SEROPELVALENCE OF HEPATITIS E VIRUS AMONG UNITED NATIONS MISSION IN HAITI (UNMIH) PEACEKEEPERS, 1995

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Abstract. Information about the prevalence of hepatitis E virus (HEV) infection is sparse in many countries. Following the identification of four cases of acute HEV infection among Bangladeshi soldiers, a serologic survey was conducted to determine the prevalence of HEV infection among other peacekeepers from the United Nations Mission in Haiti (UNMIH) and Haitian civilians. Of the 981 participants in the survey, 876 were soldiers from eight UNMIH-participating countries representing Asia, Africa, and the Americas, and 105 were Haitian civilians. The prevalence of HEV infection by country (from highest to lowest) included Pakistan (62%), India (37%), Nepal (37%), Bangladesh (27%), Djibouti (13%), Honduras (6%), Guatemala (5%), Haiti (3%), and the United States (2%). More than 90% of those surveyed from Guatemala, Haiti, and Honduras, where prevalence data has been scarce, appeared susceptible to HEV infection. Future multinational missions like the UNMIH might also present unique opportunities to study health threats of widespread interest.

Infection with hepatitis E virus (HEV) appears to be the most common form of acute viral hepatitis among young adults in the developing world. It is known to be endemic in parts of Asia, Africa, the Middle East, and North America, where outbreaks have occurred. Sporadic cases have also been identified among travelers to these regions. Since transmission principally occurs via fecally contaminated water, HEV is an especially important health threat after heavy rains in endemic areas that have inadequate or disrupted sanitation systems.

International travel may facilitate the introduction of pathogens such as HEV into new geographic areas and expose nonimmune individuals to infection. This was a concern of the United Nations Mission in Haiti (UNMIH) in 1995 when four cases of acute HEV infection with jaundice were identified among Bangladeshi soldiers whose battalion had recently deployed to Haiti as peacekeepers. Active surveillance did not identify additional cases but serologic screening of an additional 105 Bangladeshi soldiers found seven with elevated levels of IgM but without symptoms. Given the poor sanitary conditions and seasonal propensity for flooding in some parts of Haiti, there was concern about the susceptibility of other UNMIH personnel and local Haitians to HEV infection. To our knowledge, the endemicity of HEV in Haiti and other Caribbean countries has not been studied.

Since past infection elicits a detectable antibody response of unknown duration, a serologic survey was conducted to assess the relative degree of past exposure and present susceptibility to HEV infection. While a positive serologic test result suggests previous infection, a negative serologic test result suggests either no or remote infection. Joint operations involving a multinational force like that of the UNMIH presented a unique opportunity to study a potential health threat like HEV without the limitations of conducting separate country-specific studies that might be performed at varying times with differing methods. Survey participants included UNMIH military personnel representing eight countries (including Bangladesh), four of which were known to be endemic for HEV. Local Haitians who had applied for employment with the United Nations (UN) were also surveyed. Survey results might lead to a greater understanding of the risk of infection across different nationalities, reinforce the need for stricter sanitation practices in deployed settings, and help encourage the development of additional approaches for the prevention of HEV infections.

MATERIALS AND METHODS

Background. The UNMIH began its operation in March 1995 after the mission of the United States–led multinational force that helped to restore President Jean Baptiste Aristide to office was completed. At the time of this survey, approximately 80% of the 8,000 UNMIH personnel were military and predominantly from Bangladesh, Canada, Caribbean nations, Djibouti, Guatemala, Honduras, India, Nepal, the Netherlands, Pakistan, and the United States. The remaining personnel (20%) were civilians and served as police, human rights observers, and in a variety of UN administrative positions. The UNMIH forces were stationed in the capitol, Port-au-Prince, and in other strategic parts of Haiti.

Survey population. While screening the 105 Bangladeshi soldiers, a plan for a larger serosurvey of military personnel was approved by the UNMIH’s Force Surgeon and the UNMIH Commander in Port-au-Prince. The senior medical officer and contingent commanders of Djibouti, Guatemala, Honduras, India, Nepal, Pakistan, and the United States each approved the participation of approximately 100 soldiers in the serosurvey. For some company-sized contingents, 100 soldiers represented nearly the entire unit’s troop strength. Participants from other larger contingents were soldiers who were off duty and available at the time of survey.

