HHS Public Access

Author manuscript

J Viral Hepat. Author manuscript; available in PMC 2024 April 14.

Published in final edited form as:

J Viral Hepat. 2015 November ; 22(11): 948–956. doi:10.1111/jvh.12407.

An outbreak of hepatitis E in an urban area of Bangladesh

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SUMMARY.

We investigated an outbreak of jaundice in urban Bangladesh in 2010 to examine the cause and risk factors and assess the diagnostic utility of commercial assays. We classified municipal residents reporting jaundice during the preceding 4 weeks as probable hepatitis E cases and their neighbours without jaundice in the previous 6 months as probable controls. We tested the sera collected from probable cases and probable controls for IgM anti-hepatitis E virus (HEV), and the IgM-negative sera for IgG anti-HEV using a commercial assay locally. We retested the IgM-positive sera for both IgM and IgG anti-HEV using another assay at the Centre for Disease Control and Prevention (CDC), USA. Probable cases positive for IgM anti-HEV were confirmed cases; probable controls negative for both IgM and IgG anti-HEV were confirmed controls. We explored the local water supply and sanitation infrastructure and tested for bacterial concentration of water samples. Probable cases were more likely than probable controls to drink tap water (adjusted odds ratio: 3.4; 95% CI: 1.2–9.2). Fifty-eight percentage (36/62) of the case sera were IgM anti-HEV positive; and 75% of the IgM-positive samples were confirmed positive on retesting with another assay at CDC. Compared to confirmed controls, cases confirmed using either or both assays also identified drinking tap water as the risk factor. Two tap water samples had detectable thermotolerant coliforms. Research exploring decentralized water treatment technologies for sustainable safe water might prevent HEV transmission in resource-poor cities. Detection of serological markers in a majority of probable cases implied that available diagnostic assays could adequately identify HEV infection during outbreaks.

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AUTHORS' CONTRIBUTIONS

FH, SPL and MR conceived the investigation; FH, SPL, MR, IAC and SAC designed the study protocol; FH, SSB, KA, IAC and SAC carried out the epidemiological assessment; FH, SPL, SSB, KA and SK carried out the laboratory testing, and analysis and interpretation of these data. FH drafted the manuscript; SPL and SK critically revised the manuscript for intellectual content. All authors read, reviewed and approved the final manuscript. FH and SPL are guarantors of the manuscript.

CONFLICT OF INTEREST

The authors do not have any commercial or other association that might pose a conflict of interest.

Keywords

hepatitis E; outbreak investigation; risk factors; serology; urban Bangladesh

INTRODUCTION

Hepatitis E virus (HEV) is the commonest cause of enterically transmitted acute viral hepatitis in both epidemics and sporadic cases worldwide [1–3]. Although associated with a low case fatality rate in the general population, HEV causes high mortality in infected pregnant women and significant morbidity and mortality in neonates during epidemics [2,4,5]. HEV is highly endemic in the country and is responsible for 30–60% of cases hospitalized with acute viral hepatitis, but HEV is not a nationally notifiable disease in Bangladesh [6,7].

Among the available diagnostic methods, HEV-specific immunoglobulin M (IgM) detection by enzyme immunoassays (EIAs) is used for routine diagnosis and IgG anti-HEV for sero-epidemiological studies [8–11]. However, previous studies have reported considerable variability in sensitivity, specificity and interassay agreement among the commercially available IgM anti-HEV assays. Furthermore, their sensitivity, specificity and performance to accurately and reliably diagnose HEV infection during outbreaks in endemic settings have been evaluated only in limited settings [8,10,12,13]. Given the paucity of reliable diagnostic tools, data on prevalence of asymptomatic, secondary, past HEV infections, immune response and immuno-pathogenesis, and outbreak detection remain limited [8,9,12,14]. Therefore, the development of a reliable and accurate diagnostic assay has been considered critical in determining the true disease burden and understanding HEV epidemiology, although the development of such an assay is compromised by the lack of availability of well-characterized sample evaluation panels and the inability to grow HEV in tissue culture [8,15].

