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From Barnyard to Food Table: the Omnipresence of Hepatitis E virus and Risk for Zoonotic Infection and Food Safety

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Abstract

Hepatitis E virus (HEV) is an important but extremely understudied pathogen. The mechanisms of HEV replication and pathogenesis are poorly understood, and a vaccine against HEV is not yet available. HEV is classified in the family *Hepeviridae* consisting of at least four recognized major genotypes. Genotypes 1 and 2 HEV are restricted to humans and associated with epidemics in developing countries, whereas genotypes 3 and 4 HEV are zoonotic and responsible for sporadic cases worldwide. The identification and characterization of a number of animal strains of HEV from pigs, chickens, rabbits, rats, mongoose, deer, and possibly cattle and sheep have significantly broadened the host range and diversity of HEV. The demonstrated ability of cross-species infection by some animal strains of HEV raises public health concerns for zoonotic HEV infection. Pigs are a recognized reservoir for HEV, and pig handlers are at increased risk of zoonotic HEV infection. Sporadic cases of hepatitis E have been definitively linked to the consumption of raw or undercooked animal meats such as pig livers, sausages, and deer meats. In addition, since large amounts of viruses excreted in feces, animal manure land application and runoffs can contaminate irrigation and drinking water with concomitant contamination of produce or shellfish. HEV RNA of swine origin has been detected in swine manure, sewage water and oysters, and consumption of contaminated shellfish has also been implicated in sporadic cases of hepatitis E. Therefore, the animal strains of HEV pose not only a zoonotic risk but also food and environmental safety concerns.

Keywords

Hepatitis E virus (HEV); Animal reservoir; Zoonosis; Cross-species infection; Food safety

1. Introduction

Hepatitis E virus (HEV), the causative agent of hepatitis E, is an important but extremely understudied pathogen (Aggarwal and Naik, 2009; Meng, 2010b; Purcell and Emerson, 2001). HEV is a small, non-enveloped, RNA virus that is transmitted primarily via the fecal-oral route (Meng, 2008; Purcell and Emerson, 2001). The virus causes a public health concern in many developing countries of Asia and Africa where sanitation conditions are

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poor (Arankalle et al., 1995; Chandra et al., 2008; Purcell and Emerson, 2001). However, sporadic cases of acute hepatitis E have also been reported in individuals residing in many industrialized countries (Okamoto, 2007). Among the known hepatitis viruses, the relatively high mortality rate, up to 28%, in infected pregnant women is unique for HEV (Navaneethan et al., 2008; Purcell and Emerson, 2008). A vaccine against HEV is not yet available, although the experimental vaccines are promising (Krawczynski et al., 2007; Shrestha et al., 2007; Zhu et al., 2010).

Recently, significant progresses have been made in understanding the natural history and animal reservoirs of HEV (Meng, 2003, 2006, 2008; Meng and Halbur, 2006, Meng et al., 2008; Pavio et al., 2010). The discoveries of animal strains of HEV from domestic pigs (Meng et al., 1997) and wild boars (Sonoda et al., 2004), chickens (Payne et al., 1999; Haqshenas et al., 2001), rabbits (Zhao et al., 2009), rats (Johns et al., 2010), deer (Tei et al., 2003), mongoose (Nakamura et al., 2006), and possibly cattle (Hu and Ma, 2010) and sheep (Wang and Ma, 2010), and the existence of other animal species that are seropositive for HEV antibodies (Meng, 2010a, 2010b), have significantly broadened the host range and diversity of HEV. Hepatitis E is now a recognized zoonotic disease, and domestic pigs, wild boars and likely other animal species are reservoirs for HEV (Meng, 2010a). The ubiquitous nature of the virus in domestic pigs and wild boars as well as in several other animal species raises public health concern for zoonotic infection through direct contacts with infected animals or through the consumption of contaminated animal meats (Meng et al., 2009, 2010a, 2010b; Pavio et al., 2010). The focus of this review is on the animal strains of HEV with emphasis on potential zoonotic risk, food and environmental safety concerns.

2. HEV Classification

Currently, HEV is classified in the family *Hepeviridae* (Emerson et al., 2004). The species in the sole genus *Hepevirus* of the family includes the 4 recognized major genotypes of mammalian HEV: the genotype 1 (Burmese-like Asian strains), genotype 2 (a Mexican strain and some African strains), genotype 3 (strains from sporadic human cases worldwide and animal strains), and genotype 4 (strains from sporadic human cases in Asia, and animal strains). However, with the recent identification of genetically distinct HEV strains from rats (Johns et al., 2010) and wild boars (Takahashi M et al., 2011; Takahashi K et al., 2010, GenBank accession no. AB573435), the HEV taxonomy will need to be revised (Table 1). The rat HEV and the novel wild boar strain of HEV from Japan appear to belong to previously unrecognized genotypes.

The avian HEV from chickens is currently classified as a floating species in the family (Meng et al., 2008; Meng, 2010b). However, since avian HEV shares only approximately 50-60% nucleotide sequence identity with mammalian HEV strains in the genus *Hepevirus* (Meng et al., 2008), and since at least three distinct genotypes of avian HEV have now been identified worldwide (Bilic et al., 2009; Marek et al., 2010; Zhao et al., 2010), it will be more appropriate to classify avian HEV as a separate genus, instead of a floating species, within the family *Hepeviridae*. Therefore, the current classification of avian HEV will need to be revised as well.

3. Animal strains of HEV

3.1. Swine HEV from domestic pigs

In 1995, HEV antibodies and RNA were reportedly detected from domestic pigs in Nepal, although the identity of the virus in the Nepalese pigs was not known as the virus was not sequenced (Clayson et al., 1995). In 1997, the first animal strain of HEV, swine HEV, was identified and characterized from domestic pigs in the United States (Meng et al., 1997). The

authors serendipitously found out that the majority of adult pigs in the United States were positive for HEV antibodies, suggesting that the pigs were exposed to HEV. Subsequently, a prospective study was conducted in a commercial swine farm in Illinois which led to the discovery of a novel virus genetically and antigenically closely related to human HEV, designated swine HEV, in pigs (Meng et al., 1997). Since this initial identification, swine HEV has now been detected in domestic pigs from essentially all major swine-producing countries of the world (Meng and Halbur, 2006; Meng, 2010b; Okamoto, 2007). Swine HEV infection is widespread in swine farms worldwide from both developing and industrialized countries, regardless of whether HEV is endemic in the respective human populations (Meng, 2010a).

Swine HEV generally infects pigs of 2 to 4 months of age. The infected pigs generally have a transient viremia lasting for 1 to 2 weeks, and fecal virus shedding for about 3 to 7 weeks (Meng et al., 1997; Takahashi et al., 2003; Yazaki et al., 2003). At least two genotypes of swine HEV, genotypes 3 and 4, have been definitively identified and characterized from pigs worldwide. A short genotype 1 HEV-like sequence was reportedly detected in a pig from Cambodia (Caron et al., 2006), although independent confirmation of this report is lacking. The transmission route in pigs is thought to be fecal-oral (Kasorndorkbua et al., 2004). It has been shown that large amounts of infectious virus shed in feces of infected pigs (Feagins et al., 2008b), and that virus-containing feces are likely the main source of virus for transmission. Naïve pigs can become infected through direct contact with infected ones or through ingestion of feces-contaminated feed or water (Bouwknegt et al., 2008). However, experimental infection of pigs with swine or human HEV via the oral route of inoculation has been difficult (Kasorndorkbua et al., 2004), even though pigs can be readily infected via the intravenous route of inoculation (Halbur et al., 2001; Meng et al., 1998). Therefore, other route(s) of transmission cannot be ruled out.

Swine HEV infection in pigs is subclinical. In naturally infected piglets, gross pathological lesions were not detected in the liver or 18 other tissues and organs in 4 pigs necropsied during early stages of natural swine HEV infection (Meng et al., 1997), although all four piglets had microscopic lesions of hepatitis characterized by mild to moderate multifocal and periportal lymphoplasmacytic hepatitis with mild focal hepatocellular necrosis. In pigs experimentally infected with a genotype 3 swine HEV and a genotype 3 human HEV (Halbur et al., 2001), mildly-to-moderately enlarged hepatic and mesenteric lymph nodes, and microscopic lesions of hepatitis including mild-to-moderate multifocal lymphoplasmacytic hepatitis and focal hepatocellular necrosis were observed, although the infected pigs remain clinically normal.

