

# ETIOLOGY OF ENTERICALLY-TRANSMITTED HEPATITIS AMONG FOREIGNERS IN NEPAL

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**Abstract.** We report the etiology of hepatitis in travelers over a ten year period from January 1994 to December 2003. Clinics catering to expatriates and tourists in endemic Nepal provided sera for diagnostic testing from persons with signs and symptoms compatible with clinical hepatitis and alanine transaminase levels 2 1/2 times greater than normal. Hepatitis E was determined with anti-HEV IgM, and HEV RT-PCR, and hepatitis A was determined using HAV-IgM. Thirty-seven cases of hepatitis A and 30 cases of hepatitis E were diagnosed during the study period. The frequency of hepatitis A cases decreased with the increasing use of hepatitis A vaccine while the frequency of hepatitis E cases remained stable. A hepatitis E vaccine would be of benefit for travelers to high to high risk areas.

## INTRODUCTION

Travelers to developing countries are at risk for developing enteric transmitted viral hepatitis. The transmission and clinical manifestations of hepatitis A (HAV) and hepatitis E (HEV) are similar. They spread by contaminated food or water, and present after a 2-8 weeks incubation period with jaundice, usually accompanied by malaise, anorexia, abdominal discomfort and liver enlargement. Convalescence is frequently prolonged, with malaise, hyperbilirubinemia and elevated aminotransferase levels taking six or more weeks to normalize. Either virus may have a fulminant presentation resulting in death. HEV is unique among viral hepatitis in that

infection during pregnancy results in a high mortality, up to 25% or more (Krawczynski *et al*, 2005).

The HAV vaccine results in long lasting protection (Centers for Disease Control, 1995) which has the potential to eliminate travel-related HAV if put into widespread use. HEV in tourists and expatriates is under reported (Shlim and Innis, 2000; Zuckerman, 2003) and there is no commercially available vaccine against HEV.

The incidence of enterically-transmitted hepatitis is high in Nepal (Clayson *et al*, 1997). The presence of clinics catering to tourists and expatriates in Nepal in addition to a hepatitis research facility offer an opportunity to study the etiologies of hepatitis and estimate their frequencies among tourists and expatriates presenting to these clinics. A study evaluating these frequencies was carried out over a 10-year period from January 1994 to December 2003.

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## MATERIALS AND METHODS

### Patient population

The patient population consisted of tourists and foreign residents in Nepal, from nonhepatitis-endemic countries, who presented to the Canadian International Water and Energy consultants (CIWEC) Travel Medicine Center, the Nepal International Clinic, or the United States Peace Corps Medical Unit from January 1994 to December 2003 with a serum alanine aminotransferase (ALT) (Randox Laboratories)  $>2.5$  times the laboratory normal ( $>105$  IU/ml) and symptoms compatible with hepatitis. Further diagnostic tests were requested by the evaluating physician. The subjects' personal identifiers were removed on receipt of the specimens by the Department of Virology-Armed Forces Research Institute of Medical Science (VD- AFRIMS).

Annual figures of the number of non-Indian tourists visiting Nepal were provided by the Nepal Tourism Board. The estimated number of expatriates, excluding Peace Corps volunteers, was derived from a census performed by the CIWEC clinic in 1994. The number of Peace Corps volunteers in Nepal and their assignments were provided by the Peace Corps Office in Nepal or extracted from annual Peace Corps reports to the United States Congress.

### Hepatitis E surveillance

An indicator of hepatitis E activity in the Kathmandu valley was obtained from discharge statistics from six sentinel hospitals officially designated to admit jaundiced patients.

