Population Seroprevalence of Hepatitis E Virus Antibodies in Rural Bangladesh

Alain B. Labrique, K. Zaman, Zahid Hossain, Parimalendu Saha, Mohammad Yunus, Anowar Hossain, John Ticehurst, and Kenrad E. Nelson

Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland; ICDDR,B, Dhaka, Bangladesh; Applied Physics Laboratory, Johns Hopkins University, Laurel, Maryland; Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland

Abstract. Hepatitis E virus (HEV) causes a substantial burden of sporadic and epidemic disease worldwide. HEV infections result in serious morbidity and mortality, especially among pregnant women, and have significant economic costs. Few population-based studies have characterized the epidemiology of HEV. A rural Bangladeshi population was studied to determine the age- and gender-specific population seroprevalence of antibodies to HEV. Of 1,134 specimens tested from a representative, random population sample, 255 (22.5%) were anti-HEV IgG seropositive. Seroprevalence was lower among women (19.7%) than among men (25.8%). We found anti-HBc (hepatitis B core) in 380 of 1080 (35.2%) tested, anti-HCV (hepatitis C) in 14 of 917 (1.5%) tested, and anti-HAV (hepatitis A) in 116 of 124 (93.5%) tested individuals. Our data suggest that viral hepatitis, especially HEV, remains an under-recognized and significant public health problem in rural Bangladeshi populations, warranting further attention.

INTRODUCTION

Hepatitis E virus (HEV) is an emerging infectious agent, recognized globally as the leading cause of enteric hepatitis illness both in epidemics and sporadic cases. Identified in 1991, the molecular biology and some epidemiology of HEV has been characterized over the past two decades. Infections are primarily subclinical, with only 20–30% of cases experiencing symptoms. Although the disease is self-limiting and no chronic sequelae or carrier state has been documented in the general population, the feature of hepatitis E that has drawn most attention is the poorly understood case fatality rate (> 20%) in infected pregnant women, especially in their second and third trimesters. Membrane rupture, spontaneous abortion, and stillbirth, as well as increased neonatal mortality (up to 25%), are associated with HEV infections in pregnancy. This pathogenesis remains poorly understood.

The epidemiology of HEV is complex, and unlike other enteric pathogens such as hepatitis A virus (HAV), HEV infection is more common in the second and third decades of life in endemic South Asian populations, whereas Egyptian studies have shown more frequent antibody prevalence in young children. A wide range of seroprevalence estimates exist for the Indian subcontinent, where the most common human HEV genotype is prevalent (GT 1); seroprevalence varies depending on the population sampled and the assay used. Some HEV genotypes (GT 3 and 4) are primarily zoonotic, occasionally causing human infections through enteric or foodborne routes. Although the greatest historical burden of disease has been confined to South and Southeast Asia, where seasonal HEV outbreaks are documented with predictable regularity in India and Nepal, few studies have characterized the burden of HEV infections in Bangladesh. In the early 1990s, reports were published of travelers to Bangladesh acquiring HEV infections, followed by a documented outbreak of hepatitis E in a contingent of Bangladeshi UN peacekeepers that provided strong circumstantial evidence of HEV endemicity.

Several hospital-based studies have suggested that much of the acute viral hepatitis (30–60%) in Bangladesh has an HEV etiology. Despite the absence of recognized seasonal outbreaks, Bangladesh was suspected to be HEV endemic, with seroprevalence projections ranging from 27% to 60%.

This study represents the first rural community-based seroprevalence survey of HEV in rural Bangladesh, in a representative, random population sample to quantify the burden of HEV infection by age and sex. Our data confirm that HEV is endemic in Bangladesh and raise questions of whether seasonal epidemics occur, undetected, or whether population risk factors or HEV genotypic differences contribute to a different epidemiology from neighboring countries.

MATERIALS AND METHODS

The objective of this study was to determine the age-specific population seroprevalence of antibodies to HEV in a rural population of southern Bangladesh. The study was a random population serosurvey, using a cross-sectional design. Participants were selected from the Maternal and Child Health/Family Planning (MCH-FP) cohort of the Matlab Health Research Program of the International Center for Diarrheal Disease Research, Bangladesh (ICDDR,B). The population from which the representative sample was drawn consists of ~110,000 people inhabiting 67 villages, under the Matlab Health and Demographic Surveillance System (MHDSS). This is a largely agrarian, homogenous population, representative of rural Bangladesh in general.