Local Haitian nationals who were seeking UN employment and who had already been evaluated for relevant work experience and residence near a specific job donated blood as part of the required UN pre-employment health screening process. Serum remaining after required laboratory testing was frozen. Two hundred eighty-nine serum specimens from local Haitians had been collected from June to October 1995. After all personal identifiers were removed from the serum collection, 105 specimens were randomly selected for testing as part of this survey.

Informed consent. Contingent commanders have a widely recognized and overriding duty to protect the operational
readiness of their deployed forces. Consent for conducting this minimal risk serosurvey, in a possible outbreak setting, was consistent with customary practices such as military mass immunization and chemoprophylaxis programs deemed necessary to protect the operational readiness of military units.

Endorsement to test the Haitian serum specimens was obtained from the Haitian Ministry of Health and from the regional office for the Americas of the World Health Organization (PAHO/WHO). In accordance with standard minimal risk research activities, personal identifiers were dissociated from individual specimens.16

Specimen collection and questionnaire administration. The survey was conducted at the base locations of the various military contingents during the fall of 1995. A 14-item questionnaire consisting of demographic and clinical items, which focused primarily on gastrointestinal symptomatology, was administered to all military participants. Each soldier donated a blood specimen. When necessary, English-speaking interpreters were available to administer the questionnaire in the participants’ own language, to record participants’ responses in English, and to assist with blood specimen collection. Serum was then aliquoted, frozen, and shipped to the Walter Reed Army Institute of Research in Washington, DC for testing. In contrast to participating military contingents, administration of the questionnaire to Haitian participants was not feasible since at the time of the serosurvey, they were living in many different locations throughout the country.

Quantitation of antibody to HEV. Total antibody to HEV was determined by sandwich ELISA in 96-well plates. The antigen (provided by P. Yarbough, Genelabs Technologies, Inc., Redwood City, CA) was the putative capsid protein of HEV, Burma strain, expressed in Spodoptera frugiperda (SF9) cells by a recombinant baculovirus.17 The HEV sequence was open reading frame 2 (ORF-2) from which nucleotides coding for the first 111 amino-terminal amino acids had been deleted and replaced with a methionine start codon. Capsid protein (Molecular weight = 56–58 kD) was purified from infected cell extracts by column chromatography as previously described.17

The assay procedure was as follows. A single lot of purified antigen diluted to 32 U/100 μl (100 ng of protein per well) in 0.1 M carbonate buffer, pH 9.6, was used to coat plates for 1 hr at room temperature and then overnight at 4°C. Plates were washed and 300 μl of blocking solution (0.5% casein and 0.5% bovine serum albumin in phosphate-buffered saline, pH 7.9) was added to each well for 1 hr at 37°C. Plates were again washed and 100 μl of test article (serum diluted 1:1,000 or higher as necessary in blocking solution) was added to each well for 2 hr at 37°C. Plates were washed a third time and 100 μl of anti-human immunoglobulin (M + G + A) conjugated to horseradish peroxidase (Kirkegaard and Perry Laboratories, Gaithersburg, MD) diluted 1:4,000 in blocking solution was added and incubated for 1.5 hr at 37°C. Plates were finally washed and 100 μl of tetramethylbenzidine (Kirkegaard and Perry Laboratories) was added. The reaction was stopped after 10 min with acid; plates were read at 450/650 nm (Spectromax 340; Molecular Devices, Inc., Sunnyvale, CA).