3.2. Swine HEV from wild boars

Free-living wild boars (*Sus scrofa*) are indigenous in many countries of the world and pose not only ecological concerns but infectious disease concerns as well (Meng et al., 2009). Wild boars harbor many important infectious agents including HEV that are transmissible to domestic pigs and humans. Human habitation changes from rural to suburban areas due to growing world populations, increased use of lands for agricultural purposes, and deforestation have increased the chances of contact exposure of wild boars to humans and domestic animals (Meng et al., 2009). In some countries, recreational hunting of wild boars and consumption of wild boar meats further provided an ideal condition for the transmission of pathogens such as HEV from wild boars to humans (Gibbs, 1997; Sonoda et al., 2004).

HEV RNA and antibodies have been detected in wild boar populations from a number of countries including Japan, Germany, Italy, Spain, the Netherlands and Australia (Chandler, et al., 1999; de Deus et al., 2008; Kaci et al., 2008; Martelli et al., 2008; Michitaka et al., 2007; Rutjes et al., 2010). The HEV seropositivity in wild boars varied from 17% to 42%,

and the HEV RNA detection rate ranged from 3-25% in wild boars of different geographic regions. A full-length genomic sequence of HEV amplified from a wild boar shares 99.7% nucleotide sequence identity to a virus recovered from a wild deer hunted in the same forest and to four human hepatitis E patients who consumed deer meats (Takahashi *et al.* 2004). Most strains of HEV recovered from wild boars worldwide belong to genotype 3 (Meng *et al.*, 2010a). However, recently novel HEV sequences belonging to genotype 4 (GenBank accession nos. AB245437, AB245438, AB245439) and to a new unrecognized HEV genotype (Takahashi M *et al.*, 2011; Takahashi K *et al.*, 2010, GenBank accession no. AB573435) have also been detected from wild boars.

3.3. Avian HEV

In 1999, a short 523-bp HEV-like sequence with 62% nucleotide sequence identity to human HEV was amplified from chickens with big liver and spleen disease (BLS) in Australia, and the virus was named BLS virus (BLSV) (Payne *et al.*, 1999). The BLS disease mainly affects commercial broiler breeder flocks and causes decreased egg production and slight increase in mortality (Meng *et al.*, 2008). In the United States and Canada, a disease known as Hepatitis-Splenomegaly (HS) Syndrome was also reported in chickens. The cause of the HS syndrome was not known until the isolation and characterization of a virus genetically and antigenically related to human HEV from bile samples of chickens with HS Syndrome (Haqshenas *et al.*, 2001). The novel virus in chickens shares similar genomic organization with 50-60% nucleotide sequence identities across the full-length genome with human and swine HEVs (Huang *et al.*, 2004; Sun *et al.*, 2004a), and thus is designated as avian HEV to distinguish it from the mammalian HEV (Haqshenas *et al.*, 2001). Avian HEV and BLSV shared approximately 80% nucleotide sequence identity (Haqshenas *et al.*, 2001; Payne *et al.*, 1999), suggesting that BLS in Australia and HS syndrome in North America are likely caused by variant strains of the same virus (Meng *et al.*, 2008).

HS syndrome was first reported in chickens in western Canada in 1991, but the disease has now been reported in eastern Canada, the United States, Spain, China and other countries (Huang *et al.*, 2002; Peralta *et al.*, 2009a; Zhao *et al.*, 2010). BLS has been reported from chickens in Australia (Payne *et al.*, 1999; Meng *et al.*, 2008). Leghorn hens are typically affected although the disease has also been recognized in broiler breeder hens. In the United States, avian HEV infection is enzootic in chicken flocks, and approximately 71% chicken flocks and 30% chickens were seropositive for avian HEV antibodies (Huang *et al.*, 2002; Sun *et al.*, 2004a). Avian HEV infection appears to be age-dependant: approximately 17% of young chickens (less than 18 weeks of age) and 36% of adult chickens were positive for avian HEV antibodies (Huang *et al.*, 2002). It appears that avian HEV is readily transmitted within and between chicken flocks. In a prospective study of natural avian HEV transmission in a chicken flock from Virginia, at 12 weeks of age, all 14 chickens from the study were seronegative. The first chicken seroconverted at 13 weeks of age, and by 21 weeks of age, all 14 chickens from the prospective study had seroconverted (Sun *et al.*, 2004a). Avian HEV infection has been experimentally reproduced via oronasal route of inoculation in chickens (Billam *et al.*, 2005), and thus the route of transmission is presumably fecal-oral although other routes of transmission cannot be ruled out. Rodents in chicken farms may potentially serve as a mechanical vector for avian HEV (Sun and Meng, unpublished data).

Avian HEV infection in chickens is mostly subclinical (Huang *et al.*, 2002; Sun *et al.*, 2004a; Peralta *et al.*, 2009a; Zhao *et al.*, 2010). The morbidity and mortality of HS syndrome or BLS associated with avian HEV infection are relatively low. Chickens with HS syndrome display above-normal mortality in broiler breeder hens and laying hens of 30-72 week of age, with the highest incidence occurring between 40-50 weeks of age. In some cases, egg production can drop by up to 20%. Weekly mortality increases to approximately 0.3% and

may sometimes exceed 1.0%. Similar to HS syndrome, the clinical signs for BLS in Australia also vary from mostly subclinical infection to egg drops that may reach 20% with up to 1% mortality per week over a period of 3-4 weeks (Meng et al., 2008). Dead chickens associated with avian HEV infection usually have regressive ovaries, red fluid in the abdomen, and enlarged liver and spleen. Livers are enlarged with hemorrhage and some may have subcapsular hematomas. Spleens from affected birds are mild to severely enlarged. Microscopically, liver lesions varied from multifocal hemorrhage to extensive areas of necrosis and hemorrhage and infiltration of heterophils and mononuclear inflammatory cells around portal triads (Meng et al., 2008). In chickens experimentally-infected by avian HEV, subcapsular hemorrhages and enlarged livers were observed in approximately one-fourth of the infected chickens (Billam et al., 2005). The microscopic hepatitis lesions in experimentally-infected chickens are characterized by lymphocytic periphlebitis and phlebitis in the livers.

3.4. Rat HEV

Rats have long been suspected as a potential reservoir for HEV. Antibodies to HEV have been detected in various species of rats including Norway (*Rattus norvegicus*), black (*Rattus rattus*), and cotton (*Sigmodon hispidus*) rats (Arankalle et al., 2001; Favorov et al., 2000; Hirano et al., 2003; Kabrane-Lazizi et al., 1999). For example, Kabrane-Lazizi et al (1999) tested 239 wild rats trapped in different geographic regions of the United States for the presence of IgG anti-HEV, and found that approximately 44% of the rats caught in Louisiana, 77% in Maryland, and 90% in Hawaii were seropositive. Unfortunately, the source of anti-HEV seropositivity in rats could not be identified until recently (John et al, 2010a). By using a nested broad-spectrum RT-PCR, John et al (2010a) amplified two authentic HEV sequences from fecal samples of wild Norway rats (*Rattus norvegicus*) from Hamburg, Germany. The rat HEV shared only approximately 60% and 50% sequence identity with human HEV and avian HEV, respectively. Subsequently, the complete genomic sequences of two strains of rat HEV have been determined (John et al., 2010b), and sequence and phylogenetic analyses revealed that the rat HEV belongs to a previously unrecognized genotype within the genus *Hepevirus* of the family *Hepeviridae*. It remains to be determined if the rat HEV can cross species barrier and infect humans or other animal species.

3.5. Rabbit HEV

A unique strain of HEV was recently identified from farm rabbits in Gansu Province, China, and three full-length genomic sequences of the rabbit HEV were determined (Zhao et al., 2009; GenBank accession no. GU937805). The rabbit HEV shares approximately 85% nucleotide sequence identity with each other and 74%, 73%, 78-79%, 74-75%, and 46-47% nucleotide sequence identity to genotypes 1, 2, 3, 4 mammalian HEV, and avian HEV, respectively. Phylogenetic analysis revealed that the rabbit HEV is a distant member of the genotype 3 HEV. Approximately 57% of the farm rabbits in Gansu Province, China were seropositive for anti-HEV antibodies with approximately 8% of them also positive for HEV RNA. In a separate study from Beijing, China (Geng et al., 2010), approximately 55% (65/119) of the farmed rex rabbits were tested positive for anti-HEV antibody with approximately 7% of fecal samples (8/115) also positive for HEV RNA. The rabbit HEV sequences recovered from rabbits in Beijing cluster together in the genotype 3 with those from Gansu Province. Since the rabbit HEV belongs to the genotype 3, thus it is possible that the rabbit HEV may be zoonotic. Additional studies are warranted to determine the host range and species tropism of the rabbit HEV.

