Distribution of hepatitis A antibodies in US blood donors

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BACKGROUND: Recently, there has been an increase in the number of hepatitis A outbreaks in the United States. Although the presence of hepatitis A virus (HAV) RNA in blood donors is known to be low, HAV antibody prevalence in this population is unknown.

STUDY DESIGN AND METHODS: Samples from 5001 US blood donors collected primarily in the midwestern United States in 2015 were tested for the presence of HAV IgG antibodies using chemiluminescent microparticle immunoassays on the ARCHITECT platform (Abbott Laboratories).

RESULTS: The overall prevalence of IgG anti-HAV was 60%. Only one specimen was IgM anti-HAV positive, for an incidence of 0.02%. IgG anti-HAV prevalence among donors aged 16 to 19 years was 67%, decreased to 54% among donors aged 40 to 49 years and increased to 70% among donors aged 80 to 93 years. No differences were seen by sex with overall IgG anti-HAV prevalence of 61% and 60% for males and females, respectively. Among the five states (Illinois, Indiana, Kansas, Kentucky, and Missouri) with the highest number of donors tested, IgG anti-HAV prevalence in Missouri (65%) was significantly higher ($p < 0.01$) than that in Illinois (52%) or Kentucky (59%). No other significant differences between states were noted.

CONCLUSION: This study demonstrates the overall high rates of IgG anti-HAV in US blood donors, with the low associated risk of HAV transfusion transmission likely the result of low incidence and effective vaccination.

Hepatitis A is a self-limiting liver disease caused by the hepatitis A virus (HAV), which is a member of the genus *Hepatovirus* in the family *Picornaviridae*. The virus is transmitted through the fecal-oral route after consumption of contaminated food and water or contact with an infected individual. HAV infection is asymptomatic in about 70% of children aged <6 years, but 70% of adolescents and adults develop symptoms.[1]

In the United States from 1988 to 1991, 33% of the population had serological evidence of prior HAV infection on the basis of data from the Third National Health and Nutrition Examination Survey (NHANES-III). Anti-HAV prevalence was directly related to age, increasing from 10% in children aged <10 years to 75% in adults aged >70 years.[2] Further testing of NHANES specimens found a decrease in HAV seroprevalence in adults aged ≥20 years, from 29.5% during 1996 to 2006 to 24.2% during 2007 to 2012.[3]

During 1995 to 1996, effective hepatitis A vaccines were licensed for use among persons aged ≥2 years. In 1996, the Advisory Committee on Immunization Practices (ACIP) recommended vaccinating persons in groups shown to be

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at high risk of infection and children living in communities with high rates of disease.[2] In 1999, the ACIP recommended vaccinating children living in states, counties, and communities in which hepatitis A rates were consistently above the national average.[4] A report from the US Centers for Disease Control and Prevention (CDC) in 2016 indicated that the ACIP recommendation for childhood hepatitis A vaccination had resulted in increased population protection among children, but the proportion of adults with seroprotection had decreased.[5]

Transfusion transmission of HAV is extremely rare because of the short duration of viremia during acute HAV infection (approx. 10-50 days), the absence of a chronic carrier state, low incidence in the US population, and the availability of an effective vaccine.[6] However, there is the potential of HAV transmission by clotting factor concentrates, particularly because the virus is not enveloped and is resistant to inactivation.[6] Thus, US blood centers that provide plasma for further manufacture perform HAV nucleic acid screening either themselves or by their contract fractionator. Such testing is considered "in-process," with results not generated in time to interdict products for transfusion or to notify, defer, and counsel donors of their test results.[6] The rationale for this was based on the rarity of transfusion transmission, the high rate of asymptomatic resolving infection in healthy individuals, and the fact that because of the short duration of viremia, notification would occur only after infection has resolved. With the availability of real-time, automated testing platforms for HAV and parvovirus B19 by the two manufacturers of nucleic acid tests used in the United States, reexamination of policies, especially in the face of an increasing number of reported outbreaks from either contaminated food in single-sourced community outbreaks or ongoing person-to-person outbreaks for which a source has not been identified, may be warranted.[7–9]

Although the prevalence of HAV RNA in blood donors is known to be low, HAV antibody prevalence in this population is unknown. Thus, using a convenience sample available, anti-HAV prevalence was investigated.

