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Cross-species infection of specific-pathogen-free pigs by a genotype 4 strain of human hepatitis E virus

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SUMMARY

Hepatitis E virus (HEV) is an important pathogen. The animal strain of HEV, swine HEV, is related to human HEV. The genotype 3 swine HEV infected humans and genotype 3 human HEV infected pigs. The genotype 4 swine and human HEV strains are genetically related, but it is unknown whether genotype 4 human HEV can infect pigs. A swine bioassay was utilized in this study to determine whether genotype 4 human HEV can infect pigs. Fifteen, 4-week-old, specific-pathogen-free pigs were divided into 3 groups of 5 each. Group 1 pigs were each inoculated intravenously with PBS buffer as negative controls, group 2 pigs similarly with genotype 3 human HEV (strain US-2), and group 3 pigs similarly with genotype 4 human HEV (strain TW6196E). Serum and fecal samples were collected at 0, 7, 14, 21, 28, 35, 42, 49, and 56 days postinoculation (dpi) and tested for evidence of HEV infection. All pigs were necropsied at 56 dpi. As expected, the negative control pigs remained negative. The positive control pigs inoculated with genotype 3 human HEV all became infected as evidenced by detection of HEV antibodies, viremia and fecal virus shedding. All five pigs in group 3 inoculated with genotype 4 human HEV also became infected: fecal virus shedding and viremia were detected variably from 7 to 56 dpi, and seroconversion occurred by 28 dpi. The data indicated that genotype 4 human HEV has an expanded host range, and the results have important implications for understanding the natural history and zoonosis of HEV.

Keywords

Hepatitis E virus (HEV); genotypes; cross-species infection; swine HEV

INTRODUCTION

Hepatitis E, caused by hepatitis E virus (HEV), occurs predominately in Asia, Africa, and other developing countries (Emerson and Purcell, 2003; Meng, 2003), but has also been reported in industrialized countries as sporadic cases (Hsieh et al., 1999; Van der Poel et al., 2001; Takahashi et al., 2003; Yazaki et al., 2003; Clemente-Casares et al., 2003; Mizuo et al., 2005; Haagsman et al., 2007; Lewis et al., 2008; Mansuy et al., 2008). The mortality rate associated with HEV infection is generally low (< 1%), but it can reach as high as 25–30% in infected pregnant women (Hussaini et al., 1997). Transmission is primarily via the fecal-

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oral route (Emerson and Purcell, 2003), and in countries with less than optimal sanitation conditions, contaminated water has been the source of major epidemics. However, increasing evidence suggests that zoonotic transmission may be responsible for the spread of hepatitis E as well, especially for the sporadic cases (Tei et al., 2003).

HEV is a single-stranded, positive-sense RNA molecule (Purcell, 1996). The approximately 7.2 kb viral genome consists of three open reading frames (ORFs 1, 2, and 3) and short 5' and 3' untranslated regions (UTR) (Purcell, 1996; Wang et al., 2000; Huang et al., 2007). Currently, HEV isolates can be classified into one of four major genotypes (Emerson and Purcell, 2003; Meng, 2003). Many of the epidemic HEV infections occurring in Asia and Africa are caused by genotype 1 strains whereas an epidemic occurring in Mexico in 1987 was caused by a genotype 2 strain. Genotype 3 and 4 strains are responsible for sporadic cases of HEV infections worldwide (Tam et al., 1991; Huang et al., 1992; Schlauder et al., 1998; Takahashi et al., 2003, 2004; Lewis et al., 2008). The recently identified avian hepatitis E virus (avian HEV) is genetically and antigenically related to mammalian HEV strains (Haqshenas et al., 2001; Huang et al., 2004; Guo et al., 2006, 2008) but it remains to be determined whether avian HEV belongs to a new 5th genotype or a separate genus.

A significant proportion of healthy individuals from industrialized countries are found positive for HEV antibodies (Thomas et al., 1997; Mast et al., 1997; Meng et al., 2002). The detection of genotypes 3 and 4 strains of HEV in humans that are genetically closely-related, or identical in some cases, to HEV strains from pigs (Nishizawa et al., 2003; Yazaki et al., 2003) suggested that hepatitis E is a zoonotic disease and pigs and maybe other animal species are reservoir(s) for HEV (Meng, 2003). Thus far HEV has been detected in domestic and wild pigs (Meng et al., 1997), chickens (Haqshenas et al., 2001; Huang et al., 2004), and deer (Takahashi et al., 2004). Meng et al. (1997) isolated the first animal strain of HEV, swine HEV, from a pig in the United States. Since then, swine HEV has been isolated from pigs in many other countries (Van der Poel et al., 2001; Wang et al., 2002; Yazaki et al., 2003). Swine HEV isolates obtained from countries worldwide belong to either genotype 3 or 4 (Huang et al., 2002; Cooper et al., 2005).

Genotype 3 swine HEV, which is related both antigenically and genetically to human HEV, has been shown to infect nonhuman primates. Similarly, the US-2 strain of human HEV has been shown to infect pigs (Meng et al., 1998b; Halbur et al., 2001). Compared to genotype 3 HEV, genotype 4 strains of human and swine HEVs have not been well characterized experimentally. The objective of this study was to determine if a genotype 4 strain of human HEV has the ability to cross species barriers and infect pigs.

