Hepatitis E as a cause of adult hospitalization in Bangladesh: Results from an acute jaundice surveillance study in six tertiary hospitals, 2014-2017

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The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the US Centers for Disease Control and Prevention.

Abstract

In the absence of reliable data on the burden of hepatitis E virus (HEV) in high endemic countries, we established a hospital-based acute jaundice surveillance program in six tertiary hospitals in Bangladesh to estimate the burden of HEV infection among hospitalized acute jaundice patients aged ≥14 years, identify seasonal and geographic patterns in the prevalence of hepatitis E, and examine factors associated with death.

We collected blood specimens from enrolled acute jaundice patients, defined as new onset of either yellow eyes or skin during the past three months of hospital admission, and tested for immunoglobulin M (IgM) antibodies against HEV, HBV and HAV. The enrolled patients were followed up three months after hospital discharge to assess their survival status; pregnant women were followed up three months after their delivery to assess pregnancy outcomes.

From December'2014 to September'2017, 1925 patients with acute jaundice were enrolled; 661 (34%) had acute hepatitis E, 48 (8%) had hepatitis A, and 293 (15%) had acute hepatitis B infection. Case fatality among hepatitis E patients was 5% (28/589). Most of the hepatitis E cases were males (74%; 486/661), but case fatality was higher among females—12% (8/68) among pregnant and 8% (7/91) among non-pregnant women. Half of the patients who died with acute hepatitis E had co-infection with HAV or HBV. Of the 62 HEV infected mothers who were alive until the delivery, 9 (15%) had miscarriage/stillbirth, and of those children who were born alive, 19% (10/53) died, all within one week of birth.

This study confirms that hepatitis E is the leading cause of acute jaundice, leads to hospitalizations in all regions in Bangladesh, occurs throughout the year, and is associated with considerable morbidity and mortality. Effective control measures should be taken to

47 reduce the risk of HEV infections including improvements in water quality, sanitation and

48 hygiene practices and the introduction of HEV vaccine to high-risk groups.

Author summary

In the absence of reliable surveillance data on the burden of hepatitis E in endemic countries, we conducted a hospital-based acute jaundice surveillance study over a two and a half year period in six tertiary hospitals in Bangladesh. The study confirms that HEV infections occur throughout the year, and is a major (34%) cause of acute jaundice in tertiary hospitals in Bangladesh. Three-quarters of the acute hepatitis E cases were male, and HEV infection was higher among patients residing in urban areas than patients in rural areas (41% vs 32%). The overall case fatality rate of acute HEV infections in hospitals was 5%, but was higher among pregnant women (12%). Hepatitis E patients who died were more likely to have co-infection with HAV or HBV than the HEV infected patients who did not die. Fifteen percent of HEV infected mothers had miscarriage/stillbirth. Of the children who were born alive, 19% died, all within one week of birth. Considering the high burden of hepatitis E among hospitalized acute jaundice patients, Bangladesh could take control measures to reduce this risk including improvements in water quality, sanitation and hygiene practices and the introduction of hepatitis E vaccine in high-risk areas.

Introduction

Hepatitis E virus (HEV) infection causes inflammation of the liver and is an important cause of acute jaundice, especially in resource-poor countries, where fecal contamination of drinking water is common [1-3]. HEV genotypes 1 and 2 are predominantly spread by the fecal-oral route [4]. By contrast, HEV infection in high-income countries occasionally occurs as a result of zoonosis of genotypes 3 and 4, being transmitted via exposure to wild animals, and consumption of undercooked pork or game meat [5, 6]. HEV genotypes 1 and 2 in high-income countries are generally limited to travellers to hepatitis E endemic countries [7, 8].

HEV can cause sporadic cases and small outbreaks [9], but is often responsible for epidemics in Asia and many parts of Africa [1, 10-12]. The most common clinical feature of symptomatic HEV infection is jaundice; it is symptomatically indistinguishable from other causes of acute viral hepatitis [13]. Symptomatic illness due to HEV infection is infrequent in children [14, 15]; the infection primarily affects young adults, and is generally mild and self-limiting [2, 16, 17]. However, the case fatality among HEV-infected pregnant women has been reported to be as high as 6-30% [2, 18-22]. Miscarriages, stillbirths and neonatal deaths are frequently observed among HEV-infected pregnant women [20, 23-25].

The World Health Organization (WHO) identified viral hepatitis as a global health problem and set a goal to eliminate viral hepatitis by 2030 [26]. Even though an effective low-cost hepatitis E vaccine is available [27, 28], the vaccine is not recommended for general use in endemic countries, largely because the burden of disease is not quantified and so it is unclear if use of the vaccine is appropriate and cost effective [29]. Hepatitis E disease surveillance data are limited to a few high-income countries [30, 31]; most of the data from endemic countries are limited to disease outbreak and case series reports.

While hospital-based surveillance represents only the burden of severe HEV disease, it can provide valuable insights, especially regarding the contribution to mortality among severe acute jaundice patients. Patients with acute jaundice commonly present to hospitals in many resource-poor hepatitis E endemic countries, including Bangladesh [32-34]. However, there are few studies in hepatitis E endemic countries that systematically enrolled patients with acute jaundice to estimate the burden of acute HEV infection in hospital settings, especially the case fatality rate and delivery outcomes of pregnant women [35, 36]. Previous hospital-based studies in Bangladesh suggest that most of the cases of acute jaundice (22-64%) were due to HEV infection, however, those studies were limited to a single study site or a short study period which may not be a representative estimate of the burden of hepatitis E among all hospitalized acute jaundice patients in Bangladesh [34, 37, 38].

