Novel Risk Factors Associated with Hepatitis E Virus Infection in a Large Outbreak in Northern Uganda: Results from a Case-Control Study and Environmental Analysis

Christopher M. Howard,* Thomas Handzel, Vincent R. Hill, Scott P. Grytdal, Curtis Blanton, Saleem Kamili, Jan Drobeniuc, Dale Hu, and Eyasu Teshale

Division of Global Disease Detection and Emergency Response, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, Georgia; Division of Foodborne, Waterborne, and Environmental Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia; Division of Viral Hepatitis, National Center for HIV, Viral Hepatitis, TB, and STD Prevention, Centers for Disease Control and Prevention, Atlanta, Georgia

Abstract. An outbreak of hepatitis E virus (HEV) began in October 2007 in northern Uganda. To determine risk factors and sources for ongoing transmission, we conducted both a case-control study and an environmental investigation. A case patient was defined as having serologic evidence of HEV infection, whereas controls were seronegative. We identified risk factors for infection by univariable and multivariable analyses using conditional logistic regression. Several water sources were tested for HEV RNA. Among 112 cases and 145 controls, storage of drinking water in large-mouthed vessels (adjusted odds ratios [AOR] = 2.83; 95% confidence interval [CI] = 1.16–6.94) and washing hands in a group basin (AOR = 1.90; 95% CI = 1.07–3.38) were associated with HEV infection. HEV RNA was detected from communal hand-rinse and surface-water samples. The epidemiologic and environmental water-testing results suggest that household-level factors played an important role in the transmission of HEV—modalities that have been previously underappreciated.

BACKGROUND

Hepatitis E virus (HEV) is an enterically transmitted, single-stranded RNA virus that causes acute hepatitis. The virus is shed in the stool of infected persons and is primarily spread through consumption of fecally contaminated drinking water or food.¹ Direct person-to-person or zoonotic transmission has only rarely been implicated. Predominantly encountered in resource-poor settings,² infection from and sequelae of HEV are clinically similar to hepatitis A. The illness is largely self-limited, with an overall case fatality rate of 1–4%.^{1,3} The hallmark symptom is jaundice, which is most often accompanied by abdominal pain, headache, arthralgia, fever, anorexia, and fatigue. Distinguishing characteristics of HEV infection include its highest age-specific attack rate among persons aged 15–45 years, high rates of asymptomatic infection among those aged < 15 years,^{4,5} and high case fatality rates in women during the third trimester of pregnancy, generally up to 20%.^{6–9} Measures to control and prevent the transmission of hepatitis E largely include provision of clean water and proper sanitation facilities. No curative measures or commercially available vaccines exist for hepatitis E.

Beginning in October 2007, several cases of unexplained jaundice were reported by the health facility of a rural internally displaced persons (IDP) camp in the sub-county of Madi Opei (population of 13,000) in the northern Ugandan district of Kitgum (population of 304,000). These unexplained jaundice cases were eventually confirmed as being caused by HEV (genotype 1). By March 2008, new cases of hepatitis E began to be reported in four additional sub-counties in the district, including the primary site of this investigation, Paloga. At the time of this investigation in June 2008, a total of 3,350 suspected cases and 67 deaths had been reported,¹⁰ and by the end of 2008, more than 9,500 cases and 148 deaths had been reported. This analysis included a case-control study and an

environmental investigation to identify risk factors and potential sources of infection to provide guidance to local health agencies for mitigation strategies.

METHODS

Site and population. Paloga sub-county was the site of the case-control study. This site was chosen because of its recent rapid increase in cases and its proximity to Madi Opei sub-county, the first affected locality. Paloga sub-county had an estimated population of just over 11,000, including those who live in the main IDP camp, interim return sites (satellite camps), and original villages. Conditions in the main camp were crowded, and sanitation was poor, with limited numbers of communal latrines located on the periphery of the camp. The main sources of drinking water were 11 boreholes fitted with hand pumps and one mechanized system that provided up to 40,000 L of water per day. One stream, used often for bathing and drinking, was located approximately 500 m from the camp. Improvements in the camp began in mid- to late-May of 2008; those improvements included vessel chlorination of water collected at the boreholes and construction of additional latrines. Distribution of jerry cans and hand soap was just being planned and instituted at the time of the survey.

