HEPATOLOGY
Official Journal of the American Association for the Study of Liver Diseases

Current Epidemiology of Hepatitis E Virus Infection in the United States: Low Seroprevalence in the National Health and Nutrition Evaluation Survey

Ivo Ditah,[1] Fausta Ditah,[2] Pardha Devaki,[3] Calistus Ditah,[4] Patrick S. Kamath,[1] and Michael Charlton[5]

Analysis of the National Health and Nutrition Evaluation Survey (NHANES) 1988-1994 dataset found a relatively high seroprevalence (21%) of hepatitis E virus (HEV) infection in the U.S. general population. Using data obtained within the NHANES 2009-2010 survey, where a high performance assay for HEV was used, we estimated the weighted seroprevalence of HEV infection among U.S. individuals 6 years and older. We also evaluated factors associated with HEV seropositivity. A total of 8,814 individuals were included in the analysis. The median age of study participants was 37 years (interquartile range [IQR] 17-58 years), with 51.2% being female. The weighted national seroprevalence of HEV was 6% (95% confidence interval [CI] 5.1%-6.9%). About 0.5% of those with HEV had evidence of recent exposure (immunoglobulin M-positive). In the univariate analyses, factors associated with HEV seropositivity were increasing age (*P*-trend < 0.001), birth outside of the U.S., Hispanic race, and "meat" consumption (>10 times/month). No significant association was observed with low socioeconomic status, water source, or level of education. In the multivariate analysis, only older age remained predictive of HEV seropositivity. *Conclusion:* The weighted national seroprevalence of HEV in the U.S. is much less than previously reported. Using data obtained with a high performance assay, the seroprevalence of HEV was estimated at 6.0% in the U.S. Based on these results, the seroprevalence of HEV is only one-third as high as previously reported. (HEPATOLOGY 2014;60:815-822)

Hepatitis E virus (HEV) is the most common cause of acute viral hepatitis and jaundice worldwide.[1,2] It is a major public health problem in developing countries, where sporadic infections and epidemics of HEV occur periodically.[3-6] The prevalence of antibodies to HEV (anti-HEV) among adults in developing countries ranges from 30% to 80%. Infection is mainly transmitted by way of a fecal-oral route, usually through contaminated drinking water or food. HEV infection typically causes an acute, self-limited hepatitis. HEV infection can, however, be particularly severe in infants under 2 years of age, people with preexisting chronic liver disease, and is associated with 10% to 25% mortality in pregnant women.[5,7-9]

HEV infection is increasingly recognized in the developed world, where it was previously thought to be uncommon. Cases were often attributed to travel in the tropics and subtropics.[10] Recent studies indicate that most cases of HEV in the developed world are, in fact, locally acquired (autochthonous),[1,11-16] possibly related to zoonotic transmission. The reported prevalence of anti-HEV in low-incidence countries varies widely, ranging from <1% to >20%.[17-23] HEV seroprevalence in the civilian noninstitutionalized U.S. population was estimated at 21% using the National Health And Nutritional Evaluation Survey (NHANES III) data collected between 1988 and 1994.[24] This high seroprevalence suggests previous subclinical infection, unrecognized acute HEV infection, or poor

Abbreviations: CDC, Centers for Disease Control; DILI, drug-induced liver injury; FDA, Food and Drug Administration; HAV, hepatitis A virus; HCV, hepatitis C virus; HEV, hepatitis E virus; IDU, intravenous drug use; IgG, immunoglobulin G; IgM, immunoglobulin M; NCHS, National Center for Health Statistics; NH Black, non-Hispanic black; NH White, non-Hispanic white; NHANES, National Health and Nutrition Evaluation Survey.

From the [1]Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN; [2]Division of Gastroenterology Hepatology, and Nutrition, Vanderbilt University School of Medicine, Department of Medicine, Nashville, TN; [3]Wayne State University, Detroit, MI; [4]University of Michigan School of Medicine, Ann Arbor, MI; [5]Hepatology and Liver Transplantation, Intermountain Medical Center, Salt Lake City, UT.

Received March 10, 2014; accepted May 9, 2014.

performance assays with a significant proportion of false-positive results. Notably, the U.S. seroprevalence of HEV was higher than reported in other developed countries.[18,25-27]

Four major genotypes of mammalian HEV have been described. Genotypes 1 and 2 are exclusively human viruses that cause epidemic hepatitis.[28-30] Genotypes 3 and 4 are swine viruses, and appear to infect humans as an accidental host.[21] The latter have also been identified in several other mammalian species.[31-33] In the U.S., HEV3 from animals (pigs and rabbits) is the genotype most commonly associated with zoonotic infection.[1,34] All HEV genotypes comprise a single serotype.[35]

Tests for anti-HEV are available commercially, but none has formal Food and Drug Administration (FDA) approval in the U.S. The study by Kuniholm et al.[24] analyzed data obtained by using an "in-house" assay, whose specificity has been a subject of debate.[25,36] Compared to commercial assays, higher HEV seroprevalence has been reported in other studies that used the same EIA assay used by Kuniholm et al.[37,38] This assay has been found to crossreact with antibodies unrelated to HEV, e.g., in patients with hepatitis C virus infection, leading to false-positive results.[39] In the NHANES 2009-2010 cycle, blood from participants 6 years old and older was collected and tested with a more specific and sensitive assay (http://www.cdc.gov/nchs/data/nhanes/nhanes_09_10/HEPE_F_met_IgG_antibody.pdf).[40] We hypothesized that the previously published HEV prevalence in the U.S. was an overestimate of the true prevalence of anti-HEV in the United States. The main purpose of this study was to describe the current epidemiology of HEV infection in the U.S. general population based on the NHANES 2009-2010 samples that were tested using a highly specific anti-HEV assay. Specifically, we aimed to 1) estimate the national prevalence of HEV infection, and 2) identify factors associated with being HEV seropositive among U.S. residents 6 years of age and older.

