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Introduction

Hepatitis E virus (HEV), the causative agent of human hepatitis E, is an important public health disease in many developing countries [1,4,20,21,23]. At least 4 major genotypes of HEV have been identified: genotypes 1 and 2 are associated with epidemics in Asia and Mexico, and genotypes 3 and 4 are associated with sporadic cases of hepatitis E worldwide [21]. HEV is transmitted fecal-orally through contaminated water. The mortality rate associated with HEV infection is generally low (less than 1%) but it can be up to 25% in pregnant women [1,23]. Most acute cases occur as endemic or sporadic form, and epidemics are uncommon. Although only sporadic cases of acute hepatitis E with no history of traveling to endemic regions have been reported from patients in industrialized countries including the United States, a surprisingly high HEV antibody (anti-HEV) prevalence has been reported. The existence of a population of individuals in industrialized countries who are positive for anti-HEV has led to a hypothesis that an animal reservoir for HEV exists, and that hepatitis E is a zoonosis [18,21].

Discovery of Swine Hepatitis E Virus (Swine HEV) in Pigs

The first animal strain of HEV, swine HEV, was discovered from a pig in the United States in 1997 [14]. In an un-related study, pigs in the Midwestern United States were accidentally found to be positive for antibodies to the human HEV, indicating that a HEV-related virus is circulating in the pig population. To isolate swine HEV, Meng et al. [14] conducted a prospective study in a commercial swine farm in Illinois. Twenty piglets born to both anti-HEV seronegative and seropositive sows were closely monitored for more than 5 months for evidence of HEV infection. By 21 weeks of age, 16 of the 20 study piglets had seroconverted to HEV antibodies. A virus genetically related to human HEV, designated swine HEV, was identified from the acute phase sera of the naturally infected piglets. Subsequently, swine HEV infection was experimentally reproduced in specific-pathogen-free (SPF) pigs, and swine HEV was recovered from experimentally infected SPF pigs [6,12,15,16,31]. The complete genomic sequence of swine HEV has been determined, and shown to be a polyadenylated, single-stranded positive sense RNA molecule of approximately 7.2 kb in length [16]. Thus far, only genotypes 3 and 4 strains of HEV have been identified from swine. All known strains of HEV including swine HEV were classified into a new virus family Hepeviridae [11].

Seroepidemiological studies demonstrated that swine HEV is ubiquitous in pigs in the United States, and that about 80 - 100% of the pigs in commercial farms in the United States were infected [9,14]. Similar findings were also reported in pigs from more than a dozen other countries, both endemic and non-endemic [20,29,32]. In general, swine HEV infection occurs in pigs of 2 to 4 months of ages [9]. Viremia is transit and generally last only about 1 - 3 weeks, however fecal virus shedding can last for up to 5 - 7 weeks. Feces from infected pigs appear to be the principal source of the virus. Clinical signs of disease were not observed in pigs naturally infected or experimentally infected by swine HEV. However, the infected pigs did exhibit microscopic evidence of hepatitis [6,14,16].

Discovery of Avian Hepatitis E Virus (Avian HEV) in Chickens

Hepatitis-Splenomegaly (HS) syndrome, characterized by hepatitis and enlarged liver and spleen, is an emerging disease in
chickens in North America but the cause was unknown [24,26]. In 2001, Haqshenas et al. [7,8] isolated a novel virus genetically and antigenically related to human HEV from bile samples of chickens with HS syndrome in the United States, designate avian HEV. Avian HEV shares similar genomic organization and significant sequence identity with human HEV. The complete genomic sequence of avian HEV is about 6.6 kb in length, which is about 600 bp shorter than the genomes of human and swine HEV [11]. Although the overall genomic sequence identity between avian HEV and human HEV is only about 50%, motifs in the putative functional domains of ORF1 such as helicase and methyltransferase are relatively conserved between avian HEV and mammalian HEVs, supporting the conclusion that avian HEV is a member of the family Hepeviridae [11]. Avian HEV shared about 80% nucleotide sequence identity with the Australian big liver and spleen disease virus (BLSV) in a short stretch of 523 bp sequence available for BLSV [22], suggesting that BLS in Australia and HS syndrome in North America may be caused by variant strains of the same virus. Avian HEV isolates recovered from chickens with HS syndrome from 5 States displayed 78 to 100% nucleotide sequence identities to each other, suggesting that avian HEV is heterogeneous [10].

Like swine HEV, avian HEV infection is widespread in chicken flocks in the United States. Huang et al. [10] found that about 71% of chicken flocks and 30% of chickens from 5 States were positive for antibodies to avian HEV. Similar to swine HEV, avian HEV antibody prevalence in chickens is also age-dependent: about 17% of chickens younger than 18 weeks were seropositive, whereas about 36% of adult chickens were seropositive. The high prevalence of avian HEV infection in the field with low incidence of HS syndrome complicated the causal relationship between avian HEV infection and HS syndrome. It is possible that avian HEV infection is dose-dependent and only chickens infected with higher doses of the virus develop HS syndrome, however, the existence of avirulent strains of avian HEV in the field can not be ruled out [28]. It is also possible that avian HEV is the primary but not the sole causative agent of HS syndrome.

