1   **Hepatitis E as a cause of adult hospitalization in Bangladesh: Results from an acute**

2   **jaundice surveillance study in six tertiary hospitals, 2014-2017**

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24 **Abstract**

25   In the absence of reliable data on the burden of hepatitis E virus (HEV) in high

26   endemic countries, we established a hospital-based acute jaundice surveillance program in

27   six tertiary hospitals in Bangladesh to estimate the burden of HEV infection among

28   hospitalized acute jaundice patients aged ≥14 years, identify seasonal and geographic

29   patterns in the prevalence of hepatitis E, and examine factors associated with death.

30   We collected blood specimens from enrolled acute jaundice patients, defined as new onset

31   of either yellow eyes or skin during the past three months of hospital admission, and tested

32   for immunoglobulin M (IgM) antibodies against HEV, HBV and HAV. The enrolled patients

33   were followed up three months after hospital discharge to assess their survival status;

34   pregnant women were followed up three months after their delivery to assess pregnancy

35   outcomes.

36   From December'2014 to September'2017, 1925 patients with acute jaundice were

37   enrolled; 661 (34%) had acute hepatitis E, 48 (8%) had hepatitis A, and 293 (15%) had acute

38   hepatitis B infection. Case fatality among hepatitis E patients was 5% (28/589). Most of the

39   hepatitis E cases were males (74%; 486/661), but case fatality was higher among females—

40   12% (8/68) among pregnant and 8% (7/91) among non-pregnant women. Half of the

41   patients who died with acute hepatitis E had co-infection with HAV or HBV. Of the 62 HEV

42   infected mothers who were alive until the delivery, 9 (15%) had miscarriage/stillbirth, and of

43   those children who were born alive, 19% (10/53) died, all within one week of birth.

44   This study confirms that hepatitis E is the leading cause of acute jaundice, leads to

45   hospitalizations in all regions in Bangladesh, occurs throughout the year, and is associated

46   with considerable morbidity and mortality. Effective control measures should be taken to

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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.

49   **Author summary**

50   In the absence of reliable surveillance data on the burden of hepatitis E in endemic

51   countries, we conducted a hospital-based acute jaundice surveillance study over a two and a

52   half year period in six tertiary hospitals in Bangladesh. The study confirms that HEV

53   infections occur throughout the year, and is a major (34%) cause of acute jaundice in tertiary

54   hospitals in Bangladesh. Three-quarters of the acute hepatitis E cases were male, and HEV

55   infection was higher among patients residing in urban areas than patients in rural areas (41%

56   vs 32%). The overall case fatality rate of acute HEV infections in hospitals was 5%, but was

57   higher among pregnant women (12%). Hepatitis E patients who died were more likely to

58   have co-infection with HAV or HBV than the HEV infected patients who did not die. Fifteen

59   percent of HEV infected mothers had miscarriage/stillbirth. Of the children who were born

60   alive, 19% died, all within one week of birth. Considering the high burden of hepatitis E

61   among hospitalized acute jaundice patients, Bangladesh could take control measures to

62   reduce this risk including improvements in water quality, sanitation and hygiene practices

63   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.

87   While hospital-based surveillance represents only the burden of severe HEV disease, it can

88   provide valuable insights, especially regarding the contribution to mortality among severe

89   acute jaundice patients. Patients with acute jaundice commonly present to hospitals in many

90   resource-poor hepatitis E endemic countries, including Bangladesh [32-34]. However, there

91   are few studies in hepatitis E endemic countries that systematically enrolled patients with

92   acute jaundice to estimate the burden of acute HEV infection in hospital settings, especially

93   the case fatality rate and delivery outcomes of pregnant women [35, 36]. Previous hospital-

94   based studies in Bangladesh suggest that most of the cases of acute jaundice (22-64%) were

95   due to HEV infection, however, those studies were limited to a single study site or a short

96   study period which may not be a representative estimate of the burden of hepatitis E among

97   all hospitalized acute jaundice patients in Bangladesh [34, 37, 38].

