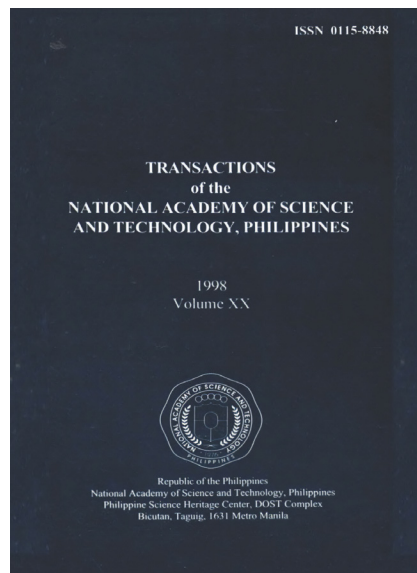


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# Hepatitis E Virus Infection Diagnosed by Serology: A Report of Cases at the San Lazaro Hospital, Manila

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## ***HEALTH SCIENCES DIVISION***

### **HEPATITIS E VIRUS INFECTION DIAGNOSED BY SEROLOGY: A REPORT OF CASES AT THE SAN LAZARO HOSPITAL, MANILA**

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#### **ABSTRACT**

Sixty five patients who presented with jaundice at the San Lazaro Hospital between July to September, 1992, were tested for the viral hepatitis markers by serology. Qualitative enzyme-linked immunosorbent assays were performed using commercial kits from Abbott or Genelabs Technologies, U.S.A. This particular study had as its primary objective, the determination of the proportion of jaundiced patients with hepatitis E virus (HEV) infection, but other serological markers were also used. The test for hepatitis A virus (HAV) and hepatitis C virus (HCV) consisted of detecting total immunoglobulins (mainly IgG) in patients' sera, whereas both IgM and IgG tests were available to detect specific antibodies to HEV. Hepatitis B virus (HBV) infection was tested using the HB s antigen detection kit. Past exposure/immunity to the hepatitis A virus as measured by total antibody against HAV was 98.46%. This confirms the high endemicity of HAV infection in our setting. As these patients are jaundiced, the ongoing inflammation of the liver may be caused by the HBV were HB s antigen positivity was 46.15% among the patients. The symptoms may be due to hepatitis C virus infection in 3% of the patients who were positive for HCV antibodies. Acute or relatively recent HEV infection was diagnosed in 6.15% of the patients who tested positive for IgM anti-HEV. Some 10.76% of the patients were positive only for IgG anti-HEV. This study therefore reports for the first time, the hepatitis E virus as a cause of viral hepatitis among our patients at the San Lazaro Hospital. This is just one of the many viral hepatitis agents that we have to consider among our patients, especially those in the lower socioeconomic group. While management is still basically supportive and the same for all viral agents, prognosis as in hepatitis A infection is much better. compared to HBV or HCV infection.

**Key words:** Hepatitis agents, enteric hepatitis, viral hepatitis, hepatitis markers.

## INTRODUCTION

Hepatitis E, like hepatitis A, is an important public health problem especially in Asia, Africa, and Central America (Bradley 1990; Zuckermann 1990). Like HAV, HEV is also transmitted via the fecal – oral route, and may cause sporadic cases as well as outbreaks of hepatitis, usually traced to contaminated water sources.

Initially described in India (Datta et al, 1987 and Ramalingaswami et al, 1988), HEV infection is now a global disease highly prevalent in developing countries where conditions of hygiene and sanitation are relatively poor.

The isolation of cDNA from the HEV in 1990 (Reyes et al.) paved the way for development of appropriate methods for its diagnosis. The HEV genome was then sequenced and subsequently, recombinant HEV antigens corresponding to the core structural regions were obtained by expression in bacterial cells (Yarborough et al. 1991). Since then, both IgM and IgG anti-HEV tests became available and were used to determine viral etiology especially among patients who were jaundiced, but were negative for the HAV, HBV, and HCV markers of infection, or the other relatively frequent causes of hepatitis like CMV or EBV.