3.6. Deer HEV

IgG anti-HEV was detected in approximately 3% of the sika deer and 35% of Yezo-deer samples in Japan (Matsuura et al., 2007; Tomiyama et al., 2009) and 5% of red deer in the Netherlands (Rutjes et al., 2010). The full-length genomic sequence of a strain of HEV was determined from a sika deer in Japan, and sequence analysis revealed that the deer HEV belongs to the genotype 3 (Takahashi et al., 2004). Genotype 3 strains of HEV were also genetically identified from roe deer in Hungary (Forgách et al., 2010; Reuter et al., 2009). Zoonotic transmission of HEV from deer to humans via the consumption of contaminated deer meats has been reported (Tei et al., 2003; Takahashi et al., 2004), and thus the deer HEV is considered zoonotic.

3.7. Mongoose HEV

It has been reported that approximately 8-21% of the mongooses in Japan were tested positive for HEV antibodies (Li et al., 2006; Nakamura et al., 2006). The full-length genomic sequence of a strain of HEV recovered from a mongoose was determined and shown to be a genotype 3 HEV that was closely related to a genotype 3 swine HEV from Japan (Nakamura et al., 2006). The ability of the mongoose HEV to infect across species is unknown, however, since the mongoose HEV belongs to genotype 3, it may be zoonotic.

3.8. Bovine HEV?

Antibodies to HEV have been detected from 4.4% to 6.9% of cattle in India (Arankalle VA et al., 2001), and 6% to 93% of cattle in China (Geng et al., 2010; Wang et al., 2002; Yu et al., 2009; Zhang et al., 2008). In a prospective study of 6 newborn calves for evidence of HEV infection in the United States (Goens et al., 2003), it was shown that the calves began to seroconvert to IgG anti-HEV at about 3 months of age, and by 7 months of age, seroconversion to IgG anti-HEV was observed in 5 out of the 6 calves. However, attempts to genetically identify HEV from these cattle were unsuccessful (Goens et al., 2003). Recently, a 189-bp ORF2 sequence of HEV was reportedly amplified from the fecal samples of 8 cows from two farms in China (Hu and Ma, 2010), and the 8 HEV sequences from cows share 96-100% nucleotide sequence identity with each other, and 76-86%, 82-84%, 79-85%, 84-96% nucleotide sequence identity with the genotypes 1, 2, 3 and 4 mammalian strains of HEV, respectively. Based on the available short sequence, it appears that the bovine HEV belongs to the genotype 4 (Hu and Ma, 2010). However, the authenticity of these genotype 4-like HEV sequences from cattle in China remains to be independently confirmed.

3.9. Sheep HEV?

Serological evidence of HEV infection in sheep has been reported: approximately 10-12% of the sheep tested in China (Chang et al., 2009; Yu et al., 2009), and 2% of sheep tested in Spain (Peralta et al., 2009b) are reportedly positive for IgG anti-HEV. A short 189-bp sequence of HEV was amplified from 6 sheep fecal samples in China by the same lab that reported the short sequences of bovine HEV (Wang and Ma, 2010), and these 6 short HEV sequences from sheep are 99-100% identical to each other but share only 79-85%, 81-83%, 79-84%, and 85-95% nucleotide sequence identity to the genotypes 1, 2, 3 and 4 mammalian HEV strains, respectively. Based on the available short sequence, the sheep HEV appears to be a genotype 4 HEV (Wang and Ma, 2010). However, like the bovine HEV, these short genotype 4-like HEV sequences in sheep have not been independently confirmed.

4. Other potential animal reservoirs for HEV

In addition to the animal species from which HEV strains have been genetically identified as discussed above, serological evidence of HEV infection has also been reported in a number of other animal species including dogs (Arankalle et al., 2001; Liu et al., 2009; Zhang et al.,

2008), cats (Kuno et al., 2003; Song et al., 2010), goats (Geng et al., 2010; Peralta et al., 2009b; Shukla et al., 2007), and rhesus monkeys (Tsarev et al., 1995). Other studies failed to detect IgG anti-HEV in some species such as goats (Arankalle et al., 2001; Wang et al., 2002). Thus far, the source of anti-HEV seropositivity in these animal species could not be definitively identified, since virus was not recovered or sequenced from these animal species. Thus, new animal strains of HEV likely exist but more studies are needed to fully understand the natural history of HEV.

5. Experimental cross-species infections by HEV

Under experimental conditions, genotypes 3 and 4 HEV can infect across species barriers (Table 1). It has been shown that non-human primates can be experimentally infected with a genotype 3 and a genotype 4 swine HEV (Meng et al., 1998a; Arankalle VA et al., 2006). The rhesus monkeys infected with the genotypes 3 and 4 swine HEV seroconverted to HEV antibodies, and had viremia, fecal virus shedding and elevation of serum liver enzymes. A chimpanzee inoculated with a genotype 3 swine HEV shed virus in feces, seroconverted to IgG anti-HEV (Meng et al., 1998), although both rhesus monkeys and the chimpanzee infected with swine HEV remained clinically normal. Conversely, it has also been demonstrated that specific-pathogen-free pigs experimentally inoculated with a genotype 3 human HEV (Halbur et al., 2001) and a genotype 4 human HEV (Feagins et al., 2008b) rapidly became infected: viremia and seroconversion to IgG anti-HEV occurred within 2 weeks post-inoculation, suggesting that the genotypes 3 and 4 human HEV have already adapted to replicate in pigs and are likely of swine origin (Xia et al., 2010) (Table 1). In fact, recently it has been demonstrated that chimeric hepatitis E viruses containing the capsid gene either alone or in combination with its adjacent region from a genotype 4 human HEV in the backbone of a genotype 3 swine HEV are infectious in pigs (Feagins et al., 2011), and thus providing experimental evidence that genotypes 3 and 4 human HEV are of swine origin. An attempt to experimentally infect pigs with genotype 1 and genotype 2 HEV was not successful (Meng et al., 2008b), suggesting that genotypes 1 and 2 HEV have a more restricted host range (Table 1).

Lambs were reportedly infected with two human isolates of HEV (Usmanov et al, 1994), and the inoculated lambs reportedly had clinical signs of hepatitis. Also, Wistar rats experimentally inoculated with a strain of human HEV became infected (Maneerat et al., 1996). However, independent studies to confirm the reported HEV transmission in rats with a genotype 1 human HEV, a genotype 3 swine HEV, a genotype 4 swine HEV, and an avian HEV were unsuccessful (Sun and Meng, unpublished data). It was also reported that Balb/c nude mice are susceptible to experimental infection with a genotype 4 strain of swine HEV (Huang et al., 2009), although others failed to infect C57BL/6 mice with genotype 1, 3 and 4 strains of HEV (Li et al., 2008). More recently, it is reported that rabbits can be experimentally infected with a genotype 1 and a genotype 4 HEV (Ma et al., 2010): six of nine rabbits inoculated with a genotype 1 HEV and all rabbits inoculated with a genotype 4 HEV seroconverted to IgG anti-HEV. However, independent confirmation of the susceptibility of rabbits to genotypes 1 and 4 HEV is still lacking. It has been demonstrated that avian HEV can cross species barriers and infect turkeys (Sun et al., 2004b). However, under experimental conditions, avian HEV failed to infect two rhesus monkeys (Huang et al., 2004), suggesting that chickens are likely not a reservoir for HEV. The expanded host ranges of HEV and its ability to infect across species raise concerns for potential zoonotic HEV infection.

6. Zoonotic HEV infection through direct contacts with infected animals

Hepatitis E is now a recognized zoonotic disease, and pigs and likely other animal species are reservoirs for HEV. In a seroepidemiological study conducted in the United States, 295 swine veterinarians from 8 States along with 400 age- and geography-matched normal blood donors were tested for the presence of IgG anti-HEV (Meng et al., 2002). The results showed that approximately 23% of swine veterinarians were positive for IgG anti-HEV (genotype 3 swine HEV antigen) compared to only 17% in normal blood donors. Swine veterinarians were 1.51 times (swine HEV antigen, $p=0.03$) more likely to be anti-HEV positive than normal U.S. blood donors. Veterinarians who reported having needle sticks while performing procedures on pigs were about 1.9 times more likely to be seropositive than those who did not (Meng et al., 2002). Also, individuals from traditionally major swine states are more likely to be seropositive than those from traditionally non-swine States. In Moldova, Drobeniuc et al (2001) tested 264 swine farmers along with 255 controls for the presence of IgG anti-HEV, and found that approximately 51% of swine farmers were positive compared to only 25% of control subjects. Withers et al (2002) reported that swine workers in North Carolina had a 4.5-fold higher rate of IgG anti-HEV prevalence than the control subjects. Taken together, the available data indicated that pig handlers such as pig farmers and swine veterinarians are at increased risk of zoonotic HEV infection.