98   The International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) in

99   collaboration with the Institute of Epidemiology, Disease Control and Research (IEDCR) of

100   the Government of Bangladesh, and the United States Centers for Disease Control and

101   Prevention (CDC), conducted a hospital-based acute jaundice surveillance study in six tertiary

102   hospitals located in five regions of Bangladesh. The objectives of this study were to estimate

103   the prevalence of HEV infection among acute jaundice patients aged ≥14 years admitted in

104   tertiary hospitals in Bangladesh, estimate the case-fatality among these patients and identify

105   factors associated with mortality, as well as describe seasonal and geographic trends in

106   prevalence of HEV disease.

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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.

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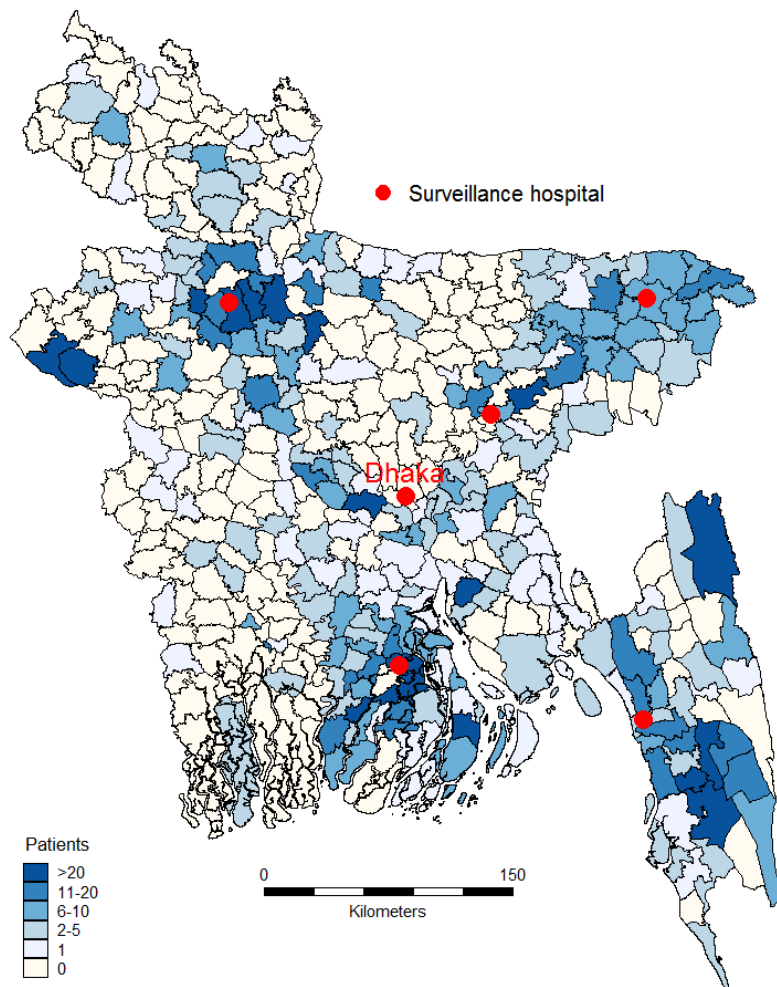
Fig 1. Location of acute jaundice surveillance hospitals.
Map of Bangladesh showing the location of surveillance hospitals and number of enrolled patients with acute jaundice by sub-district during December 2014- September 2017. Map was created using the SPMAP module for Stata 14 (StataCorp) [39].