From January to April 2010, local newspapers reported an outbreak of acute jaundice in Rajshahi City in northern Bangladesh. The Rajshahi City Corporation health team initially investigated the outbreak. Later, a collaborative investigation team from the Institute of Epidemiology, Disease Control and Research (IEDCR) and icddr,b joined the local municipal health team and conducted epidemiological, environmental and laboratory investigations to (i) determine the aetiology; (ii) identify risk factors for transmission; (iii) estimate the prevalence of primary, past and asymptomatic infections; and (iv) assess the utility of different HEV serological diagnostics in an epidemic situation.

MATERIALS AND METHODS

Case finding and determining the aetiology

During routine house-to-house visits, field workers of the municipal health team identified and listed suspected jaundice cases, defined as any municipal resident reporting yellow coloration of eyes or skin with onset between January and April 2010. The collaborative investigation team joined the local team and collected preliminary information from the

local team's line list. To generate hypotheses about the aetiology, the team conducted unstructured interviews with the local health officials and field workers. Investigators examined the clinical features and laboratory reports of seven available patients who met the definition of suspected jaundice cases. Given the epidemiological and laboratory findings, clinical features, widespread distribution of suspected jaundice cases and past experience with a similar outbreak [16], we suspected an outbreak of hepatitis E.

Respondent selection for the case–control study

From the local team's line list, field investigators identified probable HEV-infected cases, defined as any person reporting yellow coloration of eyes or skin with the onset of illness within the preceding 4 weeks from the period of sample collection between 23 and 26 April 2010 who resided in Rajshahi City, and whose symptoms (including jaundice, fever, nausea, vomiting or abdominal pain) were either verified by a health worker or supported by serum bilirubin levels. Given that some studies have shown that IgM anti-HEV may be detectable up to 40 days after illness onset, while others have shown IgM anti-HEV detectable for up to 6 months [11], for the case–control study, we only included probable cases with illness onset between 26 March and 26 April 2010. The team selected probable controls from the relatives, friends or neighbours living in the household nearest to the case household who had not reported yellowing of eyes or skin within the past 6 months until the date of interview. Assuming 50% of HEV-infected cases were exposed to municipal piped water compared to 30% controls based on preliminary investigation, we estimated a sample of 135 (60 cases and 75 controls) would provide 80% power to detect an association between municipal supplied tap water and jaundice if one truly existed. We selected 25% more probable controls to allow for the detection of asymptomatic and past HEV infections [17]. To reach the required sample size, field investigators selected six probable cases by lottery from each of the 10 wards with the highest numbers of suspected jaundice cases to interview using a standardized, pretested questionnaire [16]. If there were multiple eligible residents in a household, the team requested the household head to select one. If a probable case could not be found after another visit to the household 3–4 h after the initial visit on the same day, then the next case on the line list was approached for interview. If the relatives, friends or neighbours from the next nearest household were unavailable or failed to meet the definition of a control or refused to participate, field investigators approached the household members of the selected cases to interview.

Laboratory investigations

Trained phlebotomists collected 3 mL blood from each respondent who consented to provide a sample. All samples were tested in IEDCR's Virology Laboratory with the MP Diagnostics HEV IgM ELISA 3.0 Kit (MP Biomedicals Asia Pacific Pte. Ltd., Singapore) which had demonstrated good sensitivity (88%) and excellent specificity (99.5%) in a previous study [18]. The IgM anti-HEV negative samples were tested with the MP Diagnostics HEV IgG ELISA. As the sensitivity and specificity of commercial EIAs used in the context of outbreaks were still largely unknown [10], the team sent aliquots from the IgM anti-HEV-positive samples to the Division of Viral Hepatitis Laboratory, CDC, Atlanta, USA, where (i) IgM and IgG anti-HEV were retested with EIAs from RPC Diagnostic Systems Ltd. (DSI assay; Nizhniy Novgorod, Russia), (ii) HEV RNA was determined by

an in-house reverse-transcription polymerase chain reaction (RT-PCR), and (iii) the HEV genotype was identified after nucleotide sequencing.

