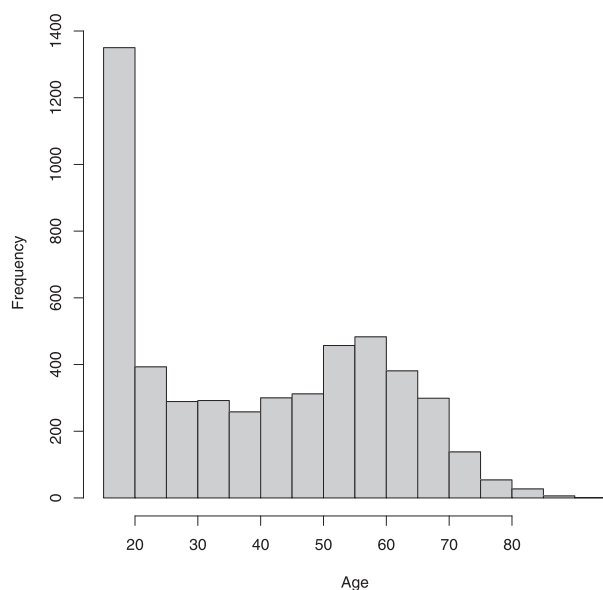

Fig. 1. Histogram of donor age distribution. Frequency distribution calculated using 5-year bins, age range (16-93).

sixth assay detected total anti-HEV antibodies: HEV ELISA 4.0 (23542-096, MP). All assays were run according to the manufacturers' instructions, except for the Wantai assays, for which initially positive samples were not retested, to standardize with the DSI and MP assays that do not require retesting. The Wantai IgG assay includes a gray-zone outcome in addition to positive and negative outcomes. A specific individual was designated to test assays from a specific vendor to minimize interoperator error potentially introduced by sample or reagent handling.

The anti-HEV assays used in this study detect antibodies using different formats. For anti-HEV IgG detection, the DSI and Wantai assays use a recombinant capsid peptide to bind total antibody, then horseradish peroxidase (HRP)-conjugated anti-human IgG is used to detect captured IgG. MP uses a recombinant capsid peptide to capture total antibody and HRP-conjugated recombinant capsid peptide to bind to total bound antibody. For the detection of anti-HEV IgM, the DSI and the MP assays use a recombinant capsid peptide as the capture antigen to

bind total antibody, and HRP-conjugated monoclonal mouse raised against anti-human IgM antibody to detect anti-IgM. The Wantai assay uses anti-μ antibody to capture total IgM and HRP-conjugated recombinant capsid peptide to bind to anti-HEV IgM.

Statistics

Pearson's chi-squared test with Yates's continuity correction, 95% confidence intervals, odds ratios, regression analysis, and Breusch-Pagan test for heteroskedasticity[28] were calculated in R (ver. 2.15.3).[29] Heteroskedasticity indicates that the variation in a variable is unequal across the range of values of a second variable used to predict the first.

RESULTS

Of the 5040 samples tested, 569 (11.29%; 95% CI, 10.43%-12.20%; DSI) and 619 (12.28%; 95% CI, 11.39%-13.22%; Wantai) were reactive for anti-HEV IgG, and 537 (10.65%; 95% CI, 9.82%-11.54%; MP) were reactive for total anti-HEV antibody, yielding an average of 11.41% (Table 1). There was no significant difference between the numbers of reactive samples detected between IgG assays. Anti-HEV IgM testing resulted in 142 reactives (2.90%; 95% CI, 2.45%-3.40%: DSI), 93 (1.85%; 95% CI, 1.49%-2.26%: MP) and 34 (0.67%; 95% CI, 0.47% to 0.94%; Wantai), yielding an average reactivity of 1.81% (Table 1). The extent of agreement between IgG anti-HEV/total anti-HEV and IgM anti-HEV assay sample detection is shown in Table S1 (available as supporting information in the online version of this paper).