Antibody levels were quantified by comparison with an HEV immunoglobulin standard that was created by pooling equal aliquots of convalescent serum from 43 donors in Nepal who during a six-month study acquired antibody to HEV that was detected with a first-generation test (Genelabs Diagnostics, Singapore). This pool was defined to contain 1,000 U of HEV antibody/ml. A four-parameter curve-fitting algorithm was used to relate optical density to antibody concentration in U/ml. Therefore, every plate included six half-log dilutions of the standard tested in duplicate, as well as additional positive and negative controls. The lower limit of detection of the assay was 1 U/ml; values greater than 300 U/ml were determined using specimens diluted 1:10,000 or more. Levels of immunoglobulin to HEV that are observed during acute hepatitis E generally exceed 1,000 U/ml; levels observed 18 months after infection generally range from 100 to 800 U/ml; the kinetics of antibody beyond 18 months are uncertain (Innis B, unpublished data).

Negative control specimens. Donors lacking exposure to HEV were required to define an assay level consistent with past infection. Therefore, we obtained from existing serum banks specimens from infants and children in Thailand. Few cases of HEV have been detected in Thailand despite intense surveillance (Clayson E, unpublished data). Two sets of negative control specimens were examined. We obtained 79 serum specimens from healthy nine-month-old infants (courtesy of S. Kalayanarooj, Queen Sirikit National Institute of Child’s Health, Bangkok, Thailand) and 196 serum specimens from 99 children (ages 2–15) residing in Bangkok who had dengue fever or dengue hemorrhagic fever (courtesy of A. Nisalak, Department of Virology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand). The latter specimens were selected because they were known to contain high levels of IgM to dengue virus. The rationale for including them was to characterize the effect of IgM elicited by intense immune activation.

RESULTS

Except for the 105 Haitian civilian participants (11%), the study population was composed primarily of young adult males from the eight military contingents. The overall average age was 29 years (range = 18–61 years). The largest proportion of survey participants 25 years old or younger were from Guatemala and the United States. Haitians composed the oldest age group with an average age of 33.8 years followed by Pakistan (31.7 years), Djibouti and Nepal (31.2 years), Bangladesh (30.9 years), and India (29.6 years). Military participants were entirely male except for the United States (96% male). Twenty-seven Haitian civilian participants were female (26%). The length of military service reflected the age distribution of each contingent. Soldiers from Pakistan, Bangladesh, and Nepal had the greatest number of years of military service. At the time of the survey, 90% or more of soldiers surveyed from Bangladesh, Djibouti, and Honduras had been in Haiti for less than eight weeks, the incubation period of HEV.

The distribution of levels of immunoglobulin to HEV determined among the 981 soldiers participating in this study is shown in the bottom panel of Figure 1. The distribution for 275 negative controls is shown in the upper panel. No serum specimen from a negative control gave a value above
the cut-off point of 20 U/ml, whereas specimens from 209 soldiers (21%) were above this level. Specimens from 772 soldiers (79%) were below the cut-off point. Donors of specimens containing immunoglobulin to HEV greater than or equal to 20 U/ml were inferred to have been previously infected with HEV. This cut-off was considered to be conservative because it represents five standard deviations (SD = 3.2 U/ml) above the mean (4.0 U/ml) of the negative controls.

Among the 209 previously infected soldiers, the range of immunoglobulin to HEV was 20–821 U/ml. The distribution of values was clustered between the cut-off and 100 U/ml (67%) with another 23% in the tail to the right. Antibody titers greater than 500 U/ml were seen in only five soldiers (two from Bangladesh and three from Nepal). We speculate that some of the highest values represent persons most recently infected.

Table 1 summarizes the serologic results by country. The prevalence of past HEV infection was greatest among soldiers from countries in the Indian subcontinent of Asia. Pakistan had the highest prevalence (62%) followed by India and Nepal (both 37%), and Bangladesh (27%). The prevalence of past infection among soldiers from non-Asian countries was 13% among the Djiboutis and 6% or less among those from Honduras, Guatemala, and the United States.