We classified probable cases with an IgM anti-HEV-positive serum sample as confirmed HEV-infected cases and apparently healthy probable controls with a sample negative for both IgM and IgG anti-HEV as confirmed controls. We categorized probable cases with samples positive for both IgM and IgG anti-HEV as primary HEV infections [12]. We classified the probable control samples, obtained from apparently healthy controls without any evidence of clinical jaundice, positive for both IgM and IgG anti-HEV as asymptomatic or subclinical infections [11,12,19–21].

Environmental investigations

Field investigators conducted unstructured interviews with purposely selected workers from the Department of Public Health Engineering (DPHE), municipality and local health department and 2-h direct observation in each of the 10 selected wards to collect information about the local water supply and sanitation. The team collected water samples from three municipal distribution pumps, from six shallow tube wells from areas with the highest concentration of cases and from five taps of households that had >3 members who met the probable case definition for bacteriological analysis using the membrane filtration method.

Statistical analysis

The team included the probable cases and probable controls in the case–control analysis to determine the risk factors for HEV transmission. We estimated unadjusted odds ratios (ORs), 95% confidence intervals (CI) and significance levels (*P*-values) for individual risk factors by unconditional logistic regression. We tested potentially confounding variables using a likelihood ratio test procedure, and we included only those terms that represented a significant ($P < 0.05$) component of the model. For the final model, we estimated adjusted odds ratios (AOR) using conditional logistic regression, combining all significant variables.

To exclude possible asymptomatic and past infections, the team repeated the case–control analysis including only the IgM anti-HEV positive confirmed HEV-infected cases with either the MP Diagnostics EIA or both MP and DSI assays at CDC and confirmed controls. To assess whether the analyses with probable cases and using cases confirmed with one or both serological tests identified variable risk factors for epidemic HEV transmission, we compared the ORs and their significance levels generated in each analysis.

Ethics

Field investigators sought verbal informed consent from the adult participants and verbal assent from child respondents before conducting interviews and collecting blood. The team ensured confidentiality of the participants through assigning random ID codes for the collected samples. As this investigation was a part of an emergency public health response to an outbreak and the primary purpose of this activity was to identify, characterize and control the illness outbreak, this investigation was exempted from review by an independent human subjects committee. However, this investigation was approved by and conducted in collaboration with the Government of the People’s Republic of Bangladesh.

RESULTS

Background information

The local team identified 2162 suspected jaundice cases in 30 of the 35 administrative blocks or wards of Rajshahi City. We identified 321 probable cases from 30 wards including 108 probable cases from the 10 wards with the highest numbers of suspected cases. Field investigators selected 138 respondents (62 probable cases and 76 probable controls) for the case–control study. Among these, the median age was 28 years (interquartile range: 18–38 years); 79 (57%) were males and 26% were university students. Twenty-two percentage (17/76) of controls came from within the same household as the cases.

Laboratory findings

Of 62 samples collected from probable cases tested by the MP Diagnostics assays at IEDCR, 36 (58%) were IgM anti-HEV positive, 14 (23%) were negative for both IgM and IgG anti-HEV, and 12 (19%) were negative for IgM anti-HEV, but positive for IgG anti-HEV (Table 1). Twenty-seven (75%) of the 36 IgM-positive samples were confirmed IgM-positive on retesting by the DSI assay at CDC, Atlanta. All of the 27 samples were also positive for IgG anti-HEV by the DSI assay. Of the 76 samples from probable controls tested using MP Diagnostics assays at IEDCR, 7 (9%) were IgM anti-HEV positive; 31 (45%) of the 69 anti-HEV IgM-negative sera had detectable IgG anti-HEV; and 38 (50%) samples from probable controls were negative for both IgM and IgG anti-HEV. When retested by the DSI assay at CDC, Atlanta, 5 (71%) of the seven IgM-positive samples were confirmed IgM positive and were also positive for IgG anti-HEV (Fig. 1). Overall, 27 of 36 (75%) of the case samples and 5 of 7 (71%) samples from probable controls positive for IgM anti-HEV with the MP Diagnostics assay were positive for both IgM and IgG anti-HEV with the DSI EIAs (Table 2). Among the 30 probable case samples available in sufficient quantity for testing, HEV RNA was detected in one sample. Sequencing analysis of the HEV RNA–positive sample showed HEV genotype 1.

