Hepatitis E viruses in humans and animals

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Abstract
Hepatitis E virus (HEV) is an emerging pathogen belonging to a newly recognized family of RNA viruses (Hepeviridae). HEV is an important enterically transmitted human pathogen with a worldwide distribution. It can cause sporadic cases as well as large epidemics of acute hepatitis. Epidemics are primarily waterborne in areas where water supplies are contaminated with HEV of human origin. There is increasing evidence, however, that many animal species are infected with an antigenically similar virus. A recently isolated swine virus is the best candidate for causing a zoonotic form of hepatitis E. The virus is serologically cross-reactive with human HEV and genetically very similar, and the human and swine strains seem to be cross-infective. Very recent evidence has also shown that swine HEV, and possibly a deer strain of HEV, can be transmitted to humans by consumption of contaminated meat. In this review, we discuss the prevalence, pathogenicity, diagnosis and control of human HEV, swine HEV, the related avian HEV and HEV in other hosts and potential reservoirs.

Keywords: hepatitis E viruses; human; pig; birds; zoonosis

Introduction
Here we review hepatitis E viruses (HEV) and their impact on human and animal health. Until recently, these viruses were thought to only infect humans. With the discovery of anti-HEV antibodies in swine and subsequently swine hepatitis E virus (Clayson et al., 1995; Meng et al., 1997) and the related big liver and spleen disease virus and avian HEV (Haqshenas et al., 2001), questions have arisen regarding the zoonotic potential of these viruses and possible wildlife reservoirs. In order to review the emergence of the animal hepatitis E viruses, it is necessary to also review what is known about hepatitis E viruses in humans, which thus far exceeds our knowledge of animal HEV and indeed directs much of our research on animal sources of the virus.

Thus far, attempts to replicate HEV in cell culture have been largely unsuccessful. One report of growth in cell culture (Huang et al., 1999) has yet to be repeated in other laboratories. HEV are single-stranded, positive-sense, non-enveloped RNA viruses. Originally considered to belong to the calicivirus family due to its virion size (approximately 30 nm) and structure (Fig. 1), genomic analysis found that the hepatitis E virus had a very different genomic organization from the caliciviruses (Purcell and Emerson, 2001b) (Fig. 2). Phylogenetic analysis of HEV sequence supports the establishment of a new taxonomic family for the hepatitis E-like viruses: Hepeviridae, genus: Hepevirus (Emerson, 2004). Phylogenetic analysis also groups the swine and human HEV together, into four or five recognized genotypes, but segregates the avian HEV from the human and swine viruses (Fig. 3).

HEV in humans

In humans, there are several hepatitis viruses, all belonging to different virus families. Hepatitis B (HBV) and hepatitis C (HCV) viruses are transmitted by hematogenous routes. Hepatitis delta viruses (HDV) are related to HBV and only occur as co-infections with HBV. There are also rarer hepatitis GB viruses (GBV),

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named from the initials of the surgeon from whom the viruses were initially isolated. GB viruses have thus far been divided into tentative species (A–C) of Flaviviridae, though not belonging to the same genus as HCV. GBV-C is also known as hepatitis G virus (HGV) (Linnen et al., 1996); GBV and HGV are also believed to be acquired parenterally (Simons et al., 1995). Prior to 1980 (before the discovery of HEV), hepatitis A virus (HAV) was the only known hepatitis virus to be transmitted by the fecal–oral route. HAV has a worldwide distribution. In developing countries, HAV is endemic and infection usually occurs at a young age, producing a seropositive adult population. As a result, epidemics of HAV are uncommon in these regions, such as Asia and Africa (Craighead, 2000).