The International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) in collaboration with the Institute of Epidemiology, Disease Control and Research (IEDCR) of the Government of Bangladesh, and the United States Centers for Disease Control and Prevention (CDC), conducted a hospital-based acute jaundice surveillance study in six tertiary hospitals located in five regions of Bangladesh. The objectives of this study were to estimate the prevalence of HEV infection among acute jaundice patients aged ≥14 years admitted in tertiary hospitals in Bangladesh, estimate the case-fatality among these patients and identify factors associated with mortality, as well as describe seasonal and geographic trends in prevalence of HEV disease.

Methods

Surveillance sites and methods

Hospital-based acute jaundice surveillance was established in six tertiary hospitals, located in five of seven divisions (the second highest level of geographic and administrative areas) in Bangladesh (Fig 1). Five of these were government teaching hospitals and one was a private teaching hospital. Severely ill patients are generally referred to these tertiary hospitals from lower level hospitals located in the adjacent districts of the surveillance hospitals. The surveillance started between 6 December 2014 and 27 March 2015 (depending on the hospital) and continued until 30 September 2017 in all six sites.

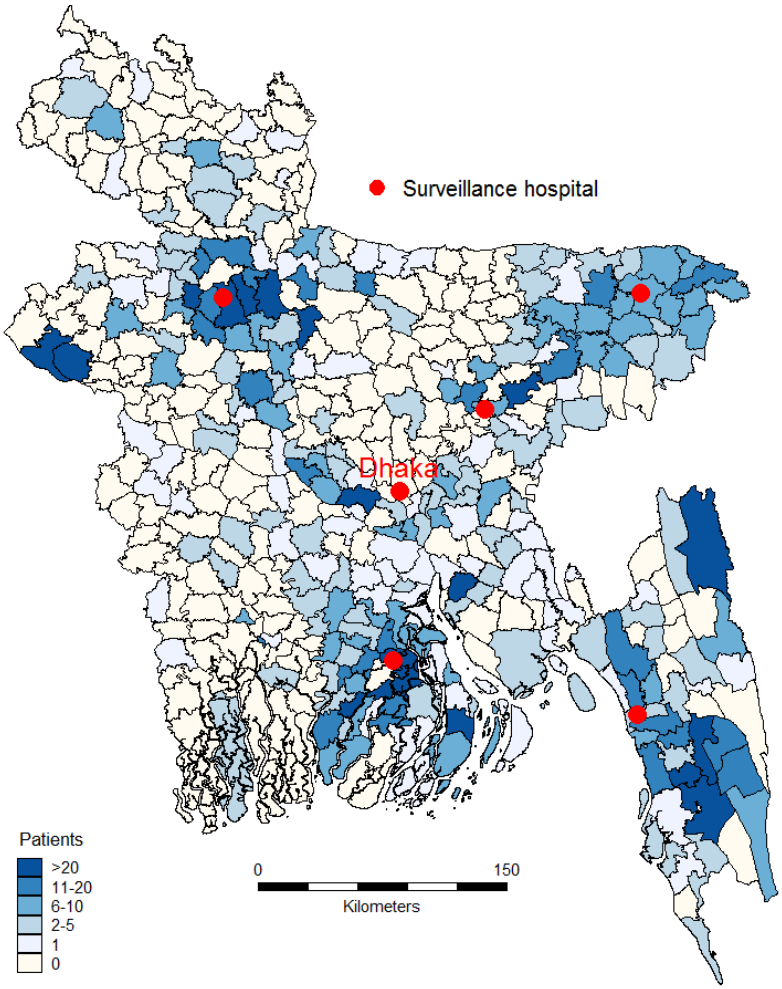

Fig 1. Location of acute jaundice surveillance hospitals.
Map of Bangladesh showing the location of surveillance hospitals and number of enrolled patients with acute jaundice by sub-district during December 2014- September 2017. Map was created using the SPMAP module for Stata 14 (StataCorp) [39].

We recruited a physician in each of the six hospitals from the existing staff to oversee the surveillance activities. A field assistant from icddr,b in each hospital assisted the surveillance physicians to collect patient information and blood specimens, and to store and transport samples to the laboratory in Dhaka. Every weekday morning, the surveillance physicians visited the obstetrics and gynaecology wards and the adult medicine wards of the hospitals and reviewed admission records from the previous day to determine if any patients aged ≥14 years met the case definition of acute jaundice. Due to resource constraints, we

were unable to conduct surveillance in all wards in each hospital; therefore we restricted enrolment to adult medicine, obstetrics and gynaecology wards (where patients aged > 14 years are admitted) as children have been reported to have less severe presentations of HEV infection [14, 15]. Patients with acute jaundice admitted on Friday were enrolled on the next working day, as study staff did not work on Fridays. Acute jaundice was defined as new onset of either yellow eyes or skin for less than 3 months, and continuing on the day of admission. The field assistants also visited the intensive care unit (ICU) every weekday to ensure that no acute jaundice cases were overlooked. If anyone met the case definition of acute jaundice, the surveillance physicians recorded enrolled patients' illness history and relevant clinical information along with basic demographic information and contact information in a handheld computer using a structured case investigation form. The surveillance physicians also collected a 5 ml blood specimen for laboratory testing.

Sample processing and laboratory testing

On the same day of sample collection, the field assistants centrifuged the blood specimen and prepared three aliquots of sera. One aliquot containing about 400µl of serum was sent to the hospital laboratory the same day of sample collection to test for bilirubin and glutamic-pyruvic transaminase to assess liver function. Two aliquots of serum were stored at the surveillance hospitals in a liquid nitrogen dry shipper and transported to the laboratory of IEDCR in Dhaka every two weeks. The samples were kept in a freezer at IEDCR at -80°C until they were tested. The samples were tested for IgM and IgG antibodies to HEV using enzyme-linked immunosorbent assay (ELISA) kits manufactured by Wantai, China (Beijing Wantai Biologic Pharmacy Enterprise Co., Ltd, Beijing, China) according to the manufacturer's instructions. Acute HEV infection was defined as a positive test result for

anti-HEV IgM antibodies. We also tested all samples for hepatitis A virus IgM antibodies (anti-HAV IgM) (a marker of HAV infection) and hepatitis B virus surface antigen (HBsAg) (a marker of acute or chronic HBV infection). The samples that were positive for HBsAg were also tested for hepatitis B virus core IgM antibodies (anti-HBc IgM; a marker of acute HBV infection). Chronic HBV infection was defined as a positive test result for HBsAg but negative test result for anti-HBc IgM. HBsAg negative samples are generally negative for anti-HBc IgM [40], therefore, only the HBsAg positive samples were tested for anti-HBc IgM. ELISA test kits manufactured by DiaSorin, Italy were used for testing anti-HAV IgM, HBsAg and anti-HBc IgM.