### Sera collection and laboratory tests

Submitted sera were stored by the Walter Reed AFRIMS Research Unit Nepal (WARUN) at  $-90^{\circ}\text{C}$  and forwarded by air express on dry ice to the VD-AFRIMS, Bangkok, Thailand, for testing. To evaluate

the sera, commercially available enzyme-linked immunosorbent assay (EIA) kits were used (Abbott Laboratories, Abbott Park, IL): hepatitis A virus (HAV) infection was diagnosed with anti-HAV-IgM, hepatitis B virus (HBV) was diagnosed with anti-HBc IgM and HBsAg, hepatitis C virus (HCV) was diagnosed with anti-HCV, and hepatitis E virus (HEV) was diagnosed with anti-HEV IgM or HEV reverse transcriptase polymerase chain reaction (RT-PCR) (Tsarev *et al*, 1999; Seriwatana *et al*, 2002). A diagnosis of HEV was made if the RT-PCR was positive and/or the anti-HEV IgM level was greater than 100 WR units per milliliter.

### Statistical analyses

Results were analyzed using Student's *t*-tests and chi-square tests performed with Analyse-it for Microsoft Excel (Analyse-It Software, Leeds, UK), incidence rates were estimated as the number of illnesses observed divided by the time at risk for the event during the observation period. Confidence limits were calculated using the Poisson distribution (Selvin, 1996).

## RESULTS

During the study period, CIWEC clinic had approximately 52,500 visits, about half were expatriates and the other half were tourists. During this period 138 patients ( $\sim 0.26\%$ ) were considered to have possible hepatitis. Due to physician preference, personnel and logistics, diagnostic assays were carried out in 76 (55.1%) of these patients: 42 expatriates and 34 tourists. Of these, enteric viral hepatitis was confirmed in 53 (69.7%) (Fig 1): 32 cases of HAV and 21 cases of HEV infection (2 of which were also HBV carriers). Three cases of HBV and 2 cases of HCV infection were also detected. Four cases had dual infections: one patient with HEV/HAV infection, one with HEV/HBV infection, one with HAV/HBV infection and one

Table 1  
Number and origin of expatriates and travelers diagnosed as having enteric hepatitis at the CIWEC Clinic from January 1994 to December 2003.

Origin of patient	Hepatitis A	Hepatitis E	Total
Africa	0	0	2
Australia/New Zealand	0	4	5
Europe	10	8	29
Japan	7	1	10
North America	15	8	30
Total	32	21	76

with HEV/HCV infection. All of the viral enteric hepatitis cases occurred in adults, with the exception of 3 cases of HAV infection which were documented in unvaccinated persons less than 18 years of age: 2 children age 6 and one child age 13. There were no cases of fulminant hepatitis and no deaths. The distribution by region of origin, for those diagnosed with HAV and HEV infection is shown in Table 1.

Of the 21 CIWEC cases diagnosed as having HEV infection, 38% (8) were tourists and 62% (13) were expatriates. Six of the 8 tourists had HEV infection, whose travel history was documented. They had been traveling in South Asia for one to four months. In the expatriates, they had been residents of Nepal for 1 to 32 years. There were no significant differences in HEV infection frequency by mean age or gender between the two groups. However, over the ten year period, the seasonality of HAV and HEV infections differed (Fig 2). HAV infection occurred throughout the year, while HEV infection occurred only during the pre-monsoon and monsoon seasons, from March through October. For HEV, the distribution of cases over the ten year period reflected the level of hepatitis activity estimated from hepatitis E surveillance (Correlation coefficient = 0.72) (Fig 3).

There were no cases of HAV infection

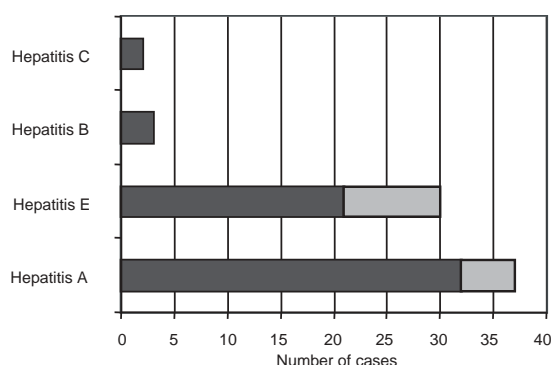
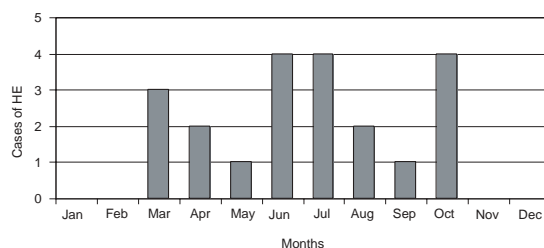
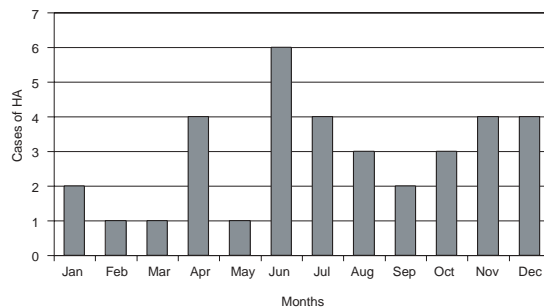