MATERIALS AND METHODS

Virus Stocks

The infectious titers of the genotype 3 human HEV stock (US-2 strain) and the genotype 4 human HEV stock (TW6196E strain) used in this study have been determined previously (Purcell, R. H., unpublished data): $10^{5.3}$ 50% monkey infectious dose (MID₅₀) per ml for the US-2 strain (Meng et al., 1998b), and 2×10^3 MID₅₀ per ml for the TW6196E strain (Huang et al., 2002; Cooper et al., 2005).

Pigs

Fifteen, two-week-old, specific-pathogen-free (SPF) pigs were purchased from a commercial source (Geneticporc Inc, Nebraska). Prior to inoculation, all pigs were confirmed to be negative for IgG anti-HEV antibodies by an enzyme-linked immunosorbent assay (ELISA) (Meng et al., 1998a, b; Halbur et al., 2001).

Experimental design for the swine bioassay

The infectious virus stocks were appropriately diluted to adjust the titer to the same for both the genotypes 3 and 4 virus stocks. The pigs were randomly divided into 3 groups of 5 pigs each. Group 1 pigs were each inoculated intravenously (I.V.) with 1 ml of sterile phosphate buffered saline (PBS) as negative controls, group 2 pigs were each inoculated I.V. with 1 ml (10^3 MID₅₀) of the genotype 3 human HEV (strain US-2) as positive controls, and group 3 pigs were each inoculated I.V. with 1 ml (10^3 MID₅₀) of the genotype 4 human HEV (strain TW6196E) as the experimental group. The pigs were monitored for evidence of HEV infections for a total of 8 weeks, and all animals were necropsied at 56 dpi.

Sample collection and processing

Serum samples and fecal swabs were collected weekly from each pig at 0, 7, 14, 21, 28, 35, 42, 49, and 56 days postinoculation (dpi). Serum samples were tested for HEV viremia by a universal reverse transcription-polymerase chain reaction (RT-PCR) (Huang et al., 2002; Cooper et al., 2005) and for anti-HEV IgG by ELISA as described previously (Meng et al., 1997, 1998a, b; Halbur et al., 2001). Fecal swabs (10% suspension in PBS buffer) were also tested for HEV RNA by RT-PCR.

In addition, samples of feces were collected from each pig in group 2 and group 3 every three days for the first 4 weeks to investigate the level of HEV viral RNA shedding in the feces during the acute stage of infection. A portion of the fecal material was made into 10% fecal suspension in sterile PBS buffer and used for the quantification of HEV RNA by a real-time RT-PCR. Samples of feces were also tested for HEV RNA by the universal RT-PCR as described previously (Huang et al., 2002; Cooper et al., 2005) for comparison purpose.

During necropsy, samples of serum, feces, bile, and liver tissue were collected and stored at -80°C until use. A portion of the liver tissue samples were homogenized in 10% (w/v) sterile PBS buffer. The liver homogenates were clarified by centrifugation at 3,000 rpm for 15 minutes at 4°C (Eppendorf centrifuge 5810) and then used for the detection of HEV RNA by RT-PCR.

ELISA to detect anti-HEV antibodies

A purified, 55 kDa truncated form of the HEV capsid protein, expressed from a recombinant baculovirus containing the ORF2 of the genotype 1 Sar-55 human strain of HEV, was used as the antigen for the ELISA as described previously (Meng et al., 1997; 1998a, b).

RT-PCR to detect HEV RNA

To detect HEV RNA in serum samples, fecal swabs, feces, bile, and liver tissue homogenates of inoculated pigs, a universal RT-PCR assay was performed as described previously (Huang et al., 2002). The universal RT-PCR assay is capable of detecting all 4 known genotypes of HEV (Huang et al., 2002; Cooper et al., 2005). Briefly, total RNAs were extracted by the use of Trizol Reagent (GIBCO-BRL) from 100 μl of the serum, fecal suspension, bile, or 10% liver homogenate. The total RNA was resuspended in 11.0 μl of DNase-, RNase-, and proteinase-free water (Invitrogen). Reverse transcription was performed at 42°C for 60 minutes with 1 μl ($10\text{ }\mu\text{M}$) of the reverse primer 3157N [5'-CCCTTA(G)TCC(T)TGCTGA(C)GCATTCTC-3'], 1 μl (200 U/ μl) of Superscript II reverse transcriptase (Invitrogen), 1 μl of 0.1 M dithiothreitol, 4 μl of $5\times$ RT buffer, 0.5 μl (40 U/ μl) of RNase inhibitor (Promega), and 1 μl of 10mM deoxynucleoside triphosphates. Ten microliters of the resulting cDNA was subsequently amplified in a 50 μl PCR reaction with AmpliTaq Gold DNA polymerase (Applied Biosystems).

