

# Virological investigation of a hepatitis E epidemic in North India

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## ABSTRACT

**Introduction:** Hepatitis E virus (HEV) infection is of major public health concern in the developing countries, including the Indian subcontinent, due to epidemics of large proportions, increased morbidity and high mortality, especially in pregnant women. This study shows the findings of two different epidemics that occurred due to HEV.

**Methods:** Blood samples were collected from 116 suspected HEV patients. Sera were separated and tested for hepatitis A virus HAV immunoglobulin M (IgM), hepatitis B virus surface antigen, hepatitis C virus (HCV) antibody and HEV IgM by Micro ELISA. 15 acute samples were subjected to reverse transcriptase polymerase chain reaction (RT-PCR) for the detection of HEV ribonucleic acid (RNA).

**Results:** Of the 116 blood samples collected, 68 (58.6 percent) were positive for HEV IgM antibodies. Mixed infections of HEV with HAV and HCV were detected in three (4.4 percent) and five (7.4 percent) cases, respectively. 15 HEV IgM-positive acute blood samples subjected to RT-PCR showed the presence of specific 343 bp amplified HEV ORF1 gene product in five cases. No untoward effects were observed in the five HEV-infected pregnant women during their follow-up.

**Conclusion:** This study confirms the HEV aetiology and highlights a major disease outbreak that occurred due to mixing of drinking water with sewerage.

**Keywords:** enterically-transmitted non-A non-B hepatitis virus, hepatitis E virus, pregnancy complications, viral epidemic

## INTRODUCTION

Enterically-transmitted non-A non-B hepatitis (hepatitis E) has been known to occur in both sporadic and epidemic forms in developing countries<sup>(1-4)</sup>. A few cases have also been reported from developed countries, following travel to a hepatitis E virus (HEV) epidemic area or contact with persons from those areas. Ever since the first 1955-56 epidemic, frequent epidemics have been reported in many parts of India<sup>(4-7)</sup>.

Hepatitis E virus, an unclassified single-stranded, positive-sense ribonucleic acid (RNA) virus with a single serotype<sup>(8)</sup>, is the leading cause of acute viral hepatitis in the world<sup>(1,2)</sup> and occurs primarily in Africa, Mexico, Central Asia and some South Asian countries<sup>(2,3)</sup>. It affects mainly young adults in the 15-40 year age group<sup>(9)</sup>, and runs a sub-clinical infection among children<sup>(10)</sup>. Being faeco-orally transmitted, HEV is often associated with poor standards of sanitation and hygiene. The highest concentration of virus is found in stools during the incubation and early symptomatic phase of the disease<sup>(5)</sup>. Infections are self-limited<sup>(5)</sup>, but severe complications with a high mortality of 20-30% have been reported during epidemics, particularly in the third trimester of pregnancy<sup>(11,12)</sup>. Unlike hepatitis A virus (HAV), HEV primarily gets transmitted through contaminated drinking water<sup>(13)</sup>, but rarely through person to person<sup>(14)</sup>. Recently, swine have been implicated as the reservoir of human infection<sup>(15)</sup>.

The present study reports the epidemiological and virological investigation of a suspected enterically-transmitted hepatitis in two districts of Punjab, a North Indian state. The two districts, Kurali and Mansa, have an approximate population of 13,000 and 15,000, respectively, where 147 cases of jaundice were reported in Kurali and 500 cases in Mansa. People live in congested colonies and thickly populated wards. Open drains, dumping of garbage at public places, poor maintenance of the sewerage disposal system, and drinking water

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**Table I. Positivity of viral markers in patients and apparently healthy subjects.**

Epidemic areas	Viral markers positivity in						RT-PCR in HEV-positive patients	
	Patients				Controls		RNA positive	Total tested
	HEV IgM	HAV IgM	HBsAg	HCV Ab	Viral markers	Total tested		
Kurali	17/40	3/68	0/68	1/68	0	10	4	9
Mansa	51/76	0/68	0/68	4/68	0	10	1	6
Grand total	68/116	3/68	0/68	5/68	0	20	5	15

supply line adjacent or through the sewerage disposal system, result in the high risk of the drinking water supplies getting contaminated due to leakage. Both districts are devoid of natural water source; hence, they depend upon the piped water supply for drinking and household needs. Lower- and middle-class people from a rural background primarily dominate both districts. We report here an epidemic of viral hepatitis that occurred in these places during the months of March and April 2003. The areas were visited for detailed epidemiological investigation.