A random list of 1,300 individuals was generated from the continuously updated MHDSS census lists, excluding children < 1 year of age, because of cultural restrictions. Two teams of field workers, consisting of an interviewer and a phlebotomist, visited listed persons to invite their participation in the survey. Individuals not found during the first attempt were visited up to four times to maximize enrollment. Consenting subjects were interviewed to collect socioeconomic status data, basic enteric risk factors, and recent morbidity history. The 3.5-month baseline enrollment of participants began December 23, 2003 and ended April 8, 2004.

A fingerstick blood specimen (~350 μL) was collected using a specialized capillary blood collection and separation microtube system (Safe-T-Fill; RAM Scientific, Needham, MA).
following procedures established by the manufacturer. Within 4 hours of collection, microtubes were centrifuged at 4,000g for 10 minutes, and the supernatant serum was aliquoted into two sterile 200-μL mini-Eppendorf tubes for testing and archival. Aliquots were stored at −20°C until shipped on dry ice to the Armed Forces Research Institute of Medical Sciences (AFRIMS, Bangkok, Thailand), a regional hepatitis reference laboratory.

Because of limited serum volumes, specimens were tested using commercial and in-house enzyme immunoassays (EIA) for antibodies in the following priority order: anti-hepatitis E virus total Ig (anti-HEV Ig), anti-hepatitis B virus core antigen total Ig (anti-HBc Ig), anti-hepatitis C IgG (anti-HCV IgG), and anti-hepatitis A total Ig (anti-HAV Ig). The anti-HEV assay (Abbott/Murex, Dartford, UK) is 100% sensitive and specific in identifying prior exposure to hepatitis A virus. The anti-HBc Ig assay (Abbott/Murex) identifies both past exposure to HBV and acute/chronic infection with >99% sensitivity and specificity. The anti-HCV assay, version 4.0 (Abbott/Murex), is a third-generation assay with a reported sensitivity and specificity >99% in detecting exposure to HCV. Initially reactive specimens were tested in duplicate. Positive and negative controls were included in every EIA run.

Although commercial anti-HEV assays are available, the appropriateness of their use for epidemiologic studies is questioned.21,22 The Walter Reed Army Institute of Research (WRAIR, Bethesda, MD) has developed a quantitative anti-HEV total Ig EIA that is highly sensitive (96%) and specific (98%) to identify HEV infections in populations.23–25 The assay uses a quantitative sandwich approach to capture and label human Ig antibodies to recombinant HEV ORF2 proteins (rHEV) of the Sar-55 Pakistani strain expressed in the baculovirus system, and the photometric result is compared with positive controls from a reference pool of Nepali HEV patients, reporting antibody concentrations in WRAIR units per milliliter (WRAIR U/mL). A cut-off of ≥ 20 U/mL is used by WRAIR to classify subjects with “definite past infections,” and ≥ 500 U/mL is used to classify subjects with definite acute clinical infection.25 This assay is described in detail elsewhere.23–25

All data were entered using custom data entry screens, with field range and data consistency checks programmed using Visual FoxPro (Microsoft, Seattle, WA). All statistical analyses were performed using Stata Version 9.0 (Stata Corporation, College Station, TX). The age distribution of the sample was compared against the mid-year Matlab HDSS census data from 2004 to determine representativeness across age categories. Seroprevalence rates were calculated based on the number of specimens meeting the defined optical density cut-off values for the commercial assays, and the WRAIR recommended cut-off of total Ig ≤ 20 U/mL described above over the total number tested. The 5-year age category-specific seroprevalence was determined and plotted, showing the number sampled from each age group, as a reference. The 95% confidence intervals (CIs) were calculated using an exact method based on the binomial distribution. The characteristics of enrolled participants were described using median and ranges for continuous variables and proportions for categorical values. Because of non-normal distributions of continuous variables, a non-parametric equality-of-medians k-sample test was used to compare age, household size, and MUAC, by sex. Univariate comparison of proportions between groups was done using the $\chi^2$ and Fisher exact tests where appropriate. To estimate strength of association, odds ratios (ORs) and associated 95% CIs were used. Variables with significant univariate associations at a $P$ value $> 0.05$ were entered in a multivariate logistic regression model. Analysis of marital status, employment, and education excluded individuals younger than 15, students or children, and those under 7 years old, respectively.

Age acted as an important confounder for these three variables, because of the strong negative association between young age and anti-HEV seropositivity. The combined antibody seropositivity between hepatitis B and anti-HEV was examined by $\chi^2$ analysis, examining whether there was greater occurrence of combined anti-HAV, anti-HBc, or anti-HEV than expected by chance. $P < 0.05$ or a 95% CI not spanning 1.0 was considered statistically significant for all analyses.