Case-control study. The case-control study was conducted from June 17–24, 2008, and it included residents living both inside and outside of the main camp. A case patient was defined as any individual with jaundice (scleral icterus), as ascertained by a team of physicians, at least one of the accompanying symptoms (fatigue, anorexia, abdominal pain, arthralgia, fever, or headache), and serological evidence of HEV infection [anti-immunoglobulin M (IgM) or -immunoglobulin G (IgG) antibody]. Case patients were recruited and enrolled over 3 days by consecutive referral from the adjacent health center. A control was defined as a person who had no symptoms, signs, or serologic evidence of HEV infection. Controls were recruited from the community and were matched to cases on the basis of age group (under 5, 5–14, 15–45, and > 45 years) and location of primary residence in the previous 8 weeks. The calculated sample size of 60 cases and 180 controls was based on the following assumptions: ratio of controls to cases of 3:1,

*Address correspondence to Christopher Howard, International Emergency and Refugee Health Branch, Centers for Disease Control and Prevention, USAID/OFDA, Room 8.07-85 RRB, 1300 Pennsylvania Avenue NW, Washington, DC 20523. E-mail: choward@usaid.gov

odds ratio (OR) worth detecting of 2.5, percentage exposed in the population sample of 50%, power of 80%, and α error of 0.05.

After consent was obtained, participants completed a structured questionnaire and underwent phlebotomy for HEV testing. The questionnaire collected demographic information, including age, sex, pregnancy status (if female), location of primary residence, household size, and education level. Through use of a recall period of 8 weeks (taking into account the incubation period for hepatitis E infection), behavioral information was also collected, including culture-specific practices such as drinking locally made, fermented alcoholic brew, engaging in communal handwashing and eating, and being exposed to various animals. Additionally, environmental information was collected, including the primary source, availability, and treatment of drinking water, the type and number of drinking water-storage vessels, handwashing practices, use and availability of soap, and latrine availability and use. Evidence of HEV infection was determined by testing for anti-HEV IgM and IgG. A 5-mL venous blood sample was collected from each participant, transported by cold chain, and mechanically centrifuged. Sera were stored at -20°C in two separate aliquots for serological testing for IgM and IgG anti-HEV (MP Biomedicals Asia Pacific, Pte, Ltd, Singapore). Laboratory testing was performed at the Hepatitis Reference Laboratory at the Centers for Disease Control and Prevention (CDC) in Atlanta, GA.

Statistical analysis. We calculated adjusted OR (AOR) and 95% confidence intervals (CI) for each risk factor using conditional logistic regression. Matching of age groups (under 5, 5–14, 15–45, and > 45 years) and location of primary residence in the past 8 weeks (main camp versus outside of the main camp) was performed for each calculation. To control for confounding, we included all significant variables from the univariate analysis ($P < 0.20$) in a conditional logistic regression model. Model reduction was performed by use of backwards selection until all remaining variables were found to be significant ($P < 0.05$). Statistical analysis was performed by use of SAS (version 9.1) and Epi Info (version 3.5.1).

Environmental analysis. Environmental samples were collected from Paloga sub-county and other nearby sub-counties that were affected by the outbreak in Kitgum District. Water was collected from boreholes with hand pumps or from the tap stands of mechanized water-distribution systems. If the water was being treated, all samples were collected before chlorination. Between 20 and 40 L of water were filtered on site by use of a peristaltic pump (Masterflex E/S Portable Sampler; Cole-Parmer Instrument Company, Vernon Hills, IL). In some locations, water from two or three boreholes was pooled and pumped through a single filter. A convenience sample of five households with a current or recent case of jaundice was taken; each contributed approximately 5 L (unchlorinated) each from their household water-storage vessels. All samples were tested for residual chlorine before collection. This pooled water was then filtered as described below.

Water was also collected from three streams and one pond. These samples included one from the stream in Paloga near the main camp and one from a stream in an adjacent sub-county near a camp where large numbers of jaundice cases had also been reported. The third sampled stream flowed between two affected sub-counties, but there were no large camps upstream of the sampling point on this stream. All three were used by local residents for bathing and washing. Two pooled hand-rinse

samples were also tested. At each location, a central point was located in which cases of jaundice were present. Individuals were asked to rinse their hands while a team member poured water from a jerry can. Water used for the hand rinses was collected from a nearby borehole that had tested negative for fecal coliforms and that subsequently tested negative for HEV RNA by reverse transcription–polymerase chain reaction (RT-PCR). The hand-rinse water sample was collected in a sanitized container. This process was repeated for 30 and 35 persons for the two samples, respectively. In both locations, a simple convenience sample of persons living in the area was conducted, and the sample included both those who had and those who had not claimed any recent jaundice. The resulting pooled water was then filtered on site and processed by use of either a positively charged Virosorb 1MDS cartridge filter (Part No. 4514402 1MDS; CUNO, Meriden, CT) for virus adsorption or a dead-end ultrafiltration technique.¹¹ RT-PCR for the detection of HEV RNA was performed by use of a TaqMan RT-PCR assay (Applied Biosystems, Foster City, CA).¹²