The nested universal RT-PCR assay amplifies a region within the ORF2 capsid gene (Huang et al., 2002; Cooper et al., 2005). The first round PCR was performed with a set of degenerate HEV primers: 3156N [forward, 5'-AATTATGCC(T)CAGTAC(T)CGG(A)GTTG-3'] and 3157N [reverse, 5'-CCCTTA(G)TCC(T)TGCTGA(C)GCATTCTC-3']. The second round PCR was performed with another set of degenerate HEV primers using the first round PCR product as the template: 3158N [forward, 5'-GTT(A)ATGCTT(C)TGCATA(T)CATGGCT-3'] and 3159N [reverse, 5'-AGCCGACGAAATCAATTCTGTC-3']. The PCR parameters for the first and second round PCR were similar with an initial denaturation step at 95°C for 9 minutes, followed by 39 cycles of denaturation for 1 minute at 94°C, annealing for 1 minute at 42°C, extension for 1 minute at 72°C, and a final extension at 72°C for 7 minutes. The expected final product of the universal nested RT-PCR was 348 bp.

Real-time RT-PCR to quantify HEV RNA in feces

Quantification of HEV RNA in the feces (10% feces suspension in PBS buffer) during the first 4 weeks of infection was performed essentially as described previously (Jothikumar et al., 2005) with a few modifications. Briefly, to generate a HEV RNA standard, a plasmid containing a region of ORF3 was constructed from the infectious cDNA clone pSHEV-3 of a genotype 3 swine HEV (Huang et al., 2005, 2007) using the TA Cloning Kit (Invitrogen) pCR®2.1 vector and the following primers: [forward, 5' – ATGCTGCCCCGCGCCACCG – 3'] and [reverse, 5' – AGGGGTTGGTTGGATGAA – 3']. Plasmid DNA was purified using the GenElute™ Plasmid Miniprep Kit (Sigma-Aldrich) and quantified using the Nanodrop ND-1000 according to the manufacturer's instructions. The mMESSAGE mMACHINE® High Yield Capped RNA Transcription Kit (Ambion) was used to generate an *in vitro* RNA transcript from the plasmid DNA template containing a T7 promoter according to the manufacturer's protocols. Plasmid DNA was removed completely with DNase I treatment and the integrity of the RNA transcript was checked by gel electrophoresis.

A standard curve was generated from 10-fold serial dilutions of the *in vitro*-synthesized HEV RNA standard and used for real-time RT-PCR with the QuantiTect Probe RT-PCR Kit (Qiagen) on an iCycler (Bio-Rad). The primers used for the real-time PCR are: forward [5' – GGTGGTTTCTGGGGTGAC – 3'] and reverse [5' – GGTGGTTGGATGAATATAGGG – 3']. The probe [5' – TGATTCTCAGCCCTTCGC – 3'] contained a 5' 6-carboxy fluorescein fluorophore (FAM) and a 3' black hole quencher (BHQ-1). The primers (Invitrogen) and the probe (MWG Biotech) were commercially synthesized. HEV RNA was extracted from 10% fecal suspensions of group 2 and group 3 pigs collected at different time points during the first 4 weeks post-inoculation. In brief, 100 µl of fecal suspension was mixed with 1 ml of Trizol Reagent and 200 µl of chloroform. The aqueous phase was added to the RNeasy Mini Kit spin column (Qiagen) for RNA extraction and purification using on-column DNase digestion according to the manufacturer's instructions and the RNA was stored at –80°C until use. Reverse transcription was carried out at 50°C for 30 minutes, followed by denaturation at 95°C for 15 minutes. Real-time PCR amplification was performed with 50 cycles at 95°C for 10 seconds, 55°C for 20 seconds and 72°C for 15 seconds. The reproducibility of the assay was determined by testing each sample in triplicate and the mean value was calculated.

RESULTS

As expected, all pigs in the negative control group 1 inoculated with sterile PBS buffer remained seronegative throughout the study, and there was no detectable HEV viremia, fecal virus shedding, or seroconversion in group 1 pigs (Fig. 1, Table 1). All pigs in the positive control group 2 inoculated with genotype 3 human HEV (strain US-2) became infected, as

evidenced by seroconversion to IgG anti-HEV as early as 2 weeks postinoculation (wpi), viremia, and fecal virus shedding (Fig. 1, Table 1).

All five pigs in the experimental group 3 inoculated with the genotype 4 human HEV strain TW6196E also became infected. Seroconversion started at 2 wpi, and by 4 wpi all pigs had seroconverted to IgG anti-HEV and remained seropositive at the end of the 8-week study (Fig. 1). Viremia and fecal virus shedding were also detected variably in group 3 pigs (Table 1). Viremia is transient and in very short duration in both genotypes 3 and 4 human HEV-infected pigs (Table 1). Fecal virus shedding in the group 2 pigs inoculated with the genotype 3 human HEV lasted approximately 4 weeks: by weeks 5 and 6, there is only one pig that still shed virus in feces. In group 3 pigs inoculated with the genotype 4 human HEV, fecal virus shedding appears to last longer: 4/5 pigs at 6 wpi and 3/5 pigs at 8 wpi still shed virus in feces (Table 1).