## METHODS

A total of 116 (76 acute and 40 convalescent) blood samples were collected from patients showing symptoms of jaundice, fever, loss of appetite, abdominal pain, scleral icterus, and fatigue. These individuals belonged to both genders within the age group of 10-70 years. Additionally, blood samples were collected from 20 apparently healthy individuals. Sera were preserved at -70°C till tested.

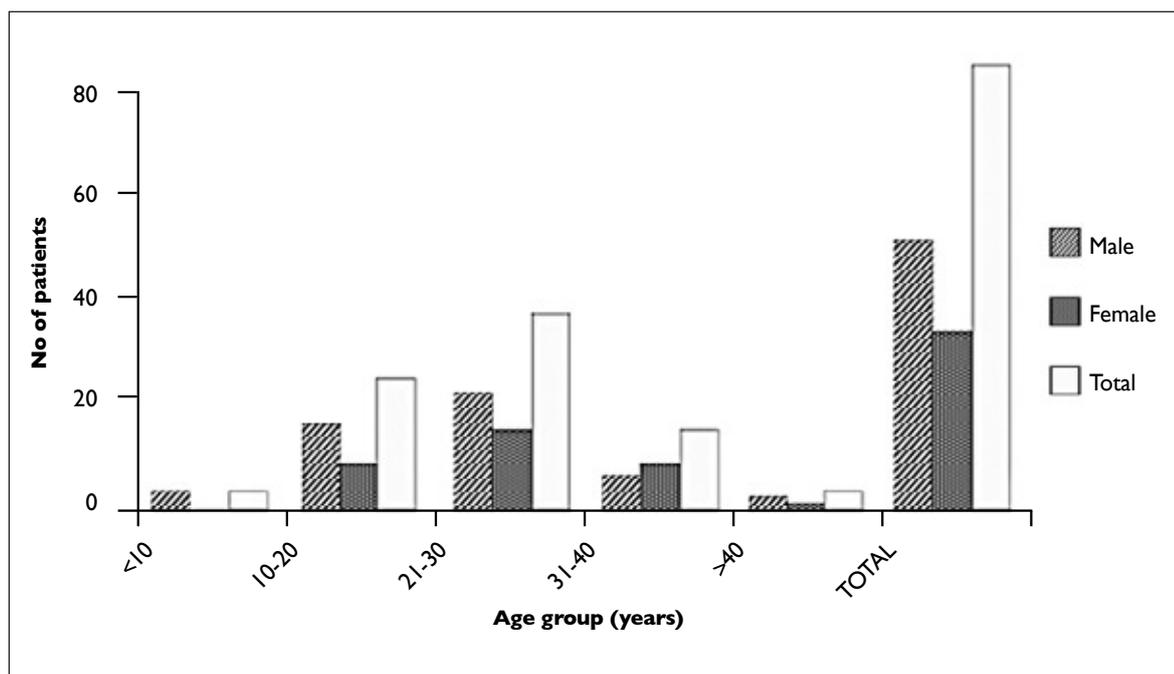
Sera were screened for HAV immunoglobulin M (IgM) (EIAgen Anti-HAV IgM, Adaltis, Spain), hepatitis B virus surface antigen (HBsAg) (Biorad Monolisa HBsAg plus), hepatitis C virus (HCV) antibody (Ab) (Innotest HCV Ab IV, Belgium) and HEV IgM (EIAgen HEV, Adaltis, Spain) by Micro ELISA. 15 acute blood samples collected within the first week of presentation, as well as five stool samples collected within ten days of symptomatology, were subjected to nested reverse transcriptase polymerase chain reaction (nRT-PCR) assay for the detection of HEV RNA. The RNA was extracted by GITC-phenol-chloroform extraction method<sup>(16)</sup> with minor modifications; i.e. for precipitation