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123   We recruited a physician in each of the six hospitals from the existing staff to oversee

124   the surveillance activities. A field assistant from icddr,b in each hospital assisted the

125   surveillance physicians to collect patient information and blood specimens, and to store and

126   transport samples to the laboratory in Dhaka. Every weekday morning, the surveillance

127   physicians visited the obstetrics and gynaecology wards and the adult medicine wards of the

128   hospitals and reviewed admission records from the previous day to determine if any patients

129   aged ≥14 years met the case definition of acute jaundice. Due to resource constraints, we

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130   were unable to conduct surveillance in all wards in each hospital; therefore we restricted

131   enrolment to adult medicine, obstetrics and gynaecology wards (where patients aged > 14

132   years are admitted) as children have been reported to have less severe presentations of HEV

133   infection [14, 15]. Patients with acute jaundice admitted on Friday were enrolled on the next

134   working day, as study staff did not work on Fridays. Acute jaundice was defined as new

135   onset of either yellow eyes or skin for less than 3 months, and continuing on the day of

136   admission. The field assistants also visited the intensive care unit (ICU) every weekday to

137   ensure that no acute jaundice cases were overlooked. If anyone met the case definition of

138   acute jaundice, the surveillance physicians recorded enrolled patients' illness history and

139   relevant clinical information along with basic demographic information and contact

140   information in a handheld computer using a structured case investigation form. The

141   surveillance physicians also collected a 5 ml blood specimen for laboratory testing.

142   **Sample processing and laboratory testing**

143   On the same day of sample collection, the field assistants centrifuged the blood

144   specimen and prepared three aliquots of sera. One aliquot containing about 400µl of serum

145   was sent to the hospital laboratory the same day of sample collection to test for bilirubin

146   and glutamic-pyruvic transaminase to assess liver function. Two aliquots of serum were

147   stored at the surveillance hospitals in a liquid nitrogen dry shipper and transported to the

148   laboratory of IEDCR in Dhaka every two weeks. The samples were kept in a freezer at IEDCR

149   at -80°C until they were tested. The samples were tested for IgM and IgG antibodies to HEV

150   using enzyme-linked immunosorbent assay (ELISA) kits manufactured by Wantai, China

151   (Beijing Wantai Biologic Pharmacy Enterprise Co., Ltd, Beijing, China) according to the

152   manufacturer's instructions. Acute HEV infection was defined as a positive test result for

153   anti-HEV IgM antibodies. We also tested all samples for hepatitis A virus IgM antibodies

154   (anti-HAV IgM) (a marker of HAV infection) and hepatitis B virus surface antigen (HBsAg) (a

155   marker of acute or chronic HBV infection). The samples that were positive for HBsAg were

156   also tested for hepatitis B virus core IgM antibodies (anti-HBc IgM; a marker of acute HBV

157   infection). Chronic HBV infection was defined as a positive test result for HBsAg but negative

158   test result for anti-HBc IgM. HBsAg negative samples are generally negative for anti-HBc IgM

159   [40], therefore, only the HBsAg positive samples were tested for anti-HBc IgM. ELISA test kits

160   manufactured by DiaSorin, Italy were used for testing anti-HAV IgM, HBsAg and anti-HBc

161   IgM.

162   We sent a subset of samples (collected between December 2014 and December

163   2016) to the Division of Viral Hepatitis Laboratory of the US Centers for Disease Control and

164   Prevention (CDC), Atlanta, USA to test for HEV RNA. All samples from patients with onset

165   within three weeks of the blood draw were selected for RNA testing. For other patients, a

166   random sample of 20% of anti-HEV IgM positive samples, 20% of anti-HEV IgG positive

167   samples, 20% of samples negative for both IgM and IgG were chosen to test for HEV RNA.

168   Serum samples were tested for HEV RNA using a quantitative real-time reverse transcriptase

169   polymerase chain reaction (PCR) assay, capable of detecting HEV genotypes 1-3 with a limit

170   of detection of 25 IU/ml, targeting a 69-bp fragment of open reading frame (ORF) 3 of HEV

171   genome [41]. For quality assurance, the samples that were sent to CDC were also retested

172   for anti-HEV IgM using the same ELISA kit as was used at the laboratory in Bangladesh.