There was no statistical difference in the gender of the donors selected (male = 2682, female = 2358). The age range for donors was from 16 to 93 years of age (Fig. 1). IgG anti-HEV detection rate increased with age, and all three assays exhibited similar uptrends (Fig. 2, upper panel) with no significant differences seen among the trends. Age and gender adjustment of these data among the four states with the highest number of donors—Missouri, Kentucky, Illinois, and Indiana—did not detect any appreciable differences in IgG anti-HEV detection rates by age group versus age- and gender-adjusted detection rates
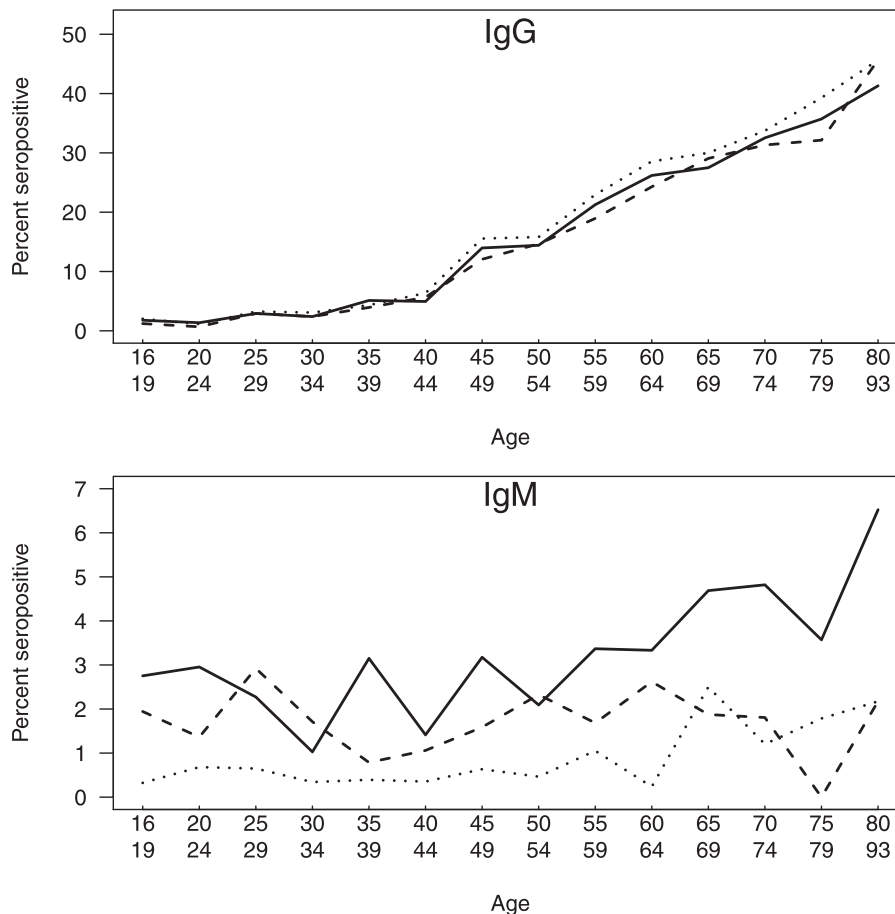
Fig. 2. Seroprevalence by age. Upper panel, anti-HEV IgG/total antibodies; lower panel, anti-HEV IgM. Solid line, DSI; dashed line, MP Biomedicals; dotted line, Wantai. Data were plotted by averaging the seroprevalence by age range from donors 15 to 80 years of age in increments of 5 years, and all ages above 80 years of age formed the last range. Seroprevalence within each age range was plotted versus the mean age within the age range.
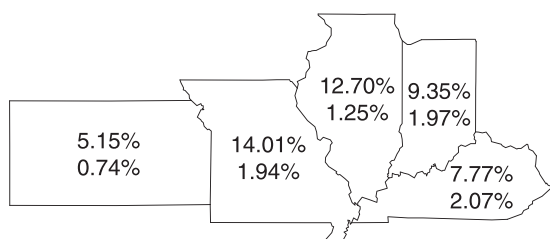

Fig. 3. Anti-HEV prevalence in the five states with the highest number of donors. The numbers within each state boundary are IgG/total antibody prevalence (upper number) and IgM prevalence (lower number). States from left to right: Kansas, Missouri, Illinois, Indiana, and Kentucky.

among the assays (Fig. S1, available as supporting information in the online version of this paper). No trend in anti-HEV IgM seroprevalence with age was evident (Fig. 2, lower panel).

Samples came from donors residing in 21 states. Differences by state of residence were analyzed in the five states with the highest number of donors; Missouri (n = 2080), Kentucky (n = 1120), Illinois (n = 887), Indiana (n = 813), and Kansas (n = 97). All other states were excluded from the state-by-state comparison because of the low donor numbers; for example, the state with the next highest number of donations was California, with eight donors. Only anti-HEV IgG/total anti-HEV was analyzed because of the low number of anti-HEV IgM positive samples by state. IgG anti-HEV/total anti-HEV prevalence percentages, as the mean among the three assays used to test specimens, across all assays within the five states having the highest numbers of donors ranged from Kansas, having the lowest (5.15%), to Illinois and Missouri, having the highest (12.70% and 14.01%, respectively) (Fig. 3); although Kansas has the lowest prevalence, due to the low number of samples tested, its 95% CI overlaps those for Illinois and Missouri and thus is not significant (Table 2 and Table S2 [available as supporting information in the online version of this paper]).