Participants and Methods

NHANES Survey Background. The NHANES is conducted by the National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention (CDC). It collates nationally representative data on the health and nutritional status of the noninstitutionalized, civilian population of the U.S. It uses a complex, stratified, and multistage probability sampling design and collects information from approximately 5,000 persons per year using standardized household interviews, physical examinations, and testing of biologic samples. More detailed information on the survey design for the NHANES, including approval from the Institutional Review Board for data collection and analysis, is available from the survey documentation.[41]

Initially, a questionnaire covering only nonsensitive topics is used to interview participants at home. Here, demographic data on age, gender, ethnicity, country of birth, etc., are collected. Information on potentially sensitive subjects, such as sexual practices and illicit drug use, is obtained later at a mobile examination center by means of a computer-assisted personal interviewing technique. The family poverty index ratio (PIR) was calculated by dividing the total family income by the poverty threshold, as defined by the U.S. Census Bureau, with adjustment for family size at the time of the interview (www.census.gov/hhes/www/poverty/definitions.html#ratio). Participants were classified as either below or at/above the poverty line with a PIR cutoff of 1. Pregnancy status was assessed by participant self-report and/or a urine pregnancy test. Questions about years of education, marital status, occupation, military service, sexual behavior, and illegal drug use including injection drug use were asked of participants 17 years of age or older.

Laboratory Testing. Tests for anti-HEV immunoglobulin M (IgM) and immunoglobulin G (IgG) were performed on all participants of the NHANES 2009-2010 cycle who were 6 years old or older. Human serum or plasma from NHANES participants were tested for IgG and IgM antibodies to hepatitis E using enzyme immunoassay (ELISA) kits DS-EIA-ANTI-HEV-G and DS-EIA-ANTI-HEV-M from Diagnostics System (Soronno, Italy). In a previous study by the CDC, this assay had the best performance characteristic, with diagnostic sensitivity and specificity of 98% and 95.2%, respectively, for detecting HEV-IgM.[42] IgG anti-HEV was tested by applying an assay from

Address reprint requests to: Ivo Ditah, M.D., M.Phil., Division of Gastroenterology and Hepatology, Mayo Clinic, 200 First St., SW, Rochester, MN 55905. E-mail: Ditah.ivo@mayo.edu or ivoditah@yahoo.com.

View this article online at wileyonlinelibrary.com.
DOI 10.1002/hep.27219
Potential conflict of interest: Nothing to report.

the same manufacturer with a specificity of 97.5%, sensitivity of 100%, and an analytic sensitivity of 1.563 units/mL of WHO reference reagent for IgG antibody to HEV (also see Supporting 1, 2).[40] The results are reported as either positive or negative. Persons with IgG anti-HEV were considered to have been exposed to HEV at some point, while participants with IgM anti-HEV were considered to have recent exposure (previous 6 months).

Statistical Analyses. Data from the 2009-2010 NHANES survey were analyzed using Stata v. 11 software according to the NCHS analytic guidelines. The NHANES uses a complex, stratified, and multistage probability sampling design and collects information from ~5,000 persons per year.[43] We used appropriate study design and published weights for all analyses. The NHANES 2009-2010 data can be found on the CDC website at http://wwwn.cdc.gov/nchs/nhanes/search/datapage.aspx?Component=Demographics&CycleBeginYear=2009. All estimates of prevalence are weighted so as to represent the total U.S. population 6 years old and older, and to account for oversampling and nonparticipation in the household interview and physical examination. Prevalence of anti-HEV is presented by various demographic factors in figures and tables. Proportions from univariate analyses were compared using the chi-square test, with factors having $P < 0.2$ included in the multivariate analyses. $P < 0.05$ was considered significant in the multivariate model.

Results

Survey Outcome and Participant Characteristics. A total of 10,537 individuals participated in the 2009-2010 NHANES survey, with 8,814 (83.6%) individuals age 6 years and older. Of those eligible for anti-HEV serology testing (8,814), 7,885 (89.5%) subjects had results reported and 929 (10.5%) had no anti-HEV results (missing). Out of the 929 individuals with missing results, 223 did not complete the Mobile Examination Center (MEC) exam and the remaining 706 did not have sufficient blood drawn for all tests. The "missing" data group was demographically similar to those with test results except that they tended to be younger ($P < 0.05$). A total of 8,814 individuals, with 490 having IgG antibodies to HEV were included in the analysis. The median age of study participants was 37 years (interquartile range [IQR] 17-58 years) with 51.2% being women. About one in six (16.2%) were born outside of the U.S.

Weighted Seroprevalence of HEV Infection in the U.S. General Population as of 2010. The overall weighted national seroprevalence of HEV in the civilian noninstitutionalized U.S. population was 6.0% (95% confidence interval [CI] 5.1%-6.9%). About 0.5% of those with HEV IgG positive results had evidence of recent exposure (IgM positive) at the time of screening. The weighted prevalence of antibodies to hepatitis C virus was 1.3% (95% CI 0.9, 1.8); hepatitis B surface antigen was 0.4% (95% CI 0.2-0.7) and hepatitis A IgG seroprevalence was 34.8% (95% CI 30.2%, 39.6%). Table 1 shows the prevalence of antibodies to HEV by the demographic characteristics of the study participants and by gender. The prevalence of anti-HEV by age, race, gender, and country of birth are shown in Fig. 1A-C.