We sent a subset of samples (collected between December 2014 and December 2016) to the Division of Viral Hepatitis Laboratory of the US Centers for Disease Control and Prevention (CDC), Atlanta, USA to test for HEV RNA. All samples from patients with onset within three weeks of the blood draw were selected for RNA testing. For other patients, a random sample of 20% of anti-HEV IgM positive samples, 20% of anti-HEV IgG positive samples, 20% of samples negative for both IgM and IgG were chosen to test for HEV RNA. Serum samples were tested for HEV RNA using a quantitative real-time reverse transcriptase polymerase chain reaction (PCR) assay, capable of detecting HEV genotypes 1-3 with a limit of detection of 25 IU/ml, targeting a 69-bp fragment of open reading frame (ORF) 3 of HEV genome [41]. For quality assurance, the samples that were sent to CDC were also retested for anti-HEV IgM using the same ELISA kit as was used at the laboratory in Bangladesh.

Patient follow-up

All enrolled patients were followed up during their hospitalization by the surveillance physicians to monitor the outcomes of the illness episode and the pregnancy outcomes for

pregnant patients. If the pregnancy ended during hospitalization and the outcome was a live birth, the surveillance physician examined the newborn to collect clinical information and note any signs or symptoms of jaundice. All enrolled patients and the newborns were followed up 3 months post hospital discharge to ascertain their vital status. If a pregnant woman with jaundice was released from the hospital before the end of her pregnancy, the field assistants followed up with her by phone one week after the expected date of delivery and three months after the date of delivery to check on the health of the mother and the newborn (S1 Table). For patients who did not have a phone, the field assistants visited their home to follow-up. Follow up three months post hospital discharge was not possible for the patients who were admitted to surveillance hospitals after June 30, 2017, as the surveillance ended on September 30, 2017.

Data analysis

We calculated the prevalence of acute HEV infection among patients with acute jaundice in each surveillance hospital and overall. Seroprevalence of acute hepatitis E was also calculated by patients' demographic characteristics and rural-urban area of residence. We compared the signs and symptoms during illness, pregnancy complications, pregnancy outcome and case fatality between acute jaundice patients with and without evidence of acute HEV infection. We examined potential risk factors of death among patients with acute hepatitis E including co-infection with other hepatitis viruses (HAV, HBV). Comparison between categorical variables was performed using the chi-squared test or Fisher exact test where appropriate and comparison between continuous variables (non-normal distribution) was performed using the Wilcoxon rank sum test. The 95% confidence interval for proportions was calculated by using the Wilson method for a binomial distribution [42]. We

199 examined the monthly trends in the number of patients with anti-HEV IgM antibodies. We
200 also calculated the proportion of patients positive for anti-HEV IgM and anti-HEV IgG by
201 duration of illness at the time of admission to hospital. The onset of illness was defined as
202 the date when the patient first reported experiencing yellow eyes or skin or
203 nausea/vomiting. Analyses were conducted in Stata 14 (StataCorp).

204 **Human subjects**

205 The surveillance physicians sought consent from the patients, or their guardians in
206 the case of severely ill patients, to enrol them in the study. Written informed consent was
207 obtained from patients aged over 17 years. For patients aged between 14 and 17 years,
208 written assent was taken from the patients as well as obtaining written consent from their
209 parents or guardians. The study protocol was reviewed and approved by the institutional
210 review board of the icddr,b. US CDC involvement in the study did not constitute engagement
211 in human subjects research and therefore, CDC relied on appropriate IRB or ethics
212 committee approval of engaged institutions.

Results

Patient characteristics

In the six surveillance hospitals, a total of 2,091 patients aged ≥14 years met the case definition of acute jaundice during December 2014 to September 2017. Of them, 1,925 (92%) agreed to be enrolled in the study and provided a blood specimen for laboratory testing (S1 Fig). Of the enrolled patients, 1,314 (68%) were male and 958 (50%) were aged between 14 and 29 years. Of the female patients, 192 (31%) were pregnant. Patients admitted to the surveillance hospitals resided in 56 out of 64 districts in Bangladesh; however, a higher number of patients lived near surveillance hospitals (Fig 1).

Laboratory testing and HEV seroprevalence

Among the 1,925 enrolled patients, 661 (34%) had IgM antibodies detected against HEV (acute HEV infection) and 652 (99%) of these also had IgG antibodies against HEV. Overall, 1,036 (54%) patients had detectable anti-HEV IgG antibodies. There were 148 (8%) patients who were positive for anti-HAV IgM antibodies and 663 (34%) who were positive for HBsAg. Of the 663 HBsAg positive patients, 293 (15% of all patients) had acute HBV infection. Among the 661 acute HEV patients, 9 (1%) also had acute HAV infection, 15 (2%) had acute HBV infection and 132 (20%) had chronic HBV infection.