This study reports four (4) cases of acute hepatitis E infection (IgM anti-HEV positive), and seven (7) cases of past infection (IgG anti-HEV positive among patients admitted at the San Lazaro Hospital.

## MATERIALS AND METHODS

### I. Study Population and Blood Collection:

Sixty five patients admitted to the San Lazaro Hospital between July to September, 1992 for jaundice, with primary viral hepatitis as the admitting diagnosis, were included in the study after appropriate informed consent was obtained. Approximately 10 cc of blood was collected aseptically from each patient for determination of the hepatitis profile, ALT/AST determinations, and in some cases, AP and Bilirubin levels. The blood was allowed to clot at room temperature for 2-3 hours and serum separated. Portions were utilized for the various serological tests.

### II. Enzyme-Linked Immunosorbent Assay for the Detection of HEV-Specific Antibodies:

We utilized IgM and IgG kits from Diagnostic Biotechnology (Pte) Ltd., a subsidiary of Genelabs Technologies, U.S.A. Briefly, the principles of the procedure consisted of the following:

Wells of polystyrene microplate strips were coated with recombinant HEV antigens, corresponding to core structural regions of the hepatitis E virus. Human sera diluted in appropriate buffer were incubated in the coated wells. If the patients' sera contained HEV-specific antibodies, these bound the solid phase

HEV antigens. The wells were then thoroughly washed to remove unbound materials and an affinity purified anti-human IgG or anti-human IgM-labelled with horseradish peroxidase added to the wells. This labelled antibody bound any antigen-antibody complexes previously formed and excess unbound labelled antibodies were removed by washing. A substrate solution containing hydrogen peroxide and O-phenylene diamine (OPD) was then added to each well. The presence of specific antibodies was indicated by formation of yellow-orange color after substrate addition. Reaction was terminated by addition of sulfuric acid. The intensity of the color was measured spectrophotometrically at 492 nm and was directly proportional to the amount of antibodies present in the patient's specimen.

The presence or absence of HEV-specific antibodies was determined by relating the absorbance of the specimens to the cut-off value (COV) of the plate.

Specimens with absorbance values less than cut-off value were considered NON-REACTIVE. Specimens with absorbance values greater than or equal to the COV were considered initially reactive and retested. Specimens found reactive on retesting were interpreted to be repeatedly reactive, or considered REACTIVE for antibodies to HEV.

- III. ELISA's for determination of HAV and HCV specific antibodies and the Hbs antigen utilized the Abbott reagents, which basically used the same principle as explained above.

## RESULTS AND CASE PRESENTATION

### I. Profile of 65 Patients Included in the Study:

Number of male patients = 47 (73%)

Number of female patients = 18 (27%)

Mean Age = 31 years (SD 16.62)

Residents of Manila belonging  
to lower socioeconomic status

### II. Summary of Reactivities of Patients' Sera for the Viral Hepatitis Markers:

Marker	Number Reactive Over Total Patients	Percent Reactive
IgG anti-HAV	64/65	98.46%
Anti-HCV	2/65	3.07%
Hbs antigen	30/65	46.15%
IgM anti-HEV	4/65	6.15%
IgG anti-HEV	7/65	10.76%