Fig 1—Number and types of hepatitis cases during the study.



HE, hepatitis E virus infection



HA, hepatitis A virus infection

Fig 2—Cases of hepatitis by type per month.

Table 2  
Estimated Annual Attack Rates of hepatitis E.

Category	Total cases (1994-2003)	$N^a$	Estimated Annual Attack Rate <sup>b</sup> (per 100,000)	95% Confidence intervals
Travelers	8	11,614 <sup>c</sup>	6.89	2.97 - 13.57
Expatriates	13	1,875 <sup>d</sup>	69.33	36.92 - 118.56
Peace Corps	2	119.5 <sup>e</sup>	167.36	20.27 - 604.54

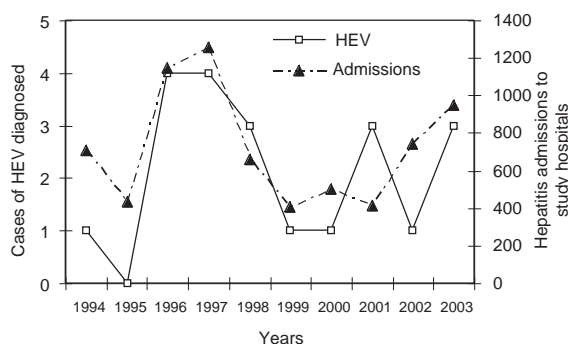
<sup>a</sup>Estimated average man years of exposure in Nepal

<sup>b</sup>Estimated Annual Attack Rate = (Total cases  $10 \times N$ )  $\times$  100,000

<sup>c</sup>Number of non-Indian tourist visits provided by the Nepal Tourism Board average tourist stay of 15 days (282,605  $\times$  15,365 = 11,614)

<sup>d</sup>Estimated expatriate population in Nepal = 2,500. The proportion seen at CIWEC Clinic = (0.75  $\times$  2,500 = 1,875)

<sup>e</sup>Extracted from Annual Reports to the US Congress



HEV, hepatitis E virus infection

Fig 3-HEV cases and admissions per year.

among travelers who received the HAV vaccine. In the HEV infection cases, of these recorded, half had not received an HAV vaccine and/or were HAV Ig negative.

In addition to the cases from the CIWEC clinic, there were 9 clinical cases of HEV infection and 5 cases of HAV infection diagnosed among expatriates and tourists seen at the Nepal International Clinic and the United States Peace Corps Medical Unit. The total number of cases from non-endemic countries detected in Nepal during the de-

cade studied was 37 for HAV infection (all HAV IgM positive) and 30 for HEV infection (12 RT-PCR positive, 11 HEV IgM positive and 7 in which by both methods were positive); both assays were not performed in all cases.

## DISCUSSION

In a previous report by the CIWEC Clinic for 1986-1988, HEV infection accounted for 9% of viral hepatitis cases out of a series of 43 patients (Clayson *et al*, 1995). During the study period from January 1994 to in December 2003, HEV infection accounted for 45% of viral hepatitis cases. Of the sera from CIWEC tested, 32% failed to yield a diagnosis of viral hepatitis. These undiagnosed cases may have resulted from the timing of single specimen collection, or from other hepatopathic agents known to be prevalent in Nepal, such as leptospirosis, scrub typhus, or murine typhus (Murdoch *et al*, 2004) or from other causes.