METHODS

Sample selection and preparation

Residual samples from blood donations made to the American Red Cross (ARC) from March 22 to April 3, 2015, were obtained. Samples from donations having reactivity to routine disease markers (e.g., hepatitis B virus, hepatitis C virus, and human immunodeficiency virus) were excluded. Approximately 5000 samples were selected randomly from approximately 50,000 samples previously screened by research-use only HEV RNA assays for a study of HEV antibody prevalence.[10,11] A total of 5001 samples with adequate volume for testing were selected, representing residents of 22 states. Blood was collected in plasma preparation tubes; the plasma from these tubes was stored at −70°C until tested.[11] Epidemiological data collected and provided with the specimens included the donor's age, sex, state of residence, and state where the donation was made. The samples were anonymized and sent to the CDC for testing.

Informed consent was obtained from all donors in this study. As part of providing consent for blood donation, all donors are informed that their surplus screening samples may be used for studies on blood safety, including those involving transfusion-transmissible infections. This HAV antibody prevalence study was approved by the ARC Institutional Review Board.

Serological testing

Samples were tested for IgM anti-HAV (list number 06 L2125) and IgG anti-HAV (list number 06 L2725) using the automated chemiluminescent microparticle immunoassays on the ARCHITECT platform (Abbott Laboratories, Abbott Park, IL). Reagents were provided from Abbott Laboratories as part of an investigator-initiated study. In the IgM anti-HAV assay, any signal-to-cutoff value within 20% of the cutoff is considered a gray-zone result; the IgG anti-HAV assay does not have a gray zone. For samples that fell in the IgM gray zone, IgM anti-HAV reactivity was further evaluated using the IgM anti-HAV chemiluminescent immunoassay (680 1812) on the Vitros ECi automated platform (Ortho Clinical Diagnostics, Rochester, NY). Samples reactive by both IgM assays were considered confirmed positive. All testing was done according to manufacturers' instructions. IgM confirmed-positive samples were also tested for HAV RNA by an in-house nested polymerase chain reaction assay, as previously described.[12]

Statistical analysis

All statistical calculations and graphic visualizations were done in R (version 3.4.0).[13] Two-sided Fisher's exact tests for count data and nonparametric local regression (loess) were performed in base R.

RESULTS

Of the 5001 samples tested, 3019 (60%) were positive for IgG anti-HAV. Remaining sample volume after IgG testing was available for further IgM anti-HAV testing for 4991 samples. Of these samples, all were IgM anti-HAV nonreactive, except one sample that was in the gray zone. The gray-zone sample was tested on an alternate assay (Vitros ECi) and was IgM positive, for an IgM incidence of 0.02%. HAV RNA was undetectable in this sample. Figure 1 shows the age distribution of the donors in this study.

An analysis of IgG anti-HAV prevalence by age showed that IgG anti-HAV prevalence among donors aged 16 to 19 years was 67%, decreased to 54% among donors aged