Prevalence

Hepatitis E was first recognized as a major cause of waterborne epidemic non-A non-B hepatitis in India in 1980 (Khuroo, 1980; Wong et al., 1980; Purcell and Emerson, 2001b). The virus was identified by electron microscopy and subsequently sequenced (Balayan et al., 1983; Reyes et al., 1990; Bradley et al., 1991). HEV was found to be the etiological agent for the majority of waterborne viral hepatitis epidemics in Asia and Africa, where hepatitis A epidemics are uncommon (Craighead, 2000; Purcell and Emerson, 2001b) (Fig. 4). However, in adults in developing countries HEV antibodies are found in less than a third of the population. It is thought that HEV immunity acquired with subclinical infections at an early age wanes, which would explain why clinical illness is seen more commonly in adolescents and young adults and the low seroprevalence in adults observed in endemic regions (Craighead, 2000). There appears to be a direct correlation between poor sanitation and a high incidence of hepatitis E. In developed countries, where HEV infections are rare or even undocumented, seroprevalence can be as high as 7% (Worm et al., 2002a). This may represent early subclinical infections, infections with less virulent HEV or the presence of a different, immuno-cross-reactive agent.

In North America, two seroepidemiological studies in humans have been conducted, prompted by the discovery of HEV in swine in the USA. Of serum samples from blood donors in eight states surveyed, 18% were seropositive. This ranged from 4% in Alabama to 36% in Indiana. These data were compared with data from samples collected from swine veterinarians in the same states. The mean percentage of HEV-seropositive swine veterinarians was 26% (ranging from 5 to 45%). However, there was a higher seroprevalence in the general blood donor population than in swine veterinarians in three of the eight states surveyed (Meng et al., 2002). A similar study in North Carolina found an increased seroprevalence in swine workers (10.9% compared with 2.4% of non-swine workers) and 35% seroprevalence in pigs (Withers et al., 2002).

The situation in Japan is interesting. Although waterborne epidemics are uncommon in Japan, a number of
Sporadic cases have been reported. Human HEV isolates have been found to be very similar to circulating swine HEV in Japan (Okamoto et al., 2003). One study by a Spanish group has investigated the prevalence of HEV in water in Spain and the USA. One of five pretreatment sewage samples collected from Washington, DC was positive for HEV by reverse transcriptase–polymerase chain reaction (RT-PCR). Results of phylogenetic analysis found this HEV sequence to be 91–92% homologous with two human HEV isolates from Fig. 3. Phylogenetic analysis of HEV. Full-length sequences of selected swine (sw) and human (hu) HEV isolates, representing the four major HEV genotypes from a range of countries. Note that swine and human isolates group by genotype and region rather than by host species and avian HEV groups as a distinct species.

Fig. 4. Epidemiology of HEV. Countries where human HEV is endemic are colored dark gray (Kumar et al., 2001; Ayoola et al., 2002; Worm et al., 2002b; Cevrioglu et al., 2004; Maila et al., 2004; Wibawa et al., 2004). Countries where HEV has been isolated from human and/or swine cases are colored light gray (Zanetti et al., 1999; Pina et al., 2000; Schlauder et al., 2000; Adhami and Angoni, 2001; Garkavenko et al., 2001; Yoo et al., 2001; van der Poel et al., 2001; Hijikata et al., 2002; Wu et al., 2002; Choi et al., 2003; Clemente-Casares et al., 2003; Banks et al., 2004; Buti et al., 2004). Selected countries where serological evidence of human or animal HEV exists are marked with an asterisk (Karetnyi et al., 1995, 1999; Meng et al., 1999, 2002; Favorov et al., 2000; Lemos et al., 2000; Arankalle et al., 2001; Withers et al., 2002; Worm et al., 2002b; Kuno et al., 2003).
the USA and 98% homologous with a swine HEV also isolated in the USA (Clemente-Casares et al., 2003). A recent study in Switzerland suggested that occupational exposure to sewage did not correspond to increased anti-HEV IgG seroprevalence. However, in this study the presence of HEV in the sewage (in the city of Zurich) was not established (Jeggli et al., 2004).