173   **Patient follow-up**

174   All enrolled patients were followed up during their hospitalization by the surveillance

175   physicians to monitor the outcomes of the illness episode and the pregnancy outcomes for

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176   pregnant patients. If the pregnancy ended during hospitalization and the outcome was a live

177   birth, the surveillance physician examined the newborn to collect clinical information and

178   note any signs or symptoms of jaundice. All enrolled patients and the newborns were

179   followed up 3 months post hospital discharge to ascertain their vital status. If a pregnant

180   woman with jaundice was released from the hospital before the end of her pregnancy, the

181   field assistants followed up with her by phone one week after the expected date of delivery

182   and three months after the date of delivery to check on the health of the mother and the

183   newborn (S1 Table). For patients who did not have a phone, the field assistants visited their

184   home to follow-up. Follow up three months post hospital discharge was not possible for the

185   patients who were admitted to surveillance hospitals after June 30, 2017, as the surveillance

186   ended on September 30, 2017.

187   **Data analysis**

188   We calculated the prevalence of acute HEV infection among patients with acute

189   jaundice in each surveillance hospital and overall. Seroprevalence of acute hepatitis E was

190   also calculated by patients' demographic characteristics and rural-urban area of residence.

191   We compared the signs and symptoms during illness, pregnancy complications, pregnancy

192   outcome and case fatality between acute jaundice patients with and without evidence of

193   acute HEV infection. We examined potential risk factors of death among patients with acute

194   hepatitis E including co-infection with other hepatitis viruses (HAV, HBV). Comparison

195   between categorical variables was performed using the chi-squared test or Fisher exact test

196   where appropriate and comparison between continuous variables (non-normal distribution)

197   was performed using the Wilcoxon rank sum test. The 95% confidence interval for

198   proportions was calculated by using the Wilson method for a binomial distribution [42]. We

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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.

213   **Results**

214   **Patient characteristics**

215   In the six surveillance hospitals, a total of 2,091 patients aged ≥14 years met the case

216   definition of acute jaundice during December 2014 to September 2017. Of them, 1,925

217   (92%) agreed to be enrolled in the study and provided a blood specimen for laboratory

218   testing (S1 Fig). Of the enrolled patients, 1,314 (68%) were male and 958 (50%) were aged

219   between 14 and 29 years. Of the female patients, 192 (31%) were pregnant. Patients

220   admitted to the surveillance hospitals resided in 56 out of 64 districts in Bangladesh;

221   however, a higher number of patients lived near surveillance hospitals (Fig 1).

222   **Laboratory testing and HEV seroprevalence**

223   Among the 1,925 enrolled patients, 661 (34%) had IgM antibodies detected against

224   HEV (acute HEV infection) and 652 (99%) of these also had IgG antibodies against HEV.

225   Overall, 1,036 (54%) patients had detectable anti-HEV IgG antibodies. There were 148 (8%)

226   patients who were positive for anti-HAV IgM antibodies and 663 (34%) who were positive for

227   HBsAg. Of the 663 HBsAg positive patients, 293 (15% of all patients) had acute HBV infection.

228   Among the 661 acute HEV patients, 9 (1%) also had acute HAV infection, 15 (2%) had acute

229   HBV infection and 132 (20%) had chronic HBV infection.

230   Of the 661 patients with acute HEV infection, 483 (73%) resided in rural areas, 486

231   (74%) were male; their median age was 25 years (IQR: 20-33). Acute hepatitis E prevalence

232   varied across the hospital sites, ranging from 17% in Sylhet to 61% in Dhaka (Table 1).

233   Among the patients with acute jaundice, those who were male, aged between 20 to 39

234   years, resided in urban areas, educated, or whose monthly family expenditure was >15,000

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235   takas (US$188) were more likely to have acute HEV infection (p<0.001). Acute HEV infection

236   was higher among the pregnant women with acute jaundice than the non-pregnant women

237   with acute jaundice (39% vs. 24%, p<0.001). The higher rate of acute HEV infection among

238   educated and higher income group patients with acute jaundice in the study hospitals was

239   consistent in the analysis by rural-urban area of residence (S2 Table).