All study procedures were approved by the Committee on Human Research at the Johns Hopkins Bloomberg School of Public Health and by the ICDDR,B Ethical Review Committee. Informed consent was obtained from all adult and emancipated participants; for children over the age of 5, the consent of the parent or guardian was sought with assent from the children.

**RESULTS**

Of the 1,300 randomly selected subjects, 1,136 (87.4%) were successfully enrolled into the study. At the end of recruitment, 57 (4.4%) were never met, 70 (5.4%) migrated out of the study area, 31 (2.4%) refused to participate, and 6 (0.5%) had died. Only two specimens were inadequate for analysis. Migration out of the study area was highest among 16–30 year olds ($N = 28$), representing 8.3% of the number selected within that age category. Refusals were higher in number in <15 year olds ($N = 17$) but represented only 3.8% of those under 15 selected. Refusals were proportionately greater in the 61- to 75-year age category ($N = 6$ of 89, 6.7%). The age distribution of the final sample closely matched that of the mid-2001 Matlab population (data not shown).

Among the 1,134 specimens, 255 (22.5%; 95% CI: 20.1–25.0) met the criteria for definite prior HCV infection. We found anti-HBc antibodies in 380 of 1,080 (35.2%; 95% CI: 32.3–38.1) tested individuals, anti-HCV in 14 of 917 (1.5%; 95% CI: 0.8–2.5) tested individuals. Because of limited specimen volumes, only 124 specimens could be tested for anti-HAV, of which 116 (93.5%; 95% CI: 87.7–97.2) were reactive (Table 1). Among anti-HEV seropositive individuals, the median antibody titer was 81.3 U/mL, and five individuals had titers exceeding 500 U/mL (0.44% population prevalence), a cut-off used to identify definite acute infection. These individuals were 9, 16, 16, 50, and 69 years old.

The age-specific seroprevalence increased in adolescence well into the second and third decades of life, with a peak 42.5% seroprevalence in the 36- to 40-year age group (Figure 1).

**Table 1**

<table>
<thead>
<tr>
<th>Assay</th>
<th>Manufacturer</th>
<th>Tested (N)</th>
<th>Positive (N)</th>
<th>Percent prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-HEV (total Ig)*</td>
<td>WRAIR</td>
<td>1134</td>
<td>146</td>
<td>22.5</td>
</tr>
<tr>
<td>Anti-HBc (total Ig)</td>
<td>Abbott-MUREX</td>
<td>1080</td>
<td>380</td>
<td>35.2</td>
</tr>
<tr>
<td>Anti-HCV (IgG)</td>
<td>Abbott-MUREX</td>
<td>917</td>
<td>14</td>
<td>1.5</td>
</tr>
<tr>
<td>Anti-HAV (total Ig)</td>
<td>Abbott-MUREX</td>
<td>124</td>
<td>116</td>
<td>93.5</td>
</tr>
</tbody>
</table>

* ≥ 20 WRAIR units/mL.
Population seroprevalence in this sample was lower among women (19.7%) than men (25.8%); this phenomenon is consistent across age categories (Figure 2).

As shown in Table 2, anti-HEV seropositive individuals were significantly older (mean ± SD = 35.9 ± 17.6 years, data not shown) than their non-reactive counterparts (mean ± SD = 25.2 ± 20.0 years, data not shown). Only male sex was significantly associated with seropositivity. Univariate differences in type of occupation were wholly explained by sex differences in employment patterns; >89% of female respondents reported “housework” as their occupation.

Although income was not significantly associated with seropositivity, stratifying by sex showed that women in the highest income class had a lower seroprevalence to HEV compared with the lowest income class after adjusting for age (OR, 0.46; 95% CI: 0.28–0.77). Seropositive individuals reported a mean frequency of travel outside the home in the past month of 2.3 ± 4.3 (SD) days in contrast to 1.6 ± 3.7 days among seronegatives, a significant difference (OR, 1.04; 95% CI: 1.01–1.08). This association (and other characteristics such as education and marital status) was largely confounded by age and sex. This association disappeared in an age/sex-adjusted analysis.

We did not find evidence of an association between anti-HEV seropositivity and religion, marital status, employment location (outdoor versus indoor), household size, education, income, or contact with “jaundiced” or sick individuals in the previous 3 months. Mid-upper arm circumference, an indicator of adult nutritional status, was not different by anti-HEV status. Among females >12 years old, current pregnancy was not associated with anti-HEV; however, there were only 16 pregnant women in the sample (5.0% versus 5.3% women were pregnant).
pregnant among HEV seropositive and seronegative women, respectively; \( P > 0.9 \).