Of the 661 patients with acute HEV infection, 483 (73%) resided in rural areas, 486 (74%) were male; their median age was 25 years (IQR: 20-33). Acute hepatitis E prevalence varied across the hospital sites, ranging from 17% in Sylhet to 61% in Dhaka (Table 1). Among the patients with acute jaundice, those who were male, aged between 20 to 39 years, resided in urban areas, educated, or whose monthly family expenditure was >15,000

takas (US$188) were more likely to have acute HEV infection (p<0.001). Acute HEV infection was higher among the pregnant women with acute jaundice than the non-pregnant women with acute jaundice (39% vs. 24%, p<0.001). The higher rate of acute HEV infection among educated and higher income group patients with acute jaundice in the study hospitals was consistent in the analysis by rural-urban area of residence (S2 Table).

Table 1. Hepatitis E serological test results by demographic characteristics of patients with acute jaundice in six tertiary hospitals in Bangladesh, December 2014–September 2017

Characteristics	Number of patients	Anti-HEV IgM Positive		*P-value*[a]
		n	Percent (95% CI)	
Total patients	*1925*	*661*	*34 (32-37)*	
Hospital sites				*<0.001*
Bogra	405	79	20 (16-24)	
Barisal	366	174	48 (42-53)	
Kishoregonj	200	36	18 (13-24)	
Chittagong	435	160	37 (32-42)	
Sylhet	237	40	17 (12-22)	
Dhaka	282	172	61 (55-67)	
Sex				*<0.001*
Male	1314	486	37 (34-40)	
Female	611	175	29 (25-32)	
Pregnancy status of women[b]				*<0.001*
Non-Pregnant	418	101	24 (20-29)	
Pregnant	192	74	39 (32-46)	
Age-group (in years)				*<0.001*
14-19	341	124	36 (31-42)	
20-29	617	294	47 (44-52)	
30-39	349	142	41 (35-46)	
40-49	236	65	28 (22-34)	
50-59	184	28	15 (10-21)	
60+	188	8	4 (2-8)	

Characteristics	Number of patients	Anti-HEV IgM Positive		*P-value*[a]
		n	Percent (95% CI)	
Residence				*<0.001*
Rural	1494	483	32 (30-35)	
Urban	431	178	41 (37-46)	
Education				*<0.001*
None	383	63	16 (13-21)	
Class 1-5	574	175	31 (27-34)	
Class 6-11	644	259	40 (36-44)	
Class 12 or more	324	164	51 (45-56)	
Monthly household expenditure in Bangladeshi taka[c]				*<0.001*
< 5000 (US$ 62)	270	48	18 (13-23)	
5000-9,999 (US$ 63-125)	717	214	30 (27-33)	
10,000-14,999 (US$ 126-187)	399	142	36 (31-41)	
≥ 15,000 (US$188)	328	138	42 (37-48)	

[a] chi-squared test
[b] Percentage was calculated among female patients
[c] Monthly household expenditure was unknown for 211 patients
Anti-HEV IgM= Immunoglobulin M antibodies against HEV

Patients who were admitted to hospital between one and two weeks of onset of illness had the highest anti-HEV IgM positivity (42%; Fig 2). The proportion who were anti-HEV IgM positive declined with time after two weeks of onset of illness ($\chi 2$ for trend: 21.3; *p <0.001)*. Patients admitted to hospital with acute HEV infection were detected every month but no specific seasonal pattern was observed during the surveillance period (Fig 3). However, the number of hepatitis E cases generally increased over time.

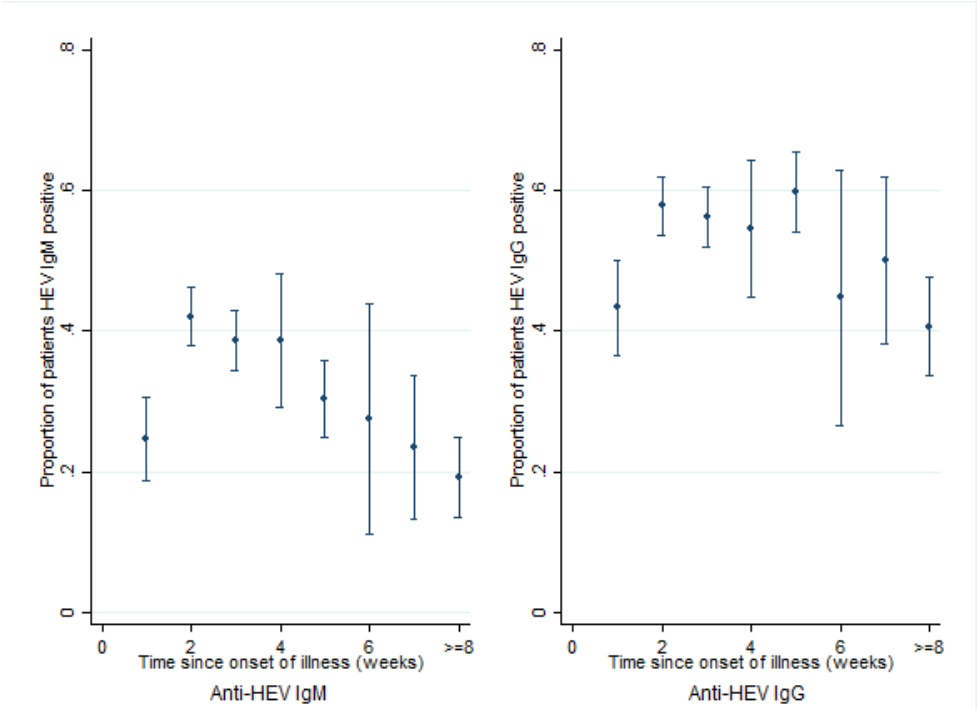

Fig 2. Proportion of patients positive for anti-HEV IgM and anti-HEV IgG by duration of illness at the time of admission to hospital in six tertiary hospitals in Bangladesh, December 2014- September 2017