240   **Table 1. Hepatitis E serological test results by demographic characteristics of patients with**
241   **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)	

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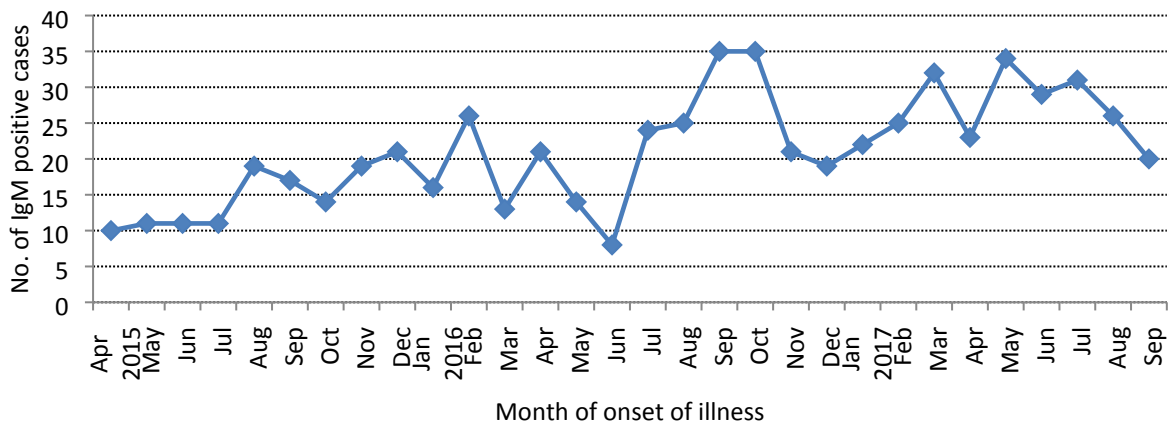
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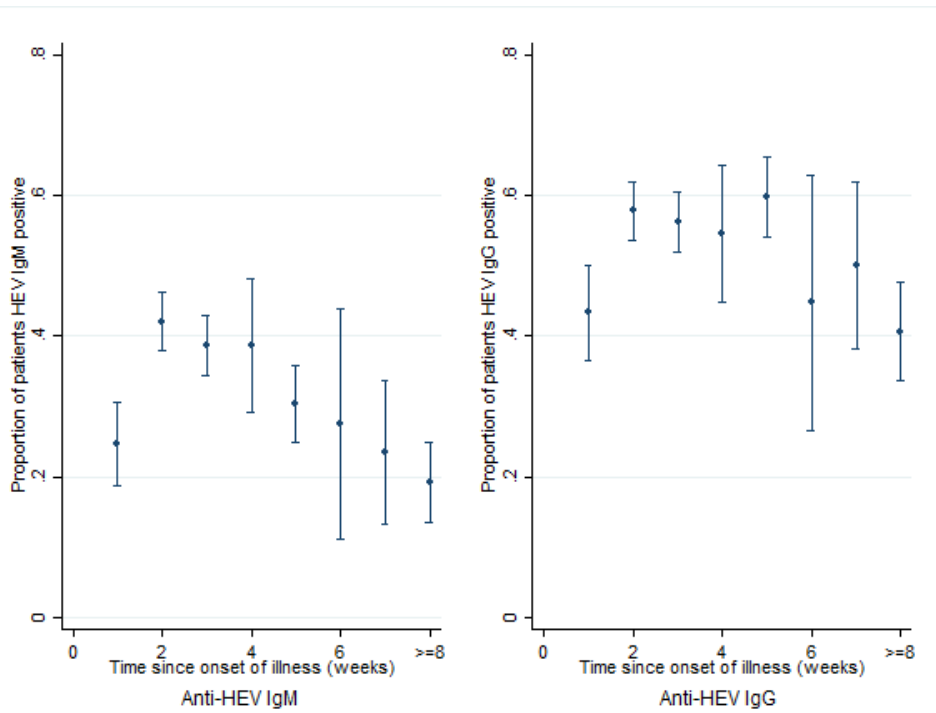
Fig 2. Proportion of patients positive for anti-HEV IgM and anti-HEV IgG by duration of illness at the time of admission to hospital in six tertiary hospitals in Bangladesh, December 2014- September 2017