**Disease**

HEV infections are self-limiting, with no chronic states. Generally, the mortality rate is low, but higher than with HAV infections: 0.5–4% compared with ~0.2% (Previsani and Lavanchy, 2001). The route of transmission is fecal–oral. However, two recent studies, one in Japan and the other in Saudi Arabia, suggest that HEV may also be transmitted parenterally in association with blood transfusions (Fukuda et al., 2004; Khuroo et al., 2004). In neither case is the primary site of virus replication known; however, with fecal–oral transmission a short viremic period precedes jaundice and elevated serum alanine transaminase (ALT) levels (Jameel, 1999). Clinical illness, if present, occurs at about this time. Signs and symptoms, which may range from subclinical to fulminant hepatitis (see section Disease in pregnancy, below), may include jaundice, anorexia, hepatomegaly, abdominal pain and tenderness, nausea and vomiting, and fever (Previsani and Lavanchy, 2001).

The incubation period between infection and clinical signs is variable, usually between 3 and 8 weeks with an average of 40 days (Craighead, 2000; Previsani and Lavanchy, 2001). This approximation is based on experimental infections of two volunteers (Purcell and Emerson, 2001b; Yarbough and Tam, 1999) and several experimental infections of cynomolgus macaques (Tsarev et al., 1992, 1994a, b). In these studies the virus was found to infect hepatocytes. Generally, the liver disease caused by HEV is more severe than with HAV (Craighead, 2000). Acute HEV infection in patients with chronic liver disease can trigger severe liver decompensation, which can lead to hepatic encephalopathy and renal failure (Kumar et al., 2004b; Monga et al., 2004).

Histologically, apoptosis and focal necrosis can be seen with only mild inflammation (Tsarev et al., 1994a; Purcell and Emerson, 2001b). There is some debate as to whether HEV is actually cytopathic or whether the hepatocellular damage is immunologically mediated (Purcell and Emerson, 2001b). Regardless, the hepatocellular damage does cause an increase in serum ALT. In addition to hepatocellular damage, chronic cholestasis can be associated with HEV (though it is not considered a feature of HAV infection) (Craighead, 2000; Purcell and Emerson, 2001b). Another histological feature of HEV infection considered to differentiate hepatitis E from hepatitis A is the tendency of liver parenchymal cells to organize in pseudoglandular formations (Craighead, 2000).

As a result of virus replication in the liver, HEV is subsequently found in bile, in large quantities (Tsarev et al., 1992; Purcell and Emerson, 2001b). Replication of HEV in the intestinal tract has not been shown. It is assumed that HEV reaches the intestines by the bile duct. Subsequently, HEV is shed in the feces. This shedding period is usually 3–4 weeks in duration, beginning just before the onset of clinical signs, coinciding with increased serum ALT and the progression of clinical signs (Tsarev et al., 1992; Jameel, 1999; Worm et al., 2002b).

**Host immune response**

Depending upon the method of detection and the virus titer of the inoculum, anti-HEV IgM is detected in experimental infections of macaques approximately 3–4 weeks after infection (Tsarev et al., 1994b). In the same study, anti-HEV IgM continued to be detectable up to 3 months. This seems to be consistent with reports in humans (Jameel, 1999; Previsani and Lavanchy, 2001; Purcell and Emerson, 2001b; Worm et al., 2002a; Emerson and Purcell, 2003). Anti-HEV IgG follows shortly after detection of IgM; however, anti-HEV IgG peaks several weeks later, and can be detected many months and years after infection (Arankalle et al., 1999; Yarbough and Tam, 1999).