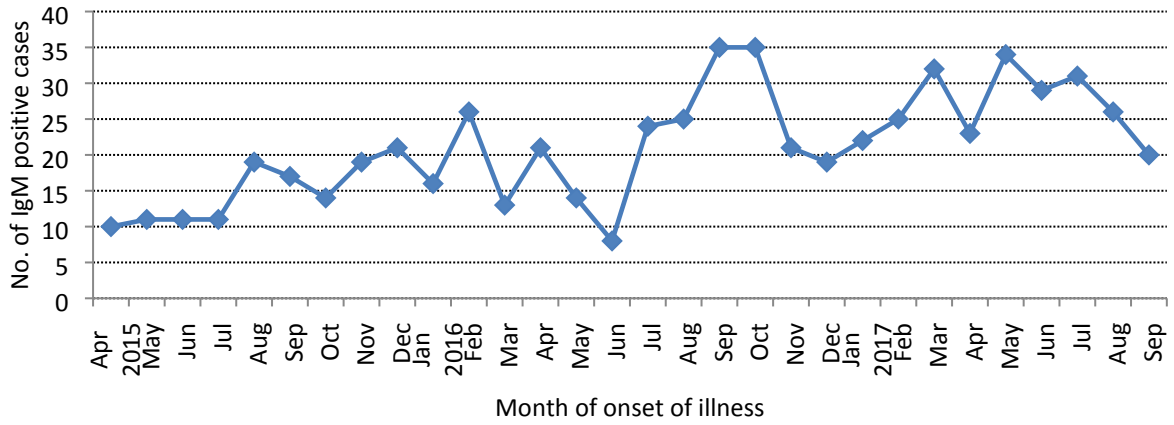

Fig 3. Number of anti-HEV IgM positive cases by the month of onset of illness among patients with acute jaundice admitted in six tertiary hospitals in Bangladesh, April 2015–September 2017

Note: Since surveillance was established in all hospitals by March, 2015, the seasonality curve covered the period from April 2015 to September 2017.

Most of the signs and symptoms were similar for anti-HEV IgM positive and negative patients (Table 2). However, oedema, distended abdomen, melaena and unconsciousness were observed more often among anti-HEV IgM negative patients than anti-HEV IgM positive patients. The median serum glutamic-pyruvic transaminase level was significantly higher among anti-HEV IgM positive patients than the anti-HEV IgM negative patients [520 (range: 221-1100) IU/L vs. 140 (range: 60-394) IU/L; p <0.001].

Table 2. Signs and symptoms during illness among anti-HEV IgM positive and negative patients admitted in six tertiary hospitals in Bangladesh, December 2014–September 2017

Signs and symptoms during illness	Anti-HEV IgM Positive (N=661) n (%)	Anti-HEV IgM Negative (N=1,264) n (%)	*P-value*[a]
Examined by physicians			
Yellow skin	660 (100)	1,258 (100)	*0.263*
Yellow eyes	660 (100)	1,262 (100)	*0.971*
Oedema	30 (5)	164 (13)	*<0.001*
Dehydration	134 (20)	237 (19)	*0.425*
Distended abdomen	69 (10)	228 (18)	*<0.001*
Reported by patients/caregivers			
Fever	596 (90)	1,121 (89)	*0.321*
Nausea/vomiting	602 (91)	1,079 (85)	*<0.001*
Anorexia	536 (81)	1,020 (81)	*0.862*
Abdominal pain	396 (60)	779 (62)	*0.450*
Melaena/ Clay-colored stools	107 (16)	260 (21)	*0.020*
Unconsciousness	37 (6)	115 (9)	*0.007*
Liver function test result			
Serum total bilirubin levels, mg/dl , median(IQR)[b]	9 (9-13)	8 (4-15)	*0.063*
Patient with above the nomal bilirubin level (Reference level: 0.2-1.2 mg/dl)	654 (100)	1,193 (95)	*<0.001*
Serum Glutamic-Pyruvic Transaminase (SGPT), IU/L, median(IQR) [†]	520 (221-1100)	140 (60-394)	*<0.001*
Patient with above the normal SGPT level (Reference level: 20-60 IU/L)	613 (94)	946 (76)	*<0.001*

[a] Chi-squared test comparing anti-HEV IgM result and patients' symptoms during illness

[b] Wilcoxon rank sum test

Of the 115 anti-HEV IgM positive samples that were sent to CDC, 73 (64%) were positive for HEV RNA; none of the anti-HEV IgM negative samples (n=403) were positive for HEV RNA. In CDC laboratory, all of the samples that tested anti-HEV IgM negative in Bangladesh except one were negative (402 of 403) and all of the anti-HEV IgM samples that tested positive in Bangladesh except two were positive (113 of 115).

Patient follow-up

Of the 1,925 patients with acute jaundice, we followed up 1,765 (92%), and of them 302 (17%) died. Among those who died, 65 (22%) died during hospitalization and the remaining died after leaving hospital but within three months of hospital discharge. Patients with acute HEV infection were significantly less likely to die than the patients who were HEV negative [4.8% (95% CI: 3.2%-6.8%) vs. 23.3% (95% CI: 20.9%-25.8%); *p<0.001,* Table 3). Among the acute hepatitis E patients, case fatality was higher among females than males (9% vs. 3%; *p<0.001*), particularly among pregnant women (12%). HEV infected patients who died were more likely to be aged ≥60 years, have a higher level of serum bilirubin (≥15 mg/dl), have co-infection with HAV or HBV than the HEV infected patients who did not die (Table 3). Fourteen of the 28 deaths in HEV infected patients (50%) and 67 of 274 deaths in HEV uninfected patients (24%) occurred within the first week of hospitalisation.