Animal species other than pigs could also serve as potential reservoir for HEV (Meng, 2010a). Karetnyi et al (1999a) tested 87 field workers from the Iowa Department of Natural Resources (DNR) and 332 normal blood donors for the prevalence of HEV antibodies, and found that the DNR workers had higher HEV antibody prevalence than normal blood donors ($P<0.05$). In addition, possible transmissions of hepatitis E from a pet cat and a pet pig to its human owners have been reported (Kuno et al., 2003; Renou et al., 2007). Therefore, direct contact with infected animals is a potential risk factor for zoonotic HEV infection.

7. Foodborne zoonotic HEV infection through the consumption of contaminated animal meats

The ubiquitous nature of HEV infection in pigs suggests that contamination of organ meats by HEV may be unavoidable. It has been shown that approximately 2% of the pig livers sold in grocery stores in Japan (Yazaki et al., 2003) and 11% in the United States (Feagins et al., 2007) were tested positive for HEV RNA. Most importantly, the contaminating virus in the pig livers sold from the grocery stores in the United States remains fully infectious (Feagins et al., 2007). The virus sequences recovered from commercial pig livers are closely related, or identical in a few cases, to the viruses recovered from human hepatitis E patients (Yazaki et al., 2003).

It has been demonstrated that the contaminating virus was completely inactivated when the HEV-contaminated pig livers were boiled or stir-fried for 5 minutes (Feagins et al., 2008a). However, incubation of the contaminated pig liver homogenates at 56°C for 1 hour, a temperature equivalent to medium-to-rare cooking conditions in a restaurant, did not inactivate the virus (Feagins et al., 2008a), indicating that consumption of undercooked or raw pig meats could potentially pose a risk of foodborne zoonotic HEV infection. In fact, sporadic cases of acute hepatitis E have been definitely linked to the consumption of contaminated raw and undercooked pig livers or grilled pork (Li et al., 2005; Masuda et al., 2005; Matsuda et al., 2003; Matsubayashi et al., 2008). Consumption of game meats is also a risk factor for HEV infection (Legrand-Abrevanel, et al., 2010). In a recent case-control study in France, 7 of 13 individuals from three families who ate raw figatelli pig liver sausages became infected by HEV, whereas 5 other individuals from the same families who did not eat figatelli sausages were not infected (Colson et al., 2010). Genotype 3 HEV RNA

was detected from 7 of 12 figatelli sausages purchased from supermarkets, and statistically significant genetic links were established between the HEV sequences from figatelli sausages and the HEV sequences recovered from hepatitis E patients who ate raw figatellu (Colson et al., 2010).

IgG anti-HEV is also highly prevalent in the wild boar populations (de Deus et al., 2008; Meng et al., 2009; Michitaka et al., 2007), and genotype 3 strains of HEV have been detected from wild boars (de Deus et al.; 2008; Sonoda et al., 2004; Takahashi et al., 2004). Sporadic cluster cases of hepatitis E have been reported in patients who ate wild boar meats (Li et al., 2005; Masuda et al., 2005; Matsuda et al., 2003; Wichmann et al., 2008). For examples, a 53-year-old Japanese man developed severe hepatitis E after eating contaminated wild boar meat (Matsuda et al., 2003). Another patient, a 70-year-old man, who also ate the same wild boar meat, died of fulminant hepatic failure. Neither patient had travelled to an HEV endemic area, but both patients ate uncooked wild boar livers on five occasions (Matsuda et al., 2003). Take together, these data provided compelling evidence that both domestic and wild pigs are the reservoirs for HEV, and that consumption of undercooked or raw domestic and wild pig meats pose a risk for foodborne zoonotic HEV infection.

In addition to pigs, other HEV-infected animal meats may also pose a potential food safety concern. It has been reported that the prevalence of IgG anti-HEV antibodies is significantly higher (18%) in subjects with experience of eating raw deer meats than in controls (2%) (Tei et al., 2004). A cluster of 4 cases of acute hepatitis E were definitively linked to the consumption of raw deer meats in two Japanese families (Takahashi et al., 2004; Tei et al., 2003). The HEV sequence recovered from the leftover frozen deer meat was 99.7 to 100% identical to the viruses recovered from the four human hepatitis E patients (Tei et al., 2003), thus confirming that foodborne zoonotic HEV transmission occurred via the consumption of infected deer meats.

7. Environmental safety concern

As a fecal-orally transmitted disease, contaminated water or water supplies are main sources of HEV infections (Meng, 2010a). Waterborne epidemics are the characteristic of hepatitis E outbreaks in humans in regions where sanitation conditions are poor (Purcell and Emerson, 2001, 2008). HEV replicates in the liver and gastrointestinal tract (Billam et al., 2008; Williams et al., 2001), and thus infected animals such as pigs excreted large amounts of HEV in feces, which poses a concern for environmental safety.

HEV has been detected in swine manure and wastewater associated with hog operations (Karenyi et al. 1999b), and in concrete pits and lagoons of swine manure storage facility in the United States (Kasorndorkbua et al, 2005). Genotype 3 HEV RNA was detected in swine manure collected from concrete holding pits on 15/22 Iowa farms, and in swine manure collected from lagoons on 3/8 Iowa farms. Most importantly, the HEV detected in pig manure slurry remains infectious when inoculated into naïve pigs (Kasorndorkbua et al, 2005). Therefore, swine manure land application and runoffs could be the source for contamination of irrigation and drinking water in nearby wells, rivers, ponds or costal water with concomitant contamination of produce or shellfish (Smith, 2001), which could subsequently lead to potential transmission of HEV to humans. For example, in Korea, genotype 3 HEV RNA closely resembling that of a Korean strain of swine HEV was detected in oysters (Song et al, 2010). Consumption of contaminated shellfish has been implicated in sporadic cases of acute hepatitis E (Cacopardo et al. 1997; Koizumi et al. 2004). It has also been shown that, in HEV endemic regions, the use of river water for bathing, waste disposal, and drinking purposes is a significant risk factor (Sedyaningsih-

Mamahit et al, 2002; Toole et al., 2006). For instance, a unique riverine transmission of HEV has been reported in Southeast Asia: epidemic foci centered in riverine environments (Corwin et al. 1999) and peaked with heavy rains and flooding, and the hepatitis E attack rate was significantly higher in villages using river water than in those using wells or ponds (Bile et al. 1994). Also, in Turkey, individuals used untreated waste water for irrigation purpose have a significant higher HEV antibody prevalence rate (34.8%) than the control subjects with similar age and socio-economic status (Ceylan et al. 2003).

HEV strains of both human and swine origins have been detected in raw sewage water (Clemente-Casares et al. 2003; Ippagunta et al., 2007; Jothikumar et al. 1993; Pina et al. 1998, 2000), and the recovered virus from sewage water is still infectious in non-human primates. Pina et al (2000) found that a HEV strain of likely pig origin recovered from sewage water of pig slaughterhouses in Spain is genetically closely related to two Spanish strains of human HEV. Also, HEV strains recovered from raw sewage water in the United States are genetically more closely related to the genotype 3 swine HEV from the United States than to the US-1 and US-2 strains of human HEV (Clemente-Casares et al. 2003). In India, it has been shown that the anti-HEV prevalence was significantly higher in sewage workers (57%) than in controls (19%). A significant rise in HEV antibody positivity was found in sewage workers working for more than 5 years (Vaidya et al. 2003). These data indicate that animal strains of HEV including human HEV pose an environmental safety concerns, as HEV-contaminated costal, drinking, irrigation and sewage water could be a source for human infection.