**Disease in pregnancy**

The most salient feature of HEV infections is the increased severity of disease in pregnant women. While there does not appear to be an increased incidence of HEV infection in pregnant women, there is a clear increase in the incidence of fulminant hepatic failure (FHF) complicated by encephalopathy and disseminated intravascular coagulation in HEV-infected pregnant women (Madan et al., 1998; Jameel, 1999; Jaiswal et al., 2001; Previsani and Lavanchy, 2001; Khuroo and Kamili, 2003). This increase in disease severity is reflected by an increase in mortality rate to over 20% in pregnant women (0.5–4% in men and non-pregnant women) (Hamid et al., 1996; Craighead, 2000; Previsani and Lavanchy, 2001; Purcell and Emerson, 2001b; Kumar et al., 2004a). This mortality rate also increases with gestation, as the incidence of FHF almost doubles after the first trimester (Khuroo and Kamili, 2003). In two independent studies 62% (Khuroo and Kamili, 2003) and 64% (Singh et al., 2003) of HEV-infected pregnant women developed FHF. While there is a 53% mortality rate in women with FHF during pregnancy in the absence of HEV infection, the mortality rate with HEV-associated FHF in the second study was 100% (Singh et al., 2003).

Transmission of HEV from mother to the fetus or
infant is 30–100% (Khuroo et al., 1995; Kumar et al., 2001, 2004a; Singh et al., 2003). A recent study in India found that two-thirds of HEV-infected pregnant women had preterm deliveries (Kumar et al., 2004a). The World Health Organization reports that the mortality rate of fetuses due to HEV-related spontaneous abortion and early neonatal death is approximately 33% (Previsani and Lavanchy, 2001). Although cynomolgus macaques are a model for hepatitis E virus infection in non-pregnant adults, the disease could not be reproduced in pregnant monkeys (Purcell and Emerson, 2001a).

One possible explanation for the high incidence of FHF in pregnant women infected with HEV is that a Schwartzman-like phenomenon occurs as an indirect result of damage to Kupffer cells (fixed tissue macrophage of the liver) by the virus (Purcell and Ticehurst, 1988). Disabling these liver macrophages may allow for damage to the liver from endotoxins produced by intestinal gram-negative bacteria. The Schwartzman phenomenon can occur in pregnant women and rabbits. A first encounter with endotoxin produces intravascular thrombi and reticuloendothelial blockage of a localized or generalized nature. The Schwartzman reaction refers to the disseminated intravascular coagulation which occurs from a second endotoxin assault, worsened by the not yet resolved reticuloendothelial blockage. The enhanced sensitivity of pregnant women to such an endotoxin-mediated effect is well recognized (Purcell and Ticehurst, 1988; Doughty et al., 2001). It is theorized that in the case of HEV-induced FHF, hepatocytes may be injured directly by the virus or endotoxins, or indirectly by the release of eicosanoids or chemokines, and that the disease is worsened by a Schwartzman-like disseminated intravascular coagulation (Purcell and Ticehurst, 1988).

**Diagnosis**

Clinically, hepatitis E is indistinguishable from hepatitis A, with elevated serum liver enzymes (total and direct serum bilirubin, ALT and aspartate transaminase). When hepatitis A has been ruled out, hepatitis E should be suspected, particularly in outbreaks of waterborne hepatitis occurring in developing countries, or with recent travel to endemic areas. HEV should be especially suspected in cases of fulminant hepatitis in pregnant women and should be considered in cases of acute fatty liver of pregnancy (Hamid et al., 1996). Acute hepatitis E can be diagnosed by amplification of HEV RNA from serum or feces or by the detection of anti-HEV IgM. Recombinant HEV proteins corresponding to ORF2 have been expressed in bacteria or insect cells and are used in enzyme immunoassays or enzyme-linked immunosorbent assays tests to detect anti-HEV antibodies (Tsarev et al., 1993; Mast et al., 1998; Anderson et al., 1999; Innis et al., 2002; Obriadina et al., 2002; Yu et al., 2003). Commercial kits are available in some countries, including Canada. In the USA, however, commercial HEV serology tests are not widely available or offered and HEV diagnosis is primarily restricted to the research community and is thus rarely requested.

