Overview of Hepatitis E

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Introduction

The Hepatitis E virus (HEV) is a leading cause of acute hepatitis worldwide. It is an enterically transmitted virus responsible for both epidemic and sporadic cases. Hyperendemic to Mexico, north Africa, much of Asia and the Indian sub-continent (Figure 1), large sustained epidemics, often associated with fecally contaminated drinking water, have been described (Wong, Purcell et al. 1980; Bradley 1992). In Egypt, communities with anti-HEV seroposivitity rates of 70% are known (Darwish, Faris et al. 1996). In the 1990s, serologic studies among blood donors in industrialized countries showed anti-HEV seropositivity among a small percentage (1%–3%) of persons without travel history to a hepatitis E–hyperendemic region (Zaaijer, Kok et al. 1993; Mast, Kuramoto et al. 1997). A more recent retrospective study of the non-institutionalized population greater than six years of age in the United States reported a surprising seroprevalence of 21% (Kuniholm, Purcell et al. 2009).

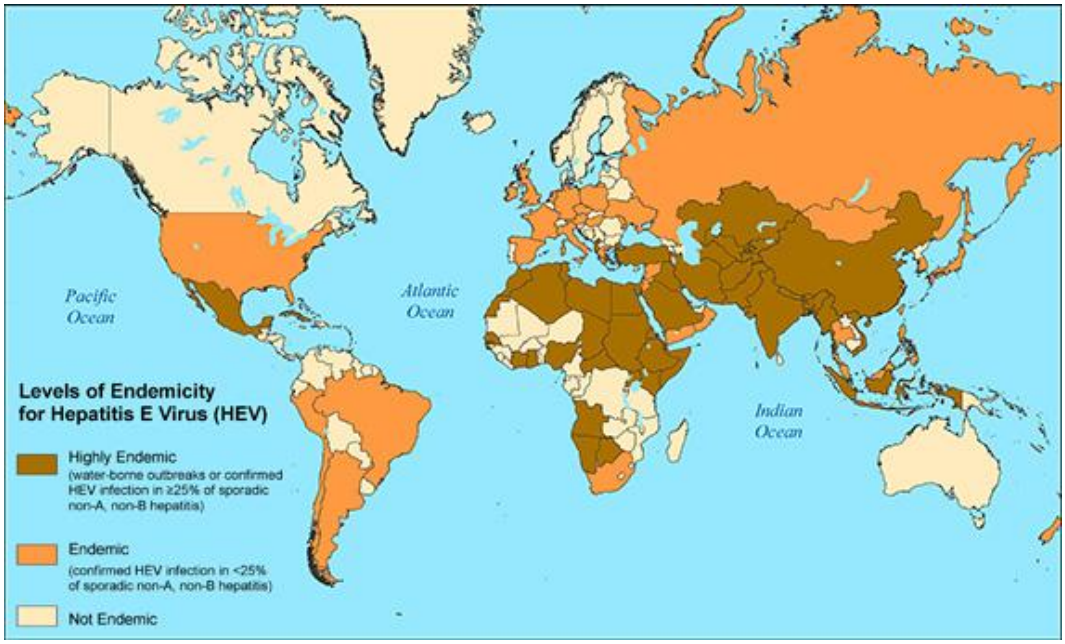

Figure 1 Geographic distribution of HEV infection (Centers for Disease Control and Prevention 2012)

Hepatitis E infection produces a clinical picture similar to hepatitis A virus (HAV) infection. While both infections are self-limited, HEV differs from hepatitis A with a lower rate of person-to-person transmission. Among adolescents and adults, HEV exhibits higher symptomatic attack and death rates (1-3% verses 0.4%) than HAV (Krawczynski, Aggarwal et al. 2000). In developing countries, more severe disease, including encephalopathy, disseminated intravascular coagulation and fulminant hepatic failure, is reported among pregnant women. The case fatality rate for this cohort can approach 20% (Navaneethan, Al Mohajer et al. 2008). As a rule, HEV does not cause chronic infections. A known exception is in organ transplantation patients (Kamar, Selves et al. 2008).