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Abbreviations

HEV	hepatitis E virus
Swine HEV	swine hepatitis E virus
Avian HEV	avian hepatitis E virus
BLSV	big liver and spleen disease virus
HS syndrome	Hepatitis-Splenomegaly Syndrome

References

- Aggarwal R, Naik S. Epidemiology of hepatitis E: current status. *J. Gastroenterol. Hepatol.* 2009; 24(9):1484–93. [PubMed: 19686410]
- Arankalle VA, Tsarev SA, Chadha MS, Alling DW, Emerson SU, Banerjee K, Purcell RH. Age-specific prevalence of antibodies to hepatitis A and E viruses in Pune, India, 1982 and 1992. *J. Infect. Dis.* 1995; 171:447–450. [PubMed: 7844387]
- Arankalle VA, Joshi MV, Kulkarni AM, Gandhe SS, Chobe LP, Rautmare SS, Mishra AC, Padbidri VS. Prevalence of anti-hepatitis E virus antibodies in different Indian animal species. *J. Viral. Hepat.* 2001; 8:223–227. [PubMed: 11380801]
- Arankalle VA, Chobe LP, Chadha MS. Type-IV Indian swine HEV infects rhesus monkeys. *J. Viral. Hepat.* 2006; 13(11):742–745. [PubMed: 17052273]
- Bile K, Isse A, Mohamud O, Allebeck P, Nilsson L, Norder H, Mushahwar IK, Magnus LO. Contrasting roles of rivers and wells as sources of drinking water on attack and fatality rates in a hepatitis E epidemic in Somalia. *Am. J. Trop. Med. Hyg.* 1994; 51(4):466–474. [PubMed: 7943574]

- Bilic I, Jaskulska B, Basic A, Morrow CJ, Hess M. Sequence analysis and comparison of avian hepatitis E viruses from Australia and Europe indicate the existence of different genotypes. *J. Gen. Virol.* 2009; 90(Pt 4):863–873. [PubMed: 19264623]
- Billam P, Huang FF, Sun ZF, Pierson FW, Duncan RB, Elvinger F, Guenette DK, Toth TE, Meng XJ. Systematic pathogenesis and replication of avian hepatitis E virus in specific-pathogen-free adult chickens. *J. Virol.* 2005; 79:3429–3437. [PubMed: 15731237]
- Billam P, Pierson FW, Li W, LeRoith T, Duncan RB, Meng XJ. Development and validation of a negative-strand-specific reverse transcription-PCR assay for detection of a chicken strain of hepatitis E virus: identification of nonliver replication sites. *J Clin Microbiol.* 2008; 46(8):2630–2634. [PubMed: 18562592]
- Bouwknegt M, Frankena K, Rutjes SA, Wellenberg GJ, de Roda Husman AM, van der Poel WH, de Jong MC. Estimation of hepatitis E virus transmission among pigs due to contact-exposure. *Vet. Res.* 2008; 39:40. [PubMed: 18367077]
- Cacopardo B, Russo R, Preiser W, Benanti F, Brancati G, Nunnari A. Acute hepatitis E in Catania (eastern Sicily) 1980–1994. The role of hepatitis E virus. *Infection.* 1997; 25(5):313–316.
- Caron M, Enouf V, Than SC, Dellamonica L, Buisson Y, Nicand E. Identification of genotype 1 hepatitis E virus in samples from swine in Cambodia. *J. Clin. Microbiol.* 2006; 44(9):3440–3442. [PubMed: 16954296]
- Ceylan A, Ertem M, Ilcin E, Ozekinci T. A special risk group for hepatitis E infection: Turkish agricultural workers who use untreated waste water for irrigation. *Epidemiol Infect.* 2003; 131(1): 753–756. [PubMed: 12948376]
- Chandler JD, Riddell MA, Li F, Love RJ, Anderson DA. Serological evidence for swine hepatitis E virus infection in Australian pig herds. *Vet. Microbiol.* 1999; 68(1-2):95–105. [PubMed: 10501166]
- Chandra V, Taneja S, Kalia M, Jameel S. Molecular biology and pathogenesis of hepatitis E virus. *J. Biosci.* 2008; 33(4):451–64. [PubMed: 19208971]
- Chang Y, Wang L, Geng J, Zhu Y, Fu H, Ren F, Li L, Wang X, Zhuang H. Zoonotic risk of hepatitis E virus (HEV): A study of HEV infection in animals and humans in suburbs of Beijing. *Hepatol Res.* 2009; 39(12):1153–1158. [PubMed: 19674158]
- Clayson ET, Myint KS, Snitbhan R, Vaughn DW, Innis BL, Chan L, Cheung P, Shrestha MP. Viremia, fecal shedding, and IgM and IgG responses in patients with hepatitis E. *J. Infect. Dis.* 1995; 172:927–933. [PubMed: 7561211]
- Clemente-Casares P, Pina S, Buti M, Jardi R, Martín M, Bofill-Mas S, Girones R. Hepatitis E virus epidemiology in industrialized countries. *Emerg. Infect. Dis.* 2003; 9:448–454. [PubMed: 12702225]
- Corwin AL, Tien NT, Bounlu K, Winarno J, Putri MP, Laras K, Larasati RP, Sukri N, Endy T, Sulaiman HA, Hyams KC. The unique riverine ecology of hepatitis E virus transmission in South-East Asia. *Trans. R. Soc. Trop. Med. Hyg.* 1999; 93(3):255–260. [PubMed: 10492753]
- de Deus N, Peralta B, Pina S, Allepuz A, Mateu E, Vidal D, Ruiz-Fons F, Martín M, Gortázar C, Segalés J. Epidemiological study of hepatitis E virus infection in European wild boars (*Sus scrofa*) in Spain. *Vet. Microbiol.* 2008; 129:163–70. [PubMed: 18082340]
- 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. *J. Infect. Dis.* 2001; 184:1594–1597. [PubMed: 11740735]
- Emerson, SU.; Anderson, D.; Arankalle, VA.; Meng, XJ.; Purdy, M.; Schlauder, GG.; Tsarev, SA. Hepevirus. In: Fauquet, CM.; Mayo, MA.; Maniloff, J.; Desselberger, U.; Ball, LA., editors. *Virus Taxonomy, VIIIth Report of the ICTV.* Elsevier/Academic Press; London: 2004. p. 851–855.
- Favorov MO, Kosoy MY, Tsarev SA, Childs JE, Margolis HS. Prevalence of antibody to hepatitis E virus among rodents in the United States. *J. Infect. Dis.* 2000; 181:449–455. [PubMed: 10669325]
- 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. *J. Gen. Virol.* 2007; 88:912–917. [PubMed: 17325364]

- Feagins AR, Opriessnig T, Guenette DK, Halbur PG, Meng XJ. Inactivation of infectious hepatitis E virus present in commercial pig livers sold in local grocery stores in the United States. *Intl. J. Food Microbiol.* 2008a; 123:32–37.
- Feagins AR, Opriessnig T, Huang YW, Halbur PG, Meng XJ. Cross-species infection of specific-pathogen-free pigs by a genotype 4 strain of human hepatitis E virus. *J. Med. Virol.* 2008b; 80:1379–1386. [PubMed: 18551597]
- Feagins AR, Córdoba L, Sanford BJ, Dryman BA, Huang YW, Leroith T, Emerson SU, Meng XJ. Intergenotypic chimeric hepatitis E viruses (HEVs) with the genotype 4 human HEV capsid gene in the backbone of genotype 3 swine HEV are infectious in pigs. *Virus Res.* Dec 30.2011 2010. [Epub ahead of print]. PMID: 21195119.
- Forgách P, Nowotny N, Erdélyi K, Boncz A, Zentai J, Szucs G, Reuter G, Bakonyi T. Detection of Hepatitis E virus in samples of animal origin collected in Hungary. *Vet. Microbiol.* 2010; 143(2-4):106–16. [PubMed: 20005644]
- Gibbs EP. The public health risks associated with wild and feral swine. *Rev. Sci. Tech.* 1997; 16(2): 594–598. [PubMed: 9501373]
- Geng Y, Wang C, Zhao C, Yu X, Harrison TJ, Tian K, Wang Y. Serological prevalence of hepatitis e virus in domestic animals and diversity of genotype 4 hepatitis E virus in china. *Vector Borne Zoonotic Dis.* 2010; 10(8):765–70. [PubMed: 20021275]
- Goens, SD.; Botero, S.; Hare, W.; Meng, XJ.; Purdue, M. Serological evidence of hepatitis E-like virus in cattle; Proceeding of the 22nd Annual Meeting of the American Society for Virology; Davis, CA. 2003. p. 179W48-1
- Halbur PG, Kasorndorkbua C, Gilbert C, Guenette DK, 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. *J. Clin. Microbiol.* 2001; 39:918–923. [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. *J. Gen. Virol.* 2001; 82:2449–2462. [PubMed: 11562538]
- Hirano M, Ding X, Li TC, Takeda N, Kawabata H, Koizumi N, Kadosaka T, Goto I, Masuzawa T, Nakamura M, Taira K, Kuroki T, Tanikawa T, Watanabe H, Abe K. Evidence for widespread infection of hepatitis E virus among wild rats in Japan. *Hepatol. Res.* 2003; 27:1–5. [PubMed: 12957199]
- Hu GD, Ma X. Detection and sequences analysis of bovine hepatitis E virus RNA in Xinjiang Autonomous Region. *Bing Du Xue Bao.* 2010; 26(1):27–32. [PubMed: 20329555]
- Huang F, Zhang W, Gong G, Yuan C, Yan Y, Yang S, Cui L, Zhu J, Yang Z, Hua X. Experimental infection of Balb/c nude mice with Hepatitis E virus. *BMC Infect. Dis.* 2009; 9:93. [PubMed: 19523236]
- 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. *J. Gen. Virol.* 2004; 85:1609–1618. [PubMed: 15166445]
- Huang FF, Haqshenas G, Shivaprasad HL, Guenette DK, Woolcock PR, Larsen CT, Pierson FW, Elvinger F, Toth TE, Meng XJ. Heterogeneity and seroprevalence of a newly identified avian hepatitis E virus from chickens in the United States. *J. Clin. Microbiol.* 2002; 40:4197–4202. [PubMed: 12409397]
- Ippagunta SK, Naik S, Sharma B, Aggarwal R. Presence of hepatitis E virus in sewage in Northern India: frequency and seasonal pattern. *J. Med. Virol.* 2007; 79(12):1827–1831. [PubMed: 17935174]
- John R, Plenge-Bönig A, Hess M, Ulrich RG, Reetz J, Schielke A. Detection of a novel hepatitis E-like virus in faeces of wild rats using a nested broad-spectrum RT-PCR. *J. Gen. Virol.* 2010; 91(Pt 3):750–758. [PubMed: 19889929]
- John R, Heckel G, Plenge-Bönig A, Kindler E, Maresch C, Reetz J, Schielke A, Ulrich RG. Novel hepatitis e virus genotype in norway rats, Germany. *Emerging Infect. Dis.* 2010b; 16(9):1452–1455. [PubMed: 20735931]

