Screening of reservoirs for hepatitis E virus

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Screening of reservoirs for hepatitis E virus

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Introduction

Hepatitis E virus (HEV) is a small non-enveloped positive sense RNA virus that causes acute hepatitis E in humans in developing countries of Asia and Africa. However is also seen sporadically in the industrialized world. HEV is divided into four genotypes where genotype 1 and 2 only infects humans; genotype 4 is found in human and swine; genotype 3 has been found in a wider host range (including human and swine). More genotypes have been characterized including a rat and an avian HEV variant. The avian HEV only resembles genotype 1-4 50% at the nucleotide level. In a recent study HEV antibodies were found in ~91% of Danish pig herds with the viral RNA present in ~50% of pigs aged 4-22 weeks (1). It is now recognized that HEV is a zoonotic disease with swine being the primary reservoir. In the present study several animal species were investigated for the possibility of an alternative reservoir. Fecal samples from dogs, cats, deer, pheasants, mink and rabbits were screened for HEV using PCR.

Materials and methods

RNA purification: A 10% suspension of each sample was made in PBS. From this 450 ul was homogenized using a Tissuelyzer (Qiagen) for 20 sec at 15 Hz. The samples were purified using Virus/bacteria kit on the QIAsymphony (Complex 200, Qiagen).

Nested PCR: The screening of samples was performed by a nested PCR (nPCR) assay developed by Reimar Johne et al. (2010) (2). The assay targets a conserved region in genotype 1-4 as well as avian HEV and rat HEV. This should ensure that distantly related HEV strains are also detected. All samples were run on 2% E-gels (Invitrogen) for 30 minutes post amplification.

Real Time PCR: An assay targeting a highly conserved region in ORF2 specific for genotype 1-4 was also used (1). The assay takes advantage of the PriProET chemistry.

Cloning: The PCR products from the nested PCR were purified from the gel using Roche High Pure PCR product purification kit as described in the protocol. A TOPO TA cloning kit using the pCR4 vector (Invitrogen) was used for cloning in *E. coli* TOP10 competent cells.

Results

A total of 124 samples have been screened with the nPCR assay (77 mink, 5 cats, 36 dogs, 2 deers and 4 pheasants). From these 124 samples one mink sample was found positive using the nPCR. This is shown in Figure 1; where the mink sample (lane 6) has the same band pattern and size as the positive control (lane 1). This sample was also tested using the real time PCR assay and was found to be negative. This result indicates that a novel HEV has been discovered in mink. The mink from which this sample was recovered showed severe damage in the liver but it has not been possible to determine if this damage was due to the HEV infection. Currently progress is being made to clone the nPCR products from the positive sample and sequencing is planned. Furthermore, screening of more samples from the species listed above, with the inclusion of rabbit samples, will be performed.

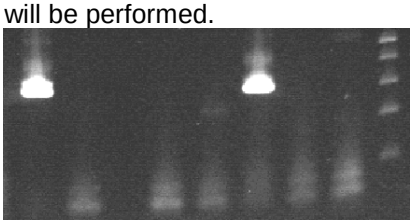

Figure 1. Gel showing the **r**esults of nPCR: Lane 1: Positive control (known genotype 3 sample from swine); lane 2: Water, lane 3: Blank; lane 4-8: Mink samples; lane 9: DNA ladder (100bp)

Discussion

This is the first time, to our knowledge, that HEV has been detected in mink. Especially mink were of interest prior to testing, as they in a period from 2007 to 2008, in some parts of Denmark, were fed with pig waste products from slaughterhouses. The negative result of the real time PCR assay suggests that this strain has not been transmitted from pigs as it is not genotype 3 or 4. Likewise from the real time PCR this mink HEV does not belong to genotype 1 or 2 either. Sequence data is needed to clarify the phylogenetic relationship of this HEV. These investigations are currently being performed along with screening of more samples. These results indicate that swine is not the sole reservoir for HEV in Denmark.

Acknowledgements

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References

1. Breum et al. (2010) Vet Mic 146, 144-149
2. Johne et al. (2010) J Gen Vir 91, 750-758