TABLE 2. Percentage of IgG antibody reactive donors by state and gender. The five states with the highest number of donors were compared

	Total*			Male			Female		
State	DSI	MP	Wantai	DSI	MP	Wantai	DSI	MP	Wantai
Illinois	12.06	11.84	14.21	13.51	12.89	16.42	10.34	10.59	11.58
Indiana	9.47	8.36	10.21	11.14	9.55	11.82	7.51	6.97	8.31
Kansas	5.15	5.15	5.15	6.67	6.67	6.67	3.85	3.85	3.85
Kentucky	7.77	7.50	8.04	8.32	8.16	9.14	7.10	6.71	6.71
Missouri	13.89	13.13	15.00	15.74‡	13.89	16.20	11.90‡	12.30	13.70
All states†	11.29	10.65	12.28	12.68‖	11.48	13.65§	9.71‖	9.71	10.73§

* Missouri (n = 2080), Kentucky (n = 1120), Illinois (n = 887), Indiana (n = 813), and Kansas (n = 97).
† Seropositivity for all 21 states in which donors resided.
‡ $p < 0.05$; § $p < 0.01$; ‖ $p < 0.005$ (chi-square for seropositivity by gender and assay).
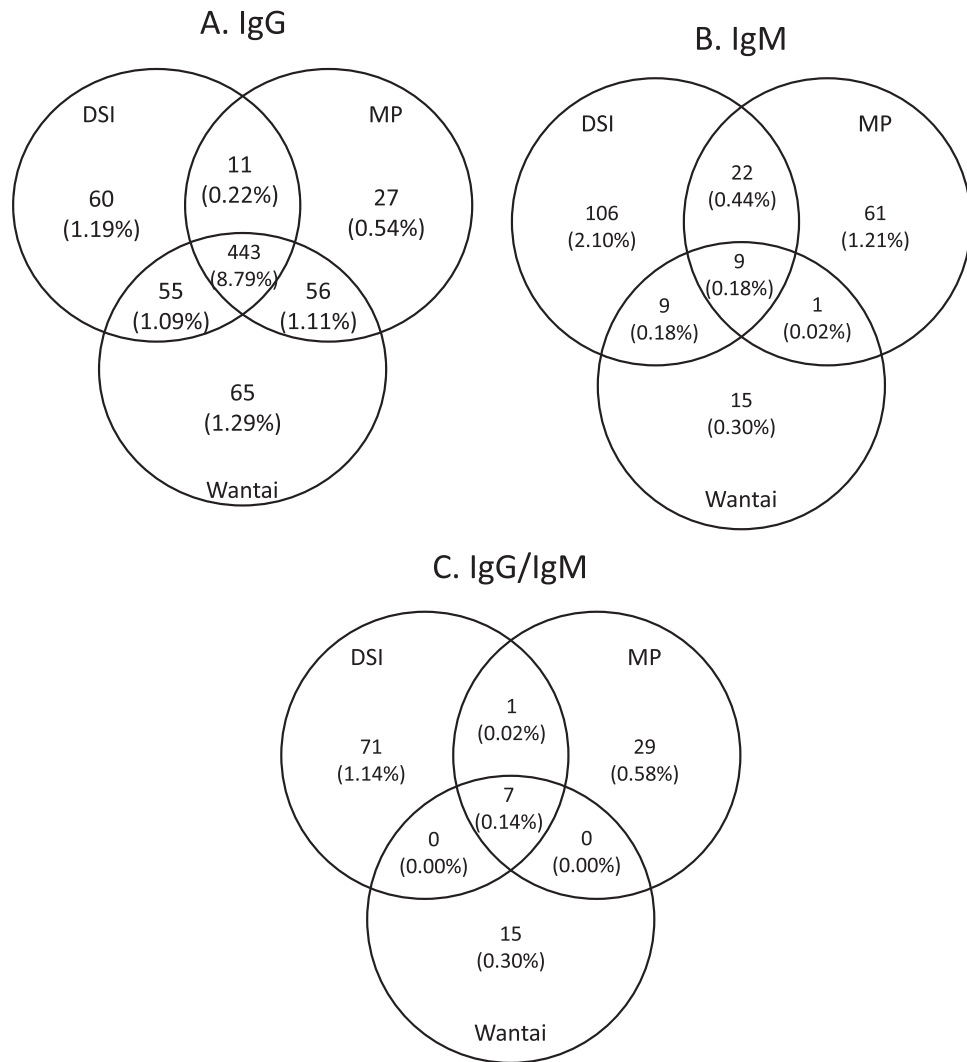
Fig. 4. Venn diagrams of anti-HEV assay concordance and discordance. (A) IgG/total assays. (B) IgM assays. (C) IgG/total antibody and IgM assays combined.