Factors Associated With HEV Seropositivity in the U.S. General Population. In the univariate analysis, factors associated with HEV seropositivity were increasing age (P-trend <0.001), birth outside of the U.S., race, marital status, ever received blood products, having antibodies to hepatitis A virus, and "meat" consumption (>10 times/month). No significant association was observed with low socioeconomic status (PIR), subject's water source, gender, substance abuse, or level of education. In the multivariate analysis (adjusting for all factors that reached statistical significance in the univariate analyses), only age remained significantly predictive of HEV seroprevalence. Table 2 shows the univariate and multivariate odds ratios for factors associated with having antibodies to HEV.

Discussion

The most important finding of this detailed national survey is a greater than 70% lower seroprevalence of HEV infection in the civilian, noninstitutionalized U.S. population in 2010, compared to that reported between 1988 and 1994.[24] We believe that the estimate found in this study is more reflective of the true seroprevalence of HEV exposure in the U.S. The lower seroprevalence observed using the NHANES 2009-2010 data, at least in part, explains why very few cases of acute HEV infection are seen in day-to-day clinical practice. This study also suggests that the association between HEV seroprevalence and age may simply be due to the accumulation of cases over time rather than a cohort effect.

HEV infection has been thought to be uncommon in developed countries,[44] with the sporadic cases that are diagnosed attributed to travel to the tropics or contact with someone from an endemic country.[10] The first national estimate of the seroprevalence of HEV in the U.S. was reported as 21% between 1988 and 1994.[24] The low frequency of clinical cases of incident

Table 1. Weighted Seroprevalence of HEV Infection By Subject Characteristics and Gender, NHANES 2009-2010

