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Hepatitis E virus infection in different groups of Estonian patients and people who inject drugs

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ABSTRACT

Background: Previously we demonstrated a high prevalence of hepatitis E virus (HEV) in domestic pigs and wild boars, the main reservoir and possible source of HEV infections in humans. But so far there are no reports about spread of HEV in Estonian human population.
Objectives: The present study aimed to determine the prevalence and genotyping of HEV in different groups of the Estonian adult population.
Study design: Totally 1426 human serum samples were tested (763 patients with clinically diagnosed nonA/B/C hepatitis, 176 hemodialysis patients, 282 patients with suspected HEV infection and 205 people who injected drugs (PWID)). Presence of anti-HEVantibodies was assessed by ELISA and confirmed by immunoblotting. All anti-HEV positive sera were analyzed for RNA by qPCR. Amplified ORF2 region was sequenced and used for phylogenetic analysis.
Results: Antibody assay revealed 49 samples from 1426 (3.4%) with acute (17) or past (32) HEV infection. HEV RNA was detected in 10 anti-HEV IgM positive samples, including 9 samples from patients with suspected HEV infection and 1 hemodialysis patient. Anti-HEV IgG were found in 7.8% patients with suspected HEV infection, in 4% hemodialysis patients, in 2.4% PWID and in 1.96% patients with nonA/B/C hepatitis. All groups demonstrated a trend to share of anti-HEV seroprevalence increasing with age. Phylogenetic analysis of 9 HEV RNA sequences revealed that 3 sequences belonged to HEV genotype 1; 6 ones to genotype 3 (1 sequence belonged to sub-genotype 3a, two ones – sub-genotype 3e, and three ones – to sub-genotype 3f).
Conclusions: Despite the high seroprevalence among domestic pigs, no evidence of HEV transmission from Estonian pigs to humans was found. The results of our study suggest that HEV infections in Estonia are most likely associated with travel or with consumption of imported food products.

1. Background

Hepatitis E virus (HEV) infection is a worldwide disease [1]. HEV is classified into at least four major mammalian genotypes and numerous subtypes. HEV genotypes appear to have a specific geographical distribution. To date, HEV genotypes 1 and 2 have only been reported to infect humans. They are commonly associated with a water-borne transmission route and are known to cause either sporadic cases or outbreaks in developing countries. Genotypes 3 and 4 appear to be responsible for acute sporadic cases and limited food-borne outbreaks in industrialized countries including Europe. Genotypes 3 and 4 are considered zoonotic and have been isolated from humans as well as from animal hosts e.g. pigs, wild boars, deer and other mammals [2]. However, the source of HEV infection in many sporadic acute hepatitis

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cases remains ambiguous [3], and the epidemiology often seems paradoxical. For instance, in Egypt, despite a nearly 100% IgG seroprevalence to HEV in pregnant women, an extremely low number of severe cases are observed [4]. In contrast, the proportion of severe cases is much higher in India, where the rate of IgG sero-prevalence does not exceed 30% [5]. In India there are no registered cases of genotype 4 infection, although it is highly prevalent in Indian swine herds and is documented to be a significant cause of the infection in China and Japan [6]. In Bolivia, a study showed that sequences from swine belonged to sub-genotype 3i, while human sequences belonged to subgenotype 3e [7]. The mechanisms of the dissociation of HEV strains between animal hosts and humans are still unknown.

Anti-HEV antibodies have been found in healthy humans living in different geographical areas, indicating that unapparent HEV infections among adults are common. In general, prevalence rates are higher in endemic areas where hepatitis E is common than in developed countries of non-endemic regions [6]. Anti-HEV IgG are frequently detected in volunteer blood donors, transfusion recipients, hemodialysis patients and people who inject drugs (particularly in HIV carriers), suggesting that HEV could be transmitted parenterally [8–10]. The vertical transmission of HEV from mother to child is well documented [11].

2. Objectives

In Estonia, HEV is not officially listed as a major biological hazard for human, and so far, there are no reports about the spread of HEV in Estonia. Our previous studies demonstrated a high prevalence of HEV in domestic pigs and wild boars which were suggested to be the main reservoir of HEV [12]. The aims of the present study included evaluation of HEV seroprevalence in humans and genotyping HEV attributed to different risk groups in Estonian adult population.

3. Study design

3.1. Serum samples

Serum samples were collected from several groups of hospitalized patients and from people who inject drugs (PWID) between 1994 and 2017.

