Veterinary Microbiology 146 (2010) 144–149

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Short communication

Hepatitis E virus is highly prevalent in the Danish pig population

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ARTICLE INFO

Article history:
Received 8 December 2009
Received in revised form 22 April 2010
Accepted 3 May 2010

Keywords:
Hepatitis E virus (HEV)
Epidemiology
Swine
Genotype 3
Zoonosis

ABSTRACT

The objective of this study was to examine the prevalence of Hepatitis E virus (HEV) in the Danish pig population. Faecal samples from 97 pigs, 1–5 months of age were analysed for HEV RNA by a new PriProET real time RT-PCR assay. In addition, serum samples from 71 sow herds were screened for the presence of anti-HEV IgG antibodies by ELISA. The genotype of the detected HEV positive samples was estimated based on the melting temperature obtained by the PriProET real time RT-PCR assay. The HEV prevalence of faecal samples was 55.0% and 49.5% for herds and animals, respectively. A HEV IgG prevalence of 91.5% was found for the sow herds which correspond to 73.2% of the sows. The PriProET assay indicated that all HEV positive samples belonged to genotype 3 or 4, which is consistent with the observation of genotype 3 as dominant in European pigs. This is the first study showing that HEV is highly prevalent in the Danish pig population. The abundant presence of HEV in Danish pigs and the known high similarity between HEV isolates from pigs and humans support previous reports indicating possible zoonotic transmission of HEV.

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1. Introduction

Hepatitis E virus (HEV) is the sole member of the genus Hepevirus in the family of Hepeviridae (Emerson et al., 2004). It is a small, round and non-enveloped particle of 27–34 nm with a genome of 7.2 kb single stranded positive sense RNA, containing three discontinuous and partially overlapping open reading frames (ORFs). ORF1 encodes for non-structural proteins such as methyl transferase, helicase and a RNA-dependent RNA polymerase. ORF2 encodes the capsid protein and ORF3 encodes the cytoskeleton-associated phosphoprotein.

HEV is responsible for major outbreaks of acute hepatitis in humans from developing countries, but evidence increases that also in industrialised countries locally acquired HEV infections occur (Ijaz et al., 2005). The disease ranges in severity from self-limited acute hepatitis with low mortality to fulminate hepatitis in pregnant women with death rates of 15–20% (Kumar et al., 2004). HEV sequences worldwide can be classified into four major genotypes. Genotypes 1 and 2 are responsible for the majority of HEV infections in humans in endemic areas such as Asia, Africa and Mexico (Chatterjee et al., 1997). HEV genotype 3 has been identified with an increasing frequency in human sporadic cases in Europe, USA, Argentina, Japan and Australia (Dalton et al., 2008). Furthermore, the HEV genotype 3 has been isolated from domestic pigs in the same countries (Meng et al., 1997; Fernández-Barredo et al., 2007). HEV genotype 4 is found mainly in Asian countries where it has been isolated from both humans and domestic pigs (Nishizawa et al., 2003). Recently a novel strain of HEV was isolated from chickens and designated avian HEV. Phylogenetic analysis showed that avian HEV was only distantly related to mammalian HEV and could be divided into different genotypes corresponding to geographical origin (Bilic et al., 2009).

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doi:10.1016/j.vetmic.2010.05.002

Although HEV is not yet considered endemic in industrialised countries, seroprevalences based on anti-HEV IgG of 2% (Netherlands) to 16% (France) have been observed in groups of the general population (Bouwknegt et al., 2008a; Mansuy et al., 2008). However, significant higher seroprevalences of 43% (China) or 45% (Egypt) have been reported for blood donors in some endemic countries (Abdel Hady et al., 1998; Li et al., 2006). Seroprevalences higher than those observed in the general population in non-endemic countries have also been reported among individuals with exposure to swine such as veterinarians, farmers, and slaughterhouse workers of these areas (Bouwknegt et al., 2008a; Galiana et al., 2008). Zoonotic spread of HEV has been suggested because isolates of human and swine HEV strains are phylogenetically closely related with nucleotide sequence identities up to 100% (Yazaki et al., 2003; Fernández-Barredo et al., 2007). Experimentally, HEV has been shown to be able to cross species by infection of non-human primates such as chimpanzee and rhesus monkeys with swine HEV and infection of pigs with a human HEV strain (Meng et al., 1998; Halbur et al., 2001). Experimentally infected pigs have been shown to shed HEV in faeces for several weeks and contact transmission have been shown to occur, indicating that swine may represent a significant reservoir for HEV (Halbur et al., 2001; Bouwknegt et al., 2008b).