Studies indicate a broad host range and potentially varied reservoir for HEV. HEV is known to infect a variety of mammals including primates, pigs, sheep, boars, deer and rabbits. HEV is also reported to infect birds including chickens (Haqshenas, Shivaprasad et al. 2001; Lu, Li et al. 2006). In some animal species such as cats, dogs, and rodents only immunoglobulin G (IgG) to HEV has been demonstrated (Mochizuki, Ouchi et al. 2006; Easterbrook, Kaplan et al. 2007). HEV RNA was not detected. Until RNA is demonstrated a cautious interpretation of antibody only studies is warranted. The HEV genotypes 3 and 4 found in swine herds across many countries are, by genetic analysis, very close to the human HEV strains found in the same geographic region (Okamoto 2007; Wibawa, Suryadarma et al. 2007). In Japan, genotype 3 infection was associated with consumption of raw wild boar liver. HEV RNA from the patient and the wild boar were nearly identical with a 99.95% nucleotide sequence match (Li, Chijiwa et al. 2005). In the United States HEV is readily detected in 11% of pig livers sold in grocery stores (Feagins, Opriessnig et al. 2007). That it was fully infections is of even greater concern.

Other studies in industrialized countries have shown HEV originating from pigs and humans is consistently present in sewage water (Pina, Buti et al. 2000; Clemente-Casares, Pina et al. 2003). In short, zoonotic transmission is involved in some human HEV infections. Potential exposure may occur through contact with infected animals, contact with contaminated animal products or other fomites, consumption of contaminated food or drink, contact with surface waters contaminated by animal waste and by other means. The full role played by zoonotic reservoirs in the occurrence of sporadic and epidemic human cases of HEV remains poorly understood.

Virus and Molecular Epidemiology

The identification of HEV began with a large outbreak of acute jaundice in New Delhi, India during 1955-1956. Over 29,000 cases were reported. The greatest attack rate was in persons 15-40 years of age with a case fatality among pregnant women of 10.5%. Infection was linked to contamination of the municipal water supply (Vishwanathan 1957). A 1980 retrospective study of specimens from this outbreak, using the newly available serologic test for HAV, provided evidence for an enterically transmitted non-A, non-B hepatitis agent (Wong, Purcell et al. 1980). This agent was first transmitted experimentally to cynomolgus macaques in 1983 and virus-like particles approximately 32 nanometers in size were recovered (Balayan, Andjaparidze et al. 1983). These particles were shown to be immunoreactive with sera from patients. Electron microscopy of the particles revealed surface features similar to those found on caliciviruses. Finally in 1990, using PCR technology, a complimentary DNA library (DNA that is complimentary to the viral genome) was created that allowed identification of a viral agent from cynomolgus bile (Reyes, Purdy et al. 1990). This virus became known as hepatitis E, genotype 1.

Over the next 15 years three additional genotypes were subsequently identified and characterized.

Based on morphology and limited analysis, HEV was initially thought to be a member of the family *Caliciviridae.* The four genera of *Caliciviridae* cover a wide host range similar to HEV. *Sapovirus* and *Norovirus* infect primarily humans, pigs and cows, *Lagovirus* targets rabbits and *Vesivirus* cats, dogs and pigs. Further analysis of HEV genomic organization, replication strategy and physicochemical characteristics finally established HEV as the only member of the genus *Hepevirus* in the family *Hepeviridae*. Figure 2 depicts the taxonomic relationship of HEV to its three closest families of single stranded, positive sense RNA viruses. *Picornaviridae,* composed of 12 genera, has the most diverse host range of the four families with members