Socio-demographic and clinical profiles of HEV cases

The socio-demographic and clinical characteristics of probable HEV cases were comparable to serologically confirmed cases (Table 3). Among the 34 (94%) cases seeking health care, 16 (47%) went to a traditional healer, 13 (36%) visited a qualified medical practitioner, and 2 (6%) took medicines from the nearest pharmacy. There was significant disruption of daily activities due to the illness as 27 of 36 (75%) cases remained absent from work or school for an average of 15 days (range = 2–30 days).

Case–control analyses of risk factors

Cases were more likely than controls to drink municipal tap water during the 1 month prior to illness (OR 2.7; CI 1.3–5.5), have less than a secondary level education (OR 1.3, CI 1.0–1.7) and report drinking foul-smelling water (OR 2.1; CI 1.0–4.7) (Table 4). After adjusting for potential confounders in the conditional logistic regression model, probable cases were still more likely to drink municipal tap water (AOR = 3.4; 95% CI = 1.2–9.2) and have less than secondary level education (AOR = 1.5; 95% CI = 1.0–2.1). We found identical risk

factors using cases selected both clinically and confirmed serologically with either or both the MP Diagnostics and DSI EIAs at CDC and sero-negative controls (Table 5).

Environmental findings

The municipality supplied untreated ground water through interconnected pipes to households within the city. According to the DPHE workers, the municipal piped water was supplied intermittently, usually for 12 h/day during spring and summer (January–April) when the water table in Rajshahi is usually lower. The local health authority and community residents reported that the pipelines often leaked. We did not find any visible leakages in the pipelines, but pipes passed through open sewers in some areas. Water from all six shallow tube wells and three source pumps were devoid of faecal coliforms, but two (40%) tap water samples connected to the municipal supply were contaminated with faecal coliforms (10–50 colony-forming units per 100 mL water).

DISCUSSION

The detection of antibodies to HEV in the majority of collected samples from probable cases confirmed that the outbreak was caused by HEV. The findings from the environmental investigation and the case–control study that compared the probable HEV cases and healthy controls identified drinking water contamination as the most likely source of this outbreak. Drinking water quality deterioration within distribution pipelines due to transient ingress of contaminants through leaks, backflow, release of particulates and sloughing of biofilms from pipe walls during intermittent flow has been frequently identified in resource-poor communities [22]. HEV outbreaks linked to faecal contamination of the piped water supply have been widely observed in South Asia, where water and sanitation infrastructure are suboptimal [2,7,12,16]. Despite the high projected sero-prevalence in Bangladesh, where a large proportion of the highly dense population live in unhygienic conditions with limited access to safe water and sanitation [23], only a few outbreaks of HEV have been reported from 2007 through 2012 in the country [2,16,24].

This outbreak was the second largest urban outbreak of HEV detected in Bangladesh within 5 years [16]. This outbreak, which disrupted the livelihoods of thousands of affected adults living within the catchment area of a tertiary government hospital and imposed substantial economic burden to both the households and the community, was not reported promptly through the conventional public health channels. Despite the high endemicity and burden in many low-income countries including Bangladesh, HEV is seldom recognized as a public health priority in South Asia [2]. This low prioritization of HEV by the public health community could translate into lack of effective surveillance to enhance detection rates of HEV outbreaks. The majority of the patients seeking care from traditional healers, the high prevalence of asymptomatic infections, the lack of demand and the poor availability of diagnostic markers capable of accurately detecting both primary and secondary infections further contributed to the paucity of HEV data in disease-endemic Bangladesh.