Because this was a cross-sectional unlinked study, there is no way to estimate declines of anti-HEV over time, or when the donors were potentially infected relative to the donation used in this study. However, looking at individuals with signal-to-cutoff (S/CO) ratios above the cutoff for each assay allows the range of S/CO ratios for individuals within age ranges to be evaluated (Fig. S2, available as supporting information in the online version

TABLE 3. Characteristics of the nine IgM anti-HEV positive specimens concordant across all three vendors

Sample*	IgG S/CO			IgM S/CO			Age (y)	Gender	State
	DSI	MP	Wantai	DSI	MP	Wantai			
1	0.01	0.08	0.13	3.03	4.61	1.42	18	M	KY
2	0.83	8.27	0.34	6.28	3.23	5.70	74	M	MO
3	6.28	8.99	14.14	1.73	1.13	2.31	68	M	MO
4	5.45	6.40	10.00	2.88	1.12	1.13	59	F	MO
5	5.18	9.00	16.60	3.33	1.44	1.12	47	M	KY
6	6.40	8.98	17.27	4.27	1.99	1.38	68	M	MO
7	5.51	8.44	14.34	4.66	2.31	7.30	81	F	MO
8	5.12	8.91	16.42	5.41	2.44	1.27	57	F	KY
9	2.29	8.91	6.33	7.84	5.09	2.65	63	M	MO

* Samples 3 to 9 are concordant for IgG and IgM across all three vendors.
KY = Kentucky; MO = Missouri.

of this paper). No significant difference or trend is observed in the range of S/CO ratios among these individuals by age group for any of the IgG/total anti-HEV assays used in this study.

Concordance between assays is shown in Fig. 4A-C and Table S1. Concordance among IgG anti-HEV/total anti-HEV–reactive specimens ranged from 443 samples reactive by all three assays, 454 to 499 samples reactive by two assays, and from 537 to 619 samples reactive by any given assay, for an overall agreement of 84%. There was a reduction in prevalence of 11.41% (reactive by any assay) to 8.79% (reactive in all three assays). The highest agreement was between MP and Wantai (Fig. 4A). For IgM, concordance ranged from 34 to 146 reactive by any given assay, 10 to 31 samples reactive by two assays, and for nine samples reactive by all three assays, for an overall agreement of 22%. There was a reduction in prevalence from 1.81% (reactive by any assay) to 0.18% (reactive in all three assays). The highest agreement was between DSI and MP (Fig. 4B). When concordance was compared between samples having both IgG anti-HEV/total anti-HEV and IgM anti-HEV reactivity, from 15 to 71 were reactive by the assays from any given manufacturer, and seven samples were reactive by all six assays, for an overall agreement of 40% and a prevalence of 0.14%. The highest agreement was again between DSI and MP (Fig. 4C). Nine samples had concordant IgM anti-HEV reactivity (including seven with concordant IgM/IgG reactivity), giving a frequency of one per 560 (0.18%; Fig. 4B). Since IgM is a marker of recent infection that develops within 2 to 6 weeks following infection, these nine IgM concordantly reactive donors were considered to be likely HEV infected recently. As Table 3 shows, the nine donors came from two states: Kentucky and Missouri; ranged in age from 47 to 81 years, except for one 18-year-old donor; and six were male.

Regression analysis (Fig. 5A-C) was initially used to assess correlations among the assays. Pearson's product-moment correlation between the MP total antibody assay and the Wantai IgG assay was r(n = 5038) = 0.89 (95% CI, 0.88-0.90), p < 0.00001, indicating strong correlation between these assays. However, this correlation was due to the large number of negative results (Fig. 5C). If samples that are negative in both assays ("true negatives") were removed, the correlation for the remaining samples was r(n = 497) = 0.74 (95% CI, 0.70-0.80), p < 0.00001. Although this value could be considered to show reasonable agreement between these two assays, a plot of S/CO ratios indicates that the variation between assays is heteroskedastic (Fig. 5A-C; p <0.00001, using the Breusch-Pagan test for heteroskedasticity among all three IgG assays, and Fig. S3 [available as supporting information in the online version of this paper]). An examination of the panels in Fig. S3 shows that no pair of assays has constant variation of data points around the regression line through the data. This result indicates that the null hypothesis of constant variance can be rejected between any two pairs of IgG assays, or, there is no relationship between S/CO ratios between pairs of assays. This outcome is similar if the DSI IgG assay was compared against either the MP or the Wantai assay (Fig. 5A, B). The lack of constant variation is still seen when samples with saturated signal with the MP assay are removed (Fig. S3). The data suggest that each assay may be detecting an epitope not detectable by the other assays and/or the effects of waning antibodies. This is further seen among the discordant samples, which are negative by one assay, but have a high S/CO ratio by another assay (Fig. 5, red and green data points). In some cases, the discrepancies are separated by more than 6 sigmas (Fig. S3).