Table 3. Survival status of patients with acute jaundice admitted in six tertiary hospitals in Bangladesh, December 2014-September 2017 (patients followed-up 3 months post hospital discharge)

	Anti-HEV IgM (+) patients				Anti-HEV IgM (-) patients				All patients	
	Patients followed-up	Died		*P-value*[a]	Patients followed-up	Died		*P-value*[a]	Patients followed-up	Died
Characteristics		n	Percent (95% CI)			n	Percent (95% CI)			n (%)
Number of patients	*589*	*28*	*5 (3-7)*		*1176*	*274*	*23 (21-26)*		*1765*	*302 (17)*
Sex				*0.001*				*0.07*		
Male	430	13	3 (2-5)		762	165	22 (19-25)		1192	178 (15)
Female	159	15	9 (5-15)		414	109	26 (22-31)		573	124 (22)
Pregnancy status of women				*0.574*				*0.017*		
Non-Pregnant	91	7	8 (3-15)		300	90	30 (25-36)		391	97 (25)
1st/2nd trimester	40	4	10 (3-24)		32	7	22 (9-40)		72	11 (15)
3rd trimester	28	4	14 (4-33)		82	12	15 (8-24)		110	16 (15)
Age-group (years)				*<0.001*				*<0.001*		
14-19	114	4	4 (1-9)		201	17	9 (5-13)		315	21 (7)
20-29	263	7	3 (1-5)		313	25	8 (5-12)		576	32 (6)
30-39	122	7	6 (2-11)		189	33	18 (12-24)		311	40 (13)
40-49	55	5	9 (3-20)		159	55	35 (27-43)		214	60 (28)
50-59	27	2	7 (1-24)		143	54	38 (30-46)		170	56 (33)
60+	8	3	38 (9-76)		171	90	53 (45-60)		179	93 (52)

Characteristics	Anti-HEV IgM (+) patients				Anti-HEV IgM (-) patients				All patients	
	Patients followed-up	Died		*P-value*[a]	Patients followed-up	Died		*P-value*[a]	Patients followed-up	Died
		n	Percent (95% CI)			n	Percent (95% CI)			n (%)
Serum total bilirubin levels				*0.001*				*<0.001*		
< 5.0 mg/dl	106	4	4 (1-9)		362	52	14 (11-18)		468	56 (12)
5.0-9.9 mg/dl	233	6	3 (1-6)		320	64	20 (16-25)		553	70 (13)
10.0-14.9 mg/dl	134	4	3 (1-7)		211	64	30 (24-37)		345	68 (20)
>= 15.0 mg/dl	116	14	12 (7-19)		283	94	33 (28-39)		399	108 (27)
Serum glutamic-pyruvic transaminase				*0.082*				*0.001*		
< 200 IU/L	140	12	9 (5-15)		696	181	26 (23-29)		836	193 (23)
200-499 IU/L	150	7	5 (2-9)		248	62	25 (20-31)		398	69 (17)
500-999 IU/L	133	3	2 (0-6)		126	21	17 (11-24)		259	24 (9)
>= 1000 IU/L	162	6	4 (1-8)		104	10	10 (5-17)		266	16 (6)
Anti-HAV IgM (+)	8	2	25 (3-65)	*0.007*	132	2	2 (0-5)	*<0.001*	140	4 (3)
Anti-HBc IgM (+)	12	3	25 (5-57)	*0.001*	258	43	17 (12-22)	*0.004*	270	46 (17)
HBsAg positive (+)	134	12[b]	9 (5-15)	*0.009*	487	98	20 (17-24)	*0.03*	621	110 (18)
Duration of illness at the time of admission to hospital				*0.191*				*<0.001*		
< 2 weeks	250	8	3 (1-6)		443	65	15 (12-18)		693	73 (11)
2-4 weeks	203	9	4 (2-8)		344	70	20 (16-25)		547	79 (14)
5-6 weeks	89	7	8 (3-16)		202	63	31 (25-38)		291	70 (24)
> 6 weeks	47	4	9 (2-20)		187	76	41 (34-48)		234	80 (34)

[a] Chi-Squared test comparing proportion of deaths by patient characteristics

[b] Three of the HBsAg positive cases were also positive for Anti-HBc IgM

Among the 192 enrolled pregnant women, we followed up 173 (90%) of them until the outcome of their pregnancy. Among the 66 mothers who were anti-HEV IgM positive, 53 (80%) had a live birth and among the 107 mothers who were anti-HEV IgM negative, 72 (67%) had a live birth (Table 4). Of the 62 HEV infected mothers who were alive until the delivery, 9 (15%) had a miscarriage or stillbirth. Nineteen percent (10/53) of the live-born babies born to mothers with acute HEV infection died compared to 7% (5/72) of babies born to women without HEV infection (p=0.038). The median age at death of the babies who were born to HEV infected mothers was two days (IQR: 1-3 days; all within one week) and was five days (IQR: 2-8 days) who were born to HEV uninfected mothers (p=0.207).

Table 4. Reported complications during pregnancy, pregnancy outcomes and survival status of newborn babies by anti-HEV IgM test status of mother in six tertiary hospitals in Bangladesh, December 2014–September 2017

	Among anti-HEV IgM (+) cases		Among anti-HEV IgM (-) cases		
Characteristics	**n**	**Percent (95% CI)**	**n**	**Percent (95% CI)**	***P-value[a]***
Complications during pregnancy/delivery	**N=66**		**N=107**		
Excessive vaginal bleeding	18	27 (18-39)	48	45 (36-54)	*0.021*
Convulsions	7	11 (5-20)	18	17 (11-25)	*0.259*
Unconscious	10	15 (8-26)	26	24 (17-33)	*0.15*
Pregnancy outcomes	**N=66**		**N=107**		*0.282*
Live birth	53	80 (59-88)	72	67 (58-75)	
Still birth	6	9 (4-18)	21	20 (13-28)	
Miscarriage (Spontaneous Abortion)	2	3 (1-10)	3	3 (1-8)	
Induced abortion	1	2 (0-8)	5	5 (2-10)	
Patient died before pregnancy outcome	4	6 (2-15)	6	6 (3-12)	
Follow-up of live-births	**N=53**		**N=72**		
Neonatal deaths	10	19 (11-31)	5	7 (3-15)	*0.038*