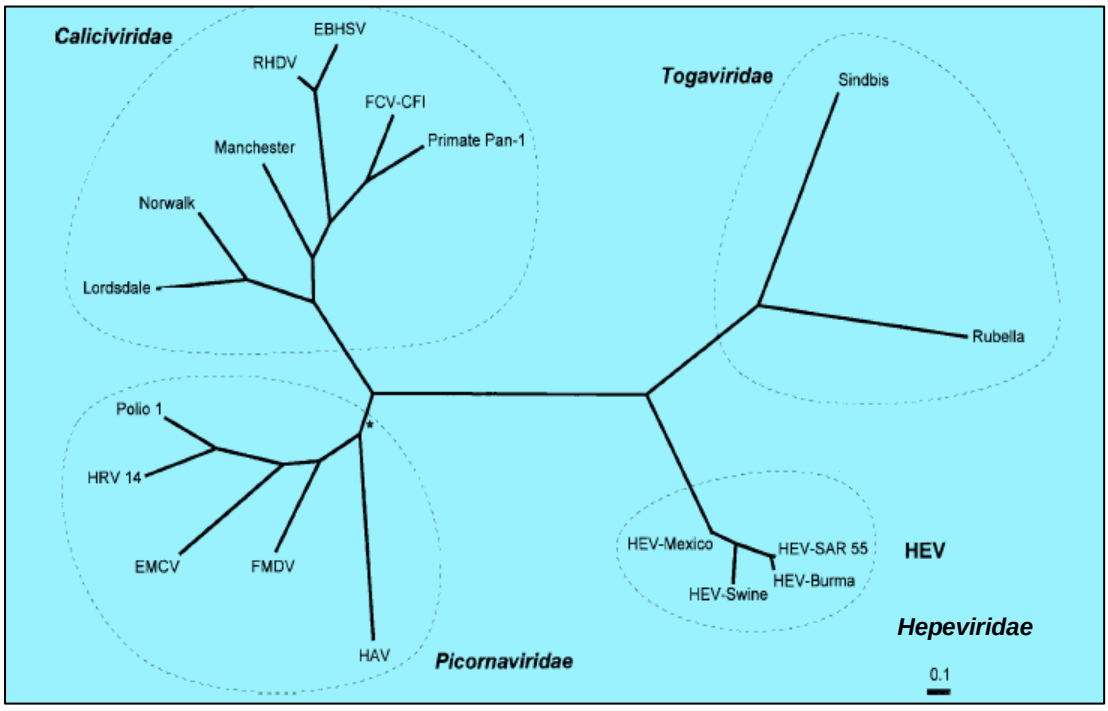

Figure 2 Partial taxonomy of single stranded, positive sense RNA viruses (Berke and Matson 2000)

infecting humans, a variety of animal and insect species as well as plants. This family includes the agents causing polio, hepatitis A, aseptic meningitis, foot-and–mouth disease in cattle and the common cold. *Togaviridae* has two genera, *Alphavirus* and *Rubivirus*. It is the only family of these four where the viruses are enveloped. (The virus acquires a lipid envelope as it buds through the host cell membrane. Disruption of the envelope renders the virus non-infectious, thus members of this family are more susceptible to environmental degradation and inactivation than the other families.) Members of the genus *Alphavirus* are spread by hematophagous vectors such as mosquitoes, making them unique within this partial taxonomy. Birds are the primary hosts for most members of this genus, humans are incidental. Rubella virus, the only human *Rubivirus*, is spread via a respiratory route. Diseases caused by viruses within this family include rubella, arthritis and equine encephalitis.

Four genotypes of HEV infecting mammals are generally recognized (Figure 3). They are represented by Burmese isolates (genotype 1), Mexican isolates (genotype 2), US isolates (genotype 3), and Chinese isolates (genotype 4) (Lu, Li et al. 2006). Genotypes 1 and 2 are limited to primate (human) hosts and associated with epidemics in developing countries. Worldwide, genotype 1 is most commonly associated with human disease. Genotypes 3 and 4 are zoonotic, infecting humans and other animals in developed as well as developing countries. The first animal strain of HEV identified and characterized was from a pig in the United States, HEV genotype 3 (Meng, Purcell et al. 1997). Non–travel-related HEV infections in developed countries are generally caused by genotype 3 (Europe, United States, Japan) and genotype 4 (Japan and China). These are typically sporadic cases with swine often the suspected reservoir. Virulent HEV strains infecting birds have been identified, but are yet to be classified.

(Haqshenas, Shivaprasad et al. 2001). Continued change in the HEV phylogenetic tree is expected as new strains are identified and characterized.