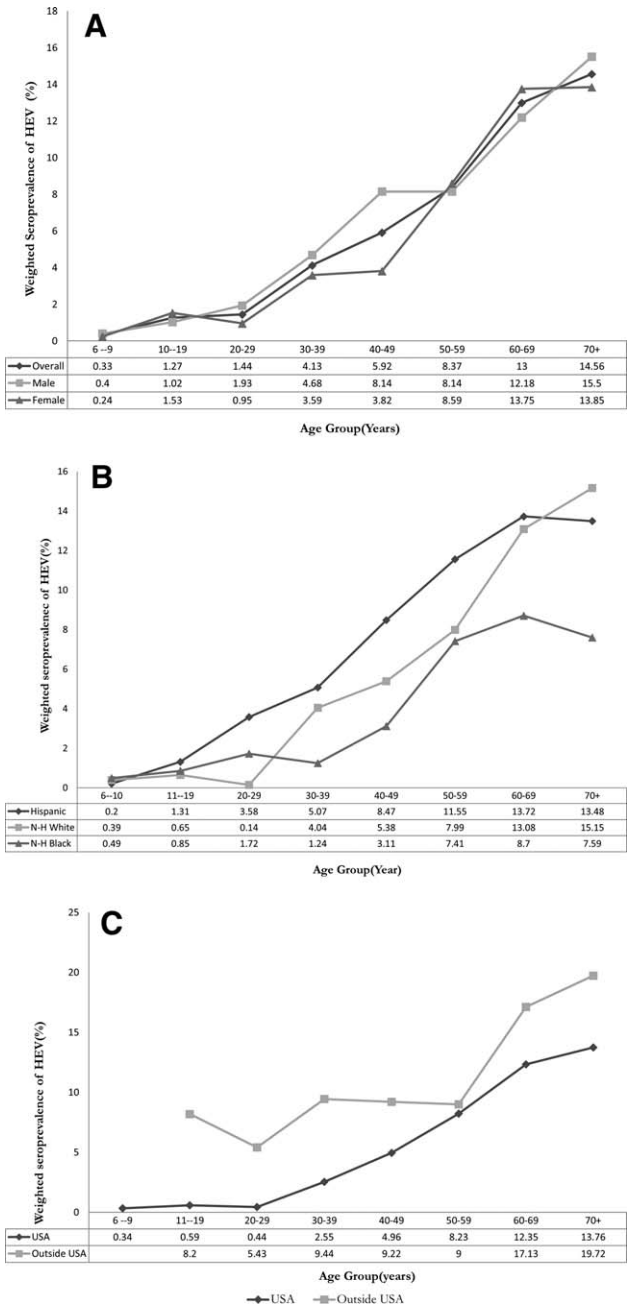
		Weighted Seroprevalence of HEV		
Characteristics	**Subjects Tested, N (%)**	**Overall (95% CI)**	**Male (95% CI) N = 3,906 (49.5%)**	**Female (95% CI) N = 3,979 (50.5%)**
All	7,885(100%)	6.0(5.1, 6.94)	6.3(4.8, 7.7)	5.7(4.7, 6.7)
Age groups	7,885			
6-19	2,162(27.4)	0.9(0.2, 1.6)	0.8(0.1, 1.7)	1.1(0.2, 1.9)
20-39	1,916(24.3)	2.7(1.9, 3.6)	3.3(1.8, 4.7)	2.2(1.3, 3.1)
40-59	1,917(24.3)	7.1(5.5, 8.7)	8.1(5.6, 10.7)	6.1(4.6, 7.6)
60-79	1,519(19.3)	13.4(10.9, 15.9)	13.1(9.1, 17.1)	13.6(10.0, 17.2)
80+	371(4.7)	15.5(11.1, 19.8)	16.9(9.8, 24.0)	14.5(9.0, 20.0)
Race	7,422			
Hispanic	2,541(32.2)	5.6(4.2, 7.0)	6.6(4.9, 8.4)	4.4(2.8, 6.1)
NH White	3,465(43.9)	6.3(5.0, 7.6)	6.2(4.3, 8.2)	6.4(5.0, 7.7)
NH Black	1,416(18.0)	3.4(2.2, 4.5)	3.2(2.2, 4.2)	3.5(1.8, 5.1)
Birthplace	7,879			
USA	6,058(76.8)	5.2(4.2, 6.2)	5.1(3.8, 6.5)	5.3(4.3, 6.2)
Outside USA	1,821(23.1)	9.7(7.0, 12.5)	11.4(8.9, 14.0)	7.9(3.7, 12.2)
Tap water source	7,708			
Private/public company	6,688(85.2)	5.57(4.5, 6.7)	5.9(4.0, 7.8)	5.3(4.1, 6.4)
Private/public well	948(12.1)	7.8(3.6, 12.1)	7.7(2.2, 13.2)	7.9(4.3, 11.6)
Other	72(0.9)	4.7(2.10, 11.4)	6.7(5.3, 18.7)	2.2(1.1, 5.6)
Eat meat (#times/month)	6,345			
<10	4,524(71.3)	4.3(3.6, 5.1)	4.6(3.2, 5.9)	4.2(3.3, 5.0)
>=10	1,821(28.7)	6.3(4.6, 8.0)	6.6(4.3, 9.0)	5.9(2.8, 8.9)
Poverty index line	7,170			
At or Above	5,356(74.7)	6.0(5.0, 6.9)	6.2(4.7, 7.8)	5.7(4.8, 6.7)
Below	1,814(25.3)	5.1(3.3, 7.0)	5.5(3.2, 7.8)	4.8(2.7, 6.9)
Military service			6,181	
Yes	718(11.6)	7.1(4.3, 9.8)	7.5(4.5, 10.4)	1.9(0.1, 6.2)
No	5,463(88.4)	6.8(5.6, 8.0)	7.1(5.2, 9.1)	6.6(5.4, 7.7)
Pregnancy	1,277			
Yes	64(4.9)	1.0(0.01, 2.9)	n/a	1.0(0.01, 2.9)
No	1,213(92.8)	2.6(1.7, 3.4)	n/a	2.6(1.7, 3.4)
Blood product transfusion	7,801			
Yes	729(9.3)	11.0(9.0, 13.0)	10.3(7.2, 13.4)	11.4(8.8, 14.1)
No	7,072(89.7)	5.4(4.5, 6.4)	5.9(4.4, 7.4)	5.0(3.9, 6.2)
IDU	4,378			
Yes	79(1.8)	4.7(2.0, 11.3)	6.8(0.4, 17.7)	1.9(0.2, 5.9)
No	4,299(98.0)	5.9(4.9, 6.8)	6.1(4.5, 7.7)	5.6(4.4, 6.8)
Drug C/H/M	4,370			
Yes	751(17.1)	5.1(2.9, 7.4)	6.9(4.0, 9.7)	2.0(0.02, 4.2)
No	3,619(82.5)	5.9(4.8, 7.0)	5.9(4.2, 7.6)	6.0(4.7, 7.2)
Hepatitis A antibody	7,621			
Yes	3,596(47.2)	7.1(5.8, 8.4)	8.0(6.2, 9.7)	6.2(4.1, 8.3)
No	4,025(52.8)	5.4(4.4, 6.4)	5.4(3.8, 6.9)	5.5(4.3, 6.8)
Hepatitis C antibody	7,871			
Yes	107(1.4)	9.3(0.2, 18.7)	9.8(1.8, 21.4)	7.8(2.8, 18.5)
No	7,764(98.5)	5.9(5.0, 6.8)	6.1(4.6, 7.7)	5.7(4.7, 6.7)
Level of education	5,711			
Above high school	1,642(28.7)	7.5(5.6, 9.4)	8.0(6.0, 10.0)	7.1(4.2, 9.9)
High school	1,305(22.8)	7.3(5.0, 9.6)	7.1(3.9, 10.3)	7.4(5.1, 9.7)
Below high school	2,764(48.3)	7.0(5.91, 8.08)	7.7(5.5, 9.8)	6.4(4.9, 7.9)
Marital status	5,719			
Single	984(17.2)	3.2(2.0, 4.4)	4.0(1.5, 6.4)	2.4(1.1, 3.8)
Separated/widow/widower	1,308(22.9)	8.9(5.8, 12.0)	8.0(5.2, 10.8)	9.4(5.5, 13.3)
Married	3,427(59.9)	7.7(6.7, 8.7)	8.5(6.5, 10.5)	6.9(5.8, 8.0)

Sum of subjects for each variable differ from total number of subjects in study due to missing data.

Questions about years of education, marital status, military service, and illegal drug use including injection drug use were asked of participants 17 years of age or older.

Questions on pregnancy were limited to females aged 20-59 years old.

Abbreviation: drug C/H/M, cocaine/heroin/marijuana.