DISCUSSION

It has been estimated that there are 20 million HEV infections worldwide annually, leading to about 3.3 million symptomatic cases of hepatitis E and 56,600 HEV-related deaths.[30,31] In the United States, an analysis of clinical cases of non–A-C acute hepatitis from 2005 to 2012 found
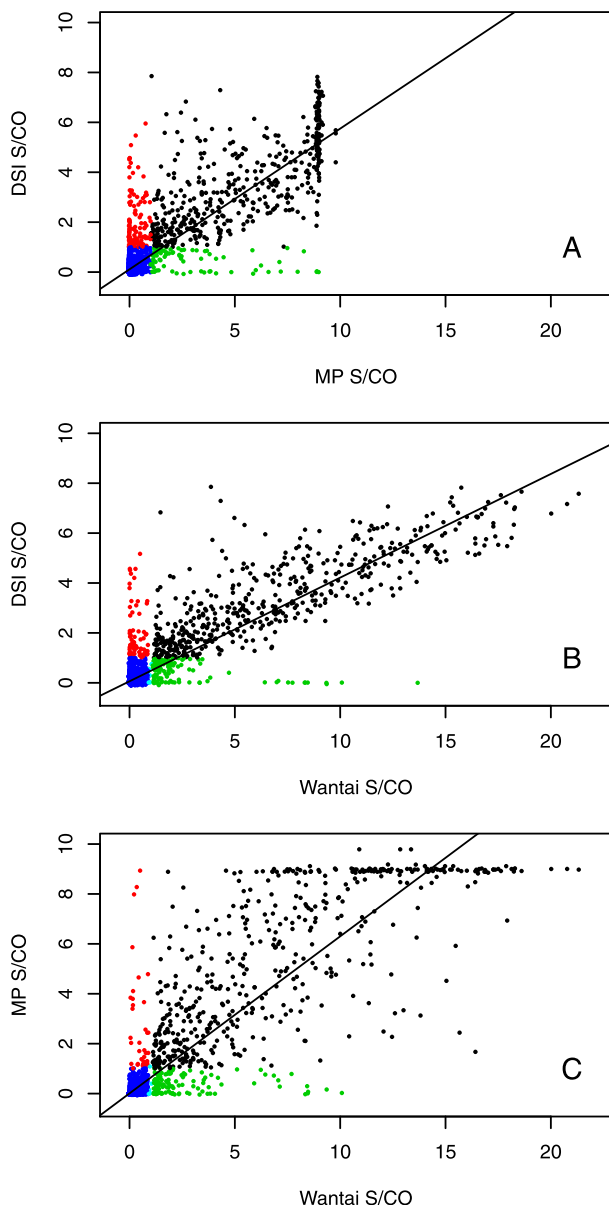
Fig. 5. Pairwise comparison of ratios (S/CO) among the DSI, MP, and Wantai IgG/total antibody assays. S/CO paired data are plotted as closed circles. The solid line is the regression line through the data between each pair of assays. Blue circles denote S/CO data pairs that are both nonreactive, black circles denote that both results are reactive. (A) Results from DSI compared with MP (red circles; DSI reactive and MP nonreactive, green circles [DSI nonreactive and MP reactive]). (B) DSI compared with Wantai (red circles; DSI reactive and Wantai nonreactive, green circles [DSI nonreactive and Wantai reactive]). (C) MP compared with Wantai (red circles; MP reactive and Wantai nonreactive, green circles [MP non-reactive and Wantai reactive]).

that 26 (17%) of 154 cases were due to HEV infection. There was a near-even split between travel-associated and autochthonous hepatitis E cases. The autochthonous cases were all infected by HEV Genotype 3 and tended to occur among older patients compared to those with travel-associated hepatitis E.[32]