We used two IgM anti-HEV assays that apparently identified HEV infection in different proportions of the genuine cases from the outbreak-affected communities of Rajshahi. The interassay agreement of 75% along with potential variations in diagnostic sensitivity and

specificity suggested that the two assays differed in their performances to serologically confirm HEV infection among affected individuals. However, the risk of individual misclassification with available diagnostic tests or even with clinical assessments was not large enough to undermine population-level risk assessments for the identification of proximal risk factors during this outbreak. Socio-demographic and clinical features of probable and confirmed cases were also similar. In addition, our estimated prevalence of symptomatic and asymptomatic infections was consistent with findings from previous studies conducted elsewhere [12,14,16], even though we used two different assays. These finding suggest that the currently available diagnostic assays can be reliably used for the detection of HEV infections especially during outbreaks in hyperendemic countries.

We observed a 41% prevalence of past infections during this outbreak [11,12], which was twice the previously detected sero-prevalence in rural Bangladesh and possibly resulted from boosting of anti-HEV following potential HEV exposure in the epidemic situation [25]. Alternatively, this could also represent a high level of asymptomatic infections in early convalescent phase who lost IgM anti-HEV faster than others. The detected prevalence of subclinical infection in 7% was consistent with past outbreaks [7,9,10,12,14,15,25]. We detected HEV RNA in only one case sample, which could be due to the collection of samples beyond the viraemic phase of infection which generally lasts up to 2 weeks. The majority of samples were collected approximately 3 weeks from illness onset [10,13,15].

The total number of HEV-infected cases estimated in this outbreak is imprecise. We could neither ascertain the consistent use of the suspected case definition nor ensure the robustness of the house-to-house search that was conducted by the local healthcare workers of the municipality during their routine visits. Our case selection strategy that lacked strict representation of the entire affected population was unlikely to affect the conclusions from our case–control study. We promptly drew neighbourhood controls in a specified pattern from the wards in which the enrolled cases lived to achieve the same potential of recall bias and similar misclassification errors in cases and controls, thereby ensuring comparability. This efficient strategy also accounted for many confounding factors – such as socioeconomic status [26].

This large HEV outbreak in a northern Bangladeshi city was caused by the contamination of municipal piped water. The available serological assays were adequate to establish the laboratory diagnosis of HEV infection during the epidemic. Syndrome surveillance can enhance outbreak detection throughout the country and improve the epidemiological evidence in HEV-endemic, low-income settings of Bangladesh.

Averting HEV transmission ultimately requires providing safe drinking water to city dwellers through a well-maintained water and sanitary infrastructure. However, ensuring microbiologically safe water is difficult in low-income communities in the context of increased water scarcity, intermittent supply and leaky pipelines [27–29]. Raising awareness to boil drinking water and/or promoting additional water treatment strategies including provision of chlorine tablets and/or filters at point-of-use could help to limit the extent of outbreaks in the short term and reduce the risk of waterborne diseases until more definitive steps can be implemented. While correct and consistent use of point-of-use technologies

can effectively improve microbial quality of drinking water, consistent uptake and use of point-of-use solutions in vulnerable, low-income communities have remained extremely low [30]. New approaches to engineering municipal water delivery systems that provide microbiologically safe water to consumers in settings where incomes are low and demand exceeds supply are an important research priority. Given that those with preexisting liver diseases and/or pregnancy tend to have a poor prognosis during hepatitis E infection, sustainable safe drinking water delivery should be considered as an urgent public health intervention for the prevention of hepatitis E in Bangladesh.

ACKNOWLEDGEMENTS

The authors would like to thank all the study participants for their contributions. We are also grateful to the Chief Medical Officer of Rajshahi City Corporation. Ms. Dorothy Southern and Ms Meghan Scott deserve special thanks for their contributions in reviewing the manuscript. This research activity was funded by the Centers for Disease Control and Prevention (CDC), Atlanta, and the Government of the People's Republic of Bangladesh (GoB). icddr,b is thankful to the Governments of Australia, Bangladesh, Canada, Sweden and the UK for providing core/unrestricted support.