	6--9	10--19	20-29	30-39	40-49	50-59	60-69	70+
Overall	0.33	1.27	1.44	4.13	5.92	8.37	13	14.56
Male	0.4	1.02	1.93	4.68	8.14	8.14	12.18	15.5
Female	0.24	1.53	0.95	3.59	3.82	8.59	13.75	13.85

	6--10	11--19	20-29	30-39	40-49	50-59	60-69	70+
Hispanic	0.2	1.31	3.58	5.07	8.47	11.55	13.72	13.48
N-H White	0.39	0.65	0.14	4.04	5.38	7.99	13.08	15.15
N-H Black	0.49	0.85	1.72	1.24	3.11	7.41	8.7	7.59

	6--9	11--19	20-29	30-39	40-49	50-59	60-69	70+
USA	0.34	0.59	0.44	2.55	4.96	8.23	12.35	13.76
Outside USA		8.2	5.43	9.44	9.22	9	17.13	19.72

Fig. 1. (A) shows sero-prevalence of HEV infection in US general population by age and gender, as of 2010. The sero-prevalence of HEV increased significantly with age ($P < 0.001$). The gender differences were not significant. ($P > 0.05$) Numbers shown are weighted sero-prevalence for overall, and by gender for each age group. (B) shows the sero-prevalence of HEV infection in the US general population by Age and Race as of 2010. The Highest prevalence was noted in the Hispanics. Compared to Non-Hispanic Blacks, this difference was statistically significant. ($P = 0.01$) N-H White-Non Hispanic White; N-H Black-non Hispanic Black. Numbers shown are sero-prevalence by race for each age group. (C) shows sero-prevalence of HEV infection in the US general population by place of birth and age as of 2010. People born outside of the USA had a significantly hgher sero-prevalence of HEV infection ($P = 0.003$) and ths increased significantly with age ($P < 0.001$). Numbers shown are sero-prevalence by place of birth for each age group.

HEV infection diagnosed on a national basis resulted in some degree of skepticism by the medical community regarding the 21% estimated seroprevalence of HEV in the U.S. Of the samples sent to the CDC between June 2005 and March 2012 from 154 patients suspected to have HEV infection, only 27(22%) were confirmed.[42] The high estimated seroprevalence raised questions about a possible high incidence of subclinical infection or unrecognized acute HEV infection in clinical settings due to lack of physician awareness. It has been shown that a subset of patients with supposedly drug-induced liver injury (DILI) actually had evidence of HEV infection when stored sera were retested.[45,46] More important, the high seroprevalence is thought to be related to the low specificity and poor performance of the in-house assay that was used in the NHANES 1988 to 1994 samples.[25,36]

Commercially available and "in-house" serological assays vary widely and have poor interassay concordance.[36,38,47] Other studies using the assay used in the NHANES 1988-1994 study found higher seroprevalence of HEV when compared to commercially available assays.[37,38] It was hypothesized that, perhaps, the "in-house" assay detected remote infections better than commercial assays which were developed to diagnose more recent exposures, during which time antibody titers are typically higher. In the current study, samples from the NHANES 2009-2010 survey were tested with a high performing assay with a specificity of 97.5% and sensitivity of 100%.[40] Supporting Material 1 provides detail information on the performance of the assay used in this study. Unfortunately, while the IgG anti-HEV used in this study has not been validated outside of the validation studies, the IgM anti-HEV assay produced by the same company has been compared to other commercially available assays by the CDC and found to have the best performance characteristics.[42,48] Our results show a significantly lower seroprevalence of HEV from 21% in 1994 to 6% in 2010. The duration of persistence of IgG anti-HEV is uncertain.[49,50] If the 3.5-fold drop in seroprevalence seen in this study was due to a decline in the IgG anti-HEV titers over time, we would have seen a lower seroprevalence among the older age groups. Furthermore, given that the U.S. is not endemic for HEV infection, assays with low specificity are more likely to include a good proportion of false positives, and may partially account for the high seroprevalence previously reported. We believe that the 6% seroprevalence found in this study more closely represents the reality in the U.S. and partially explains the small number of clinical

Table 2. Adjusted Odds Ratios for the Presence of HEV Antibodies, NHANES 2009-2010*

Potential Risk Factor or Exposure	Univariate OR (95% CI)	*P* Value	Multivariate OR (95% CI)	*P* Value
Age (years)	1.1(1.0, 1.1)	*trend* <0.001	1.1(1.0, 1.1)	*trend* <0.001
Race				
NH White	1.0 (Reference)			
NH Black	1.7(1.0, 2.8)	0.006	1.9(0.8, 4.2)	0.11
Hispanic	1.9(1.2, 3.0)	0.49	1.5(0.8, 2.9)	0.17
Birthplace				
USA	1.0 (Reference)			
Outside USA	2.0(1.3, 2.9)	0.003	2.2(0.9, 5.2)	0.08
Eat Meat (times/month)				
<10	1.0 (Reference)			
>=10	1.5(1.1, 1.9)	0.007	1.3(1.0, 1.8)	0.07
Blood Product Transfusion				
No	1.0 (Reference)			
Yes	2.2(1.7, 2.7)	<0.001	1.1(0.9, 1.3)	0.56
Anti-HAV				
No	1.0 (Reference)			
Yes	1.3(1.0, 1.7)	0.03	0.8(0.6, 1.2)	0.24
Marital Status				
Single	1.0 (Reference)			
Separated/widow/widower	2.9(1.9, 4.4)	<0.001	1.2(0.6, 2.1)	0.53
Married	2.5(1.7, 3.6)	<0.001	1.3(0.7, 2.2)	0.34

*Only factors (from Table 1) that had a *P* value < 0.2 in the univariate analyses were included in the multivariate model and shown on this table. Particularly, water source, low socio-economic status, gender, substance abuse, military service & level of education were not associated to HEV sero-positivity in the univariate analyses.

cases seen. A good proportion of these individuals likely acquired infections locally through exposure or contact with animals or watercourses contaminated by runoff from outdoor pig farms.[14,15,51] A recent study by Drobeniuc et al.[42] found that out of 26 confirmed cases of HEV in the U.S., 15 had no history of overseas travel. These patients without a history of travel were more likely to be older, solid organ transplant recipients, and eight of them had genotype 3 (zoonotic) infection.