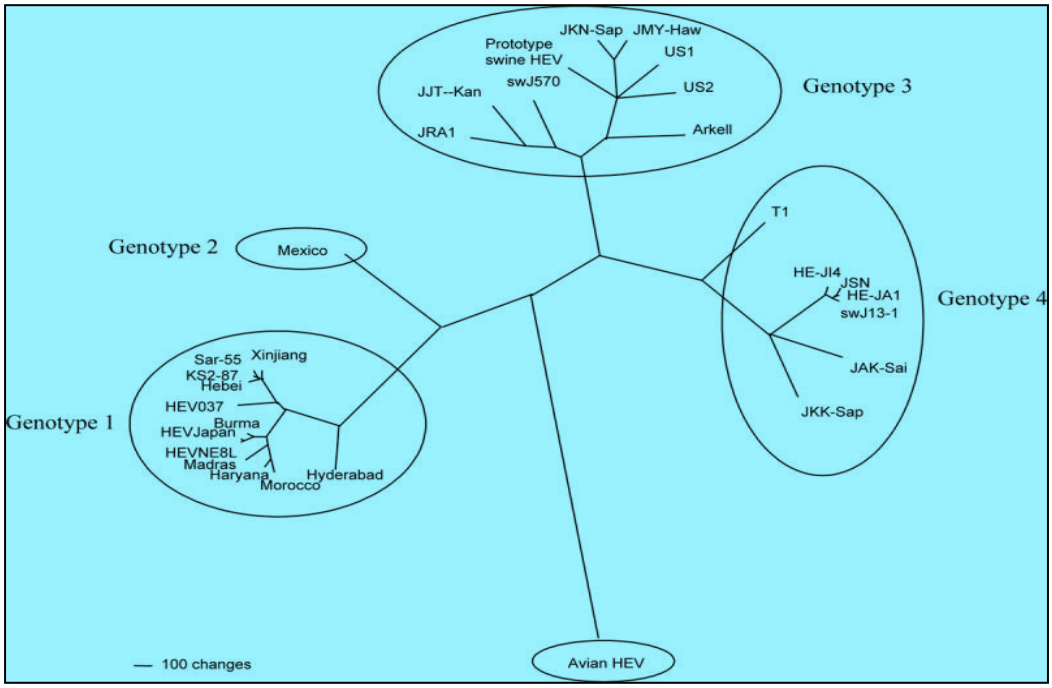

Figure 3 Unrooted HEV phylogeny (Meng 2010)

HEV is a spherical, non-enveloped, single stranded, positive sense RNA virus. (Positive sense means the viral RNA can be directly translated into proteins by ribosomes within the cell without the need for intermediaries.) The genome is approximately 7,200 bases (7.2kb) in length (Figure 4). There are short non-translated regions (NTR) at both the 5' and 3' ends. The genome