The determination of anti-HEV serostatus remains enigmatic, and the results of various assays are often divergent. This is particularly true of healthy individuals like blood donors.[27] Recently pairwise concordance among three commercially available IgG anti-HEV assays and one laboratory-developed assay ranged from 56% to 87%, with a concordance of 52% observed in all samples tested among all four assays.[26] Another study of five assays for the detection of IgM and IgG found concordances of 71 and 70%. The limit of detection varied up to 19-fold for the IgM assays and 17-fold for the IgG assays.[33] A study of healthy US citizens using NHANES specimens found a decrease in IgG prevalence from 10.2% during 1988 to 1994 to 6% during 2009 to 2010 using the DSI assay.[19] IgG anti-HEV seroprevalence in NIH blood donors was 21.8% in 2006, with a decrease to 16.0% in 2012 using the Wantai assay[20] and 7.7% in ARC blood donors in 2013 using the MP assay.[21] The decreases in anti-HEV IgG rates observed in the NHANES and NIH studies are similar to observations seen in Germany, where a study of 45 subjects found that anti-HEV IgG concentrations decreased significantly after 5 years,[34] and a study of Dutch blood donors showed that IgG seroprevalence decreased from 19.8% in 1998 to 12.7% in 2011.[18] These last two studies observed seroreversion and HEV reinfections in some individuals despite preexisting HEV antibodies.

This study examined the anti-HEV IgG/total anti-HEV and IgM anti-HEV detection percentages in US blood donors and the performance of three commercial assays for IgG/total and IgM antibodies in this donor population. The assays used were from DSI, MP, and Wantai. Overall, the three assays yielded similar results. Within the total donor population, the IgG anti-HEV/total anti-HEV positivity was 11.41% (range by assay of 10.65%-12.28%), and the IgM positivity was 1.81% (range by assay of 0.67%-2.90%) (Table 1). An increase in anti-HEV IgG positivity was seen with increasing age, regardless of gender, as has been seen in other studies (Fig. 2, top panel).[19-21] However, no trend was seen for IgM seropositivity with increasing age (Fig. 2, bottom panel). Differences were seen between genders, regardless of age, where more males than females were IgG anti-HEV reactive (Table 2). These results are in agreement with the earlier ARC study that used only the MP assay to test anti-HEV IgG.[21] However, in our study, the gender difference was significant in the total donor population with the DSI ($p < 0.005$) and Wantai ($p < 0.01$) assays, but not significant in the MP assay. When the five states with the most donors were compared individually, the differences between males and

females were not significant except in Missouri, and only with the DSI assay ($p < 0.05$).

Agreement among the assays was better for the IgG/total anti-HEV than the IgM assays. The overall agreement among the IgG/total anti-HEV assays was 84% but was only 22% for the IgM assays (Fig. 4A-C). Some of the poor agreement among the IgM assays appears to be due to the discordance between the DSI and MP assays (Fig. 4B). The difference between discordant and concordant results was 2 logs higher in the IgM assays than the IgG/total anti-HEV assays. The wide range in these values is due to the low concordance between the IgM assays (Fig. 4B), which in turn is probably due to difference in the epitopes and assay formats used to detect IgM anti-HEV.

Despite the overall 84% agreement between the IgG/total antibody assays, the disparity in sample detection as well as S/CO ratios among discordant samples seen among all three assays could indicate that each assay is detecting an epitope(s) not detectable by the other assays (Fig. 5, red and green data points). The presence of heteroskedasticity among the IgG/total assays indicates that the null hypothesis of constant variance should be rejected, further supporting the conclusion of differing epitope detection. Alternatively, these differences could be due in part to the varying formats used in these assays or waning or less avid antibodies in the donor population. Differences in detection rates between the IgG anti-HEV assays (DSI and Wantai) and the total antibody assay could be due to IgM-positive detection by the total antibody assay (the MP assay) not detected by the IgG-only assays. However, MP IgM anti-HEV positivity did not correlate with total antibody positivity. Detection of IgA-containing samples cannot be excluded, but there is no way to test for IgA anti-HEV. As has been previously suggested, these data indicate that a panel of well-characterized plasma samples from HEV-infected individuals needs to be created to validate anti-HEV assay performance. Additionally, some as-yet-undiscovered factor, as with protein Fv, may be interfering with detection of HEV epitopes.[35] Fv, an Fab-binding factor, was found to interfere with an early in-house anti-HEV assay, leading to false-negative results.

The major limitations to this study include the fact that the assays use different antigens and detection formats, with no established method to determine their absolute performance characteristics. Blood donors are a select low-risk population that does not mirror the general US population.[36] The assays used have been validated for clinical purposes in symptomatic persons but not for epidemiologic studies in asymptomatic individuals. In addition, the performance of antibody assays is much better in patients with acute infection than in those with past infection.[37] Also, specimens initially positive by the Wantai assays were not retested as recommended in the manufacturer's instructions; however, one would expect a high

correlation between initially reactive and repeatedly reactive samples in a commercial assay that has been widely used for anti-HEV studies worldwide. In addition, any potential reductions in IgG or IgM reactivity due to the absence of repeat testing are not expected to reduce the heteroskedasticity seen in the assays or to significantly alter the lack of concordance seen among the IgM assays.