In general, fecal virus shedding in pigs infected by swine HEV or genotype 3 human HEV occur in the first 4 to 5 wpi (Halbur et al., 2001; Meng et al., 1998). Therefore, to evaluate the onset and pattern of fecal virus shedding between genotype 3 and genotype 4 human HEV-infected pigs, the amounts of fecal viral shedding during the first 4 weeks of the infection were determined by a real-time PCR. HEV RNA was detected in fecal samples from group 2 and group 3 pigs as early as 3 days postinoculation (dpi). In group 2 pigs infected with genotype 3 human HEV, viral RNA levels in feces ranged from 4.49 to 10.10 log copies per mg of feces during the first 4 weeks. For group 2 pigs, the virus titers peaked in the feces of most pigs at approximately 12 to 18 dpi (Table 2, Fig. 2). Fecal virus shedding disappeared in one pig (ID 32) at 24 dpi, whereas another pig (ID 44) stopped shedding virus at 27 dpi. The remaining 3 pigs in group 2 still shed virus in feces at the end of the 4 week observation period (Table 2). In group 3 pigs infected by the genotype 4 human HEV, viral RNA levels in feces ranged from 5.21 to 10.26 log copies per mg of feces (Table 2). The fecal virus titers of most group 3 pigs peaked at approximately 9 to 21 dpi (Table 2, Fig. 2). Samples with detectable HEV RNA in feces by real-time RT-PCR (Table 2) were also determined to be positive by the universal RT-PCR (data not shown).

All pigs from each group were necropsied at 8 wpi. The PCR products amplified from serum, fecal swabs, bile, and liver samples from two selected pigs from group 2 and group 3 were sequenced for the ORF2 gene region. Sequence analyses confirmed that the viruses recovered from the experimentally infected pigs originated from the respective inoculum (data not shown).

DISCUSSION

The rare incidence of human-to-human HEV transmission in endemic regions suggests that animal reservoirs of HEV may exist (Meng, 2003). Human HEV strains (US-1 and US-2) recovered from hepatitis E patients in the United States are closely related to the genotype 3 swine HEV strain isolated from a pig in the same geographical area (Meng et al., 1998b; Schlauder et al., 1998). Similarly, genotype 4 human HEV isolates obtained from hepatitis E patients in China and Taiwan (Hsieh et al., 1999; Wang et al., 2002; Wu et al., 2002) are very similar to swine HEV isolates obtained from pigs in the same area. These findings strongly suggest that pigs are reservoirs for genotype 3 and genotype 4 strains of HEV.

Meng et al. (1998b) has demonstrated, experimentally, that the genotype 3 swine HEV was able to cross species barriers and infect rhesus monkeys and a chimpanzee and, in a reciprocal experiment, that SPF pigs were experimentally infected with the US-2 strain of genotype 3 human HEV. However, a genotype 1 human HEV strain (Sar-55) and the genotype 2 human HEV strain (Mex-14) were not able to infect SPF pigs under

experimental conditions (Meng et al., 1998a). The failure to infect pigs with the Sar-55 (genotype 1) and Mex-14 (genotype 2) HEV strains suggest that the transmissibility of human HEV to other species such as pigs varies from HEV genotype to genotype. Therefore, it is important to determine whether or not the genotype 4 human HEV strain identified recently has the ability to infect across species barriers.

The genomes of genotype 4 human HEV strains have been shown to be closely related to genotype 4 swine HEV strains obtained from the same geographic region (Nishizawa et al., 2003; Takahashi et al., 2003). A genotype 4 swine HEV strain isolated from a pig on a swine farm in Hokkaido, Japan was found to have 99% nucleotide sequence identity with a genotype 4 human HEV isolate obtained from a patient with sporadic acute hepatitis E in Hokkaido (Nishizawa et al., 2003). Similar results have also been reported with genotype 4 swine and genotype 4 human HEV strains in Bali, Indonesia (Wibawa et al., 2007). In addition, Yazaki et al. (2003) found that a genotype 4 swine HEV isolate obtained from pig livers purchased in local grocery stores in Hokkaido, Japan shared 97.8–100% nucleotide sequence identity with genotype 4 human HEV strains obtained from patients with sporadic acute or fulminant hepatitis E who had consumed undercooked pig livers prior to the onset of the disease. The seroprevalence of IgG anti-HEV, when tested with a genotype 4 HEV capsid protein as the antigen, was found to be 58% in pigs from commercial herds in Hokkaido (Takahashi et al., 2003). These data strongly suggest that, similar to genotype 3 HEV, genotype 4 HEV may also have an expanded host range. In fact, recently Arankalle et al. (2006) were able to infect rhesus monkeys with a genotype 4 Indian strain of swine HEV.