of the RNA, the aqueous layer was incubated at -20°C overnight instead of three hours. On the next day, the solution was centrifuged and the pellet was resuspended in sol-D (4% guanidinium isothiocyanate, 25 mM sod. citrate, 0.5% sarkosyl, 0.1M β-mercaptoethanol) and absolute ethanol and again incubated at -20°C overnight.

After cDNA synthesis using MuLV RT enzyme and random hexamer, PCR amplification was carried out using the specific primers against the ORF1 gene, (sense: 5'-CGGGAT CCACACACAT CTGAGCTACATTCGTGAGCT-3' antisense: 5'-CGGAATTCAAAGGCATCCATGGTGTGTTGAG AATGAC-3' for first cycle and sense: 5'-GGAAT TCGACTCCACCCAGAATAACTT-3' antisense: 5'-GGAATTCACAGCCGGCGATCAGGACAG-3' for second cycle) which gave rise to a product of 343 bp visualised by agarose gel electrophoresis according to the protocol mentioned by Jameel et al<sup>(17)</sup>.

## RESULTS

In the present study, among the 116 patients studied, males outnumbered females with a ratio of 8:5. The majority of patients were adults (Fig. 1). Of the 116 samples tested for the HEV serology, 68 (58.6%) were positive for HEV IgM antibodies (Table I). Among the positives, five were pregnant women distributed in the proportion of one, two and two in the first, second and third trimester of pregnancy, respectively. HEV-positive samples were further tested for evidence of HAV IgM, HBsAg and HCV Ab. None of the samples were positive for HBsAg. However, dual infection of HAV and HEV was detected in three (4.4%) patients belonging to the 9-10 year age group, whereas HCV and HEV positivity could be found



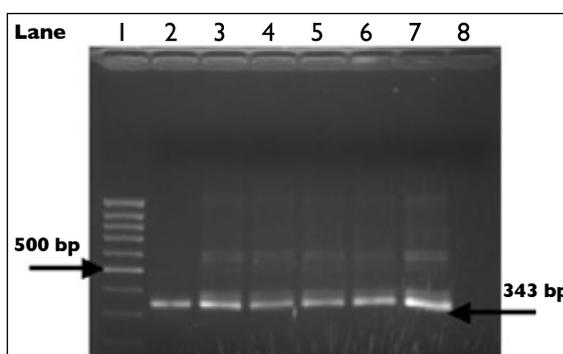
**Fig. 1** Age and gender distribution of HEV-positive patients.

in five (7.4%) patients. None of the apparently healthy individuals were positive for any viral markers tested (Table I). Five of the 15 acute HEV IgM positive blood samples tested by RT-PCR were positive, whereas none of the stool samples yielded HEV RNA (Fig. 2).

## DISCUSSION

Hepatitis E was first recognised during an epidemic of hepatitis in Kashmir valley in 1978<sup>(18)</sup>. It is an ecologically-determined disease that spreads through faecal contamination of drinking water. It exists as sporadic hepatitis with periodic resurgence. According to the South East Asia Regional Office of the World Health Organisation (WHO), hepatitis E is widespread in several countries of this region, accounting for up to 90% of all sporadic cases of acute viral hepatitis<sup>(19)</sup>. Contamination of water sources usually occurs either via recession of flood waters from sewerage pipes and open drains, or in crowded living conditions with unsafe water supply and disposal of human waste, as in refugee camps and rapidly-growing urban slums<sup>(20)</sup>.