The HAV vaccine became available in Europe in 1992 and in the United States in 1995. The study period can be divided into 2 periods based on the use of the HAV vac-

cine: January 1993 to December 1998 and January 1999 to December 2003. Comparing the two time periods, the number of foreigners diagnosed with HAV infection in Nepal fell from 24 cases to half that level. The halving of HAV cases after the introduction of the vaccine, coupled with the absence of HAV cases in those who were fully immunized demonstrates the efficacy of the HAV vaccine under field conditions. Therefore, the appropriate application of the HAV vaccine in travelers could ultimately eliminate HAV in this group. There is currently no vaccine available for HEV, which occurred at the same frequency (averaging 3 cases/year) through both time periods represented 56% of the enteric hepatitis cases diagnosed.

The risk for HEV infection has been underrated by travelers. No cases of HEV have been reported in Israeli backpackers to the developing world (Potasman *et al*, 2000) or in North American missionaries, mostly to sub-Saharan Africa (Smalligan *et al*, 1995). However, as was noted by these authors, the studies examined relatively small populations, and no information was provided about the endemicity of hepatitis E or the seasonality of travel in the areas visited. Additionally, recent GeoSentinel findings indicate that HEV infection is seldom detected in travelers to areas outside of South Asia (Freedman *et al*, 2006).

In 2002 Piper-Jenks' exhaustively reviewed the travel medical literature, finding only 12 reports (Piper-Jenks *et al*, 2000), totaling 161 cases of HEV infection. Most of these were acquired in South Asia (of which 17 from Nepal are also recorded here). Our 10-year study of 30 cases of HEV infection, from only one of the world's endemic destination countries, increases by over 20% the total number of travel-related cases reported in the world's literature. Wider surveillance would likely identify many more cases.

In Nepal, the incidence of HEV infection is high (Clayson *et al*, 1997), similar to that seen in returnees from India to Australia (Cowie *et al*, 2005). The frequency of HEV infection cases among travelers mirrors the epidemic situation in the indigenous population. With increased HEV testing, increases in the frequency of HEV infection diagnoses in travelers are expected and are likely to reflect the timing and degree of transmission in the regions visited.

HEV infection attack rates among travelers are not available, as insufficient cases have been studied with known exposure times in destinations with known high attack rates. Although our data regarding tourists and expatriates are by necessity approximations, we attempted to estimate attack rates for the groups studied (Table 2). For tourists, this is clearly an underestimation as the period of travel is usually during the non-epidemic season. The exposure time is usually limited to an average of two weeks in Nepal. The incubation period for HEV infection means the majority of tourist cases will occur after leaving Nepal. Expatriates are at much higher risk for infection due to continuous presence in an endemic country. However, our findings are again an underestimation because laboratory results were available in only 53% of patients meeting the study criteria for viral hepatitis. Despite wide confidence intervals due to small numbers, a more realistic estimate comes from a study of 1,195 United States Peace Corps volunteers. Two HEV cases were detected in this group giving an annual attack rate per 100,000 of 167 (95% confidence interval 27.7-604.5).

Changes in the epidemiology of viral hepatitis, due to the introduction of HAV vaccine are described in this paper. The decrease in the proportion of HAV cases over the study period indicate that HAV may be avoided by appropriate vaccination. Cur-

rently, no vaccine is commercially available for HEV; dietary precautions and hand washing are the only prophylaxis available. Recently, in a proof of principal trial, a recombinant HEV vaccine was found to be highly effective in preventing HEV infection (Shrestha *et al*, 2007). The vaccine was designed for the developing world where HEV is endemic, however, if it becomes available, tourists, diplomats and expatriates in HEV endemic areas are at sufficient risk from HEV disease for vaccination to be considered.

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The opinions and assertions contained herein are the private views of the authors and are not to be construed as official or reflecting the views of the US Department of

the Army or the US Department of Defense. The use of trade names is for identification only and does not constitute endorsement by the US Government.

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