One of the objectives of this study was to identify factors associated with HEV seropositivity in the U.S. The association between HEV seroprevalence and age seen in this study could have several explanations. First, this could be a reflection of the continually improving environmental hygiene in the U.S. and worldwide, and consequently, a decrease risk of exposure and overall seroprevalence of HEV in the U.S. This hygiene hypothesis is supported by the lower seroprevalence seen in the younger age groups and the sharp takeoff thereafter as shown on Fig. 1A. Second, it is possible that repeated exposure of the immune system to HEV may be necessary to accumulate sufficient antibodies to reach a threshold titer that is detectable with current anti-HEV assays. Finally, this linear rising trend in seroprevalence with age could simply be a reflection of exposed cases accumulated over time. In other words, the longer a person has lived, the higher the probability that they would have been exposed at some point in their lifetime to HEV, and thus the higher prevalence seen with increasing age.

Participants of Hispanic origin and those born outside of the U.S. were associated with higher anti-HEV prevalence. This association, however, disappears when age is taken into account. Similarly, an association with military service, which often involves traveling to endemic countries, was noted. Together, the above results suggest that at least some of those with evidence of HEV infection in the U.S. may have been exposed to HEV abroad. In contrast to the study by Kuniholm et al.,[24] we did not observe an association between anti-HEV and "meat consumption" in our multivariate model. This may be on the basis of the smaller number of persons reporting meat consumption in the current study, leading to insufficient power to detect such an association. It is important to note, however, that the study by Kuniholm et al.[24] found the association between anti-HEV and "meat consumption" only among U.S.-born individuals, and they did not adjust for all potential confounders that were included in our study. Unfortunately, the NHANES data does not contain data on other potential confounders including occupations such as animal (swine, rabbits, etc.) farmers or veterinarians, which are well known to increase the risk of autochthonous HEV infection.[1,52] No association was found with blood transfusion. Although cases of blood transfusion-related HEV infections are well documented, the contribution of this source appears to be negligible.[53]

This NHANES-based study has a few shortcomings. It was not possible to map out the genotypic distribution of HEV in the U.S. since NHANES does not routinely perform genotype testing. Perhaps a more feasible approach would be to consider confirmatory polymerase chain reaction (PCR) or genotypic testing in individuals who were identified as being IgM-positive. HEV genotyping in the NHANES survey would help to identify the probable sources of HEV exposure in the U.S. In a recent study by Drobeniuc et al.,[42] all cases of HEV infection in patients with no travel history were seen to derive exclusively from HEV genotype 3. The precise impact of the choice of anti-HEV assay on the results of the current and previous NHANES datasets is difficult to know, as head-to-head comparisons of different assays on same samples are known to yield discordant results.[48,54] This issue has been highlighted recently by Traore et al.[55] and Rossi-Tamisier et al.[56] There has never been a head-to-head comparison between the assay used in this study and the in-house assay used in the NHANES III analysis.[24] The high performance characteristics of the assay used in the current NHANES analysis make it highly likely that the results of this survey more closely reflect the actual seroprevalence of HEV exposure in the U.S. Furthermore, confirmatory testing with PCR in individuals testing IgM anti-HEV-positive would have added more credibility to the performance of the assay used in this study. Extensive validation of existing anti-HEV antibody assays or development of assays with superior performance is critical for reproducible epidemiological surveillance of HEV infection.

In conclusion, this study represents the most updated data on the epidemiology of HEV in the U.S. Using data obtained within the NHANES 2009-2010 survey with a high performance assay for HEV, we were able to show that the seroprevalence of HEV in the U.S. may not be as high as previously reported. Compared to the 1988-1994 NHANES survey, the seroprevalence of HEV has decreased from 21% to 6% in the U.S. population 6 years and older; a 3.5-fold decline. The only factor significantly associated with HEV seropositivity is increasing age.

Acknowledgment: We thank the viral hepatitis section of the National Center for Health Statistics at the Centers for Disease Control for their immense work in processing the hepatitis E data and making it available online to researchers.