- Jothikumar N, Aparna K, Kamatchiammal S, Paulmurugan R, Saravanadevi S, Khanna P. Detection of hepatitis E virus in raw and treated wastewater with the polymerase chain reaction. *Appl. Environ. Microbiol.* 1993; 59(8):2558–2562. [PubMed: 8368844]
- Kabrane-Lazizi Y, Fine JB, Elm J, Glass GE, Higa H, Diwan A, Gibbs CJ Jr, Meng XJ, Emerson SU, Purcell RH. Evidence for widespread infection of wild rats with hepatitis E virus in the United States. *Am J Trop Med Hyg.* 1999; 61:331–5. [PubMed: 10463689]
- Kaci S, Nöckler K, John R. Detection of hepatitis E virus in archived German wild boar serum samples. *Vet. Microbiol.* 2008; 128(3-4):380, 385. [PubMed: 18068914]
- Karenyi YV, Gilchrist MJ, Naides SJ. Hepatitis E virus infection prevalence among selected populations in Iowa. *J. Clin. Virol.* 1999a; 14:51–55. [PubMed: 10548130]
- Karenyi, YV.; Moyer, N.; Gilchrist, MJR.; Naides, SJ. Workshop on the Effects of Animal Feeding Operations on Hydrologic Resources and the Environment. United States Geological Survey Animal Feedlot Operations; Fort Collins, CO: August 3. 1999b Swine hepatitis E virus contamination in hog operation waste streams. An emerging infection?. 1999
- Kasorndorkbua C, Guenette DK, Huang FF, Thomas PJ, Meng XJ, Halbur PG. Routes of transmission of swine hepatitis E virus in pigs. *J. Clin. Microbiol.* 2004; 42:5047–5052. [PubMed: 15528694]
- Kasorndorkbua C, Opriessnig T, Huang FF, Guenette DK, Thomas PJ, Meng XJ, Halbur PG. Infectious swine hepatitis E virus is present in pig manure storage facilities on United States farms, but evidence of water contamination is lacking. *Appl Environ Microbiol.* 2005; 71(12):7831–7837. [PubMed: 16332757]
- Koizumi Y, Isoda N, Sato Y, Iwaki T, Ono K, Ido K, Sugano K, Takahashi M, Nishizawa T, Okamoto H. Infection of a Japanese patient by genotype 4 hepatitis e virus while traveling in Vietnam. *J. Clin. Microbiol.* 2004; 42(8):3883–3885. [PubMed: 15297556]
- Kuno A, Ido K, Isoda N, Satoh Y, Ono K, Satoh S, Inamori H, Sugano K, Kanai N, Nishizawa T, Okamoto H. Sporadic acute hepatitis E of a 47-year-old man whose pet cat was positive for antibody to hepatitis E virus. *Hepato. Res.* 2003; 26:237–242. [PubMed: 12850697]
- Krawczynski K. Hepatitis E vaccine--ready for prime time? *N. Engl. J. Med.* 2007; 356(9):949–951. [PubMed: 17329703]
- Legrand-Abravanel F, Kamar N, Sandres-Saune K, Garrouste C, Dubois M, Mansuy JM, Muscari F, Sallusto F, Rostaing L, Izopet J. Characteristics of autochthonous hepatitis E virus infection in solid-organ transplant recipients in France. *J. Infect. Dis.* 2010; 202(6):835–844. [PubMed: 20695798]
- Li TC, Chijiwa K, Sera N, Ishibashi T, Etoh Y, Shinohara Y, Kurata Y, Ishida M, Sakamoto S, Takeda N, Miyamura T. Hepatitis E virus transmission from wild boar meat. *Emerg. Infect. Dis.* 2005; 11:1958–60. [PubMed: 16485490]
- Li TC, Saito M, Ogura G, Ishibashi O, Miyamura T, Takeda N. Serologic evidence for hepatitis E virus infection in mongoose. *Am. J. Trop. Med. Hyg.* 2006; 74(5):932–936. [PubMed: 16687706]
- Li TC, Suzaki Y, Ami Y, Tsunemitsu H, Miyamura T, Takeda N. Mice are not susceptible to hepatitis E virus infection. *J. Vet. Med. Sci.* 2008; 70(12):1359–1362. [PubMed: 19122406]
- Liu J, Zhang W, Shen Q, Yang S, Huang F, Li P, Guo X, Yang Z, Cui L, Zhu J, Hua X. Prevalence of antibody to hepatitis E virus among pet dogs in the Jiang-Zhe area of China. *Scand. J. Infect. Dis.* 2009; 41(4):291–295. [PubMed: 19229761]
- Ma H, Zheng L, Liu Y, Zhao C, Harrison TJ, Ma Y, Sun S, Zhang J, Wang Y. Experimental infection of rabbits with rabbit and genotypes 1 and 4 hepatitis E viruses. *PLoS One.* 2010; 5(2):e9160. [PubMed: 20161794]
- Maneerat Y, Clayson ET, Myint KS, Young GD, Innis BL. Experimental infection of the laboratory rat with the hepatitis E virus. *J. Med. Virol.* 1996; 48:121–128. [PubMed: 8835343]
- Marek A, Bilic I, Prokofieva I, Hess M. Phylogenetic analysis of avian hepatitis E virus samples from European and Australian chicken flocks supports the existence of a different genus within the Hepeviridae comprising at least three different genotypes. *Vet. Microbiol.* 2010; 145(1-2):54–61. [PubMed: 20399575]
- Martelli F, Caprioli A, Zengarini M, Marata A, Fiegna C, Di Bartolo I, Ruggeri FM, Delogu M, Ostanello F. Detection of hepatitis E virus (HEV) in a demographic managed wild boar (*Sus scrofa scrofa*) population in Italy. *Vet. Microbiol.* 2008; 126(1-3):74–81. [PubMed: 17706898]