1. Patients with nonA/B/C hepatitis: 763 serum samples from patients submitted to the National Institute for Health Development (NIHD) for laboratory diagnosis of acute or chronic hepatitis were analyzed for the presence of viral antigens or/and antibodies to hepatitis A virus (HAV), hepatitis B virus (HBV) or hepatitis C virus (HCV). All patients negative for HAV, HBV and HCV, were assigned to the nonA/B/C hepatitis group. Samples were collected from 1994 to 2000 and retrospectively analyzed for the presence of antibodies against HEV.
2. Hemodialysis patients included 176 sera from all patients undergoing hemodialysis in West Tallinn Hospital from 1996 to 2006. All sera retrospectively analyzed for HEV infection were negative for HAV, HBV and HCV.
3. Patients with suspected HEV infection included 282 sera from individuals who addressed to NIHD for laboratory diagnosis of HEV in period 2011–2017. All patients had clinical signs of acute hepatitis, but were negative for HAV, HBV and HCV. Serum samples were analyzed immediately.
4. PWID group included 205 sera collected in 2007, 2009 and 2010 and retrospectively analyzed for HEV. The samples negative for antibodies to HBV, HCV and HIV only were examined. HIV-positive sera were excluded from the study due to reasons of biosafety.

Data on each group are shown in Table 1. All sera were stored at −80°C.

3.2. Serological assays

Sera were tested by ELISA for anti-HEV IgM and IgG antibodies using commercial kits (recomWell HEV IgM or IgG, Mikrogen GmbH, Germany). Immunoblotting for anti-HEV IgG and IgM (recomLine HEV IgG/IgM, Mikrogen GmbH, Germany) was used for confirmation. The samples were considered to be positive if both assays rendered positive results. All manipulations and interpretation of data were carried out according to the manufacturer's instructions. Serum samples with the presence of anti-HEV IgM with or without IgG were classified as acute infection. The sera positive for anti-HEV IgG but not for IgM were regarded as past infection.

3.3. Detection and genotyping of hepatitis E virus RNA

HEV RNA was extracted from 140 ml of serum by QIAamp Viral RNA mini kit (QIAGEN, Germany). Extracted RNA was stored at −80 °C until use. Real-time PCR and PCR amplification in ORF2 region were performed as described earlier [12]. Purification and subsequent sequencing of nested PCR products (1011 bp) were carried out at the Estonian Biocentre (Tartu, Estonia).

3.4. Phylogenetic analysis

Phylogenetic analysis of the obtained sequences was performed with MEGA7 software. Although we determined 1011 bp from each HEV isolate, the whole sequences were not used for phylogenetic analysis. In order extend number of comparable sequences, 865 bp fragment from 9 obtained sequences was aligned with the respective region of 38 reference sequences accessible from GenBank. Complete sequences were deposited in GenBank (Accession No MF983529-MF983537). The General Time-Reversible model with Gamma distribution and invariance (GTR + G + I) was chosen by the likelihood ratio test (LRT) as appropriate for the construction of a maximum-likelihood tree with the given data. Bootstrap testing of phylogeny was performed across 1000 replicates.

3.5. Statistical analysis

Statistical analyses were performed using Yates corrected Chi-square test ($\chi 2$). Differences were considered to be statistically significant when P-value was ≤ 0.05.

4. Results

4.1. Seroprevalence of anti-HEV antibodies and presence of HEV RNA

Totally 1426 serum samples were analyzed for the presence of anti HEV antibodies and 49 (3.4%) of them exhibited either acute (17 sera) or past (32 sera) HEV infection (Table 2). HEV RNA was found in 10 anti-HEV IgM positive samples (with or without anti-HEV IgG) while all anti-HEV IgG positive samples without detectable anti-HEV IgM were negative for HEV RNA in real-time PCR.

Out of 763 samples from nonA/B/C patients, 15 anti-HEV IgG positive samples (1,96%, CI 0.98%–2.96%) were detected. No HEV RNA was detected by real-time PCR in this group.

In the group with suggested HEV infection, 22 samples out of 282 (7.8%, CI 4.67% to 10.93%) contained anti-HEV IgG and/or IgM. Acute HEV infection was detected in 16 patients; among them 4 were positive only for anti-HEV IgM while 12 were positive for both IgM and IgG. Six sera were positive for anti-HEV IgG only. Noteworthy, there was found an anti-HEV IgM positive patient who did not exhibit IgG seroconversion when observed 6 months after the primary examination. This patient was excluded from the analysis. HEV RNA was revealed by real-time PCR in 1 from 4 anti-HEV IgM-positive individuals and in 8 of 12 anti-HEV IgM-IgG positive ones.