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Short communication

Detection of hepatitis E virus in archived German wild boar serum samples

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Abstract

Hepatitis E is a rare human disease in Central Europe commonly imported from endemic regions. For autochthonous infections a zoonotic transmission from pigs, deer and wild boar is assumed. Using three different RT-PCR protocols, hepatitis E virus (HEV) RNA was detected in 10 out of 189 (5.3%) serum samples collected in 1995/1996 from wild boars in Germany. Sequence analysis indicates a close relationship with genotype 3 isolates of pigs and humans from the Netherlands and Japan. The results indicate that HEV is present in Germany since more than 10 years and that wild boar may function as a reservoir for HEV.

Keywords: Hepatitis E virus; Wild boar; Zoonotic transmission; Phylogenetic analysis; Archived samples

1. Introduction

Infection with hepatitis E virus (HEV) could lead to a severe human disease with acute hepatitis as the major clinical symptom. In overall, the case/fatality rates of HEV are low, however, for pregnant women rates up to 25% have been recorded due to fulminant hepatitis (Khuroo et al., 1981). Epidemics of hepatitis E have been recorded in Central and South East Asia, North and West Africa, and Mexico (Worm et al., 2002). Most of the hepatitis E cases in North America and Central Europe could be traced to imported infections from endemic regions. However, there is increasing evidence for several autochthonous HEV infections in these non-endemic regions (Dalton et al., 2007; Herremans et al., 2007; Preiss et al., 2006; Schlauder et al., 1998).

The detection of strains closely related to human HEV in pigs, deer and wild boar have led to the assumption that zoonotic transmission of HEV is possible (Michitaka et al., 2007; Takahashi et al., 2004; Tei et al., 2003; Worm et al., 2002). Reports on human hepatitis E cases after consumption of uncooked meat from deer and wild boar strengthened the hypothesis of zoonotic food-borne HEV infection of humans (Masuda et al., 2005; Tei et al., 2003; Li et al., 2005). Recently, HEV strains isolated from pigs in the Netherlands have been shown to be very closely

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related to HEV strains from human cases of hepatitis E of the same region indicating that autochthonous HEV infections are acquired from pigs in Central Europe (Herremans et al., 2007). In Japan, wild boars have been suggested to serve as a reservoir for HEV infections as a broad variety of strains including those closely related with human HEV strains has been detected in this animal species (Michitaka et al., 2007).

HEV is a non-enveloped virus with a single-stranded RNA genome. It is the only member of the unassigned genus *Hepevirus*. Until now, four genotypes have been defined. Genotypes 1, 2 and 4 are found only in distinct geographical regions of the world whereas genotype 3 seems to have a worldwide distribution and is found in humans as well as in pigs (Lu et al., 2006). The avian HEV-like viruses are only distantly related to the mammalian HEV types and a grouping into a new genotype 5 has been suggested (Huang et al., 2004).

In order to determine whether animals from Germany could also be a reservoir for HEV, samples of wild boars were chosen for examination. Due to availability, archived serum samples originally collected in 1995/1996 for serological surveillance purposes in Northern Germany (Mecklenburg-Western Pomerania) have been used. The obtained results indicate that HEV is present in Germany since more than 10 years and that wild boar may function as a reservoir for HEV in Central Europe.

2. Materials and methods

2.1. Samples

A total of 189 sera of wild boar were collected in 1995/1996 for serological surveillance purposes in Northern Germany (Mecklenburg-Western Pomerania). For all of the samples, data on gender, geographic region and date of sampling were available (Table 1 shows the data for the positive tested samples). The sera had been stored at −20 °C until use. An RNA extract of a HEV-positive pig liver sample from the Netherlands (kindly provided by van der Poel, Wageningen University, The Netherlands) served as positive control for RT-PCR analyses.

2.2. RNA extraction and (real-time) RT-PCR

RNA was isolated from the sera using the QIAamp Viral RNA Mini Kit (Qiagen, Germany) according to the manufacturer's protocol, however, using the two-fold volume of serum and reagents before application to the column. The higher amount of starting material was because of the expected low virus concentration in the serum samples. The extracted RNA was tested in parallel by real-time RT-PCR according to Jothikumar et al. (2006), RT-PCR according to Schlauder et al. (1999) and modified by Herremans et al. (2007), and nested RT-PCR according to Huang et al. (2002) amplifying PCR products of 69, 197 and 348 base pairs, respectively. The real-time PCR was performed in an ABI PRISM 7700 cyler using the Quantitect Probe RT-PCR Kit (Qiagen, Germany). Conventional RT-PCR was performed using the One-Step RT-PCR Kit (Qiagen, Germany) and nested PCR using the TITANIUM Taq Polymerase (Clontech, France). PCR products obtained by conventional RT-PCR were separated on ethidium bromide-stained 1.5% agarose gels and visualized by UV light.

2.3. Sequence analysis

RT-PCR products considered for sequence analysis were purified using the Qiaquick DNA purification kit (Qiagen, Hilden) and subsequently cloned into the vector pCR4-TOPO using the TOPO TA Cloning Kit for Sequencing (Invitrogen, The Netherlands). The inserts of the plasmids were sequenced using M13 Forward and M13 Reverse primers (Invitrogen, The Netherlands) in an ABI 377 DNA sequencer (Applied Biosystems, USA). Sequence similarity searches were performed using the BLAST 2.2.14 search facility (Altschul et al., 1997) and the GenBank database. A phylogenetic tree was constructed on the basis of the nucleotide sequences using the MegAlign module of the DNASTAR software package (LASERGENE, USA).

3. Results

3.1. Detection of HEV RNA in sera of wild boars from Germany

A total of 189 sera derived from wild boars originating from the North of Germany and sampled