In conclusion, our data indicate that these HEV IgG/total anti-HEV assays are useful for examining seroprevalence and associated trends in seroprevalence with an interassay agreement of 84%; however, the disagreement among these assays indicates that there is an associated discordance rate or difference in target detection driving variability as seen by the heteroskedasticity evident among the IgG assays. The case for the IgM assays is worse since no IgM assay evaluated here demonstrated clinical utility in the blood donor population tested, although an IgM response in HEV RNA-positive blood donors has been well characterized.[38] These data suggest that more reliable information on prevalence may be obtained from using concordant reactive results from multiple assays. Despite these limitations, and assuming that the IgM-positive period exceeds that for HEV RNA detection, we calculated a rate of recent infection of 0.18% (1:560) based on concordance among all three anti-HEV IgM assays, noting that none of the nine IgM-reactive samples was HEV-RNA positive (data not shown). A recent study in 20,000 US blood donors found low HEV-RNA positivity (0.01%) and thus a low burden of new infection in US blood donors.[21]

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

REFERENCES

1. Khuroo MS. Discovery of hepatitis E: the epidemic non-A, non-B hepatitis 30 years down the memory lane. Virus Res 2011;161:3-14.
2. Purdy MA, Smith DB, Simmonds P, et al. New classification scheme for Hepeviridae ICTV taxonomic proposal 2014.008a-hV.A.v6.Hepeviridae (approved). 2014 [cited 2018 Feb 20]. Available from: https://talk.ictvonline.org/files/ictv_official_taxonomy_updates_since_the_8th_report/m/vertebrate-official/5150.
3. Smith DB, Simmonds P, Jameel S, et al. Consensus proposals for classification of the family Hepeviridae. J Gen Virol 2014; 95:2223-32.

4. Lee G-H, Tan B-H, Teo EC-Y, 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.

5. Teo C-G. Fatal outbreaks of jaundice in pregnancy and the epidemic history of hepatitis E. Epidemiol Infect 2012;140: 767-87.

6. Krawczynski K, Kamili S, Aggarwal R. Global epidemiology and medical aspects of hepatitis E. Forum (Genova) 2001;11: 166-79.

7. Izopet J, Kamar N. Hepatite E: de la transmission zoonotique du virus a l'evolution chronique de l'infection chez l'immunodeprime [Hepatitis E: from the zoonotic transmission of the virus to the chronic evolution of infection in immunocompromised patients]. Med Sci (Paris) 2008;24: 1023-5.

8. Tavitian S, Peron JM, Huynh A, et al. Hepatitis E virus excretion can be prolonged in patients with hematological malignancies. J Clin Virol 2010;49:141-4.

9. Legrand-Abravanel F, Kamar N, Sandres-Saune K, et al. Characteristics of autochthonous hepatitis E virus infection in solid-organ transplant recipients in France. J Infect Dis 2010; 202:835-44.

10. Colson P, Borentain P, Queyriaux B, et al. Pig liver sausage as a source of hepatitis E virus transmission to humans. J Infect Dis 2010;202:825-34.

11. Tei S, Kitajima N, Takahashi K, et al. Zoonotic transmission of hepatitis E virus from deer to human beings. Lancet 2003; 362:371-3.

12. Mizuo H, Yazaki Y, Sugawara K, et al. Possible risk factors for the transmission of hepatitis E virus and for the severe form of hepatitis E acquired locally in Hokkaido, Japan. J Med Virol 2005;76:341-9.

13. Gotanda Y, Iwata A, Ohnuma H, et al. Ongoing subclinical infection of hepatitis E virus among blood donors with an elevated alanine aminotransferase level in Japan. J Med Virol 2007;79:734-42.

14. Lapa D, Capobianchi MR, Garbuglia AR. Epidemiology of hepatitis E virus in European countries. Int J Mol Sci 2015; 16:25711-43.

15. Guillois Y, Abravanel F, Miura T, et al. High proportion of asymptomatic infections in an outbreak of hepatitis E associated with a spit-roasted piglet, France, 2013. Clin Infect Dis 2015;62:351-7.

16. 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: pii:20550.

17. Mansuy J-M, Bendall R, Legrand-Abravanel F, et al. Hepatitis E virus antibodies in blood donors, France. Emerg Infect Dis 2011;17:2309-12.

18. Hogema BM, Molier M, Slot E, et al. Past and present of hepatitis E in the Netherlands. Transfusion 2014;54:3092-6.

19. 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.