### Table 1

<table>
<thead>
<tr>
<th>Country</th>
<th>n</th>
<th>≥20 U/ml</th>
</tr>
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<tbody>
<tr>
<td>Pakistan</td>
<td>109</td>
<td>68 (62%)</td>
</tr>
<tr>
<td>India</td>
<td>107</td>
<td>40 (37%)</td>
</tr>
<tr>
<td>Nepal</td>
<td>114</td>
<td>42 (37%)</td>
</tr>
<tr>
<td>Bangladesh</td>
<td>105</td>
<td>28 (27%)</td>
</tr>
<tr>
<td>Djibouti</td>
<td>112</td>
<td>14 (13%)</td>
</tr>
<tr>
<td>Honduras</td>
<td>109</td>
<td>6 (6%)</td>
</tr>
<tr>
<td>Guatemala</td>
<td>111</td>
<td>6 (5%)</td>
</tr>
<tr>
<td>Haiti*</td>
<td>105</td>
<td>3 (3%)</td>
</tr>
<tr>
<td>United States</td>
<td>109</td>
<td>2 (2%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>981</td>
<td>209 (21%)</td>
</tr>
</tbody>
</table>

* Civilians participating in a United Nations pre-employment health screening program.
More than 90% of participants from the Americas, including the military contingents and Haitian civilians, had no serologic evidence of past HEV infection. Of the 31 women who participated in the survey, none had serologic evidence of past HEV infection.

Survey questionnaires were completed by the 876 soldiers. Of the 36 soldiers (4%) who listed a current medical problem, one reported jaundice as a result of hepatitis B infection. Two other soldiers reported hepatitis-like symptoms and were subsequently diagnosed with appendicitis and malaria, respectively. No other participants reported symptoms suggestive of hepatitis. None of the five soldiers (< 1%) with the highest antibody titers (> 500 U/ml) reported a current medical problem. One of the five recalled a single episode of jaundice eight years prior to coming to Haiti. Another one of the five soldiers who had been in Haiti for six weeks described a recent hepatitis-like illness that lasted at least five days with loss of appetite, nausea, pain in the right upper quadrant, and weakness but without jaundice. Twenty-nine (14%) of the 206 soldiers who had serologic evidence of past HEV infection in contrast to 13 (2%) of the 670 soldiers who did not have evidence of past HEV infection could recall ever experiencing jaundice (P < 0.01, by chi-square test).

**DISCUSSION**

Following the diagnosis of acute HEV infection with jaundice in four Bangladesh soldiers, an epidemiologic investigation was conducted to better understand the threat of HEV to UNMIH personnel and local Haitians. Not surprisingly, serologic results showed that the prevalence of HEV was highest among those military contingents from the Indian subcontinent of Asia where HEV is known to be endemic. In HEV endemic countries, anti-HEV has been detected in 10–40% of adults more than 25 years of age.18–20 The prevalence of HEV infection for the four Asian contingents was within this range except for Pakistan (prevalence of HEV infection = 62%). Pakistan had the highest mean age of the eight UNMIH military contingents.

Several studies have shown that increasing age is associated with an increasing prevalence of HEV.5,21,22 This association might be due to a relatively even accumulation of infections in a population or to different rates of infection among various age cohorts. We had no information to clarify this specific point or other issues related to previous relevant exposures of the groups surveyed. However, it seems unlikely that the relatively small age differences between the Asian contingents alone could account for the magnitude of the disparity in the prevalence of HEV infection among them. Our results show that a relatively large proportion of their ranks (38–73%) had no evidence of past infection.

Epidemic and sporadic HEV infections also occur in many parts of Africa including the northeast23 where Djibouti is located. In our survey, the prevalence of HEV infection in the Djibouti military contingent was 16%. A study in 1993 that involved a smaller number of Djibouti adults (n = 26) found a prevalence of 15%.24 Both surveys indicated that nearly 85% of Djibouti adults had no serologic evidence of past HEV infection. This is in marked contrast to the apparently low susceptibility of adult Djiboutis to hepatitis A virus (HAV) infection, which is also enterically transmitted. A survey in 1987 showed that among 656 Djibouti adults, the prevalence of antibody to HAV was 99%.25 In countries such as Djibouti where HAV and HEV coexist, HEV infection (and clinical disease) appears to pose a far greater health threat to the local adult population than HAV infection, which is primarily acquired early in childhood.