In this present study, we demonstrated that SPF pigs experimentally inoculated with genotype 4 human HEV (strain TW6196E) became infected. Fecal virus shedding and viremia were detected variably from 7 to 56 dpi, seroconversion occurred by 28 dpi. There are a few minor discrepancies in HEV RNA positivity detected in the weekly fecal swabs by RT-PCR (Table 1) and in the feces samples collected every three days during the first 4 weeks (Table 2). For example, the fecal swabs from weeks 1 and 2 in pig number 28 were tested negative for HEV RNA (Table 1), whereas the same pig was tested positive for HEV RNA during the first two weeks when the feces samples (instead of swabs) were tested (Table 2). This is not surprising since the amounts of fecal materials collected weekly by fecal swabs are variable (Meng et al., 1999a) and sometimes only a small amount of fecal materials can be obtained by swabs in some pigs, and thus likely reflecting the minor discrepancies of the results between fecal swabs (Table 1) and feces (Table 2). The fecal virus shedding in pigs infected with genotype 4 human HEV appears to last longer than pigs infected with the genotype 3 human HEV. With the exception of one pig, the fecal virus shedding in all other 4 genotype 3 HEV-infected pigs disappeared after 4 wpi (Table 1). In contrast, three of the 5 genotype 4 HEV-infected pigs still shed virus at the end of the 8 week study. However, the titers of viral RNA levels quantified by real-time PCR were comparable in pigs infected with genotype 3 human HEV and genotype 4 human HEV, and there is no significant difference in virus RNA titers in feces (Fig. 2). It remains to be determined whether there exist virulence differences between genotype 3 and genotype 4 human HEV strains. Since both genotypes 3 and 4 human HEV strains are now shown to infect pigs, future experiments to compare the pathogenicity of these strains in pigs can now be performed.

This is the first report of a genotype 4 human HEV strain crossing species barriers and infecting pigs, thereby supporting field observations that genotypes 1 and 2 HEV strains are restricted to humans, whereas genotypes 3 and 4 HEV strains have a broader host range that include both humans and swine. The results from this study have important implications for understanding the natural history and zoonosis of HEV. The ubiquitous nature of genotype 3 and 4 swine HEV worldwide (Banks et al., 2004; Cooper et al., 2005; Wang et al., 2002,

Yazaki et al., 2003;) and the demonstrated ability to infect across species raises potential public health concerns. Approximately 60–80% of swine herds in the United States are infected with swine HEV and swine veterinarians in the United States have an increased risk for zoonotic infection (Meng et al., 2002). Similar results have also been reported amongst pig handlers in Moldova (Drobeniuc et al., 2001) and Taiwan (Hsieh et al., 1999). Sporadic cases of hepatitis E have been linked to the consumption of raw or undercooked pig livers (Yazaki et al., 2003). Therefore, individuals who consume raw or undercooked pig livers are also at an increased risk for zoonotic HEV infection (Yazaki et al., 2003; Feagins et al., 2007). On the other hand, since both genotypes 3 and 4 human HEV can infect pigs, thus pigs may play an important role in the survival and transmission of genotypes 3 and 4 human HEV in human populations in endemic areas. Future studies are warranted to determine the mechanism of cross-species infection by genotypes 3 and 4 HEV strains.

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REFERENCES

- Arankalle VA, Chobe LP, Chadha MS. Type-IV Indian swine HEV infects rhesus monkeys. *Journal of viral hepatitis*. 2006; 13(11):742–745. [PubMed: 17052273]
- Banks M, Heath GS, Grierson SS, King DP, Gresham A, Girones R, Widen F, Harrison TJ. Evidence for the presence of hepatitis E virus in pigs in the United Kingdom. *The Veterinary Record*. 2004; 154(8):223–227. [PubMed: 15005446]
- Clemente-Casares P, Pina S, Buti M, Jardi R, Martín M, Bofill-Mas S, Girones R. Hepatitis E virus epidemiology in industrialized countries. *Emerging Infectious Diseases*. 2003; 9(4):448–454. [PubMed: 12702225]
- Cooper K, Huang FF, Batista L, Rayo CD, Bezanilla JC, Toth TE, Meng XJ. Identification of genotype 3 hepatitis E virus (HEV) in serum and fecal samples from pigs in Thailand and Mexico, where genotype 1 and 2 HEV strains are prevalent in the respective human populations. *Journal of Clinical Microbiology*. 2005; 43(4):1684–1688. [PubMed: 15814985]
- Drobeniuc J, Favorov MO, Shapiro CN, Bell BP, Mast EE, Dadu A, Culver D, Iarovoi P, Robertson BH, Margolis HS. Hepatitis E virus antibody prevalence among persons who work with swine. *The Journal of Infectious Diseases*. 2001; 184(12):1594–1597. [PubMed: 11740735]
- Emerson SU, Purcell RH. Hepatitis E virus. *Reviews in Medical Virology*. 2003; 13(3):145–154. [PubMed: 12740830]
- Feagins AR, Opriessnig T, Guenette DK, Halbur PG, Meng XJ. Detection and characterization of infectious Hepatitis E virus from commercial pig livers sold in local grocery stores in the USA. *The Journal of General Virology*. 2007; 88(Pt 3):912–917. [PubMed: 17325364]
- Guo H, Zhou EM, Sun ZF, Meng XJ, Halbur PG. Identification of B-cell epitopes in the capsid protein of avian hepatitis E virus (avian HEV) that are common to human and swine HEVs or unique to avian HEV. *The Journal of General Virology*. 2006; 87(Pt 1):217–223. [PubMed: 16361434]
- Guo H, Zhou EM, Sun ZF, Meng XJ. Immunodominant epitopes mapped by synthetic peptides on the capsid protein of avian hepatitis E virus are non-protective. *Viral Immunology*. 2008; 21(1):61–67. [PubMed: 18355123]
- Haagsman A, Reuter G, Duizer E, Nagy G, Herremans T, Koopmans M, Szücs G. Seroepidemiology of hepatitis E virus in patients with non-A, non-B, non-C hepatitis in Hungary. *Journal of Medical Virology*. 2007; 79(7):927–930. [PubMed: 17516521]