- Masuda J, Yano K, Tamada Y, Takii Y, Ito M, Omagari K, Kohno S. Acute hepatitis E of a man who consumed wild boar meat prior to the onset of illness in Nagasaki, Japan. *Hepatol. Res.* 2005; 31:178–83. [PubMed: 15792639]
- Matsuda H, Okada K, Takahashi K, Mishihiro S. Severe hepatitis E virus infection after ingestion of uncooked liver from a wild boar. *J. Infect. Dis.* 2003; 188:944. [PubMed: 12964128]
- Matsuura Y, Suzuki M, Yoshimatsu K, Arikawa J, Takashima I, Yokoyama M, Igota H, Yamauchi K, Ishida S, Fukui D, Bando G, Kosuge M, Tsunemitsu H, Koshimoto C, Sakae K, Chikahira M, Ogawa S, Miyamura T, Takeda N, Li TC. Prevalence of antibody to hepatitis E virus among wild sika deer, *Cervus nippon*, in Japan. *Arch Virol.* 2007; 152(7):1375–81. [PubMed: 17431737]
- Matsubayashi K, Kang JH, Sakata H, Takahashi K, Shindo M, Kato M, Sato S, Kato T, Nishimori H, Tsuji K, Maguchi H, Yoshida J, Maekubo H, Mishihiro S, Ikeda H. A case of transfusion-transmitted hepatitis E caused by blood from a donor infected with hepatitis E virus via zoonotic food-borne route. *Transfusion.* 2008; 48(7):1368–1375. [PubMed: 18651907]
- 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. *Proc. Natl. Acad. Sci. USA.* 1997; 94:9860–9865. [PubMed: 9275216]
- 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. *J. Virol.* 1998a; 72:9714–9721. [PubMed: 9811705]
- 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. *Arch Virol.* 1998b; 143(7):1405–1415. [PubMed: 9722883]
- 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. *J. Clin. Microbiol.* 2002; 40:117–122. [PubMed: 11773103]
- Meng XJ. Swine hepatitis E virus: cross-species infection and risk in xenotransplantation. *Curr. Top. Microbiol. Immunol.* 2003; 278:185–216. [PubMed: 12934945]
- Meng, XJ. Hepatitis E as a zoonosis. In: Thomas, H.; Zuckermann, A.; Lemon, S., editors. *Viral Hepatitis*. 3rd edition. Blackwell Publishing Ltd; Oxford, U.K.: 2006. p. 611–623.
- Meng, XJ.; Halbur, PG.; Straw, BE., et al. *Diseases of Swine*. 9th Edition. Blackwell Publishing Press; 2006. Swine hepatitis E virus; p. 537–545.
- Meng, XJ. Hepatitis E virus (*hepevirus*). In: Mahy, BWJ.; van Regenmortel, MHV., editors. *Encyclopedia of Virology*. 3rd edition. Vol. 5. Elsevier; Oxford: 2008. p. 377–383.
- Meng, XJ.; Shivaprasad, HL.; Payne, C. Hepatitis E virus infections. In: Saif, M., et al., editors. *Diseases of Poultry*. 12th Edition. Blackwell Publishing Press; 2008. p. 443–452.
- Meng XJ, Lindsay DS, Sriranganathan N. Wild boars as sources for infectious diseases in livestock and humans. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 2009; 364(1530):2697–707. [PubMed: 19687039]
- Meng XJ. Hepatitis E virus: animal reservoirs and zoonotic risk. *Vet. Microbiol.* 2010a; 140(3–4):256–65. [PubMed: 19361937]
- Meng XJ. Recent advances in Hepatitis E virus. *J. Viral. Hepat.* 2010b; 17(3):153–61. [PubMed: 20040046]
- Michitaka K, Takahashi K, Furukawa S, Inoue G, Hiasa Y, Horiike N, Onji M, Abe N, Mishihiro S. Prevalence of hepatitis E virus among wild boar in the Ehime area of western Japan. *Hepatol. Res.* 2007; 37:214–20. [PubMed: 17362304]
- Nakamura M, Takahashi K, Taira K, Taira M, Ohno A, Sakugawa H, Arai M, Mishihiro S. Hepatitis E virus infection in wild mongooses of Okinawa, Japan: Demonstration of anti-HEV antibodies and a full-genome nucleotide sequence. *Hepatol. Res.* 2006; 34:137–40. [PubMed: 16413822]
- Navaneethan U, Al Mohajer M, Shata MT. Hepatitis E and pregnancy: understanding the pathogenesis. *Liver Int.* 2008; 28(9):1190–9. [PubMed: 18662274]
- Okamoto H. Genetic variability and evolution of hepatitis E virus. *Virus Res.* 2007; 127:216–228. [PubMed: 17363102]

- Payne CJ, Ellis TM, Plant SL, Gregory AR, Wilcox GE. Sequence data suggests big liver and spleen disease virus (BLSV) is genetically related to hepatitis E virus. *Vet. Microbiol.* 1999; 68:119–125. [PubMed: 10501168]
- Pavio N, Meng XJ, Renou C. Zoonotic hepatitis E: animal reservoirs and emerging risks. *Vet. Res.* 2010; 41(6):46. PMID: 20359452. [PubMed: 20359452]
- Peralta B, Biarnés M, Ordóñez G, Porta R, Martín M, Mateu E, Pina S, Meng XJ. Evidence of widespread infection of avian hepatitis E virus (avian HEV) in chickens from Spain. *Vet. Microbiol.* 2009a; 137(1-2):31–6. [PubMed: 19136224]
- Peralta B, Casas M, de Deus N, Martín M, Ortuño A, Pérez-Martín E, Pina S, Mateu E. Anti-HEV antibodies in domestic animal species and rodents from Spain using a genotype 3-based ELISA. *Vet Microbiol.* 2009b; 137(1-2):66–73. [PubMed: 19200664]
- Pina S, Jofre J, Emerson SU, Purcell RH, Girones R. Characterization of a strain of infectious hepatitis E virus isolated from sewage in an area where hepatitis E is not endemic. *Appl Environ Microbiol.* 1998; 64(11):4485–4488. [PubMed: 9797311]
- Pina S, Buti M, Cotrina M, Piella J, Girones R. HEV identified in serum from humans with acute hepatitis and in sewage of animal origin in Spain. *J Hepatol.* 2000; 33(5):826–833. [PubMed: 11097493]
- Purcell, RH.; Emerson, SU. Hepatitis E virus. In: Knipe, D.; Howley, P.; Griffin, D.; Lamb, R.; Martin, M.; Roizman, B., et al., editors. *Fields Virology*. 4th edition. Lippincott: Williams and Wilkins; Philadelphia, PA: 2001. p. 3051-3061.
- Purcell RH, Emerson SU. Hepatitis E: an emerging awareness of an old disease. *J Hepatol.* 2008; 48(3):494–503. [PubMed: 18192058]
- Renou C, Cadranet JF, Bourlière M, Halfon P, Ouzan D, Rifflet H, Carencu P, Harafa A, Bertrand JJ, Boutrouille A, Muller P, Igual JP, Decoppet A, Eloit M, Pavio N. Possible zoonotic transmission of hepatitis E from pet pig to its owner. *Emerg. Infect. Dis.* 2007; 13:1094–6. [PubMed: 18214190]
- Reuter G, Fodor D, Forgách P, Kátai A, Szucs G. Characterization and zoonotic potential of endemic hepatitis E virus (HEV) strains in humans and animals in Hungary. *J. Clin. Virol.* 2009; 44(4): 277–81. [PubMed: 19217346]
- Rutjes SA, Lodder-Verschoor F, Lodder WJ, van der Giessen J, Reesink H, Bouwknegt M, de Roda Husman AM. Seroprevalence and molecular detection of hepatitis E virus in wild boar and red deer in The Netherlands. *J. Virol. Methods.* 2010; 168(1-2):197–206. [PubMed: 20510298]
- Sedyaningsih-Mamahit ER, Larasati RP, Laras K, Sidemen A, Sukri N, Sabaruddin N, Didi S, Saragih JM, Myint KS, Endy TP, Sulaiman A, Campbell JR, Corwin AL. First documented outbreak of hepatitis E virus transmission in Java, Indonesia. *Trans. R. Soc. Trop. Med. Hyg.* 2002; 96(4):398–404. [PubMed: 12497976]
- Shrestha MP, Scott RM, Joshi DM, Mammen MP Jr, Thapa GB, Thapa N, Myint KS, Fourneau M, Kuschner RA, Shrestha SK, David MP, Seriwatana J, Vaughn DW, Safary A, Endy TP, Innis BL. Safety and efficacy of a recombinant hepatitis E vaccine. *N. Engl. J. Med.* 2007; 356:895–903. [PubMed: 17329696]
- Shukla P, Chauhan UK, Naik S, Anderson D, Aggarwal R. Hepatitis E virus infection among animals in northern India: an unlikely source of human disease. *J. Viral. Hepat.* 2007; 14(5):310–317. [PubMed: 17439520]
- Smith JL. A review of hepatitis E virus. *J. Food Prot.* 2001; 64(4):572–586. [PubMed: 11307901]
- Song YJ, Jeong HJ, Kim YJ, Lee SW, Lee JB, Park SY, Song CS, Park HM, Choi IS. Analysis of complete genome sequences of swine hepatitis E virus and possible risk factors for transmission of HEV to humans in Korea. *J. Med. Virol.* 2010; 82(4):583–591. [PubMed: 20166181]
- Sonoda H, Abe M, Sugimoto T, Sato Y, Bando M, Fukui E, Mizuo H, Takahashi M, Nishizawa T, Okamoto H. Prevalence of hepatitis E virus (HEV) infection in wild boars and deer and genetic identification of a genotype 3 HEV from a boar in Japan. *J. Clin. Microbiol.* 2004; 42:5371–4. [PubMed: 15528746]
- Sun ZF, Larsen CT, Dunlop A, Huang FF, Pierson FW, Toth TE, Meng XJ. Genetic identification of avian hepatitis E virus (HEV) from healthy chicken flocks and characterization of the capsid gene