Abbreviations:

AOR	adjusted odds ratio
CI	confidence interval
EIA	enzyme immunoassay
HEV	hepatitis E virus
OR	odds ratio
RT-PCR	reverse-transcription polymerase chain reaction

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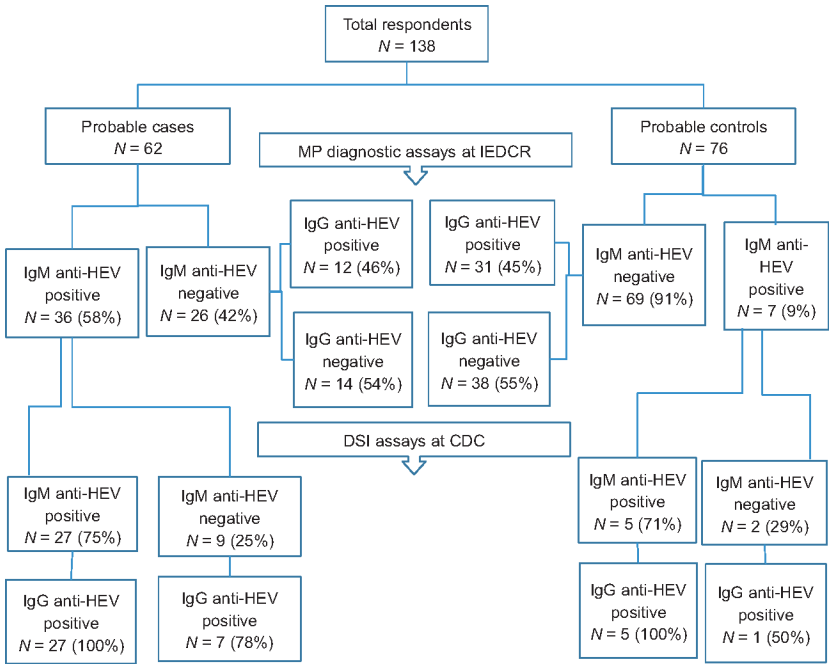

Fig. 1.

Flow chart of different serological tests performed on serum samples from 138 respondents of the jaundice outbreak-affected communities in Rajshahi City, Bangladesh, April 2010.

Table 1

IgM and IgG anti-HEV status of 138 specimens collected from Rajshahi City, April 2010, during the HEV outbreak

	MP Diagnostics IgM and IgG EIAs		
	IgM anti-HEV positive (%)	**IgG anti-HEV positive (%)**	**IgM and IgG negative (%)**
Probable cases [*] (*N* = 62)	36 (58)	12 (19)	14 (23) [†]
Probable controls [‡] (*N* = 76)	7(9)	31 (41) [§]	38 (50)
Total (*N* = 138)	43 (31)	43 (31)	52 (38)

EIAs, enzyme immunoassays; HEV, hepatitis E virus.

[*] A person of any age reporting yellow coloration of eyes or skin with onset between 26 March and 26 April 2010 who resided in Rajshahi City and whose symptoms were either verified by a health worker or supported by serum bilirubin levels.

[†] 14 of 62 (23%) case samples negative for both IgM and IgG anti-HEV using the MP Diagnostics EIAs were non-HEV infections.

[‡] A relative, friend or neighbour living in the household nearest to the case household who had not reported yellowing of eyes or skin within the past 6 months until the date of interview.

[§] 31 of 76 (41%) IgG anti-HEV-positive controls using the MP Diagnostics assay represented past HEV infections [11,12].

Table 2

Comparison of sero-positivity of anti-HEV IgM measured using the DSI EIAs for the 43 IgM anti-HEV-positive samples (MP Diagnostics) collected from the jaundice outbreak-affected communities in Rajshahi City, Bangladesh, April 2010

	Anti-HEV IgM test result with the DSI EIAs (no. of samples)		
Anti-HEV IgM positive with the MP Diagnostics EIA (no. of samples)	**Positive *n* (%)**	**Negative (%)**	**Total**
Probable case *	27 (75)	9 (25)	36
Probable control †	5 (71)	2 (29)	7
Total	32 (74)	11 (26)	43

EIAs, enzyme immunoassays; HEV, hepatitis E virus.

* A person of any age reporting yellow coloration of eyes or skin with onset between 26 March and 26 April 2010 who resided in Rajshahi City and whose symptoms were verified either by a health worker or supported by laboratory evidence, for example serum bilirubin.