RT-PCR can also be used to test for HEV RNA. HEV RNA can be detected in acute-phase feces by RT-PCR in approximately 50% of cases (Kurstak et al., 1996; Previsani and Lavanchy, 2001). Viremia is shorter-lived and in serum the virus is found at lower titers than in feces. Again, however, HEV RT-PCR is not routinely performed in diagnostic laboratories in North America, though available through the Centers for Disease Control. Less sensitive than RT-PCR, immune electron microscopy used to visualize viral particles in feces is positive in only about 10% of cases (Previsani and Lavanchy, 2001).

**Prevention (vaccination)/treatment**

Both passive immunization with late convalescent plasma and active immunization using whole virus (Tsarev et al., 1994a; Arankalle et al., 1999) have been accomplished. Because HEV cannot be replicated in cell culture, live attenuated or killed virus vaccines cannot be used for vaccine development. However, several groups have developed DNA (Kamili et al., 2004) or a recombinant protein (Safary, 2001; Purcell et al., 2003; Li et al., 2004) vaccine based on the capsid protein (ORF2), and these have been successful in protecting monkeys from HEV challenge. Two reviews specifically addressing recent advances in HEV vaccine research are available (Wang and Zhuang, 2004; Worm and Wirnsberger, 2004).

Since there is evidence that passive immunization is protective, seroprophylaxis should be considered in pregnant women during epidemics (Pillot et al., 1995).
also been found in research animals and wild boar (Matsuda et al., 2003). Sequence analysis of swHEV isolated from North America (Meng et al., 1997; Pei and Yoo, 2002), Taiwan (Hsieh et al., 1998, 1999), the Netherlands (van der Poel et al., 2001), Japan (Nishizawa et al., 2003) and the UK (Banks et al., 2004) reveals the closest genetic relationship to be to the human HEV in the same region, rather than other swHEV in different regions of the world.

**Disease in pigs**

Prior to the discovery of swHEV there was no clinical evidence of viral hepatitis in pigs. Experimental infections of pigs by intravenous inoculation with US swHEV isolates produced viremia, but no noticeable clinical disease or increase in serum liver enzymes could be detected (Meng et al., 1998a, 1998b; Halbur et al., 2001). In addition to serum and feces, swHEV could be detected by RT-PCR in the liver, a pool of lymph nodes, small intestine and colon as well as, in some animals, in the stomach, spleen, kidney, tonsil, salivary gland and lung (Williams et al., 2001).

Immunohistochemistry of experimentally infected pigs was able to detect HEV antigen primarily in the liver and small intestine (Ha and Chae, 2003). Other tissues where HEV antigen was detected less consistently and in lesser amounts and with less intense immunostaining were lymph nodes (specific lymph nodes not specified), tonsil, spleen and large intestine. Although the authors speculate that the infected cells were hepatocytes, lymphocytes and macrophages, identification of these cell types with cell type-specific antibodies was not performed.

Some experimental infection studies also examined the ability of human HEV to infect pigs. While the first attempt to infect pigs with human HEV strains (Sar-55 isolated in Pakistan and Mex-14 isolated in Mexico) was unsuccessful (Meng et al., 1998a), in subsequent experiments the human HEV isolates from the USA did infect pigs, with clinical results comparable to those found when pigs were infected with swHEV (Meng et al., 1998a; Halbur et al., 2001; Williams et al., 2001). The mode of swHEV transmission has also been studied in an effort to mimic the consumption of undercooked pork meat and the risk of transmission by xenotransplantation of HEV-infected pig tissue (Kasorndorkbua et al., 2002). In this study, naive pigs were fed by stomach tube suspensions of skeletal muscle and feces and intravenously inoculated with extracts of liver, heart, pancreas and skeletal muscle from pigs infected with swHEV. Liver homogenates and fecal suspensions, regardless of the inoculation route, resulted in the naive pigs becoming viremic and seroconverting. Preparations from other tissues did not infect the naive pigs. This study highlights several possible modes of transmission among pigs; however, in terms of the risk of zoonotic transmission, a better model would involve different species, such as a non-human primate.