- of 14 avian HEV isolates from chickens with hepatitis-splenomegaly syndrome in different geographical regions of the United States. *J. Gen. Virol.* 2004a; 85:693–700. [PubMed: 14993655]
- Sun ZF, Larsen CT, Huang FF, Billam P, Pierson FW, Toth TE, Meng XJ. Generation and infectivity titration of an infectious stock of avian hepatitis E virus (HEV) in chickens and cross-species infection of turkeys with avian HEV. *J Clin Microbiol.* 2004b; 42:2658–62. [PubMed: 15184448]
- Takahashi M, Nishizawa T, Miyajima H, Gotanda Y, Iita T, Tsuda F, Okamoto H. Swine hepatitis E virus strains in Japan form four phylogenetic clusters comparable with those of Japanese isolates of human hepatitis E virus. *J. Gen. Virol.* 2003; 84(Pt 4):851–862. [PubMed: 12655086]
- Takahashi K, Kitajima N, Abe N, Mishiro 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; 330:501–5. [PubMed: 15567444]
- Takahashi M, Nishizawa T, Sato H, Sato Y, Jirintai D, Nagashima S, Okamoto H. Analysis of the full-length genome of a hepatitis E virus isolate obtained from a wild boar in Japan that is classifiable into a novel genotype. *J. Gen. Virol.* Jan 12.2011 2011. [Epub ahead of print]. PMID: 21228128.
- Tei S, Kitajima N, Takahashi K, Mishiro S. Zoonotic transmission of hepatitis E virus from deer to human beings. *Lancet.* 2003; 362:371–373. [PubMed: 12907011]
- Tei S, Kitajima N, Ohara S, Inoue Y, Miki M, Yamatani T, Yamabe H, Mishiro S, Kinoshita Y. Consumption of uncooked deer meat as a risk factor for hepatitis E virus infection: an age- and sex-matched case-control study. *J. Med. Virol.* 2004; 74(1):67–70. [PubMed: 15258970]
- Tomiyama D, Inoue E, Osawa Y, Okazaki K. Serological evidence of infection with hepatitis E virus among wild Yezo-deer, *Cervus nippon yesoensis*, in Hokkaido, Japan. *J Viral Hepat.* 2009; 16(7):524–528. [PubMed: 19215576]
- Toole MJ, Claridge F, Anderson DA, Zhuang H, Morgan C, Otto B, Stewart T. Hepatitis E virus infection as a marker for contaminated community drinking water sources in Tibetan villages. *Am. J. Trop. Med. Hyg.* 2006; 74(2):250–254. [PubMed: 16474079]
- Tsarev SA, Tsareva TS, Emerson SU, Rippey MK, Zack P, Shapiro M, Purcell RH. Experimental hepatitis E in pregnant rhesus monkeys: failure to transmit hepatitis E virus (HEV) to offspring and evidence of naturally acquired antibodies to HEV. *J. Infect. Dis.* 1995; 172:31–7. [PubMed: 7797939]
- Usmanov RK, Balaian MS, Dvořnikova OV, Alymbaeva DB, Zamiatina NA, Kazachkov IuA. Belov VI. An experimental infection in lambs by the hepatitis E virus. *Vopr. Virusol.* 1994; 39:165–168. [PubMed: 7998395]
- Vaidya SR, Tilekar BN, Walimbe AM, Arankalle VA. Increased risk of hepatitis E in sewage workers from India. *J. Occup. Environ. Med.* 2003; 45(11):1167–1170. [PubMed: 14610398]
- 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. *J Med Virol.* 2002; 67(4):516–521. [PubMed: 12115997]
- Wang Y, Ma X. Detection and sequences analysis of sheep hepatitis E virus RNA in Xinjiang autonomous region. *Wei Sheng Wu Xue Bao.* 2010; 50(7):937–941. [PubMed: 20815242]
- Wichmann O, Schimanski S, Koch J, Kohler M, Rothe C, Plentz A, Jilg W, Stark K. Phylogenetic and case-control study on hepatitis E virus infection in Germany. *J. Infect. Dis.* 2008; 198(12):1732–1741. [PubMed: 18983248]
- Williams TP, Kasorndorkbua C, Halbur PG, Haqshenas G, Guenette DK, Toth TE, Meng XJ. Evidence of extrahepatic sites of replication of the hepatitis E virus in a swine model. *J Clin Microbiol.* 2001; 39(9):3040–3046. [PubMed: 11526125]
- Withers MR, Correa MT, Morrow M, Stebbins ME, Seriwatana J, Webster WD, Boak MB, Vaughn DW. Antibody levels to hepatitis E virus in North Carolina swine workers, non-swine workers, swine, and murids. *Am. J. Trop. Med. Hyg.* 2002; 66:384–388. [PubMed: 12164292]
- Xia H, Wahlberg N, Belák S, Meng XJ, Liu L. The emergence of genotypes 3 and 4 hepatitis E virus in swine and humans: a phylogenetic perspective. *Arch. Virol.* Oct 7.2010 2010. [Epub ahead of print]; PMID: 20927637.
- 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. *J. Gen. Virol.* 2003; 84:2351–2357. [PubMed: 12917455]

- Yu Y, Sun J, Liu M, Xia L, Zhao C, Harrison TJ, Wang Y. Seroepidemiology and genetic characterization of hepatitis E virus in the northeast of China. *Infect. Genet. Evol.* 2009; 9(4): 554–61. [PubMed: 19460321]
- Zhao C, Ma Z, Harrison TJ, Feng R, Zhang C, Qiao Z, Fan J, Ma H, Li M, Song A, Wang Y. A novel genotype of hepatitis E virus prevalent among farmed rabbits in China. *J. Med. Virol.* 2009; 81(8):1371–9. [PubMed: 19551838]
- Zhao Q, Zhou EM, Dong SW, Qiu HK, Zhang L, Hu SB, Zhao FF, Jiang SJ, Sun YN. Analysis of avian hepatitis E virus from chickens, China. *Emerg. Infect. Dis.* 2010; 16(9):1469–1472. [PubMed: 20735936]
- Zhang W, Shen Q, Mou J, Gong G, Yang Z, Cui L, Zhu J, Ju G, Hua X. Hepatitis E virus infection among domestic animals in eastern China. *Zoonoses Public Health.* 2008; 55(6):291–298. [PubMed: 18638181]
- Zhu FC, Zhang J, Zhang XF, Zhou C, Wang ZZ, Huang SJ, Wang H, Yang CL, Jiang HM, Cai JP, Wang YJ, Ai X, Hu YM, Tang Q, Yao X, Yan Q, Xian YL, Wu T, Li YM, Miao J, Ng MH, Shih JW, Xia NS. Efficacy and safety of a recombinant hepatitis E vaccine in healthy adults: a large-scale, randomised, double-blind placebo-controlled, phase 3 trial. *Lancet.* 2010; 376(9744):895–902. [PubMed: 20728932]

Table 1

Host range and cross-species infection of the hepatitis E virus

HEV strains	Natural host	Susceptible experimental host
Mammalian HEV		
Genotype 1	Humans, domestic pigs*	Non-human primates, rats*, lambs*, rabbits*
Genotype 2	Humans	Non-human primate
Genotype 3	Humans, domestic pigs, wild boars, deer, mongoose, rabbits	Non-human primates, domestic pigs
Genotype 4	Humans, domestic pigs, wild boars, cattle*, sheep*	Non-human primates, domestic pigs, Balb/C mice*, rabbits*
Novel unclassified genotype, Rat HEV	Rats	Unknown
Novel unclassified genotype, Boar HEV	Wild boars in Japan	Unknown
Avian HEV		
Genotype 1	Chickens (Australia)	Chickens
Genotype 2	Chickens (USA, Canada)	Chickens, Turkeys
Genotype 3	Chickens (Europe, and China)	

* Reported but not independently confirmed.