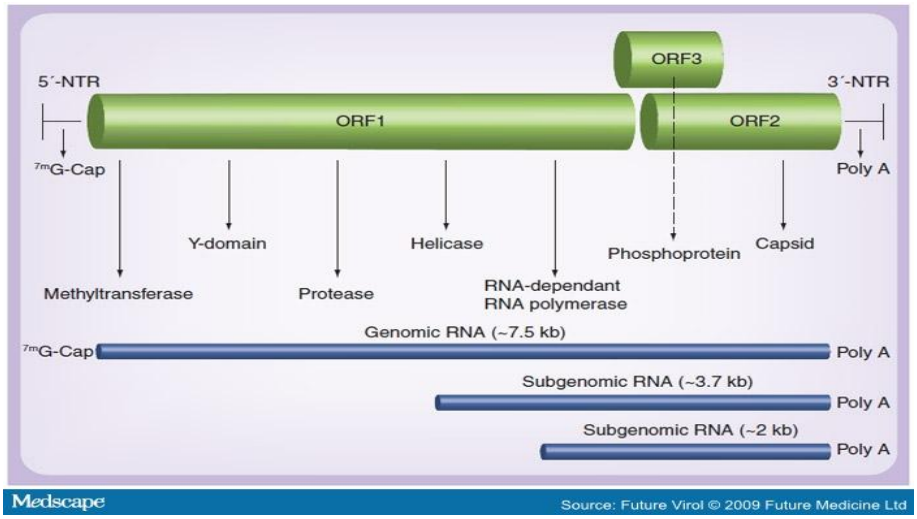

Figure 4 Organization of HEV genome

contains three open reading frames (ORF's). The largest, ORF1, extends approximately 5.1 kb in length from the 5' end of the genome. It encodes the nonstructural proteins used in replication (RNA helicase, RNA dependent RNA polymerase). ORF2 starts very near the end of ORF1 and extends nearly 2.0kb toward the 3' end of the genome, overlapping ORF3 It encodes the only structural protein, the capsid protein. This protein contains the epitopes that induce neutralizing antibodies and thus is one target for vaccine development. The function of ORF3 is unclear, but may play a role in modulating host cell response to infection (Meng 2010).

The genome of avian HEV is only 6.6 kb in length, slightly shorter than mammalian HEV. Although avian HEV shares just 50% nucleotide sequence identity with mammalian HEV the genomic organization and functional motifs are conserved between the two (Haqshenas, Shivaprasad et al. 2001). Antigenic epitopes in the capsid protein that are unique to avian HEV as well as common to both mammalian and avian HEV have been identified (Guo, Zhou et al. 2006).

Due to the lack of an efficient cell culture system and a practical animal model for HEV the mechanisms of HEV replication is poorly understood (Figure 5). Models based on similarities and sequence homology with better characterized positive sense, single stranded RNA viruses are typically used (Jameel 1999). Little is known about the initial binding or cellular receptors for entry into the cell. Once inside, the genomic RNA is uncoated and ORF1 translated in the cytosol producing the encoded nonstructural protein. Cellular proteases, with help from a viral

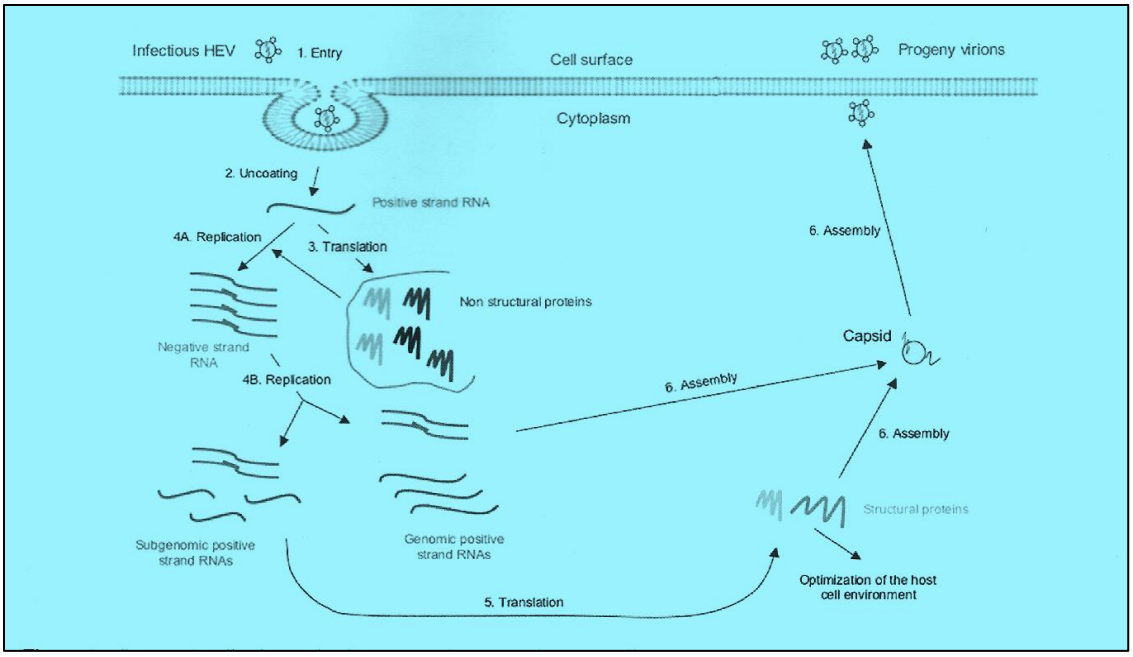

Figure 5 Proposed HEV replication cycle (Chandra, Taneja et al 2008)