**Disease in pregnancy**

In order to assess, in pigs, the health and reproductive issues that HEV presents in humans and also to assess the suitability of a swine model of FHF in pregnancy, pregnant gilts were intravenously inoculated with swHEV (Kasorndorkbua et al., 2003). Although HEV RNA was detected in the feces of all gilts and at various times in serum and liver, clinical signs of disease were not evident. In addition, unlike the high degree of vertical transmission of human HEV from mother to fetus/child, there was no vertical transmission of swHEV. Reproduction also appeared not to be affected.

**Diagnosis and prevention**

Since swHEV has not been shown to be virulent or to be associated with disease in pigs (at least in North America) and combined with the fact that it appears to be endemic, there is little or no demand for diagnostic approaches or for vaccination against swHEV. However, as more is learned about the zoonotic potential of HEV this situation may change.

Although reagents are not commercially available, swHEV can be diagnosed by amplification of HEV RNA (with species and perhaps geographically specific HEV primers) from serum or feces (Meng, 2000; Yoo et al., 2001) or by the detection of anti-HEV IgM using the same recombinant capsid protein from human HEV (Meng et al., 2001). SwHEV can also be detected, post-mortem, by immunohistochemistry using anti-human HEV antibodies (Ha and Chae, 2004).

**HEV in birds**

In 2001 a virus associated with a distinct syndrome in chickens in the USA, hepatitis-splenomegaly syndrome (HS), was partially sequenced and shown to be genetically related to hepatitis E viruses (Haqshenas et al., 2001). The HS syndrome was first described in Canada (Ritchie and Riddell, 1991) and subsequently in the USA (Riddell, 1997). It probably occurs worldwide and is presumably related to big liver and spleen disease (BLSD) described in Australia (Riddell, 1997; Payne et al., 1999). A short region of BLS virus (BLSV) sequence was first shown to be 61% identical to human hepatitis E (Payne et al., 1999) and the RT-PCR primers used to fish out the HS-related virus were designed based on the BLSV sequence. These two avian strains cosegregated in a distinct clade when compared with partial helicase gene
sequences from human and other animal strains (Huang et al., 2002) and are thus presumably closely related genetically. They also share antigenic epitopes with each other and the swine and human strains (Haqshenas et al., 2002). These are the only avian virus strains known thus far to be related to hepatitis E viruses. The HS-related virus segregates quite distinctly from the other animal strains of HEV (Fig. 3) and inferences made regarding the genomic organization or the replication of the avian strain are based solely upon the genome organization of the human strains. All attempts to culture the virus have thus far failed (Shivaprasad and Woolcock, 1995).

In terms of the molecular organization and phylogenetic status of the avian strains, recently the capsid genes of an additional 14 isolates taken from HS-affected chickens were sequenced and compared (Sun et al., 2004a). These 14 isolates exhibited considerable sequence diversity, sharing 76–100% identity with each other. This is a similar heterogeneity to that seen among the clustered swine and human strains. The complete sequence of the prototype US avian strain was also recently published (Huang et al., 2004). The avian strain is some 600 base pairs shorter than the swine and human strains, shares 50% sequence identity with them over the whole genome and is organized into a short 5’ non-coding region (NCR) followed by three ORFs and a 3’ NCR in the same genetic arrangement as the other Hepeviridae.

Prevalence

Serological evidence unequivocally implicates an HEV-like agent widespread among poultry, with 71% of flocks from five US states positive (Huang et al., 2002) and the presence of cross-reacting antibodies in over 40% of the chickens in Vietnam (Tien et al., 1997).