References

1. Kamar N, Bendall R, Legrand-Abravanel F, Xia NS, Ijaz S, Izopet J, et al. Hepatitis E. Lancet 2012;379:2477-2488.
2. Purcell RH, Emerson SU. Hepatitis E: an emerging awareness of an old disease. J Hepatol 2008;48:494-503.
3. Bile K, Isse A, Mohamud O, Allebeck P, Nilsson L, Norder H, et al. Contrasting roles of rivers and wells as sources of drinking water on attack and fatality rates in a hepatitis E epidemic in Somalia. Am J Trop Med Hyg 1994;51:466-474.
4. Naik SR, Aggarwal R, Salunke PN, Mehrotra NN. A large waterborne viral hepatitis E epidemic in Kanpur, India. Bull World Health Organ 1992;70:597-604.
5. Teshale EH, Howard CM, Grytdal SP, Handzel TR, Barry V, Kamili S, et al. Hepatitis E epidemic, Uganda. Emerg Infect Dis 2010;16:126-129.
6. Zhuang H, Cao XY, Liu CB, Wang GM. Epidemiology of hepatitis E in China. Gastroenterol Jpn 1991;26(Suppl 3):135-138.
7. Sharapov MB, Favorov MO, Yashina TL, Brown MS, Onischenko GG, Margolis HS, et al. Acute viral hepatitis morbidity and mortality associated with hepatitis E virus infection: Uzbekistan surveillance data. BMC Infect Dis 2009;9:35.
8. Tsega E, Hansson BG, Krawczynski K, Nordenfelt E. Acute sporadic viral hepatitis in Ethiopia: causes, risk factors, and effects on pregnancy. Clin Infect Dis 1992;14:961-965.
9. Tsega E, Krawczynski K, Hansson BG, Nordenfelt E. Hepatitis E virus infection in pregnancy in Ethiopia. Ethiop Med J 1993;31:173-181.
10. Schwartz E, Jenks NP, Van DP, Galun E. Hepatitis E virus infection in travelers. Clin Infect Dis 1999;29:1312-1314.
11. Dalton HR, Bendall R, Ijaz S, Banks M. Hepatitis E: an emerging infection in developed countries. Lancet Infect Dis 2008;8:698-709.
12. Chapman BA, Burt MJ, Wilkinson ID, Schousboe MI. Community acquired viral hepatitis in New Zealand: a case of sporadic hepatitis E virus infection. Aust N Z J Med 1993;23:722-723.
13. Heath TC, Burrow JN, Currie BJ, Bowden FJ, Fisher DA, Demediuk BH, et al. Locally acquired hepatitis E in the Northern Territory of Australia. Med J Aust 1995;162:318-319.
14. Ijaz S, Arnold E, Banks M, Bendall RP, Cramp ME, Cunningham R, et al. Non-travel-associated hepatitis E in England and Wales: demographic, clinical, and molecular epidemiological characteristics. J Infect Dis 2005;192:1166-1172.
15. Mansuy JM, Peron JM, Abravanel F, Poirson H, Dubois M, Miedouge M, et al. Hepatitis E in the south west of France in individuals who have never visited an endemic area. J Med Virol 2004;74:419-424.
16. Sainokami S, Abe K, Kumagai I, Miyasaka A, Endo R, Takikawa Y, et al. Epidemiological and clinical study of sporadic acute hepatitis E caused by indigenous strains of hepatitis E virus in Japan compared with acute hepatitis A. J Gastroenterol 2004;39:640-648.
17. Boutrouille A, Bakkali-Kassimi L, Cruciere C, Pavio N. Prevalence of anti-hepatitis E virus antibodies in French blood donors. J Clin Microbiol 2007;45:2009-2010.
18. Buti M, Dominguez A, Plans P, Jardi R, Schaper M, Espunes J, et al. Community-based seroepidemiological survey of hepatitis E virus infection in Catalonia, Spain. Clin Vaccine Immunol 2006;13:1328-1332.
19. Dalton HR, Stableforth W, Thurairajah P, Hazeldine S, Remnarace R, Usama W, et al. Autochthonous hepatitis E in Southwest England: natural history, complications and seasonal variation, and hepatitis E virus IgG seroprevalence in blood donors, the elderly and patients with chronic liver disease. Eur J Gastroenterol Hepatol 2008;20:784-790.
20. Ding X, Li TC, Hayashi S, Masaki N, Tran TH, Hirano M, et al. Present state of hepatitis E virus epidemiology in Tokyo, Japan. Hepatol Res 2003;27:169-173.
21. Lewis HC, Wichmann O, Duizer E. Transmission routes and risk factors for autochthonous hepatitis E virus infection in Europe: a systematic review. Epidemiol Infect 2010;138:145-166.
22. Mansuy JM, Legrand-Abravanel F, Calot JP, Peron JM, Alric L, Agudo S, et al. High prevalence of anti-hepatitis E virus antibodies in blood donors from South West France. J Med Virol 2008;80:289-293.
23. Thomas DL, Yarbough PO, Vlahov D, Tsarev SA, Nelson KE, Saah AJ, et al. Seroreactivity to hepatitis E virus in areas where the disease is not endemic. J Clin Microbiol 1997;35:1244-1247.

