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# Seroepidemiology of Hepatitis E Virus in Patients With Non-A, Non-B, Non-C Hepatitis in Hungary

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Many cases of acute hepatitis remain undiagnosed and the hepatitis E virus (HEV) is emerging in industrialized countries. The aim of this study was to assess the role HEV as causative agent in acute non-A, non-B, and non-C hepatitis patients in Hungary. 10.5% of the 264 acute non-A, non-B, and non-C hepatitis patients tested had anti-HEV IgG and 1.9% had anti-HEV IgM as tested by ELISA. After confirmation by Western blot 6.1% of the acute non-A, non-B, and non-C hepatitis patients had anti-HEV IgG antibodies only and 1.1% of the patients had both IgG and IgM. All 19 patients that were positive for anti-HEV IgG and/or IgM tested negative for HEV RNA by PCR. Only a small proportion of the acute hepatitis cases in the southwest of Hungary are assumed to be attributed to HEV infection, however, hepatitis E should be considered along with hepatitis A, B, and C in the diagnosis of acute hepatitis. ***J. Med. Virol. 79:927–930, 2007.*** 

**KEY WORDS:** hepatitis E virus; HEV; seroprevalence; non-A, non-B, non-C hepatitis; anti-HEV antibodies; Hungary

## INTRODUCTION

Acute hepatitis is a major public health problem that is caused by a variety of viruses. In developed countries hepatitis E virus (HEV) infection is considered an emerging disease. Infections with HEV are recognized mostly as imported cases, in relation to travel to countries where HEV is endemic [Aggarwal and Krawczynski, 2000]. HEV spreads predominantly by the faecal-oral route, although zoonotic foodborne transmission and transmission by blood transfusion is described [Arankalle and Chobe, 2000; Tei et al., 2003; Yazaki et al., 2003; Khuroo et al., 2004; Mitsui et al., 2004; Takahashi et al., 2004].

The diagnosis of an HEV infection is dependent mainly on HEV-specific IgM and IgG detection at the onset of acute hepatitis. The aim of this study was to investigate the presence of anti-HEV IgG and IgM antibodies in hepatitis patients in Hungary.

## MATERIALS AND METHODS

Serum samples were obtained from 264 acute hepatitis patients from the years 2003 and 2004 from the southwest region of Hungary. The serum samples were negative for hepatitis A, B, C, cytomegalovirus and Epstein-Barr virus. The average age of the patients was 49 years (range 2–87 years). The gender ratio male/female was 46.6/53.4. No information was available on travel history to endemic regions. The samples had been stored at −20°C until testing. For comparability within he Foodborne Viruses in Europe (FBVE) network, testing for HEV specific IgG, IgM, and RNA was undertaken in the same way as in the Netherlands using published and validated methods [Waar et al., 2005; Herremans et al., 2007].

## RESULTS

A total of 264 serum samples from acute non-A, non-B, and non-C hepatitis patients tested for the presence of anti-HEV IgG or IgM by ELISA, 28 (10.6%) were positive for IgG, and 5 (1.9%) were positive for IgM. One of these 5 IgM positive samples was also positive for IgG. Thirty-two samples were examined further by Western blot (Table I).

Of the 28 (10.6%) anti-HEV IgG positive serum samples screened by ELISA, 19 (7.2%) were confirmed by the Western blot assay. Of the 5 (1.9%) originally anti-HEV IgM ELISA-positive serum samples, only 1 (0.4%)

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TABLE I. Characteristics of Serum Samples that Tested Positive in the Anti-HEV IgG and/or IgM ELISA