- Halbur PG, Kasorndorkbua C, Gilbert C, Guenette D, Potters MB, Purcell RH, Emerson SU, Toth TE, Meng XJ. Comparative pathogenesis of infection of pigs with hepatitis E viruses recovered from a pig and a human. *Journal of Clinical Microbiology*. 2001; 39(3):918–923. [PubMed: 11230404]
- Haqshenas G, Shivaprasad HL, Woolcock PR, Read DH, Meng XJ. Genetic identification and characterization of a novel virus related to human hepatitis E virus from chickens with hepatitis-splenomegaly syndrome in the United States. *The Journal of General Virology*. 2001; 82(Pt 10): 2449–2462. [PubMed: 11562538]
- Hsieh SY, Meng XJ, Wu YH, Liu ST, Tam AW, Lin DY, Liaw YF. Identity of a novel swine hepatitis E virus in Taiwan forming a monophyletic group with Taiwan isolates of human hepatitis E virus. *Journal of Clinical Microbiology*. 1999; 37(12):3828–3834. [PubMed: 10565892]
- Huang CC, Nguyen D, Fernandez J, Yun KY, Fry KE, Bradley DW, Tam AW, Reyes GR. Molecular cloning and sequencing of the Mexico isolate of hepatitis E virus (HEV). *Virology*. 1992; 191(2): 550–558. [PubMed: 1448913]
- Huang FF, Haqshenas G, Guenette DK, Halbur PG, Schommer SK, Pierson FW, Toth TE, Meng XJ. Detection by reverse transcription-PCR and genetic characterization of field isolates of swine hepatitis E virus from pigs in different geographic regions of the United States. *Journal of Clinical Microbiology*. 2002; 40(4):1326–1332. [PubMed: 11923352]
- Huang FF, Sun ZF, Emerson SU, Purcell RH, Shivaprasad HL, Pierson FW, Toth TE, Meng XJ. Determination and analysis of the complete genomic sequence of avian hepatitis E virus (avian HEV) and attempts to infect rhesus monkeys with avian HEV. *The Journal of General Virology*. 2004; 85(Pt 6):1609–1618. [PubMed: 15166445]
- Huang YW, Haqshenas G, Kasorndorkbua C, Halbur PG, Emerson SU, Meng XJ. Capped RNA transcripts of full-length cDNA clones of swine hepatitis E virus are replication competent when transfected into Huh7 cells and infectious when intrahepatically inoculated into pigs. *Journal of Virology*. 2005; 79(3):1552–1558. [PubMed: 15650181]
- Huang YW, Opriessnig T, Halbur PG, Meng XJ. Initiation at the third in-frame AUG codon of open reading frame 3 of the hepatitis E virus is essential for viral infectivity in vivo. *Journal of Virology*. 2007; 81(6):3018–3026. [PubMed: 17202216]
- Hussaini SH, Skidmore SJ, Richardson P, Sherratt LM, Cooper BT, O'Grady JG. Severe hepatitis E infection during pregnancy. *Journal of Viral Hepatitis*. 1997; 4(1):51–54. [PubMed: 9031065]
- Jothikumar N, Cromeans TL, Robertson BH, Meng XJ, Hill VR. A broadly reactive one-step real-time RT-PCR assay for rapid and sensitive detection of hepatitis E virus. *Journal of Virological Methods*. 2006; 131(1):65–71. [PubMed: 16125257]
- Lewis HC, Boisson S, Ijaz S, Hewitt K, Ngui SL, Boxall E, Teo CG, Morgan D. Hepatitis E in England and Wales. *Emerging Infectious Diseases*. 2008; 14(1):165–167. [PubMed: 18258100]
- Mansuy JM, Legrand-Abravanel F, Calot JP, Peron JM, Alric L, Agudo S, Rech H, Destruel F, Izopet J. High prevalence of anti-hepatitis E virus antibodies in blood donors from South West France. *Journal of Medical Virology*. 2008; 80(2):289–293. [PubMed: 18098159]
- Mast EE, Kuramoto IK, Favorov MO, Schoening VR, Burkholder BT, Shapiro CN, Holland PV. Prevalence of and risk factors for antibody to hepatitis E virus seroreactivity among blood donors in Northern California. *The Journal of Infectious Diseases*. 1997; 176(1):34–40. [PubMed: 9207347]
- Meng XJ. Swine hepatitis E virus: cross-species infection and risk in xenotransplantation. *Current Topics in Microbiology and Immunology*. 2003; 278:185–216. [PubMed: 12934945]
- Meng XJ, Halbur PG, Haynes JS, Tsareva TS, Bruna JD, Royer RL, Purcell RH, Emerson SU. Experimental infection of pigs with the newly identified swine hepatitis E virus (swine HEV), but not with human strains of HEV. *Archives of Virology*. 1998a; 143(7):1405–1415. [PubMed: 9722883]
- Meng XJ, Halbur PG, Shapiro MS, Govindarajan S, Bruna JD, Mushahwar IK, Purcell RH, Emerson SU. Genetic and experimental evidence for cross-species infection by swine hepatitis E virus. *Journal of Virology*. 1998b; 72(12):9714–9721. [PubMed: 9811705]
- Meng XJ, Purcell RH, Halbur PG, Lehman JR, Webb DM, Tsareva TS, Haynes JS, Thacker BJ, Emerson SU. A novel virus in swine is closely related to the human hepatitis E virus. *Proceedings*