† A relative, friend or neighbour living in the household nearest to the case household who had not reported yellowing of eyes or skin within the past 6 months until the date of interview.

Table 3

Socio-demographic characteristics of probable cases and controls and confirmed cases and sero-negative controls in the hepatitis E outbreak in Rajshahi City Corporation, Bangladesh during January-April 2010

Characteristics	Probable case* (*N* = 62)	Probable control† (*N* = 76)	Confirmed case‡ (*N* = 36)	Confirmed control§ (*N* = 38)
Age in years				
Mean (range)	28 (2–65)	28 (3–65)	31 (8 –65)	27 (3–65)
Median	27	29	28	29
Education in completed years of schooling				
Mean (range)	6 (0–18)	9 (0–18)	7 (0–18)	9.5 (0–16)
Median	6	10	7	10
Sex				
Male (%)	54	59	64	66
Female (%)	46	41	36	34
Average household members sharing the same stove				
Mean (range)	5 (2–22)	7 (1–44)	5 (2–22)	7 (2–30)
Median	4	5	4	5
Average monthly expenditure				
Mean (range)	US $ 78 (14–239)	US $ 94 (24–323)	US $ 83 (14–241)	US $ 96 (30–325)
Median	US $ 60	US $ 72	US $ 60	US $ 84
Clinical symptoms				
Yellowing of eyes, *n* (%)	60 (97)	N/A	34 (94)	N/A
Yellowing of skin, *n* (%)	48 (77)	N/A	29 (81)	N/A
Severe weakness, *n* (%)	61 (98)	N/A	35 (97)	N/A
Vomiting, *n* (%)	55 (89)	N/A	34 (94)	N/A
Loss of appetite, *n* (%)	58 (94)	N/A	33 (92)	N/A
Fever, *n* (%)	48 (77)	N/A	27 (75)	N/A
Itching, *n* (%)	30 (48)	N/A	17 (47)	N/A
Mental irritability, *n* (%)	19 (31)	N/A	15 (42)	N/A
Diarrhoea, *n* (%)	11 (17)	N/A	6 (17)	N/A
Loss of consciousness, *n* (%)	2 (3)	N/A	1 (3)	N/A
Disruption of daily activities, *n* (%)	42 (67)	N/A	27 (75)	N/A

Characteristics	Probable case* (*N* = 62)	Probable control† (*N* = 76)	Confirmed case‡ (*N* = 36)	Confirmed control§ (*N* = 38)
Mean duration of disruption in days (range)	14 (2–30)	N/A	15 (2–30)	N/A
Healthcare seeking (%)				
Soughtany health care	52 (82)	N/A	34 (94)	N/A
Qualified medical practitioner	19 (37)	N/A	13 (36)	N/A
Traditional healer	27 (52)	N/A	17 (47)	N/A
Took medicines from pharmacy	33 (53)	N/A	2 (6)	N/A

HEV, hepatitis E virus; N/A, not applicable.

*A person of any age reporting yellow coloration of eyes or skin with onset between 26 March and 26 April 2010 who resided in Rajshahi City and whose symptoms were either verified by a health worker or supported by laboratory evidence, for example serum bilirubin.

†A relative, friend or neighbour living in the household nearest to the case household who had not reported yellowing of eyes or skin within the past 6 months until the date of interview.

‡A probable case with an IgM anti-HEV-positive sample with either the MP Diagnostics or the DSI assay.

§A probable control with a sample negative for both IgM and IgG anti-HEV.

Table 4

Results of bivariate analyses of risk factors for outbreak-associated hepatitis E, Rajshahi City, 2010, defining confirmed HEV cases using different EIAs and comparing with HEV case and controls selected solely on clinical criteria