protease cleave this protein. Among the products, the newly formed viral replicase (RNA-dependent RNA polymerase) creates a negative strand image of the genome. This serves as a template for positive genomic and subgenomic copies. The newly minted structural components are assembled and the genomic RNA encapsidated in the cytosol. The virion exits the cell through an undefined pathway (Chandra, Taneja et al. 2008).

Diagnosis and Laboratory Testing

The diagnosis of HEV infection depends on clinical and epidemiological features and exclusion of other causes of hepatitis, particularly HAV. Hepatitis E is diagnosed by screening for IgM anti-HEV in patients presenting with signs and symptoms of acute hepatitis. Early tests for anti-HEV antibodies developed in various laboratories and used in seroprevalence studies showed wide variation in sensitivity (Mast, Alter et al. 1998). Currently, tests developed for use in countries with the high rates of HEV infection are often based on genotypes other than genotype

3. This hinders standardization and ascertainment of infection particularly in regions where genotype 3 may predominate. A major challenge for HEV screening in the United States is the varying sensitivities and specificities of current commercially available tests and the fact none are approved by the US Food and Drug Administration (Drobeniuc, Meng et al. 2010). It would be desirable in clinical practice and for disease surveillance to have a screening assay that identifies incident HEV infection regardless of the genotype.

Robust molecular assays with reliable performance characteristics are needed not only for confirmation of acute infection, but also for monitoring food and water supplies for contamination. Reverse transcriptase polymerase chain reaction (RT-PCR) is used to confirm the presence of HEV and identify genotype. Serum viremia is detected before alanine aminotransferase (ALT) elevation and lasts one to four weeks (Chauhan, Jameel et al. 1993). RT-PCR identifies virus in stool approximately four weeks after ingesting contaminated food or water and about 14 days after the onset of signs and symptoms. RNA in the feces can be found in about 50% of cases. The duration of viral shedding is about two weeks.

The incubation period for HEV ranges from 15-64 days with a mean incubation during epidemics of 26-42 days (Heymann 2008). Clinical disease is typically found in young and middle aged adults, 15-40 years of age. Anicteric infections have also been reported (Smith 2001). The expression of icterus appears to increase with age. Lower disease rates in younger age groups may be the result of anicteric or subclinical infections. Such age related differential in disease expression is not uncommon in the hepatitides, HAV being a good example. No pathognomonic sign exists for hepatitis E infection. The signs and symptoms of HEV infection

are representative of acute hepatitis. Persons presenting with jaundice, vomiting, hepatalgia, fever, hepatomegaly or a distended abdomen should be screened with a hepatitis panel. In developing countries this panel should include hepatitis E. In tropical countries uncomplicated malaria and yellow fever would be part of the initial differential diagnosis.

Epidemiology

An estimated 2 billion people live in areas hyperendemic for HEV. Highest infection rates occur in regions with poor sanitation. Outbreaks are frequently associated with the rainy season (Goumba, Konamna et al. 2011). Fecal contamination of wells and water supply networks is the most common mode of transmission documented in large outbreaks. Person to person transmission via fecal-oral route may occur. However, the lack of secondary household cases during outbreaks suggests this is not a major route of transmission (Somani, Aggarwal et al. 2003). A limited role for other modes of transmission in hyperendemic areas, including mother to child at birth and blood transfusions, has been suggested (Khuroo, Kamili et al. 1995; Khuroo, Kamili et al. 2004).