Note: Out of 192 pregnant women, 173 women could be followed-up for pregnancy outcome; out of 125 live-births, 124 could be followed up

[a] Chi-squared test comparing anti-HEV IgM result and different characteristics

Discussion

This study confirms that HEV is a major cause of acute jaundice, is often serious enough to require hospitalization in all regions in Bangladesh, occurs throughout the year, and is associated with considerable mortality, especially among pregnant women and those co-infected with other hepatitis viruses (HAV or HBV). Fifteen percent of HEV infected pregnant women had a miscarriage or stillbirth, and of the children who were born alive, 19% died, all within one week of birth. Three-quarters of the acute hepatitis E cases in hospitals were male, and HEV infection was higher among patients residing in urban areas than patients in rural areas (41% vs 32%).

Previous estimates of hepatitis E prevalence among hospitalized acute jaundice patients in other endemic countries ranges from 10-70% [35, 36]. One study in Bangladesh tested 22 fulminant hepatitis patients admitted in a tertiary hospital and detected acute HEV infection among 64% of the patients [37]. Another study in Bangladesh tested 69 retrospectively collected samples from admitted acute-on-chronic liver failure patients in the hepatology unit of a tertiary hospital and identified acute HEV infection among 22% of the samples [34]. The large variation of hepatitis E prevalence might be due to the small number of patients recruited, geographic location of hospitals, and the types of patients recruited. One study that recruited a large number of acute hepatitis patients (685 patients during four years) from the liver clinic of a tertiary hospital in India reported the prevalence of hepatitis E as 39% [43], which is similar to the estimate in our study.

About three-quarters of laboratory-confirmed HEV infected patients were males. One explanation for this may be that males are more involved in outdoor activities than females in low- and middle-income countries [44], putting them at higher risk for exposure to

337 contaminated drinking water. Other reasons for this observation could be due to gender
338 differences in health-seeking behaviour and access to health services. A recent study noted
339 that healthcare expenditure on females is significantly lower than on males in low-income
340 settings [45].

341 Hepatitis E patients were more likely to be more educated, reside in urban areas and
342 from a high-income group than HEV negative acute jaundice patients. Higher HEV infection
343 rates among educated and high-income group patients might be associated with higher
344 exposure to contaminated drinking water due to their greater involvement in jobs outside
345 the home as reported in previous studies [12, 46]. An alternative explanation might be that
346 children in low socio-economic groups are exposed to pathogens frequently at a young age
347 and so develop robust immunity that protects them from clinically severe illness later in life
348 [47]. This pattern of higher risk of illness among wealthier adolescents and young adults has
349 been observed in some other fecal-oral transmitted diseases, most notably hepatitis A and
350 typhoid fever [48-50]. Higher HEV infection among patients who resided in urban areas
351 might be related to higher possibility of fecal contamination of drinking water sources in
352 urban settings. In large cities in Bangladesh, municipal water pipes are commonly exposed to
353 sewerage lines which may lead to fecal contamination of drinking water [51].

354 Although most reported hepatitis E epidemics have been related to fecally
355 contaminated drinking water in the rainy season and hot summer months [13], we did not
356 observe any strong seasonal trend. There were no reported hepatitis E outbreaks in
357 Bangladesh during the surveillance period. A previous study conducted in a private
358 laboratory in Dhaka identified small outbreaks of HEV infections throughout the year [9]. The

patients in our surveillance hospitals might be sporadic cases of hepatitis E or cases from small outbreaks that occurred throughout the year, as was observed in that study [9].

There are a number of limitations of our study. We observed a declining trend in HEV infection positivity by the duration of onset of illness at the time of hospitalization (Fig 2). Anti-HEV IgM antibody titres might have waned for patients who were admitted to hospital in the later stage of their illness [52]; this may lead to an underestimate the rate of HEV infection among acute jaundice patients. In our study, we used Wantai ELISA which is largely accepted as the most sensitive among the available commercial assays [53]. However, the study conducted by Huang et al. observed that about 3% of acute hepatitis cases (defined as acute liver damage evidenced by a 2.5 fold upper limit of the normal level of alanine aminotransferase) were negative for anti-HEV IgM (using the Wantai kit) but had a 4-fold rise in anti-HEV IgG in their convalescent sera [52], which indicates an acute HEV infection [54-56]. Therefore, our results likely underestimate the true prevalence of acute hepatitis E due to the performance of the test kit. HEV infections are generally self-limiting and do not require hospitalization [2] and the patients admitted in tertiary hospitals are severely ill; therefore, the findings in our study represent only the prevalence of hepatitis E among severely ill patients. Finally, in our study, the etiological agent of acute jaundice was unknown for about one-third of the patients, and the samples were not tested for hepatitis C virus.

This study provides a reliable estimate of the prevalence of HEV infection among hospitalized patients with acute jaundice in a hepatitis E endemic country by enrolling patients admitted from a wide geographic area through systematic surveillance over a two and a half year period. It confirms that hepatitis E is the leading cause of acute jaundice in

Bangladesh. We identified a high prevalence of hepatitis E among patients with acute jaundice in all of the hospitals included in the study and throughout the multiple ecological zones in Bangladesh. Hepatitis E is therefore a considerable public health problem in Bangladesh. Considering the high burden of HEV among hospitalized patients with acute jaundice, Bangladesh could consider to take control measures to reduce this risk including improvements in water quality, sanitation and hygiene practices and the introduction of hepatitis E vaccine in high-risk areas. An improved understanding of the burden of hepatitis E is required in other endemic countries to plan for effective global actions to prevent HEV infections including the introduction of hepatitis E vaccine.

Supporting information

S1 Checklist. STROBE checklist.

S1 Table. Post hospital discharge follow-up schedule of enrolled patients in the surveillance hospitals.