**III. Case Presentation of Four Patients Positive for IgM Anti-HEV:**

1. **CASE 1** = A 22 year old female housewife from Manila consulted because of moderate grade fever of a few days' duration, accompanied by headache and body malaise, followed by yellowing of the sclerae and tea-colored urine. RUQ tenderness was noted on admission, with icteric sclerae and hepatomegaly. ALT and AST taken on two occasions were between 462-596 IU per liter. Liver ultrasound revealed diffuse liver disease consistent with hepatitis. The patient was positive for IgG anti-HAV, also HB S Ag(+), and negative for anti-HCV. She stayed in the hospital for 12 days, and was discharged uneventfully.
2. **CASE 2** = A one year, 3 month-old baby boy from UP Diliman, was brought to SLH because of moderate grade fever of one week duration, followed by yellowing of the sclerae and skin. On admission, the baby was observed to have icteric sclerae, with liver palpable 2-3 cm below the right costal margin. ALT/AST were only slightly elevated to 146-166 IU/liter. At this young age, the baby was already positive for IgG anti-HAV, and also positive for HBsAg. He was negative for anti-HCV, but liver ultrasound showed patterns consistent with hepatobiliary TB. No further tests were done, and the patient was discharged after 28 days.
3. **CASE 3** = A 29 year-old male worker from Manila was admitted for high grade fever with chills, sweating, and headache. This was observed since one month PTC, later on accompanied by dark-colored urine and terminal dysuria. Malarial smear done 3x was negative and empiric therapy for typhoid fever provided no relief. On admission, his temperature was 39 °C, but he had anicteric sclerae, and no hepatomegaly. He was found to have a tender abdomen in the hypogastric area. Liver ultrasound on admission revealed normal findings, but a repeat ultrasound 10 days later showed diffuse liver disease consistent with hepatitis. IgM anti-HAV done at SLH was non-reactive, Hbs antigen and Anti-HB c were non-reactive, and anti-HCV was also non-reactive. He absconded on the 21st day of hospitalization.
4. **CASE 4** = A 32 year-old male worker from Manila consulted because of on and off moderate grade fever since one month PTC, associated with RUQ pain and icteric sclerae of 3 weeks' duration. On admission, the patient was febrile, with a temperature of 39°C, with liver palpable 9 cm below the RCM, with direct tenderness. ALT/AST were only slightly elevated at 80-109 IU/liter. Malarial smear was negative, while ultrasound was consistent with an acute pancreatitis. The patient was reactive for IgG anti-HAV and anti-HB s, and non-reactive for HB s antigen, anti-HBc, and anti-HCV. He stayed for 11 days in the hospital, having been managed as a case of acute pancreatitis.

**IV. Profile of Seven Patients Positive for IgG Anti-HEV:**

1. EA, 49 years old, male, IgG anti-HAV (+) also
2. AO, 15 years old, male, IgG anti-HAV (+) and HB s Ag (+)
3. MN, 43 years old, male, IgG anti-HAV (+)
4. MI, 30 years old, male, IgG anti-HAV (+)
5. LR, 24 years old, male, IgG anti-HAV (+)
6. CD, 31 years old, female, IgG anti-HAV (+) and HB s Ag (+)
7. DF, 50 years old, male, IgG anti-HAV (+) and HB s Ag (+)

All are from the Metro Manila area.

**DISCUSSION AND CONCLUSIONS:**

This study has shown that approximately 6-7% of acute viral hepatitis cases seen at the San Lazaro Hospital are due to the hepatitis E virus. This figure is somewhat lower compared to HAV being the cause of acute hepatitis in some 25-30% of jaundiced patients. It appears that HAV is more ubiquitous than HEV, although HEV also has the potential to cause large epidemic outbreaks of viral hepatitis, in particular, in geographic areas with inadequate water supply and toilet facilities and where public sanitation is generally poor.

Based on the presenting symptoms and duration of the illness, HEV clinically resembles hepatitis A. Thus, it is also usually a self-limited syndrome of fever, malaise, fatigue, nausea, transaminase elevation, and hyperbilirubinemia. Most cases resolve within weeks, leaving no evidence of chronicity. The patients we presented here were all discharged uneventfully, despite some staying just a bit too long in the wards.

The 10-11% of patients who were found to have IgG anti-HEV could have had their infection or exposure within the year. It has been observed that IgG antibodies directed against HEV usually disappear within one year after infection (Goldsmith et al. 1992). All these patients were also positive for IgG anti-HAV, (even the younger - 15 year-old male patient), possibly reflecting high exposure to these fecally-orally acquired agents in environments that are substantially lacking in hygienic practices and conditions of sanitation.

The rest of the findings, e.g., that of the Hbs antigen positivity only confirms the high endemicity of HBV infection in our setting. The 3% anti-HCV positivity is also consistent with reports on the general population/healthy blood donors of approximately 2.3% anti-HCV positivity (Katayama et al. 1996).

With etiologic diagnosis of primary cases of viral hepatitis being available to us, we can then monitor and advise patients accordingly, and plan appropriate programs for intervention and control.

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