RESULTS

Case-control study. A total of 112 case patients and 145 controls were enrolled in the study. Table 1 details the breakdown by age category and primary residence. When adjusting for age and primary residence, basic demographics such as sex, household size, and education level were not significantly associated with disease. Adjusted univariate analysis revealed several variables with levels of association at $P < 0.20$: bathing in the local river, storing drinking water in a large-mouthed container, preparation of rats for consumption, consumption of homemade alcoholic brew (often made from river water), washing hands in a common basin with others, eating from a common plate with others, promiscuous defecation, and improper disposal of children's feces (Table 2).

Several variables that were theorized to have potential contribution to transmission before the analysis were not significant in the matched univariate analysis, including having recently drank water from the river, having a borehole as a primary source of drinking water, having pigs or goats at home, having chlorine tablets distributed with drinking water, and having been to a funeral where communal handwashing was common. After inclusion of variables with $P < 0.20$ in a conditional logistic regression model, two factors remained significant ($P < 0.05$): storage of drinking water in a large-mouthed container (AOR = 2.8; 95% CI = 1.16–6.94) and communal handwashing in a common basin with others (AOR = 1.9; 95% CI = 1.07–3.38; Table 2).

TABLE 1
Distribution of matched variables between cases and controls in Paloga sub-county, Kitgum, Uganda (June 2008)

	Cases (N = 112)	Controls (N = 145)
Age (years)		
0–4	4 (3.6%)	20 (13.8%)
5–14	21 (18.8%)	26 (17.9%)
15–45	67 (59.8%)	74 (51.0%)
> 45	20 (17.9%)	25 (17.2%)
Primary residence (in the past 8 weeks)		
Main camp	88 (80.7%)	104 (72.2%)
Satellite camp, original village, or other	21 (19.3%)	40 (27.8%)

TABLE 2

Behaviors and practices associated with symptomatic HEV infection on univariate ($P < 0.2$) and multivariable ($P < 0.05$) analyses (Paloga sub-county, Kitgum, Uganda; June 2008)

Variable	Cases (N = 112)	Controls (N = 145)	Univariate analysis (crude OR; 95% CI)	Multivariable analysis (AOR; 95% CI)
Storage of drinking water in large-mouthed container				
No	91 (82%)	131 (94.2%)	3.51; 1.47–8.39	2.83; 1.16–6.94
Yes	20 (18%)	8 (5.8%)	$P = 0.005$	$P = 0.023$
Washing hands in a common basin				
No	28 (25%)	59 (41%)	2.17; 1.25–3.79	1.90; 1.07–3.38
Yes	84 (75%)	85 (59%)	$P = 0.006$	$P = 0.028$
Eating from a common plate				
No	18 (16.1%)	42 (29%)	2.11; 1.12–3.99	N/A
Yes	94 (83.9%)	103 (71%)	$P = 0.021$	N/A
Involved in the preparation of rats for consumption				
No	97 (87.4%)	135 (93.1%)	1.90; 0.80–4.50	N/A
Yes	14 (12.6%)	10 (6.9%)	$P = 0.144$	N/A
Bathing in the river				
No	59 (52.7%)	97 (66.9%)	1.89; 1.11–3.23	N/A
Yes	53 (47.3%)	48 (33.1%)	$P = 0.019$	N/A
Defecation				
Latrine	77 (68.8%)	112 (77.8%)	1.85; 1.02–3.32	N/A
Open area/bush or other	35 (31.3%)	32 (22.2%)	$P = 0.042$	N/A
Consumption of homemade alcoholic brew				
No	82 (73.2%)	122 (84.1%)	1.83; 0.99–3.37	N/A
Yes	30 (26.8%)	23 (15.9%)	$P = 0.056$	N/A
Disposal of child's feces				
Place in latrine	73 (65.2%)	104 (72.7%)	1.49; 0.87–2.59	N/A
None or throw into open area/bush	39 (34.8%)	39 (27.3%)	$P = 0.157$	N/A

Environmental study. HEV RNA was detected from water filtered from two of four surface-water sources, including the stream in Paloga. One of two hand-rinse samples also tested positive. HEV RNA was not detected in any of the 15 drinking water samples (430 L were tested).