Disease and host immune responses

It is somewhat difficult to assess the importance of these viruses within the context of the human and swine strains. The sporadically occurring HS syndrome has apparently been reproduced via the fecal-oral route using material from infected chickens (Huang et al., 2002) and measured seroconversion among healthy birds clearly suggests that the virus is enzootic among US chicken flocks (Huang et al., 2002; Sun et al., 2004a). It is speculated that the generation of the sporadic disease may be dose-dependent (Sun et al., 2004a) and most chickens are subclinically infected; this is similar to the situation in swine. The HS syndrome in US chickens is characterized by increased mortality in birds (primarily broiler breeder) from 30 to 72 weeks of age, the presence of an enlarged liver and spleen, regressive ovaries and red fluid in the abdomen (Riddell, 1997), whereas young birds are more often asymptomatic or subclinical (Sun et al., 2004a, b). In an experimental infection, seroconversion was found to occur at between 12 and 21 weeks in healthy chickens. Lesions in the liver ranged from multifocal patches to extensive necrosis and hemorrhage (Sun et al., 2004a).

In a study of BLSD, seroconversion occurred between 45 and 50 weeks, but it was not clear that this was to a hepatitis E-specific antigen (Crerar and Cross, 1994b). Reports on BLSD in Australia have indicated drops in egg production and enlargement of kidneys in many cases, and occasionally lesions in the pancreas (Crerar and Cross, 1994a, b).

In terms of animal health, avian strains probably have limited clinical significance. The question remains as to whether the disease syndromes caused by the avian hepatitis E strains are dose-dependent or are die to distinct differences in virulence. If there are different virulence phenotypes there should perhaps be increased concern.

Recently avian HEV from chickens has been shown to infect turkeys (Sun et al., 2004b). In this study, 1-week-old specific-pathogen-free turkeys were inoculated intravenously with infected chicken bile or feces suspensions. While clinical signs were not observed, these turkeys became viremic and seroconverted. Moreover, the uninoculated contact control turkey also seroconverted. Further studies will be required to determine if turkeys are naturally infected and whether avian HEV is pathogenic in these animals.

Since the HEV have shown a propensity to infect a wide variety of animals (at least in terms of seroprevalence) and we know the potential importance of emerging animal pathogens for public health (witness the recent SARS and avian influenza outbreaks in Asia), it is prudent to continue to search for and characterize these viruses among avian and mammalian populations. With these facts in mind, a recent study evaluated the experimental inoculation of non-human primates with an avian HEV (Huang et al., 2004). It was concluded that, unlike swine and human HEV strains, avian HEV was unable to cause seroconversion or viremia in rhesus macaques.

Diagnosis and prevention

There are currently no rapid diagnostic tests for HS syndrome in the USA, although the data recently published from Meng’s laboratory (Huang et al., 2004; Sun et al., 2004b) would certainly provide sufficient information for the development of a test. Diagnosis is by observation of typical signs and lesions, and prevention is currently by thorough cleaning and disinfection after depletion of an affected flock, increased biosecurity and all-in/all-out production.
HEV in other species and potential for zoonotic transmission of HEV

There has been considerable speculation that animal strains of HEV have given or can give rise to human strains of the virus. The most commonly suggested reservoirs are swine (Meng, 2000, 2003) and rodents (Favorov et al., 2000; Arankalle et al., 2001; He et al., 2002; Emerson and Purcell, 2003). In the case of swine, studies have shown that swine viruses are phylogenetically closely linked to human strains (Meng et al., 1997), and are capable of infecting primates (Meng et al., 1998b). Direct human association with swine results in statistically significant increases in seroconversion to hepatitis E-specific antibodies (Meng et al., 2002; Withers et al., 2002). Furthermore, in some cases virus has been detected in sewage containing pig feces (Pina et al., 2000). Since the infection is subclinical in pigs and presumably can also be subclinical in humans, it has been difficult to assess the likelihood of sporadic cases of zoonotic transmission through animal waste. As mentioned earlier, it is assumed that transmission of epidemic cases of hepatitis E in developing countries has been due to fecal–oral transmission of human strains due to deficiencies in sanitation rather than to zoonotic transmission.