We found a significant univariate association between a reported history of injection receipt in the past 3 months and seropositivity (OR, 1.89; 95% CI: 1.35–2.66). Overall, 17% of respondents reported recent injection exposure, more common among females (23.8%) than males (9.0%). However, this association was not significant in an age- and sex-adjusted model.

Table 3 examines the most significant overall characteristics associated with anti-HEV seropositivity in the Matlab population. Individuals < 15 were much less likely (OR, 0.15; 95% CI: 0.10–0.23) to have antibodies to HEV. The likelihood of seropositivity increased with each age category, dropping slightly in the older age groups. Females were significantly less likely to be antibody seropositive across all age strata.

Subjects who were seropositive to HEV were significantly more likely to be anti-HCV or HBc seropositive (Table 4).

Although not statistically different by seropositivity, recent (past 3 month) self-reported morbidity in this population was common (data not shown). A substantial number of respondents claimed at least one episode of high fever (45%), anorexia (35%), extreme weakness (29%), liver (or upper right quadrant) pain (29%), nausea, or vomiting (26%). A smaller proportion claimed to have had dark urine (14%), yellow eyes (7%), or ash-colored stools (6%) in the past 3 months. Women were ~30% more likely to report weakness and anorexia and 86% more likely to report liver (upper right quadrant) pain.

**DISCUSSION**

The data suggest a previously undescribed, substantial burden of hepatitis virus infections within a typical rural community of southern Bangladesh, likely representative of similar communities across the country. Clearly, HEV is an under-recognized
problem of significance, which likely contributes to population morbidity and perhaps to the high level of maternal and neonatal mortality documented in Bangladesh. The 22.5% prevalence of antibodies to HEV using a highly sensitive and specific research assay documents that HEV is endemic in this population.

These findings confirm recent clinical studies identifying HEV as an etiologic agent contributing to substantial hepatitis morbidity in Bangladesh. The rural population prevalence is lower than the 60.1% proposed by a recent urban study of 273 apparently healthy adults, but similar to the 27% estimates of anti-HEV IgG prevalence in a study of 105 Bangladeshi peacekeepers participating in the United Nations Mission in Haiti in 1996. A 1992 population serosurvey in southern India found an overall anti-HEV seroprevalence of 26%, whereas other India estimates range from 4% to 64%, with varying sample sizes and age ranges using different assays. Studies in Nepal estimate a population seroprevalence of anti-HEV to be between 10% and 37%. These data contrast sharply from the higher seroprevalence rates (> 60%) shown in a rural Egyptian population. Thus, despite the absence of reported large seasonal outbreaks of HEV in this population, our seroprevalence estimates in Bangladesh are remarkably similar to neighboring epidemic-prone countries.

The age-specific seroprevalence estimates seen in our data reflect an unusual epidemiologic distribution for what is thought to be an enterically transmitted virus, yet has been consistently described in similar studies in Nepal and India. The 66.7% peak in seroprevalence in the 85-year age category is likely an artifact of the small sample for this age category. The paucity of infections in infancy and childhood, reported in other South Asian populations, was similar in our population despite a large representation of participants ≤ 15 years of age (N = 6) for this age category. The paucity of infections in infancy and childhood, reported in other South Asian populations, was similar in our population despite a large representation of participants ≤ 15 years of age (N = 6) for this age category. The recent introduction of the virus into this population, or even waning immune markers with time. Small numbers of older subjects makes the estimation of seroprevalence in subjects older than 65 years difficult, as seen in the wide CIs of Figure 1. Stratification by sex disclosed a similar pattern in both male and female participants with seroprevalence peaking between ages 16 and 40 (Figure 2). This antibody pattern contrasts sharply with that of other enteric pathogens in developing countries, such as HAV, where antibody seropositivity appears early in life, and, by the end of childhood, most individuals are seropositive. Higher infection rates in young and middle-aged adults has been a characteristic of both sporadic and epidemic HEV in South Asia.

None of the expected proxies for socioeconomic status or education were clearly associated with increased seropositivity, and the initial associations with occupational categories were explained by confounding by sex and age.