Swine HEV infection (genotypes 3 and 4) is widespread in herds worldwide and generally occurs in pigs 2–4 months of age. Approximately 80–100% of pigs in commercial farms are infected (Meng, Purcell et al. 1997). The infected pigs remain clinically normal, although microscopic evidence of hepatitis was found (Halbur, Kasorndorkbua et al. 2001). They excrete large amounts of HEV in their feces for 2-7 weeks raising concerns about environmental (watershed) contamination (Kasorndorkbua, Opriessnig et al. 2005). While it is believed pigs acquire the infection by a fecal-oral route, experimental reproduction of swine HEV infection via oral

inoculation has been difficult even though pigs can be readily infected via intravenous inoculation (Kasorndorkbua, Guenette et al. 2004; Bouwknegt, Frankena et al. 2008). Swine strains are very close genetically to the human HEV strains found in the same geographic region (Okamoto 2007; Wibawa, Suryadarma et al. 2007). The infection cycle is potentially reciprocal. Experimental infection of swine with human HEV has been demonstrated (Feagins, Opriessnig et al. 2008).

Avian HEV is commonly found in flocks of chickens. A serosevey of 76 different flocks found 71% of the flocks and 30% of the birds were positive for anti-avian HEV (Huang, Haqshenas et al. 2002). HEV infection produces hepatitis-splenomegaly syndrome in commercial chicken flocks across the United States and Canada (Haqshenas, Shivaprasad et al. 2001).

In developed countries, zoonotic transmission may be an underappreciated public health issue. Clinically manifested HEV infection is seldom reported outside a small number of travel related cases. Assuming their anti-HEV test performance was not plagued by false positives, it is unclear how to account for the reported seroprevalence of 21% in the United States (Kuniholm, Purcell et al. 2009). The disparity between clinical disease and seroprevalence may be due to low-dose exposure to HEV, asymptomatic infection by relatively avirulent HEV strains, or missed or under-diagnosed HEV infection. Increased prevalence has been observed among veterinarians, pig farmers, men who have sex with men, and injecting drug users (Thomas, Yarbough et al. 1997; Meng, Wiseman et al. 2002). In the United Kingdom HEV genotype 3 infections have become more common (Ijaz, Arnold et al. 2005). While the majority of infections are asymptomatic in developed countries, the suggestion that a sustained cycle of

zoonotic transmission may lead to the evolution of more virulent HEV strains has been made (Zheng, Ge et al. 2006). This thought worthy suggestion parallels our understanding of influenza dynamics in several respects, cross species infection and evolution of virulence through swine and avian strains. However, influenza's segmented genome permits reassortment, an evolutionary mechanism unavailable to HEV.

Hepatitis E mortality in the general population ranges from about 1-3% (Krawczynski, Aggarwal et al. 2000). Not surprisingly increased morbidity and mortality is found in patients with underlying liver disease (Hamid, Atiq et al. 2002). Uncomplicated HEV infection is frequently associated with more severe disease in pregnant women, particularly from regions in India (Navaneethan, Al Mohajer et al. 2008). Other studies suggest the occurrence of severe disease, including death, in pregnant women is comparable to that in similarly aged non-pregnant women. A retrospective study in India of 1015 patients in acute liver failure (ALF) of reproductive age found 249 (38.5%) were pregnant. HEV related ALF was not dependent on gender or pregnancy status. Among the pregnant women with HEV related ALF, mortality was similar to that found in non-HEV related ALF, 51% verses 54.7% (Bhatia, Singhal et al. 2008). Using experimental animals, infection with HEV genotypes 1 and 3 did not produce more severe disease during pregnancy (Tsarev, Tsarev et al. 1995; Kasorndorkbua, Thacker et al. 2003). The reason for geographic differences in disease severity and mortality rates reported with HEV infection during pregnancy may reflect socio-economic variables, availability of healthcare resources, co-infection with other agents, hormonal and immunologic changes during pregnancy, or simply a reporting bias. A fully understanding awaits more complete and better designed multi-regional studies.