| | | Anti-HEV IgG | | | Anti-HEV IgM | | | |
|---|---|---|---|---|---|---|---|---|
| Age | Gender (m, male; f, female) | ELISA ratio | Western blot score | Result | ELISA ratio | Western blot score | Result | Interpretation |
| 56 | m | **6,18** | **11** | + | **2,10** | **8** | + | Acute HEV infection[a] |
| 33 | f | **2,24** | **9** | + | 0,05 | **7** | + | Acute HEV infection but missed by IgM ELISA[b] |
| 48 | f | **1,52** | **5** | + | 0,03 | **6** | + | Acute HEV infection but missed by IgM ELISA |
| 32 | m | **5,64** | **9** | + | 0,05 | 0 | − | HEV infection in the past[c] |
| 55 | m | **6,50** | **9** | + | 0,33 | 2 | − | HEV infection in the past |
| 79 | f | **1,66** | **7** | + | 0,03 | 0 | − | HEV infection in the past |
| 56 | m | **5,14** | **6** | + | 0,30 | 3 | − | HEV infection in the past |
| 61 | m | **6,04** | **9** | + | 0,23 | 2 | − | HEV infection in the past |
| 63 | m | **4,06** | **8** | + | 0,25 | 2 | − | HEV infection in the past |
| 70 | m | **6,22** | **7** | + | 0,03 | 5 | − | HEV infection in the past |
| 57 | f | **3,10** | **6** | + | 0,03 | 0 | − | HEV infection in the past |
| 54 | m | **1,90** | **6** | + | 0,00 | 4 | − | HEV infection in the past |
| 82 | m | **4,08** | **6** | + | 0,03 | 4 | − | HEV infection in the past |
| 69 | m | **3,76** | **5** | + | 0,05 | 1 | − | HEV infection in the past |
| 57 | f | **2,34** | **5** | + | 0,03 | 2 | − | HEV infection in the past |
| 64 | m | **1,92** | **5** | + | 0,00 | 5 | − | HEV infection in the past |
| 58 | f | **4,98** | **5** | + | 0,43 | 2 | − | HEV infection in the past |
| 77 | f | **1,68** | **4** | + | 0,03 | 0 | − | HEV infection in the past |
| 50 | f | **2,72** | **4** | + | 0,03 | 0 | − | HEV infection in the past |
| 81 | m | **5,30** | 3 | − | 0,03 | 0 | − | No indication for acute HEV infection[d] |
| 50 | m | 0,02 | 3 | − | **1,20** | 5 | − | No indication for acute HEV infection |
| 39 | m | 0,04 | 2 | − | **1,13** | 5 | − | No indication for acute HEV infection |
| 37 | m | **1,68** | 3 | − | 0,05 | 3 | − | No indication for acute HEV infection |
| 57 | f | **2,38** | 3 | − | 0,45 | 5 | − | No indication for acute HEV infection |
| 50 | f | **5,80** | 3 | − | 0,05 | 1 | − | No indication for acute HEV infection |
| 70 | f | **1,48** | 2 | − | 0,00 | 0 | − | No indication for acute HEV infection |
| 25 | f | 0,04 | 2 | − | **1,13** | 5 | − | No indication for acute HEV infection |
| 51 | m | **1,58** | 1 | − | 0,03 | 0 | − | No indication for acute HEV infection |
| 79 | f | **1,12** | 1 | − | 0,03 | 2 | − | No indication for acute HEV infection |
| 26 | m | **2,96** | 0 | − | 0,03 | 1 | − | No indication for acute HEV infection |
| 34 | f | 0,04 | 0 | − | **1,48** | 3 | − | No indication for acute HEV infection |
| 19 | f | **1,70** | * | ? | 0,08 | 0 | − | Retest, request new sample |

**Bold fonts** are considered positive in that test.
[a]Confirmed positive for IgG and IgM indicates an acute HEV infection.
[b]Confirmed positive for IgG and positive in the IgM Western blot but negative in the IgM ELISA; this could indicate an acute HEV infection with false negativity in the IgM ELISA, it could however also indicate a past infection with false positivity in the IgM Western blot.
[c]Positive for IgG and negative for IgM: indicates HEV infection in the past (high IgG values may however indicate a recent infection).
[d]No confirmed positive result for either IgG or IgM means there are no serological indications for an acute HEV infection.
*Blot could not be read.

was confirmed by Western blot and this patient was also positive for anti-HEV IgG. In addition, two initially ELISA IgG positive but IgM negative samples were positive for anti-HEV IgM and IgG by the Western blot assay.

Using the combined testing regime of ELISA and Western blot, a total of three patients (1.1%) were diagnosed as acute HEV patients since they were positive for both IgM and IgG antibodies. In 16 patients (6.1%) only a solitary anti-HEV IgG response was detected. No patients with confirmed anti-HEV IgM in the absence of IgG were detected.