To our knowledge, the prevalence of HEV infection in the Central American countries of Guatemala and Honduras has not been described. Previous reports from this region have documented the prevalence of past HEV infection in Nicaragua (5–8%)26 and the two HEV outbreaks that occurred in Mexico in 1986.27 Similar to the survey in Nicaragua, we found that 5–6% of the soldiers sampled from Guatemala and Honduras had evidence of past HEV infection. Surveys conducted in neighboring South American countries showed the following prevalences: Brazil (6%),28 Chile (7%),29 Peru (14%),30 and Venezuela (2–5%).31 In light of the prevalence of 1–5% found in blood donors from nonendemic countries as a baseline,32 these data indicate a relatively low-to-intermediate level of HEV transmission among the populations surveyed. Travel to HEV-endemic areas such as parts of Mexico may help account for these findings, which are consistent with the prevalence of HEV infection found among Mexican migrants who worked in California (16%) and U.S. Peace Corps volunteers who had spent two years in Africa (10%).32

Only two soldiers from the United States had evidence of HEV infection and neither one reported a history of jaundice. Both soldiers listed another country, Vietnam and Mexico, respectively, in addition to the United States as their country, and the possibility exists that their infection might have been acquired in these HEV-endemic countries. Overall, the prevalence of HEV infection for the United States contingent (2%) was within the range (1–5%) reported from other nonendemic countries. Still, this appears to be a relatively high degree of seropositivity given the virtual absence of autochthonous cases in nonendemic countries such as the United States. Several factors should be considered when interpreting these findings. In populations with a low prevalence of infection, it is important to know the operating characteristics of the serologic tests used since highly specific tests help to avoid false-positive results. Further studies are needed to determine these test characteristics and refinements may be necessary. Given the geographic spread of participants in our survey, cross-reacting caliciviruses or less pathogenic strains of HEV might also elicit variable clinical responses in the human host and affect serologic testing.33,34

Along with the three military contingents from the Americas, a small proportion (3%) of Haitian civilians displayed serologic evidence of past HEV infection. These results suggest that HEV is not endemic in Haiti and that concern about its importation by multinational peacekeepers with acute disease was justified. However, introduction of a pathogen such as HEV into a new geographic area is but just one part of a complex process that may lead to disease emergence. Survival and proliferation of the pathogen in the environment and its transmission into a susceptible host are critical additional steps.35 Routine and proper handling of human waste by the UNMIH was likely a significant factor in preventing secondary cases.
Unfortunately, if HEV was introduced into Haiti’s ecosystem, conditions appear favorable for disease emergence since many communities have poor sanitary conditions and contaminated water supplies. Moreover, Haiti lies within the Caribbean’s hurricane belt. Environmental health conditions can be made even worse by fresh water flooding and mud slides after natural disasters with heavy rainfalls such as occurred in southern Haiti with Hurricane Gordon in 1994. Epidemics due to HEV in other countries caused by the contamination of fresh water supplies have been well documented.4 5

Several factors should be considered when interpreting these results. Demographic differences existed between each military contingent and its country’s general population. Also, no data regarding socioeconomic status and other characteristics were available to assess to what extent Haitian survey participants were representative of Haitians in general. Therefore, caution is warranted when extrapolating study findings to the general population of the participating countries.

In conclusion, this cross-sectional serologic survey suggests a wide range of susceptibilities to HEV infection among participants from nine countries. The prevalences of HEV infection that we observed were remarkably consistent with those published from those countries in which HEV had been studied previously. More than 90% of those surveyed from Guatemala, Haiti, and Honduras where data has been scarce appeared susceptible to HEV infection. Safe water sanitation practices will help prevent HEV transmission among military and civilian travelers, and local populations alike as other countermeasures are being developed. Health planners and care providers for multinational deployments must be sensitive to the potential for the transmission of emerging pathogens such as HEV to susceptible mission personnel and host country nationals. Future multinational missions like the UNMIH might also present rare opportunities to study a variety of diseases because of the assembly of unique and varied groups in a relatively small geographic area.

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