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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.

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266   Most of the signs and symptoms were similar for anti-HEV IgM positive and negative

267   patients (Table 2). However, oedema, distended abdomen, melaena and unconsciousness

268   were observed more often among anti-HEV IgM negative patients than anti-HEV IgM

269   positive patients. The median serum glutamic-pyruvic transaminase level was significantly

270   higher among anti-HEV IgM positive patients than the anti-HEV IgM negative patients [520

271   (range: 221-1100) IU/L vs. 140 (range: 60-394) IU/L; p <0.001].

272   **Table 2. Signs and symptoms during illness among anti-HEV IgM positive and negative**
273   **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

274   [a] Chi-squared test comparing anti-HEV IgM result and patients' symptoms during illness
275   [b] Wilcoxon rank sum test
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277   Of the 115 anti-HEV IgM positive samples that were sent to CDC, 73 (64%) were

278   positive for HEV RNA; none of the anti-HEV IgM negative samples (n=403) were positive for

279   HEV RNA. In CDC laboratory, all of the samples that tested anti-HEV IgM negative in

280   Bangladesh except one were negative (402 of 403) and all of the anti-HEV IgM samples that

281   tested positive in Bangladesh except two were positive (113 of 115).

282   **Patient follow-up**

283   Of the 1,925 patients with acute jaundice, we followed up 1,765 (92%), and of them

284   302 (17%) died. Among those who died, 65 (22%) died during hospitalization and the

285   remaining died after leaving hospital but within three months of hospital discharge. Patients

286   with acute HEV infection were significantly less likely to die than the patients who were HEV

287   negative [4.8% (95% CI: 3.2%-6.8%) vs. 23.3% (95% CI: 20.9%-25.8%); $p<0.001$, Table 3).

288   Among the acute hepatitis E patients, case fatality was higher among females than males

289   (9% vs. 3%; $p<0.001$), particularly among pregnant women (12%). HEV infected patients who

290   died were more likely to be aged ≥60 years, have a higher level of serum bilirubin (≥15

291   mg/dl), have co-infection with HAV or HBV than the HEV infected patients who did not die

292   (Table 3). Fourteen of the 28 deaths in HEV infected patients (50%) and 67 of 274 deaths in

293   HEV uninfected patients (24%) occurred within the first week of hospitalisation.

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

| 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 (%)
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**			Patients followed-up	**Died**			Patients followed-up	**Died**
		n	Percent (95% CI)	P-value[a]		n	Percent (95% CI)	P-value[a]		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

298   Among the 192 enrolled pregnant women, we followed up 173 (90%) of them until

299   the outcome of their pregnancy. Among the 66 mothers who were anti-HEV IgM positive, 53

300   (80%) had a live birth and among the 107 mothers who were anti-HEV IgM negative, 72

301   (67%) had a live birth (Table 4). Of the 62 HEV infected mothers who were alive until the

302   delivery, 9 (15%) had a miscarriage or stillbirth. Nineteen percent (10/53) of the live-born

303   babies born to mothers with acute HEV infection died compared to 7% (5/72) of babies born

304   to women without HEV infection (p=0.038). The median age at death of the babies who

305   were born to HEV infected mothers was two days (IQR: 1-3 days; all within one week) and

306   was five days (IQR: 2-8 days) who were born to HEV uninfected mothers (p=0.207).