The age dependent anti-HEV IgG prevalence shows a significant increase ($P < 0.05$) from none for people younger than 30 years (n = 48) to 11.6% for people older than 59 (n = 69). The highest prevalence found was 17.6% for men over 60 years of age (n = 34) but this was not significantly different from the seroprevalence observed in the matching female group (5.7%, n = 35). There were also no significant differences detected in the seroprevalence of anti-HEV IgG antibodies within the female and male group and between the other age groups.

The 19 acute non-A, non-B, and non-C hepatitis patients considered serologically positive for either anti-HEV IgG, IgM, or both, all tested negative with RT-PCR for the presence of HEV RNA.

## DISCUSSION

After the combined testing regime of ELISA and Western blot, a total of 19 acute non-A, non-B, and non-C hepatitis patients (7.2%) were considered positive for anti-HEV IgG. This seroprevalence is comparable to cohorts of comparable patients in countries such as the United States, Italy, and the Netherlands were, 4.9% (10/204), 10.1% (22/218), and 8.8% (27/305) HEV patients were identified, respectively [Karetnyi et al., 1999; Zanetti et al., 1999; Herremans et al., 2007].

In this study, only 68% of the anti-HEV IgG positive samples by ELISA could be confirmed by Western blot and of the five samples positive for IgM by ELISA, only 20% could be confirmed by this blot test. These confirmation rates are lower than those found in a comparable study in the Netherlands where, respectively, 90 and 49% of the ELISA positive anti-HEV IgG and IgM samples were confirmed by Western blot [Herremans et al., 2007]. False negativity in this Hungarian study with the IgM Western blot assay might be explained by omission of IgG depletion prior to IgM testing. In the Dutch study, IgG antibodies were depleted from the serum prior to IgM testing, in order to reduce interference of IgG antibodies with IgM assay [Herremans et al., 2007]. However, both studies do show that confirmation of ELISA positive results by Western blot is important to exclude false anti-HEV IgG and IgM responses.

The seroprevalence of anti-HEV antibodies is significantly higher among acute non-A, non-B, and non-C hepatitis patients aged $\geq$60 years then among those aged $\leq$29 years. An age-related increase in the prevalence of anti-HEV antibodies has also been shown in serosurveys of hepatitis patients in endemic [Arankalle et al., 1995; Jameel, 1999; Aggarwal and Krawczynski, 2000] and non-endemic areas [Mizuo et al., 2002; Daniel et al., 2004]. Nonetheless, it is remarkable that in the present study none of 48 acute hepatitis patients younger than 30 years of age had anti-HEV IgG.

It was found that the prevalence of anti-HEV IgG antibodies was identical in both sexes up to the age of 59 years. In the patient group of 60 years and older, however, three times as many males as females with anti-HEV IgG were found. Even though this was not statistically significant it might indicate that older men are at risk of HEV infection.

None of the 19 acute hepatitis patients that were positive serologically for a past or acute HEV infection, had HEV RNA. Based on serological results only three cases of acute HEV infections were detected (i.e., IgM positive). In previous studies in the Netherlands a PCR positive rate of 51% was found in samples positive for anti-HEV IgG and IgM (n = 45) [Herremans et al., 2007]. This indicates that the fact that no HEV RNA was found in the three serologically positive samples is not an unexpected result. It is known that HEV disappears from the bloodstream soon after symptoms become apparent and antibodies appear. However, patients with faecal shedding of HEV without detectable viraemia have been described [Aggarwal et al., 2000], indicating that faecal samples might be a valuable specimen in acute hepatitis diagnosis.

In this retrospective study no data on travel history could be obtained, neither were any HEV sequences obtained. However, in a recent study in Hungary, hepatitis patients without travel history were positive by RT-PCR for HEV [Reuter et al., 2005]. The HEV sequences were identified as genotype 3 strains, indicating that HEV genotype 3 viruses are endemic in Hungary.

In conclusion, based on serological data, HEV infections do occur in the human population of Hungary and the seroprevalence is comparable to that of other industrialized countries [Psichogiou et al., 1995; Karetnyi et al., 1999; Zanetti et al., 1999; Herremans et al., 2007]. Furthermore, confirmation of ELISA positive samples by Western blot is recommended for both anti-HEV IgG and IgM responses. This study shows that hepatitis E should be considered along with hepatitis A, B, and C in the diagnosis of acute hepatitis.

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