20. 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.

21. Stramer SL, Moritz ED, Foster GA, et al. Hepatitis E virus: seroprevalence and frequency of viral RNA detection among US blood donors. Transfusion 2015;56:481-8.

22. Chalupa P, Vasickova P, Pavlik I, et al. Endemic hepatitis E in the Czech Republic. Clin Infect Dis 2014;58:509-16.

23. Ijaz S, Said B, Boxall E, et al. Indigenous hepatitis E in England and Wales from 2003 to 2012: evidence of an emerging novel phylotype of viruses. J Infect Dis 2014;209:1212-8.

24. Centre National de Reference. Plan du rapport annuel d'activite: virus des hepatites a transmission enterique [Annual activity report plan: enteric transmission hepatitis virus; monograph on the Internet]. 2015. Available from: http://www.cnrvha-vhe.org/wp-content/uploads/2012/03/2014-Rap-Act-VHE-VHA.pdf

25. Drobeniuc J, Meng J, Reuter G, et al. Serologic assays specific to immunoglobulin M antibodies against hepatitis E virus: pangenotypic evaluation of performances. Clin Infect Dis 2010;51:e24-e7.

26. Kodani M, Ahmed N, Tejada-Strop A, et al. Variability in the performance characteristics of IgG anti-HEV assays and its impact on reliability of seroprevalence rates of hepatitis E. J Med Virol 2016;89:1055-61.

27. Wenzel JJ, Preiss J, Schemmerer M, et al. Test performance characteristics of anti-HEV IgG assays strongly influence hepatitis E seroprevalence estimates. J Infect Dis 2012;207: 497-500.

28. Breusch TS, Pagan AR. A simple test for heteroskedasticity and random coefficient variation. Econometrica 1979;47: 1287-94.

29. R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2013.

30. 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.

31. Lozano R, Naghavi M, Foreman K, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2010;380:2095-128.

32. Drobeniuc J, Greene-Montfort T, Le N-T, et al. Laboratory-based surveillance for hepatitis E virus infection, United States, 2005-2012. Emerg Infect Dis 2013;19:218-22.

33. Norder H, Karlsson M, Mellgren A, et al. Diagnostic performance of five assays for anti-HEV IgG and IgM in a large cohort study. J Clin Microbiol 2016;54:549-55.

34. Schemmerer M, Rauh C, Jilg W, et al. Time course of hepatitis E-specific antibodies in adults. J Viral Hep 2017;24:75-9.

35. Diallo A, Lazizi Y, Le Guenno B, et al. Hepatitis-E-virus-associated antigen: improved detection in stools by protein Fv removal. Res Virol 1991;142:449-59.

36. Goldman M, Steele WR, Di Angelantonio E, et al. Comparison of donor and general population demographics over time: a BEST Collaborative group study. Transfusion 2017;57:2469-76.

37. Aggarwal R, Goel A. Advances in hepatitis E-I: virology, pathogenesis and diagnosis. Expert Rev Gastroenterol Hepatol 2016;10:1053-63.

38. Tedder RS, Tettmar KI, Brailsford SU, et al. Virology, serology, and demography of hepatitis E viremic blood donors in South East England. Transfusion 2016;56:1529-36. ◖

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website.

Fig. S1. Comparison of age- and gender-adjusted seropositivity among the four states with the highest number of donors (Missouri, Kentucky, Illinois, and Indiana upper panel) versus the actual data from the same four states (lower panel).

Fig. S2. Comparison of signal to cutoff (S/CO) ratios for IgG reactive specimens by age group and IgG assay. Age ranges were created as described in Fig. 2. Data are shown as box plots by age group for each assay. Top panel, DSI; middle panel, MP; and bottom panel, Wantai.

Fig. S3. Pairwise comparison of signal-to-cutoff (S/CO) specimen ratios among the DSI, MP Biomedicals, and Wantai IgG assays after removal of saturated signal samples from the MP assay. Samples with an S/CO ratio greater than or equal to 8.0 with the MP assay were removed from analysis. The data from each assay were normalized with respect to the sample with the highest S/CO from each assay. Solid line, regression line through the data; dashed lined, normalized cutoff for each assay and dotted line, the six sigma lines around the regression line.

Table S1. 2 × 2 tables for IgG and IgM results among the three assays. IgM results are irrespective of IgG results. *6 Wantai IgM results fell into the gray zone and were removed from the calculation.

Table S2. 95% confidence intervals for IgG antibody positivity by state and gender. These data are for the values presented in Table 2. See Table 2 for additional information. *95% confidence intervals for antibody positivity for all 21 states in which donors resided.