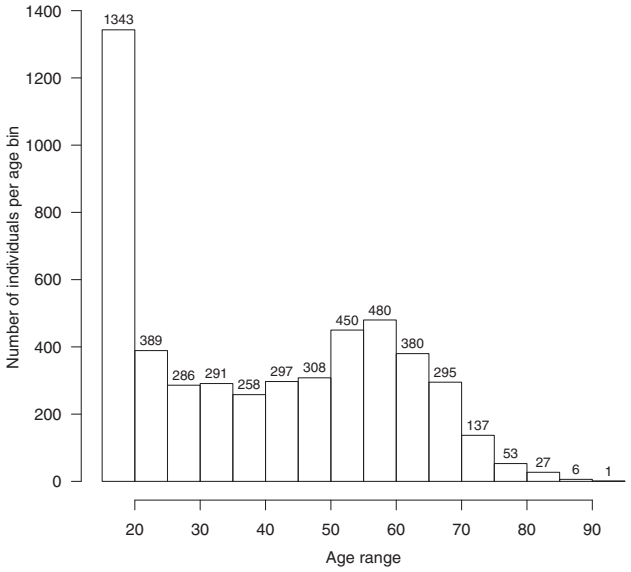

Fig. 1. Age distribution for participants (n = 5001). The number of participants were grouped into 5-year age bins, except for the youngest age group, which covered individuals aged from 16 to 19 years, inclusive, and the oldest age group, which included individuals aged from 90 to 93 years, inclusive. The numbers at the top of each bar are the number of individuals in that age range.

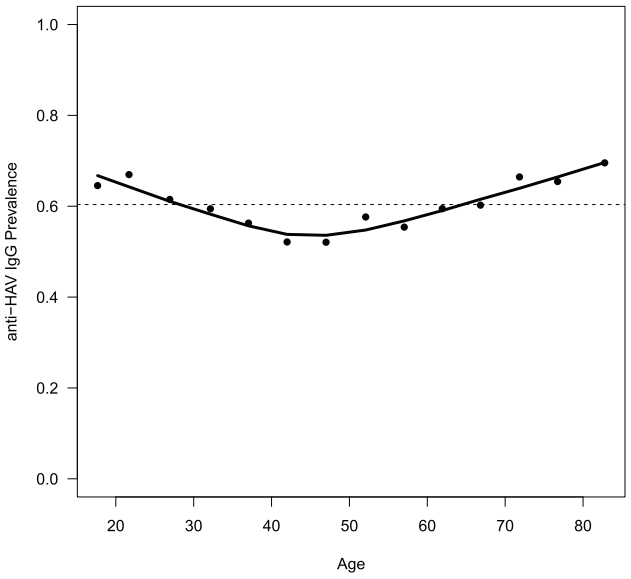

Fig. 2. IgG Anti–hepatitis A virus (HAV) prevalence by age (n = 5001). The fractional IgG anti-HAV reactivity by age range bin is plotted against the mean age within each age bin. Each bin covers 5 years of age, except for the first bin, which covered individuals aged from 16 to 19 years, inclusive, and the oldest age group, which included individuals aged from 80 to 93 years, inclusive. The solid black line is the nonparametric local regression line for the data calculated with the loess function in R. The horizontal dashed line is the mean fractional IgG anti-HAV reactivity among all individuals tested.

40 to 49 years, and then increased to 70% among donors aged 80 to 93 years (Figure 2). There are statistically significant differences between the prevalence rates for the donors aged 40 to 49 years versus either the donors aged 16 to 24 years or the donors aged 70 to 74 years ($p < 0.05$, two-sided Fisher's exact test). The percentage of IgG anti-HAV tested donors by sex was 53% male (n = 2662) and 47% female (n = 2339).

Among the five states (Illinois, Indiana, Kansas, Kentucky, and Missouri) with the highest number of donors on the basis of donor residential zip code, the overall IgG anti-HAV prevalence among male and female donors was 61% and 60%, respectively, and ranged from 50–67% (Table 1). Donors from the remaining states were not included in this analysis because none of these states had more than eight donors. Although some significant differences in overall prevalence among the five states were observed, there was little overall variability (52% for Illinois to 65% for Missouri). An examination of IgG anti-HAV by age and sex showed that there was no difference between male and female donors, with the exception of the group aged 40 to 44 years ($p < 0.0001$, Fisher's exact test, two-tailed; odds ratio = 3.0 [95% confidence interval, 1.7–5.2]), with 70% of males (n = 136) and 44% of females (n = 118) testing IgG anti-HAV positive.