In the case of potential transmission by rodents, the majority of the evidence is serological. There is widespread occurrence of hepatitis E-specific antibodies in rodents around the world (Kabrane-Lazizi et al., 1999; Favorov et al., 2000; He et al., 2002; Smith et al., 2002; Hirano et al., 2003). One report demonstrated the presence of viral sequences by PCR in trapped rodents in Nepal. Analysis of a 40-base pair segment of the capsid gene placed these sequences in the same clade as the isolates from human cases in the area during the same time period, suggesting the rodents were a source (He et al., 2002). In this study, 12% (n = 675) of rodents trapped in a region of Nepal hyperendemic for human HEV were found to be HEV-seropositive. This is a lower seroprevalence than found in rodents in many other regions (i.e. 78–91% in urban Maryland, USA) (Favorov et al., 2000). Of the 78 seropositive rodent sera from Nepal, four were RT-PCR-positive. Although the investigators suggest that the rodents provided a source of the epidemic virus, another plausible reason for both the relatively low seroprevalence in the rodents and the derived nucleotide sequence being so very closely related to Nepalese human HEV is that the four rodents were infected with human HEV as well as or rather than a rodent HEV which contributes to the commonly found seroprevalent antigen. The high HEV seroprevalence reported in rodents in places where human HEV is rare and the inability to amplify RNA from these rodents may reflect a different situation and a genetically diverse rodent HEV.

In India, where human HEV is endemic, anti-HEV IgG have also been identified in several other animal species. Seropositivity varied from 54.6 to 74.4% in pigs and 2.1–21.5% in rodents, but was also found to be 4.4–6.9% in cattle and 22.7% of dogs; however, none of the 250 goats tested were found to be anti-HEV positive (Arankalle et al., 2001). Similar evidence of HEV in various animal species has also been reported in China. Again, the majority of pigs (79%), 6% of cattle and none of the goats tested were seropositive for IgG anti-HEV. HEV sequence obtained from the pigs belonged to genotype 4 and were most closely related to viruses isolated from Chinese patients with acute hepatitis (Wang et al., 2002). There is also evidence that cattle in the USA may be infected with an HEV. Recent data in our laboratory have shown that Holstein calves clearly seroconvert to an HEV-related agent between 3 and 6 months of age (Goens et al., 2003). Serological evidence for HEV infection among these different species certainly suggests a zoonotic reservoir of HEV in food and other animals, though direct proof is lacking.

Recently, however, more distinct cases of likely zoonotic transmission of HEV have indeed been documented. In the UK, an HEV isolated from a human was shown to share 100% amino acid identity with a circulating swine strain, although there was little evidence of direct association of the patient with swine. The patient had reported consumption of raw sausage and bacon, although not within 3 months of the infection (Banks et al., 2004). Other cases of potential transmission by consumption of contaminated meat were reported recently in Japan involving raw pig liver (Yazaki et al., 2003) and raw Sika deer meat. In the latter case, the human isolate was shown to be identical to the virus detected in the un eaten portion of the same deer (Tei et al., 2003). In another sporadic human case in Japan, the only prior association with a source of HEV was with the family cat, which had antibody titers to the virus (Kuno et al., 2003). This was followed by a seroprevalence study in Japan which found that 44 of 135 cats examined were anti-HEV IgG positive (Okamoto et al., 2004). So, the evidence is quite strong that we should be concerned about the mammalian strains of HEV as potential sporadic zoonotic agents. The distinct phylogenetic status of the avian strain(s) and recent failed attempts to transmit the avian virus to non-human primates (Huang et al., 2004) suggest that avian HEV is unlikely to have zoonotic potential.

Summary

Current HEV research is focusing on vaccines for human use and the potential for zoonotic transmission. The general belief is that viruses that are antigenically similar to human and swine HEV will be found in other species. It is likely that they will also be difficult to culture and will be genetically divergent, and this contributes to difficulties in identifying these viruses.
Whether they will be found to be pathogenic in their hosts or whether they will be zoonotic also remains to be determined.

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