The transmission of HEV in humans by the faecal–oral route has been well documented in large water borne epidemics in endemic regions (Panda et al., 2007) whereas transmission routes in non-endemic regions for individuals with no history of travel to endemic areas are uncertain (Ijaz et al., 2005). Direct evidence of zoonotic HEV transmission has recently been reported in Japan where cases of acute hepatitis E were linked epidemiologically and genetically to the consumption of uncooked pig livers and wild deer meat (Yazaki et al., 2003; Tei et al., 2003). Furthermore, HEV RNA has been detected in pig livers sold in grocery stores in countries like the Netherlands, United States, and Japan and further some livers were shown to contain infectious virus (Yazaki et al., 2003; Bouwknegt et al., 2007; Feagins et al., 2007). In summary, all these observations suggest that swine may be an animal reservoir for HEV.

In the present study, the prevalence of HEV in the Danish pig population was investigated by analysis of faeces and serum samples obtained from pigs and sows.

2. Materials and methods

2.1. Animal and samples

In total, 97 faecal samples of Danish pigs from 4 to 22 weeks of age collected from September 2007 to May 2008 were studied. Of these, 54 were randomly selected among samples submitted to the National Veterinary Institute, Denmark, for routine diagnostic analyses. The faecal samples originated from 26 herds. The remaining 43 faeces samples were collected from pigs at 14 different farms. The final number of pigs tested per farm was between 1 and 4. All faecal samples were analysed for HEV RNA by real time RT-PCR.

A total of 213 serum samples from sows (3 per farm) were collected at 71 Danish farms and analysed for anti-HEV IgG by ELISA. From 22 HEV seropositive herds having at least 2 out of 3 positive sows, serum samples from 2 piglets aged 8–12 weeks were analysed for the presence of HEV RNA.

2.2. RNA extraction

Viral RNA was extracted from 140 μl of 10% faecal suspensions (diluted in PBS) or 140 μl of serum samples by use of QIAamp Viral RNA Mini Kit (QIAGEN GmbH, Hilden, Germany) according to the kit instruction. The recovered RNA was stored at −80 °C until analysis.

2.3. Detection of HEV RNA by real time RT-PCR assay

An alignment of full-length HEV sequences of the four known genotypes from humans and swine showed a short highly conserved region within HEV *orf*2. A real time RT-PCR assay based on the PriProET technique was designed against this conserved region by use of the Beacon Designer 4 software (PREMIER Biosoft International, Palo Alto, CA, USA). The PriProET real time RT-PCR assay was designed to detect all four HEV genotypes based on the sequence alignment and further to provide an indication of the HEV genotype since the probe target region showed perfect match to genotypes 3 and 4 but contained two mismatches to genotypes 1 and 2 which affects the melting temperature of the probe (Fig. 1).

Names, composition and T_m of the primers and probe were:

Forward primer HEV2-F 5′-GTGGTTTCTGGGGTGAC-3′, T_m = 52 °C;
Reverse primer HEV2-R-FAM 5′-FAM-AAGGGGTTGG-TTGGATG-3′, T_m = 51 °C;
Probe HEV2-P-Cy5 5′-TCTCAGCCCTTCGCCCTCC-Cy5-3′, T_m = 61 °C.

The primers and probe were obtained from DNA Technology (Århus, Denmark).

The real time RT-PCR was performed on a Rotor-Gene 3000 machine (Corbett Research, Sidney, Australia) in a total volume of 25 μl using Qiagen OneStep RT-PCR kit (Qiagen) and 4 μl of extracted RNA, 300 nM of HEV2-F, 600 nM of HEV2-R-FAM and 700 nM of HEV2-P-Cy5. The amplification temperature profile was 48 °C for 30 min for reverse transcription followed by 95 °C for 15 min and 40 cycles of 94 °C for 20 s, 52 °C for 10 s and 72 °C for 30 s. This was immediately followed by a melting point analysis: 95 °C for 45 s, 45 °C for 30 s, followed by ramping from 45 to 80 °C by 1 °C increment and hold for 7 s at each step. The acquisition of fluorescence signal was performed at 52 °C during each PCR cycle and at each temperature increment step during the melting analysis. Fluorescence was generated by excitation of the donor fluorophor (FAM) followed by collection of emission signals from the acceptor fluorophor (Cy5). All fluorescence measurements were analysed with the Rotor-Gene Software Version 6.0.

In each real time RT-PCR run the plasmid pORF2 containing a part of the *orf*2 gene (kindly provided by Dr.

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