Cases	**Probable cases (based on case definition with clinical and epidemiological criteria) *N* = 62**		**Confirmed case (IgM positive with the MP Diagnostics EIA) *N* = 36**		**Confirmed case (IgM positive with the MP Diagnostics and CDC in-house EIAs) *N* = 27**	
Controls	**Probable controls, *N* = 76**		**Sero-negative controls, *N* = 38**		**Sero-negative controls, *N* = 38**	
	Unadjusted OR (95% CI)	***P*-value**	**Unadjusted OR (95% CI)**	***P*-value**	**Unadjusted OR (95% CI)**	***P*-value**
Risk factors						
Currently drinking municipal tap water	2.7 (1.3–5.5)	0.01	3.1 (1.2–8.0)	0.02	3.4 (1.2–9.2)	0.02
Less than any secondary education	1.3 (1.0–1.7)	0.03	1.9 (1.1–3.2)	0.02	1.7 (1.1–2.6)	0.01
Foul smell in drinking water (last 1 month)	2.1 (1.0–4.7)	0.07	3.0 (1.0–9.1)	0.05	3.3 (1.0–10.8)	0.04
Never wash hands with soap and water before meal	1.9 (0.8–4.9)	0.07	3.9 (1.0–15.8)	0.06	2.8 (0.6–12.3)	0.17
Drinking or eating outside home (last 1 month)	2.3 (0.9–6.4)	0.10	6.6 (0.8–57.5)	0.09	5.6 (0.6–49.5)	0.12
Visible dirt in drinking water (last 1 month)	1.7 (0.9–3.5)	0.13	2.2 (0.8–6.0)	0.11	3.3 (1.1–9.4)	0.06
Having another household member affected with jaundice (last 2 months)	1.3 (0.7–2.7)	0.16	0.4 (0.2–1.2)	0.11	0.4 (0.2–1.2)	0.11
Monthly household expenditure <4000 taka (US $ 58.4)	1.6 (0.8–3.3)	0.24	2.1 (0.8–5.6)	0.16	2.0 (0.7–5.8)	0.18
Colour change in drinking water (last 1 month)	2.0 (1.0–4.0)	0.35	2.0 (0.8–5.3)	0.17	2.0 (0.7–5.6)	0.17
Having no soap/detergent inside the toilet	1.4 (0.6–3.3)	0.39	1.8 (0.6–5.6)	0.33	1.6 (0.5–5.2)	0.48
Never wash hands with soap and water after toilet	0.9 (0.5–1.8)	0.42	0.7 (0.3–1.8)	0.50	0.7 (0.3–1.7)	0.38
Having no water inside toilet	1.9 (0.7–5.2)	0.46	0.8 (0.3–2.0)	0.58	0.7 (0.3–2.0)	0.54
No drinking water purification	1.1 (0.4–3.2)	0.79	0.7 (0.2–3.0)	0.66	0.6 (0.2–2.5)	0.50
Sharing toilet with a case outside of own household in last 6 months	1.4 (0.7–2.7)	0.81	1.2 (0.5–3.2)	0.68	0.5 (0.2–1.3)	0.14
Having no sanitary/septic tank/advanced latrine	1.4 (0.7–2.7)	0.89	0.7 (0.1–4.4)	0.69	0.8 (0.1–5.1)	0.82

CI, confidence interval; EIA, enzyme immunoassays; HEV, hepatitis E virus; OR, odds ratio.

Table 5

Results of multivariate analysis of risk factors for outbreak-associated hepatitis E, Rajshahi City, 2010

Cases	**Probable cases (based on case definition with clinical and epidemiological criteria) *N* = 62**		**Confirmed case (IgM positive with the MP Diagnostics EIA) *N* = 36**		**Confirmed case (IgM positive with the MP Diagnostics and DSI EIAs) *N* = 27**	
Controls	**Probable controls, *N* = 76**		**Sero-negative controls, *N* = 38**		**Sero-negative controls, *N* = 38**	
Risk factors	**AOR (95% CI)**	***P*-value**	**AOR (95% CI)**	***P*-value**	**AOR (95% CI)**	***P*-value**
Currently drinking municipal tap water	3.4 (1.2–9.2)	0.02	3.1 (1.2–8.0)	0.03	3.3 (1.1–10)	0.03
Less educated (below secondary)	1.5 (1.0–2.1)	0.03	1.9 (1.1–3.2)	0.02	1.9 (1.1–3.5)	0.03

AOR, adjusted odds ratio; CI, confidence interval; EIA, enzyme immunoassays.