DISCUSSION

The overall prevalence for IgG anti-HAV among a population of blood donors predominantly from the Midwest is 60%. This is higher than the 33% rate seen for individuals tested from 1988 to 1991 using NHANES-III samples.[2] The rate in adults aged ≥20 years was 24% (total anti-HAV) in NHANES samples collected between 2007 and 2012.[3] There are several differences between these two populations. NHANES collects blood from healthy individuals aged ≥5 years, whereas the US donor population is restricted to individuals screened for risk behavior and who are aged ≥16 years. NHANES samples were collected from across the country using a sampling method

TABLE 1. The Five States With the Highest Number of Donors Were Compared (n = 4960 From Five States) With the Fraction of IgG Antibody Reactive Donors by State and Sex Shown

State	Population		
	All	Female	Male
Illinois	0.52*	0.50	0.54
Indiana	0.60	0.61	0.59
Kansas	0.61	0.67	0.53
Kentucky	0.59*	0.60	0.57
Missouri	0.65	0.62	0.67
All states	0.60	0.60	0.61

All states show the data for all states with donors (n = 5001).
* $p < 0.01$ (vs. Missouri; Fisher's exact test for count data, and confidence intervals do not overlap).

meant to be representative of the nation, whereas the studied blood donor population was not representative of the entire United States and was predominantly from the Midwest. The (1988-1991) NHANES population is a prevaccination population, whereas the current 2015 blood donor population and the (2007-2012) NHANES population were sampled at least 11 years after the licensure of HAV vaccines, some in combination with hepatitis B vaccines (e.g., Twinrix; Glaxo Smith Kline, May 2001), thus likely increasing their penetrance in the population at large. The most likely explanation for the prevalence curve seen in Figure 2 is that younger donors have been vaccinated as a result of the ACIP recommendations,[5] as mentioned in Klevens et al.[3] Prevalence then decreases in older donors up to those from the age of 40 to 49 years because of the lack of concerted vaccination programs for older children and adults. After this point, donors in the population >49 years of age have an increase in IgG anti-HAV because of natural infection, as was seen in the NHANES study. We were not able to test these specimens to resolve the issue of IgG prevalence resulting from vaccination versus natural immunity after infection.

Among the five states with the highest numbers of donors, only the IgG anti-HAV prevalence in Missouri (65%) was significantly higher than the prevalence in Illinois (52%) and Kentucky (59%) (Table 1, $p < 0.01$). The only difference in IgG anti-HAV prevalence between males and females was in the 40- to 44-year age range ($p < 0.0001$). The blood donor population used in this study did not represent the entire United States and was restricted mainly to the Midwest. In addition, blood donors generally represent a low-risk population regarding drug use and sexual behaviors, and donors include many young individuals who would have the opportunity to be vaccinated and thus differ from the general US population.[14] Another limitation to this study is that not all samples were tested for HAV RNA; however, its frequency in blood donors is extremely low (on the order of less than 1 per 2 million donors tested annually; ARC internal data).

In summary, this study demonstrates overall high background rates of IgG anti-HAV in the general blood donor population, particularly in younger aged, presumably vaccinated donors and those aged >60 years. The presence of antibody in 60% of the donor population undoubtedly affords protection from infection in an HAV-exposed recipient.[15] The low risk of HAV transfusion transmission was further demonstrated by the absence of acute HAV infection identified in this data set and the low rate of recent infection, as measured by IgM anti-HAV (estimated at 2 per 10,000 from the single IgM anti-HAV confirmed-positive sample). All of these findings further confirm the low transfusion transmission risk of HAV in the United States.

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CONFLICT OF INTEREST

S. Stramer has received laboratory support from Abbott Laboratories, Roche, and Grifols. All the other authors have disclosed no conflicts of interest.

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