In the present study, among the 116 serum samples obtained from acute and convalescent hepatitis cases with jaundice, 58.6% (68/116) had detectable levels of HEV IgM antibodies. HEV



**Fig. 2** Agarose gel electrophoresis shows HEV-specific 343 bp amplified product in positive control and HEV IgM positive samples by nRT-PCR. (Lane 1: 100 bp mol. marker; Lane 2: positive control; Lane 3-7: HEV IgM positive samples; Lane 8: negative control).

affects the young adult population (15-40 years) and has a lower attack rate in children below 14 years of age. This is in contrast to HAV in developing countries, where HAV is a childhood disease, and the majority of children below ten years of age has been exposed to the infection<sup>(21)</sup>. In this study, the majority of those infected with HEV were adults within the age group of 21-30 years (Fig.1).

In the case of dual infections, there is potential worsening or sudden onset of liver disease among people contracting hepatitis A, if they already have chronic hepatitis B or C viral infections. However, to date, there has been little data available to determine the degree of HEV infection accelerating or worsening liver damage in people with such chronic hepatitis. In this study, dual infections with HAV and HCV in acute HEV patients have been observed in 4.4% (3/68) and 7.4% (5/68) of cases, respectively, without any sequelae. All the HAV and HEV dual infections were possibly co-infections (having a common route of transmission) and all infected patients were less than 14 years of age. The HCV and HEV were superinfections, with all the cases having a history of prior exposure to procedures relating to a parenteral route, i.e., two had surgery, two had blood transfusion, and one was a drug abuser. Similarly, Nanda et al<sup>(22)</sup> had reported a dual infection of HEV and HCV in 11% of cases.

A high case fatality rate (15-25%) among pregnant women is a characteristic hallmark of HEV infections being observed in many large epidemics reported worldwide. HEV has been associated with cholestatic jaundice, hepatocyte necrosis, and inflammatory cell infiltration in the liver. Miscarriages or premature delivery can also occur following HEV infection<sup>(11)</sup>. Although the attack rate among women of childbearing age was not different from that observed in the general population, the incidence among pregnant women was significantly higher. Hormonal changes and immune complex-mediated vasculitis or glomerulonephritis might be critical factors in deciding the course of HEV infection in pregnancy<sup>(23)</sup>.

Infected pregnant women in the present epidemic had normal deliveries, except for one patient who had to undergo caesarean section, and cord blood of another baby born from an HEV-infected mother tested negative for HEV IgM antibodies. 15 HEV-positive blood samples that were subjected to viral RNA detection by RT-PCR using specific primers against the ORF1 region yielded positive for five patients (including one from a pregnant woman) (Fig. 2). HEV viraemia is transient and HEV RNA remains for one week in the serum after onset of jaundice. For this reason, a few acute cases with a history of 5-6 days of jaundice were included for RT-PCR analysis.

The distribution of cases within the tributaries of common water supply, affecting young adults and pregnant women with HEV IgM and RNA positivity suggests the classic, common source,

waterborne epidemic of HEV. Thus, this study highlights a major disease outbreak that occurred due to contamination of sewerage water with the piped drinking water supply. The sewerage line and drinking water pipeline were closely laid and due to leakage in the sewerage line, the faecally-contaminated wastewater was sucked into the intermittently-supplied drinking water system. In the absence of effective antiviral therapy, control measures for HEV outbreaks should focus on ensuring a safe water supply. Water at the source of distribution and at the consumer end should, therefore, be regularly examined for faecal/sewerage contamination and the preventive measures thereof. Because of the potentiality of extensive outbreaks and associated mortality in pregnant women, concerted efforts must be taken to have an effective vaccine at hand.

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## The 4th Asian Breast Diseases Association (ABDA) Teaching Course

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Spread over two days, the course programme will consist of lectures that will cover comprehensively all aspects of breast imaging and management of breast diseases.

### Course Objectives

At the conclusion of this course, participants will have received substantial knowledge of the newest methods in diagnosis and management of breast diseases.

**Date** : November 3-4, 2006  
**Venue** : The Imperial Mae Ping Hotel, Chiang Mai, Thailand  
 (Venue details at [www.imperialmaeping.com](http://www.imperialmaeping.com))  
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