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

Characteristics	Among anti-HEV IgM (+) cases		Among anti-HEV IgM (-) cases		
	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

310   Note: Out of 192 pregnant women, 173 women could be followed-up for pregnancy outcome; out of 125 live-births,
311   124 could be followed up
312   [a] Chi-squared test comparing anti-HEV IgM result and different characteristics
313

Discussion

315   This study confirms that HEV is a major cause of acute jaundice, is often serious

316   enough to require hospitalization in all regions in Bangladesh, occurs throughout the year,

317   and is associated with considerable mortality, especially among pregnant women and those

318   co-infected with other hepatitis viruses (HAV or HBV). Fifteen percent of HEV infected

319   pregnant women had a miscarriage or stillbirth, and of the children who were born alive,

320   19% died, all within one week of birth. Three-quarters of the acute hepatitis E cases in

321   hospitals were male, and HEV infection was higher among patients residing in urban areas

322   than patients in rural areas (41% vs 32%).

323   Previous estimates of hepatitis E prevalence among hospitalized acute jaundice

324   patients in other endemic countries ranges from 10-70% [35, 36]. One study in Bangladesh

325   tested 22 fulminant hepatitis patients admitted in a tertiary hospital and detected acute HEV

326   infection among 64% of the patients [37]. Another study in Bangladesh tested 69

327   retrospectively collected samples from admitted acute-on-chronic liver failure patients in the

328   hepatology unit of a tertiary hospital and identified acute HEV infection among 22% of the

329   samples [34]. The large variation of hepatitis E prevalence might be due to the small number

330   of patients recruited, geographic location of hospitals, and the types of patients recruited.

331   One study that recruited a large number of acute hepatitis patients (685 patients during four

332   years) from the liver clinic of a tertiary hospital in India reported the prevalence of hepatitis

333   E as 39% [43], which is similar to the estimate in our study.

334   About three-quarters of laboratory-confirmed HEV infected patients were males. One

335   explanation for this may be that males are more involved in outdoor activities than females

336   in low- and middle-income countries [44], putting them at higher risk for exposure to

22

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

359   patients in our surveillance hospitals might be sporadic cases of hepatitis E or cases from

360   small outbreaks that occurred throughout the year, as was observed in that study [9].

361   There are a number of limitations of our study. We observed a declining trend in HEV

362   infection positivity by the duration of onset of illness at the time of hospitalization (Fig 2).

363   Anti-HEV IgM antibody titres might have waned for patients who were admitted to hospital

364   in the later stage of their illness [52]; this may lead to an underestimate the rate of HEV

365   infection among acute jaundice patients. In our study, we used Wantai ELISA which is largely

366   accepted as the most sensitive among the available commercial assays [53]. However, the

367   study conducted by Huang et al. observed that about 3% of acute hepatitis cases (defined as

368   acute liver damage evidenced by a 2.5 fold upper limit of the normal level of alanine

369   aminotransferase) were negative for anti-HEV IgM (using the Wantai kit) but had a 4-fold

370   rise in anti-HEV IgG in their convalescent sera [52], which indicates an acute HEV infection

371   [54-56]. Therefore, our results likely underestimate the true prevalence of acute hepatitis E

372   due to the performance of the test kit. HEV infections are generally self-limiting and do not

373   require hospitalization [2] and the patients admitted in tertiary hospitals are severely ill;

374   therefore, the findings in our study represent only the prevalence of hepatitis E among

375   severely ill patients. Finally, in our study, the etiological agent of acute jaundice was

376   unknown for about one-third of the patients, and the samples were not tested for hepatitis

377   C virus.

378   This study provides a reliable estimate of the prevalence of HEV infection among

379   hospitalized patients with acute jaundice in a hepatitis E endemic country by enrolling

380   patients admitted from a wide geographic area through systematic surveillance over a two

381   and a half year period. It confirms that hepatitis E is the leading cause of acute jaundice in