DISCUSSION

Results from the case-control and environmental investigations underscore the role of poor personal and environmental hygiene in HEV transmission. Communal handwashing was significantly associated with acute hepatitis E in the case-control analysis. This practice was relatively common in Paloga, especially during funeral services when large numbers of relatives and friends congregated. In addition, storing water in large-mouthed containers was also associated with acute hepatitis E, suggesting post-collection contamination of drinking water, most likely at the household level (storage of drinking water in large-mouthed containers has also been associated with diarrheal disease).¹³ The epidemiologic findings of usage of wide-mouth water-storage vessels and communal handwashing, together with detection of HEV RNA in hand-rinse water samples, suggested that poor hygiene and poor water-storage practices played an important role in transmission of HEV during this outbreak. Unlike other HEV outbreaks implicating contaminated drinking-water sources, transmission in Kitgum seems to have been multifactorial and may have also included person-to-person transmission.

Outbreaks linked to contaminated water supplies have been widely documented. In fact, the first documented epidemic of hepatitis E was likely because of exposure to raw sewage.¹⁴ Other outbreaks in nearby regions have even been linked to exposure to melting snow that contained feces that had accumulated over the previous winter.¹⁵ An outbreak in Somalia was linked to exposure to one of many contaminated water sources, including rivers, ponds, and wells, but there was no

evidence of person-to-person transmission.¹⁶ The most recent report of a hepatitis E outbreak among displaced populations comes from the Darfur region of Sudan in 2004.⁹ No specific sources of infection were identified, but the report alluded to exposure to a previously chlorinated surface-water source. Furthermore, secondary transmission has largely been considered negligible or non-existent in previous outbreaks, irrespective of the size or location of the epidemic. This point is critical, because suspected modes of transmission dictate the scale and type of control strategies implemented by health agencies. Because HEV has historically been perceived to be transmitted through exposure to contaminated sources of water and/or food as opposed to through household-level transmission, water and sanitation services may get emphasized over individual- and household-level hygiene promotion. These data suggest that transmission is likely multi-factorial and would require that these hygiene promotion activities be just as aggressively supported and promoted.

This investigation had some limitations. The questionnaire used for the case-control study may have been susceptible to responder bias. For example, because of recently enforced laws making use of river water for bathing and drinking illegal, such behaviors may have been underreported; as is common in many settings, proper hygiene and sanitation practices may have been overreported by respondents. Also, considering the novelty of testing water sources for HEV, there may be some insensitivity of detection. More specifically, the volumes collected for each sample may have been inadequate and could have missed sources with low levels of contamination. As well, water sources reported as negative could have been contaminated earlier in the outbreak. Additionally, obtaining water samples for detection of HEV RNA is a labor-intensive effort, one that is attempted only rarely, even in more conducive settings.^{17–19} Therefore, the collection of various water samples, while appropriate, was based on convenience and was not intended to be comprehensive.

This outbreak caused significant morbidity and mortality, and it was the largest outbreak of hepatitis E ever reported among a displaced population.^{3,20,21} Overall, our findings suggest that, in crowded living conditions, HEV transmission may be multifactorial and include household-level or person-to-person transmission. Factors that were responsible for the onset of the outbreak may have been different from those that allowed it to continue spreading over time. Although no single source of infection was identified for this large epidemic, the identification of such specific risk factors such as improper water storage and handwashing practices and the isolation of HEV RNA from hand-rinse samples do give critical insights into the nuances of this outbreak. On the basis of our findings, we conclude that, for future HEV epidemics, conventional control efforts at the community level, such as water decontamination and provision of proper sanitation resources, must also be supplemented by supplies, services, and education related to personal and household hygiene measures. Additionally, in such situations, the availability of a safe and effective vaccine is very important. The most recent trials of an HEV vaccine are certainly promising,^{22,23} and in outbreaks, vaccination may be particularly useful for high-risk groups, such as pregnant women (if the vaccine is proven safe), or in settings such as Kitgum, where pre-existing immunity among exposed populations is believed to be non-existent.

Received July 6, 2010. Accepted for publication July 22, 2010.

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention and the Agency for Toxic Substances and Disease Registry.

Authors' addresses: Christopher Howard, Thomas Handzel, and Curtis Blanton, International Emergency and Refugee Health Branch, Centers for Disease Control and Prevention, USAID/OFDA, Washington, DC, E-mail: choward@usaid.gov. Vincent Hill, Division of Parasitic Diseases, National Center for Zoonotic, Vector-Borne, and Enteric Diseases, Centers for Disease Control and Prevention, Atlanta, GA. Scott Grytdal, Saleem Kamili, Jan Drobeniuc, Dale Hu, and Eyasu Teshale, Division of Viral Hepatitis, National Center for HIV, Viral Hepatitis, TB, and STD Prevention, Centers for Disease Control and Prevention, Atlanta, GA.

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