Female participants were up to 58% less likely to be seropositive (Table 3). Lower infection rates with HEV has been reported from HEV outbreaks in Nepal, Pakistan, and India, where women were 2-fold less likely to report clinical illness during outbreaks. In much of rural Bangladesh, conservative norms often restrict the mobility and employment of women outside of the home or neighborhood, which may, in turn, limit their exposures to potential sources of HEV. Women in the higher income category were also less likely to be seropositive, possibly because of better sanitary conditions and limited exposures to contaminated water.

Limited access to clean drinking water, exacerbated by annual floods and combined with generally poor sanitary conditions in rural Bangladesh, likely contribute to high levels of risk for enteric infections such as HEV. The 93.5% anti-HAV seroprevalence (95% CI: 87.7–97.2%) provides strong evidence that this population is highly exposed to risk factors for enteric viral infections. As many others have shown, HAV infections in developing countries are ubiquitous by age 10, and provide long-term immunity. This discrepancy between the age-specific distributions of the two enterically transmitted hepatitis viruses has been noted in a recent HEV study of healthy urban residents of the capital city of Bangladesh (Dhaka).

The increase in evidence of infection around age 15, peaking roughly around 30, is somewhat difficult to explain. Other infectious agents with similar epidemiologic patterns have clear sexual risk factors, but this has not been shown with HEV. The role of hormonal changes in adolescence and the possibility that host characteristics may influence the immune susceptibility or course of infection with this hepatotropic virus needs further examination. There may also be behavioral characteristics of early adulthood such as travel outside the home to school or increased contact with infective livestock, which results in either novel exposure to sources of HEV or in larger infective doses of HEV. Others have shown the increased propensity for clinical disease when individuals are acutely

**Table 4**

<table>
<thead>
<tr>
<th>Antibodies to</th>
<th>HEV + Hbc</th>
<th>HEV + HCV</th>
<th>Hbc + HCV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number positive (%)</td>
<td>117 (10.8%)</td>
<td>9 (1.0%)</td>
<td>6 (0.7%)</td>
</tr>
<tr>
<td>Number tested for both</td>
<td>1,080</td>
<td>917</td>
<td>866</td>
</tr>
<tr>
<td>$\chi^2$ P value</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0.559</td>
</tr>
</tbody>
</table>

*Anti-Hbc total Ig, anti-HCV IgG, and anti-HEV total Ig.
co-infected with multiple hepatotropic viruses. Our analysis of co-existing antibody reactivity to multiple hepatitis viruses (Table 4) found that there are more individuals than expected by chance with prior infection by HEV and either HBV or HCV. Although the infections may be temporally distant from each other, this raises the question whether physiologic changes induced by one hepatotropic virus increases susceptibility to another. Both these possibilities require further study.

The anti-HBe prevalence of 35.2% represents a high rate of individuals in this rural community with historical exposure to HBV. This study did not distinguish between acute, chronic, or resolved hepatitis B infections. Previous studies of hepatitis B in Bangladesh have focused on high-risk or vulnerable populations such as drug users or professional blood donors. Bangladesh is clearly endemic for HBV, providing further support for the recently introduced hepatitis B vaccine in the national EPI program.

Like neighboring India and parts of Nepal, Bangladesh is networked with rivers that flood annually. Generally poor sanitary conditions and enteric risk factors exist across the rural populations of the greater Gangetic floodplain, yet the absence of reported HEV outbreaks in Bangladesh is difficult to explain, given the high seroprevalence we found this study.

Given the high infant and maternal mortality rates in Bangladesh, HEV may be an important contributor to poor pregnancy outcomes, deaths in late pregnancy, and infant mortality in early life. Because an HEV vaccine exists and was found to be highly efficacious in a controlled trial, it is important to better understand the epidemiology of this pathogen to develop public health strategies that target the most vulnerable and high-risk groups in endemic countries.

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Authors’ addresses: Alain B. Labrique, John Ticehurst, and Konrad E. Nelson, Global Disease Epidemiology and Control Department of International Health, Bloomberg School of Public Health, Johns Hopkins University, 615 N. Wolfe Street, E5543, Baltimore, MD 21205. K. Zaman, Zahid Hossain, Parimalendu Saha, Mohammad Yunus, and Anowar Hossain, ICDDR,B, Centre for Health and Population Research, Mohakhali, GPO Box 128, Dhaka 1000, Dhaka 1212, Bangladesh.

Reprint requests: Alain B. Labrique, Global Disease Epidemiology and Control Department of International Health, Bloomberg School of Public Health, Johns Hopkins University, 615 N. Wolfe Street, E5543, Baltimore, MD 21205.

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