24. Kuniholm MH, Purcell RH, McQuillan GM, Engle RE, Wasley A, Nelson KE. 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.
25. Li RC, Ge SX, Li YP, Zheng YJ, Nong Y, Guo QS, et al. Seroprevalence of hepatitis E virus infection, rural southern People's Republic of China. Emerg Infect Dis 2006;12:1682-1688.
26. Mansuy JM, Legrand-Abravanel F, Calot JP, Peron JM, Alric L, Agudo S, et al. High prevalence of anti-hepatitis E virus antibodies in blood donors from South West France. J Med Virol 2008;80:289-293.
27. Tanaka E, Matsumoto A, Takeda N, Li TC, Umemura T, Yoshizawa K, et al. Age-specific antibody to hepatitis E virus has remained constant during the past 20 years in Japan. J Viral Hepat 2005;12:439-442.
28. Buisson Y, Grandadam M, Nicand E, Cheval P, van Cuyck-Gandre H, Innis B, et al. Identification of a novel hepatitis E virus in Nigeria. J Gen Virol 2000;81(Pt 4):903-909.
29. van Cuyck-Gandre H, Zhang HY, Tsarev SA, Clements NJ, Cohen SJ, Caudill JD, et al. Characterization of hepatitis E virus (HEV) from Algeria and Chad by partial genome sequence. J Med Virol 1997;53: 340-347.
30. Tam AW, Smith MM, Guerra ME, Huang CC, Bradley DW, Fry KE, et al. Hepatitis E virus (HEV): molecular cloning and sequencing of the full-length viral genome. Virology 1991;185:120-131.
31. Meng XJ. From barnyard to food table: the omnipresence of hepatitis E virus and risk for zoonotic infection and food safety. Virus Res 2011; 161:23-30.
32. Shukla P, Nguyen HT, Torian U, Engle RE, Faulk K, Dalton HR, 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;108:2438-2443.
33. Takahashi H, Tanaka T, Jirintai S, Nagashima S, Takahashi M, Nishizawa T, et al. A549 and PLC/PRF/5 cells can support the efficient propagation of swine and wild boar hepatitis E virus (HEV) strains: demonstration of HEV infectivity of porcine liver sold as food. Arch Virol 2012;157:235-246.
34. Zhao C, Ma Z, Harrison TJ, Feng R, Zhang C, Qiao Z, et al. A novel genotype of hepatitis E virus prevalent among farmed rabbits in China. J Med Virol 2009;81:1371-1379.
35. Tang X, Yang C, Gu Y, Song C, Zhang X, Wang Y, et al. Structural basis for the neutralization and genotype specificity of hepatitis E virus. Proc Natl Acad Sci U S A 2011;108:10266-10271.
36. Bendall R, Ellis V, Ijaz S, Ali R, Dalton H. 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.
37. Ghabrah TM, Tsarev S, Yarbough PO, Emerson SU, Strickland GT, Purcell RH. Comparison of tests for antibody to hepatitis E virus. J Med Virol 1998;55:134-137.
38. Mast EE, Alter MJ, Holland PV, Purcell RH. Evaluation of assays for antibody to hepatitis E virus by a serum panel. Hepatitis E Virus Antibody Serum Panel Evaluation Group. Hepatology 1998;27:857-861.
39. Herremans M, Bakker J, Duizer E, Vennema H, Koopmans MP. Use of serological assays for diagnosis of hepatitis E virus genotype 1 and 3 infections in a setting of low endemicity. Clin Vaccine Immunol 2007; 14:562-568.
40. Centers for Disease Control. Available at: http://www.cdc.gov/nchs/data/nhanes/nhanes_09_10/HEPE_F_met_IgG_antibody.pdf. Division of Viral Hepatitis, National Center for HIV/AIDS, Viral Hepatitis, STD and TB Prevention, 2012.
41. Centers for Disease Control and Prevention. About the National Health and Nutrition Examination Survey (NHANES) Available at: www.cdc.gov/nchs/about/major/nhanes/intro_mec.htm. Accessed May 13, 2013. N/A 2009.
42. Drobeniuc J, Greene-Montfort T, Le NT, Mixson-Hayden TR, Ganova-Raeva L, Dong C, et al. Laboratory-based surveillance for hepatitis E virus infection, United States, 2005-2012. Emerg Infect Dis 2013;19:218-222.
43. Centers for Disease Control and Prevention. About the National Health and Nutrition Examination Survey (NHANES). 2009.
44. Hoofnagle JH, Nelson KE, Purcell RH. Hepatitis E. N Engl J Med 2012;367:1237-1244.
45. Dalton HR, Fellows HJ, Stableforth W, Joseph M, Thurairajah PH, Warshow U, et al. The role of hepatitis E virus testing in drug-induced liver injury. Aliment Pharmacol Ther 2007;26:1429-1435.
46. Davern TJ, Chalasani N, Fontana RJ, Hayashi PH, Protiva P, Kleiner DE, et al. Acute hepatitis E infection accounts for some cases of suspected drug-induced liver injury. Gastroenterology 2011;141: 1665-1672.
47. Wenzel JJ, Preiss J, Schemmerer M, Huber B, Jilg W. Test performance characteristics of Anti-HEV IgG assays strongly influence hepatitis E seroprevalence estimates. J Infect Dis 2013;207:497-500.
48. Drobeniuc J, Meng J, Reuter G, Greene-Montfort T, Khudyakova N, Dimitrova Z, et al. Serologic assays specific to immunoglobulin M antibodies against hepatitis E virus: pangenotypic evaluation of performances. Clin Infect Dis 2010;51:e24-e27.
49. Khuroo MS, Kamili S, Dar MY, Moecklii R, Jameel S. Hepatitis E and long-term antibody status. Lancet 1993;341:1355.
50. Myint KS, Endy TP, Shrestha MP, Shrestha SK, Vaughn DW, Innis BL, et al. Hepatitis E antibody kinetics in Nepalese patients. Trans R Soc Trop Med Hyg 2006;100:938-941.
51. Dalton HR, Bendall R, Ijaz S, Banks M. Hepatitis E: an emerging infection in developed countries. Lancet Infect Dis 2008;8:698-709.
52. Wichmann O, Schimanski S, Koch J, Kohler M, Rothe C, Plentz A, et al. Phylogenetic and case-control study on hepatitis E virus infection in Germany. J Infect Dis 2008;198:1732-1741.
53. Khuroo MS, Khuroo MS. Hepatitis E virus. Curr Opin Infect Dis 2008;21:539-543.
54. Aggarwal R. Diagnosis of hepatitis E. Nat Rev Gastroenterol Hepatol 2013;10:24-33.
55. Traore KA, Rouamba H, Nebie Y, Sanou M, Traore AS, Barro N, et al. Seroprevalence of fecal-oral transmitted hepatitis A and E virus antibodies in Burkina Faso. PLoS One 2012;7:e48125.
56. Rossi-Tamisier M, Moal V, Gerolami R, Colson P. Discrepancy between anti-hepatitis E virus immunoglobulin G prevalence assessed by two assays in kidney and liver transplant recipients. J Clin Virol 2013;56:62-64.

Supporting Information

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