Swine and Chicken Models for HEV

Non-human primates have been used as animal models for HEV [4,23]. However, due to the limited resources, ethical concerns and restricted experimental procedures available for the use of non-human primates, little has been learned about the pathogenesis of HEV. In addition, extrapolating from or interpreting the significance of human HEV pathogenesis in non-human primates could be difficult as non-human primates are not the natural hosts of human HEV. The discoveries of swine HEV from pigs and avian HEV from chickens afforded an opportunity to develop small homologous animal models for HEV.

SPF pigs were successfully infected with both swine HEV and a genotype 3 strain of human HEV (US-2) [6,12,15,16,31]. However, evidence of clinical disease or elevation of liver enzymes or bilirubin was not observed. Domestic pigs experimentally infected with a Central Asian strain of human HEV (presumably genotype 1) reportedly developed jaundice [2], but others failed to reproduce the results [15]. The only gross lesions observed in infected pigs were mildly-to-moderately enlarged hepatic and mesenteric lymph nodes from 7 to 55 days postinoculation (DPI) [6,31]. Microscopic evidence of hepatitis was evident in the livers of both swine HEV- and human HEV-infected pigs. Hepatic inflammation and hepatocellular necrosis, characterized by multifocal lymphoplasmacytic hepatitis and focal hepatocellular necrosis, peaked in severity at 20 DPI [6,31]. Although swine clearly is a useful model to study certain aspects of HEV replication and pathogenesis, the swine model is limited by the fact that swine HEV and human HEV causes only a subclinical infection in pigs.

The discovery of avian HEV and its association with a hepatic disease (HS syndrome) provides a homologous model system to study HEV pathogenesis and replication. SPF adult chickens were experimentally infected by avian HEV via both intravenous route and the natural fecal-oral route (Billam et al., unpublished data). By 21 DPI, all oronasally- and IV-inoculated chickens had seroconverted to avian HEV antibodies. Fecal virus shedding was detected variably from 1 to 20 DPI in IV group, and 10 to 56 DPI in oronasal group. Avian HEV RNA was detected in serum, bile, and liver samples in both IV- and oronasally-inoculated chickens. Gross liver lesions, characterized by subcapsular hemorrhages or enlargement of right intermediate lobe, were observed in 7/28 oronasally- and 7/29 IV-inoculated chickens. Microscopic liver lesions were mainly lymphocytic periphlebitis and phlebitis. Slight elevations of plasma liver enzyme lactate dehydrogenase were also observed in infected chickens. The results suggested that chicken could be a useful model for studying HEV replication and pathogenesis.

Implication for Zoonosis

Swine HEV is genetically very closely related to, and in some cases indistinguishable from, genotypes 3 and 4 strains of human HEV, suggesting that swine HEV may infect humans [20,21,25]. Under experimental conditions, rhesus monkeys and a chimpanzee have been successfully infected by swine HEV [16]. The infected rhesus monkeys had a slight elevation of
serum liver enzymes and exhibited focal necroinflammatory changes in the liver. Infection of non-human primates, the surrogates of man, with swine HEV demonstrated the ability of swine HEV to infect across species barriers and the possibility of human infection with swine HEV. Several seroepidemiological studies have shown that swine handlers such as pig farmers and swine veterinarians are at increased risk of HEV infection compared to normal blood donors [3,17,19]. For example, using swine HEV capsid antigen for the serological assay, Meng et al. found that swine veterinarians in the United States were 1.51 times (p=0.03) more likely to be anti-HEV positive than normal U.S. blood donors in the same geographic region [19]. More recently, sporadic cases of acute hepatitis E were linked to the consumption of grilled or undercooked pig livers or intestines [13,32]. About 2% of the packaged raw pig livers sold in local grocery stores were found to be positive for swine HEV RNA [32]. Most importantly, the sequences of 7 swine HEV isolates recovered from packaged pig livers in grocery stores are very closely related, or identical in a few cases, to the viruses recovered from human hepatitis E patients [32]. Also, a cluster of 4 cases of human hepatitis E were recently linked to the consumption of raw deer meats in two families [30]. A HEV sequence was detected from the leftover frozen deer meat, and found to have 99.7 to 100% nucleotide sequence identity to the viruses recovered from the four patients [30]. Taken together, these data indicated that hepatitis E is a zoonotic disease, and swine (and maybe other species) are animal reservoir(s) for HEV [5,21].

Under experimental conditions, avian HEV recovered from a chicken with HS syndrome infected turkeys [27], demonstrating that avian HEV also has the ability to infect across species. However, an attempt to experimentally infect two rhesus monkeys with avian HEV was unsuccessfully [11]. Thus, it appears that, unlike swine HEV, avian HEV may not infect humans but additional studies are warranted to further assess the zoonotic potential of avian HEV.

References


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