Table 1 summarizes the similarities and differences among the four HEV genotypes recognized to infect mammals. While much is known, some key details surrounding the basic epidemiology

Characterstics	Genotype 1	Genotype 2	Genotype3	Genotype 4
Viral discovery	1983	1986	1995	2003
Geographic distribution	Developing countries	Mexico, West Africa	Developed countries	China, Taiwan, Japan
Food-borne transmission	No	No	Yes	Yes
Fecal-oral transmission	Yes	Yes	?	No
Water-borne transmission	Yes	Yes	?	No
Person-to-person transmission	Yes	Unknown	Yes	Unknown
Zoonotic transmission	No	No	Yes	Yes
Occurrence of epidemics	Common	Smaller scale epidemics	No epidemics	Uncommon
Highest attack rate	Young adults	Young adults	Persons ⩾ 40 yr of age	Young adults
Gender	Male preponderance	Not discriminatory	Mostly male	Not discriminatory
Mortality rate	0.5%-3%	0.5%-3%	Not determined	0.5%-3%
Mortality among pregnant women	High	High	Not determined	High
Chronic infection	None	None	Yes	None
Severe disease among immuno-compromised	Not reported	Not reported	Yes	Not reported
Interspecies transmission	Only humans and non-human primates	Only humans and non-human primates	Humans Pigs	Humans Pigs
Subtypes	5	2	10	7

Table 1 Comparison of HEV genotypes (Teshale and Hu 2011)

of genotypes found in developed counties are still lacking.

Although HEV infection in the United States appears widespread, clinically manifested HEV infection is seldom reported (Kuniholm, Purcell et al. 2009). Only seven cases of clinically apparent, hepatitis E infections have been confirmed among U.S. residents with no history of international travel. Three of the cases occurred in Texas. In 2004 a 69 year old woman from El Paso was diagnosed with acute non-A, non-B hepatitis (Amon, Drobeniuc et al. 2006). She was admitted to the hospital four days after the onset of an acute illness that included fever, chills, abdominal pain, bloating, jaundice, dyspnea, and decreased urine output. The patient's medical history included autoimmune hepatitis. Biopsy found bridging fibrosis and cirrhosis. Further testing revealed both IgM and IgG anti-HEV. The HEV isolated from the patient was genotype 3. Despite a wide ranging investigation that included analysis of dog food, rodent droppings, and

bird droppings no source of infection was identified. The patient recovered and was discharged home. In 2009 genotype 3a, which is closely related to swine HEV, was isolated from one of two patients in San Antonio (Tohme, Drobeniuc et al. 2011). The two patients were unrelated, but both presented to the same hospital with signs and symptoms of acute hepatitis at about the same time. Both had serologic evidence of acute HEV infection, IgM and IgG anti-HEV. The first patient, a 21 year old female developed fulminant liver failure and died a month after admission. The second patient, a 44 year old female had severe hepatitis with centrilobular necrosis on biopsy. HEV RNA was detected in her stool specimen. She recovered and was discharged. Despite an extensive investigation of contacts, food consumption, drinking water sources, animal exposures, travel history and social contacts no common link between the two cases was found, nor an exposure source identified.

Even though persons in endemic areas carry anti-HEV antibodies protective immunity is not developed. This may be because the limited fidelity of the RNA viral replication process promotes selection for epitopes that are poorly recognized by the immune system, or protective titers simply wane. Immune globulin prepared from plasma in non-and high-endemic areas has not been demonstrated effective in preventing disease during outbreaks. (Similar observations have been made concerning hepatitis C, another RNA virus from the family *Flaviviridae*. Immune globulin is ineffectual as post exposure prophylaxis and the evolution of quasi-species thwarts immune system clearance of the virus resulting in chronic infection.) However, a successful recombinant vaccine has been developed and tested in China, though it is not currently approved for use in the United States (Zhu, Zhang et al. 2010).

Conclusion

Globally HEV is an important, but understudied pathogen. Understanding its ecology and natural history are key for effective prevention and control of human infections. For genotypes 1 and 2, prevention is accomplished by good sanitation and clean drinking water. Accumulating evidence for genotypes 3 and 4 indicates HEV has a zoonotic component with swine and perhaps other animal species acting as reservoirs. Reciprocal infection by swine and human strains has been demonstrated. Improved screening tests, particularly for use in developed countries, are needed to identify infection trends and understand the disparity between the number of clinical cases and seroprevelance. HEV poses a potential public health risk as a zoonosis and a food safety issue.

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