- of the National Academy of Sciences of the United States of America. 1997; 94(18):9860–9865. [PubMed: 9275216]
- Meng XJ, Wiseman B, Elvinger F, Guenette DK, Toth TE, Engle RE, Emerson SU, Purcell RH. Prevalence of antibodies to hepatitis E virus in veterinarians working with swine and in normal blood donors in the United States and other countries. *Journal of Clinical Microbiology*. 2002; 40(1):117–122. [PubMed: 11773103]
- Mizuo H, Yazaki Y, Sugawara K, Tsuda F, Takahashi M, Nishizawa T, Okamoto H. Possible risk factors for the transmission of hepatitis E virus and for the severe form of hepatitis E acquired locally in Hokkaido, Japan. *Journal of Medical Virology*. 2005; 76(3):341–349. [PubMed: 15902701]
- Nishizawa T, Takahashi M, Mizuo H, Miyajima H, Gotanda Y, Okamoto H. Characterization of Japanese swine and human hepatitis E virus isolates of genotype IV with 99 % identity over the entire genome. *The Journal of General Virology*. 2003; 84(Pt 5):1245–1251. [PubMed: 12692290]
- Purcell, RH. Hepatitis E Virus. In: Fields DMK, BN.; Howley, PM., editors. *Fields Virology*. 3rd ed.. Philadelphia: Lippincott-Raven; 1996. p. 2831-2843.
- Schlauder GG, Dawson GJ, Erker JC, Kwo PY, Knigge MF, Smalley DL, Rosenblatt JE, Desai SM, Mushahwar IK. The sequence and phylogenetic analysis of a novel hepatitis E virus isolated from a patient with acute hepatitis reported in the United States. *The Journal of General Virology*. 1998; 79(Pt 3):447–456. [PubMed: 9519822]
- Takahashi K, Kang JH, Ohnishi S, Hino K, Miyakawa H, Miyakawa Y, Maekubo H, Mishihiro S. Full-length sequences of six hepatitis E virus isolates of genotypes III and IV from patients with sporadic acute or fulminant hepatitis in Japan. *Intervirology*. 2003; 46(5):308–318. [PubMed: 14555851]
- Takahashi K, Kitajima N, Abe N, Mishihiro S. Complete or near-complete nucleotide sequences of hepatitis E virus genome recovered from a wild boar, a deer, and four patients who ate the deer. *Virology*. 2004; 330(2):501–505. [PubMed: 15567444]
- Tam AW, Smith MM, Guerra ME, Huang CC, Bradley DW, Fry KE, Reyes GR. Hepatitis E virus (HEV): molecular cloning and sequencing of the full-length viral genome. *Virology*. 1991; 185(1):120–131. [PubMed: 1926770]
- Tei S, Kitajima N, Takahashi K, Mishihiro S. Zoonotic transmission of hepatitis E virus from deer to human beings. *Lancet*. 2003; 362(9381):371–373. [PubMed: 12907011]
- Thomas DL, Yarbough PO, Vlahov D, Tsarev SA, Nelson KE, Saah AJ, Purcell RH. Seroreactivity to hepatitis E virus in areas where the disease is not endemic. *Journal of Clinical Microbiology*. 1997; 35(5):1244–1247. [PubMed: 9114415]
- van der Poel WH, Verschoor F, van der Heide R, Herrera MI, Vivo A, Kooreman M, de Roda Husman AM. Hepatitis E virus sequences in swine related to sequences in humans, The Netherlands. *Emerging Infectious Diseases*. 2001; 7(6):970–976. [PubMed: 11747723]
- Wang Y, Zhang H, Ling R, Li H, Harrison TJ. The complete sequence of hepatitis E virus genotype 4 reveals an alternative strategy for translation of open reading frames 2 and 3. *The Journal of General Virology*. 2000; 81(Pt 7):1675–1686. [PubMed: 10859372]
- Wang YC, Zhang HY, Xia NS, Peng G, Lan HY, Zhuang H, Zhu YH, Li SW, Tian KG, Gu WJ, Lin JX, Wu X, Li HM, Harrison TJ. Prevalence, isolation, and partial sequence analysis of hepatitis E virus from domestic animals in China. *Journal of Medical Virology*. 2002; 67(4):516–521. [PubMed: 12115997]
- Wibawa ID, Suryadarma IG, Mulyanto, Tsuda F, Matsumoto Y, Ninomiya M, Takahashi M, Okamoto H. Identification of genotype 4 hepatitis E virus strains from a patient with acute hepatitis E and farm pigs in Bali, Indonesia. *Journal of Medical Virology*. 2007; 79(8):1138–1146. [PubMed: 17596841]
- Wu JC, Chen CM, Chiang TY, Tsai WH, Jeng WJ, Sheen IJ, Lin CC, Meng XJ. Spread of hepatitis E virus among different-aged pigs: two-year survey in Taiwan. *Journal of Medical Virology*. 2002; 66(4):488–492. [PubMed: 11857526]
- Yazaki Y, Mizuo H, Takahashi M, Nishizawa T, Sasaki N, Gotanda Y, Okamoto H. Sporadic acute or fulminant hepatitis E in Hokkaido, Japan, may be food-borne, as suggested by the presence of