S1 Fig. Flow chart of patient enrolment, laboratory testing and follow-up in the surveillance hospitals.

S2 Table. Hepatitis E serological test results by educational status and residence (rural-urban) of patients with acute jaundice in six tertiary hospitals in Bangladesh, December 2014–September 2017.

Supporting information legends

S1 Table. Enrolled patients and the newborns were followed up post hospital discharge to ascertain their vital status.

S1 Fig. Number of patients diagnosed with acute jaundice, provided a blood specimen and followed up post hospital discharge in the six tertiary hospitals in Bangladesh.

406 **Acknowledgements**

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572 **S1 Checklist. STROBE checklist.**

573 STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No.
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2-3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5-6
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7-11
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	8-9
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	9-10
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7-11
Bias	9	Describe any efforts to address potential sources of bias	N/A
Study size	10	Explain how the study size was arrived at	N/A
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	11-12
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	1-12
		(*b*) Describe any methods used to examine subgroups and interactions	N/A
		(*c*) Explain how missing data were addressed	N/A

		(*d*) If applicable, describe analytical methods taking account of sampling strategy	N/A
		(*e*) Describe any sensitivity analyses	N/A
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	13
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	32
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	13-14
		(b) Indicate number of participants with missing data for each variable of interest	N/A
Outcome data	15*	Report numbers of outcome events or summary measures	13
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	13-15
		(*b*) Report category boundaries when continuous variables were categorized	N/A
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	18-21
Discussion			
Key results	18	Summarise key results with reference to study objectives	22
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	24
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from	22-25

		similar studies, and other relevant evidence	
Generalisability	21	Discuss the generalisability (external validity) of the study results	24-25
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	N/A

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

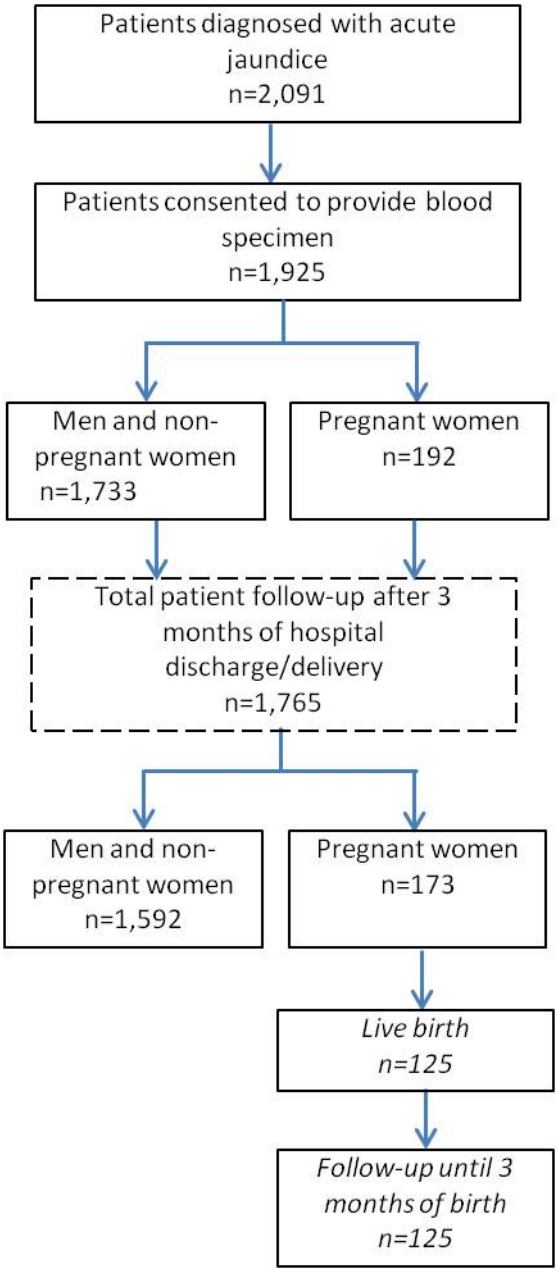
583 **S1 Table. Post hospital discharge follow-up schedule of enrolled patients in the**
584 **surveillance hospitals**

Patient type	Timing of follow-up		
	1 week after delivery date	3 months after delivery date	3 months after hospital discharge
Non-pregnant women and adult men			√
Pregnant women delivered in hospital	√	√	
Pregnant women discharged from hospital before 3rd trimester	√	√	√
Pregnant women discharged from hospital during 3rd trimester	√	√	

585

586 **S1 Fig. Flow chart of patient enrolment, laboratory testing and follow-up in the**
587 **surveillance hospitals**

588



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S2 Table. Hepatitis E serological test results by educational status and residence (rural-urban) of patients with acute jaundice in six tertiary hospitals in Bangladesh, December 2014–September 2017

Characteristics	Rural (N=1494)		Urban (N=431)		Total (N=1925)	
	Patient tested	Anti-HEV IgM Positive	Patient tested	Anti-HEV IgM Positive	Patient tested	Anti-HEV IgM Positive
		n (%)		n (%)		n (%)
Education						
None	316	51 (16)	67	12 (18)	383	63 (16)
Class 1-5	482	137 (28)	92	38 (41)	574	175 (31)
Class 6-11	460	175 (38)	184	84 (46)	644	259 (40)
Class 12 or more	236	120 (51)	88	44 (50)	324	164 (51)
Monthly household expenditure in Bangladeshi taka						
< 5000 (US$ 62)	236	40 (17)	34	8 (24)	270	48 (18)
5000-9,999 (US$ 63-125)	595	171 (29)	122	43 (35)	717	214 (30)
10,000-14,999 (US$ 126-187)	314	110 (35)	85	32 (38)	399	142 (36)
≥ 15,000 (US$ 188)	201	79 (39)	127	59 (46)	328	138 (42)