24

382   Bangladesh. We identified a high prevalence of hepatitis E among patients with acute

383   jaundice in all of the hospitals included in the study and throughout the multiple ecological

384   zones in Bangladesh. Hepatitis E is therefore a considerable public health problem in

385   Bangladesh. Considering the high burden of HEV among hospitalized patients with acute

386   jaundice, Bangladesh could consider to take control measures to reduce this risk including

387   improvements in water quality, sanitation and hygiene practices and the introduction of

388   hepatitis E vaccine in high-risk areas. An improved understanding of the burden of hepatitis

389   E is required in other endemic countries to plan for effective global actions to prevent HEV

390   infections including the introduction of hepatitis E vaccine.

391   **Supporting information**

392   **S1 Checklist. STROBE checklist.**

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

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

397   **S2 Table. Hepatitis E serological test results by educational status and residence (rural-**
398   **urban) of patients with acute jaundice in six tertiary hospitals in Bangladesh, December**
399   **2014–September 2017.**

400

401   **Supporting information legends**

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

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

25

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

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		(*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

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575   *Give information separately for exposed and unexposed groups.

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577   **Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological

578   background and published examples of transparent reporting. The STROBE checklist is best used in conjunction

579   with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of

580   Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the

581   STROBE Initiative is available at www.strobe-statement.org.

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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	√	√	

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

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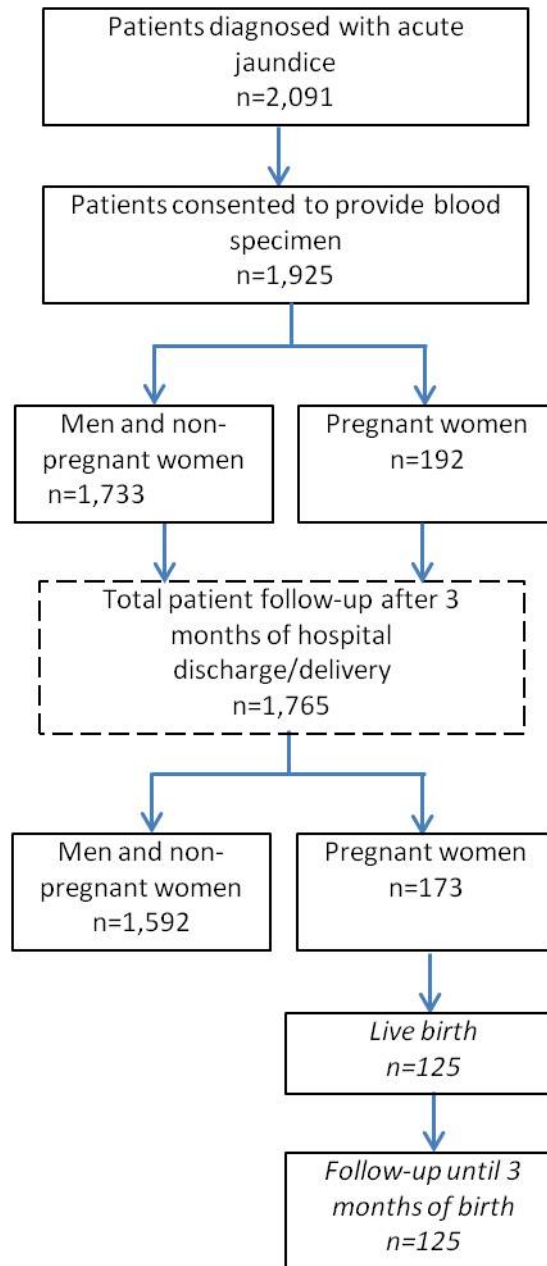
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591 **S2 Table. Hepatitis E serological test results by educational status and residence (rural-**
592 **urban) of patients with acute jaundice in six tertiary hospitals in Bangladesh, December**
593 **2014–September 2017**

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)

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