hepatitis E virus in pig liver as food. The Journal of General Virology. 2003; 84(Pt 9):2351–2357. [PubMed: 12917455]

FIG. 1. Seroconversion to IgG anti-HEV in HEV-inoculated and control pigs. **Panel A**, pigs inoculated with sterile phosphate buffered saline (PBS) as negative controls (group 1); **Panel B**, pigs inoculated with a genotype 3 human HEV (strain US-2) as positive controls (group 2); **Panel C**, pigs inoculated with a genotype 4 human HEV (strain TW6196E) as the experimental group (group 3). The ELISA cutoff OD value is 0.30.

FIG. 2.

Average titers of HEV RNA in feces (expressed as log copies per mg of feces) from all five pigs in each group at the indicated days post-inoculation (dpi) during the first 4 weeks of infection. The open bars represent pigs inoculated with the genotype 3 human HEV (Strain US-2) and the filled bars represent pigs inoculated with genotype 4 human HEV (Strain TW6196E). The error bars represent $\pm$ standard deviation.

TABLE 1

Detection of HEV RNA by RT-PCR in samples (fecal/serum) collected weekly from pigs inoculated with PBS buffer, with a genotype 3 human HEV, and a genotype 4 human HEV

Group	Pig ID	positive (+) or negative (-) in fecal / serum samples at indicated wpi							
		1	2	3	4	5	6	7	8
1 ^a	3	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
	15	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
	16	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
	18	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
	22	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
2 ^b	25	+/-	+/+	+/+	+/+	+/+	+/+	-/-	-/-
	26	+/+	+/+	+/+	+/+	-/-	-/-	-/-	-/-
	32	+/+	+/+	+/+	+/+	-/-	-/-	-/-	-/-
	42	-/-	+/+	+/+	+/+	-/-	-/-	-/-	-/-
	44	-/-	+/+	+/+	-/-	-/-	-/-	-/-	-/-
3 ^c	28	-/-	-/-	+/+	+/+	+/+	-/-	-/-	-/-
	29	+/+	-/+	+/+	+/+	-/-	+/+	+/+	+/+
	34	+/+	-/-	+/+	+/+	+/+	+/+	+/+	+/+
	39	-/-	-/-	+/+	+/+	-/-	+/+	+/+	-/-
	40	+/+	-/-	+/+	+/+	+/+	+/+	-/-	+/+

^a inoculated with sterile phosphate buffered saline, PBS (negative controls)

^b inoculated with a genotype 3 human HEV strain US-2 (positive controls)

^c inoculated with a genotype 4 human HEV strain TW6196E (experimental group)

Quantification by Real-Time RT-PCR of HEV RNA is Fecal Samples Collected Every 3 Days From Pigs Inoculated With a Genotype 3 (G3, Group 2) and a Genotype 4 (G4, Group 3) Human HEV Between 3 and 27 Days Post-Inoculation

Table II

Group 2—G3 US-2 Strain					
HEV RNA in fecal sample (log copies/mg)					
Days postinoculation	Pig 25	Pig 26	Pig 32	Pig 42	Pig 44
3	3.49	5.21	4.96	4.40	5.94
6	7.56	7.55	7.01	6.55	7.04
9	7.93	8.54	7.77	7.58	8.17
12	8.86	8.56	8.05	7.11	8.14
15	9.00	6.58	7.98	8.20	8.11
18	8.85	4.49	6.51	8.99	6.84
21	8.93	5.45	6.50	8.04	6.25
24	9.10	5.20	— ^a	6.05	8.86
27	8.95	4.81	—	4.46	—

Group 3—G4 TW6196E Strain					
HEV RNA in fecal sample (log copies/mg)					
Days postinoculation	Pig 28	Pig 29	Pig 34	Pig 39	Pig 40
3	5.15	5.70	4.21	4.40	6.79
6	6.71	7.52	5.83	4.74	7.09
9	7.82	8.62	7.25	5.90	8.16
12	7.33	8.34	7.31	6.45	6.35
15	6.29	8.95	8.50	7.46	6.86
18	4.57	8.34	7.99	7.13	8.23
21	4.80	8.75	8.71	8.52	8.48
24	5.04	7.53	7.97	7.91	7.25
27	4.99	8.51	9.26	6.89	4.42

^aNot detected.