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Review

In vivo models for studying Hepatitis E virus infection; Updates and applications

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ABSTRACT

Hepatitis E virus (HEV) is the most common cause of acute viral hepatitis globally. HEV belongs to the *Hepeviridae* family and at least five genotypes (gt) infect humans. Several animal species are reservoirs for different HEV strains, and they are the source of infection for humans. Some HEV strains are species specific, but other strains could cross species and infect many hosts. The study of HEV infection and pathogenesis was hampered due to the lack of an *in vitro* and *in vivo* robust model system. The cell culture system has been established for certain HEV strains, especially gt3 and 4, but gt1 strains replicate poorly *in vitro*. To date, animal models are the best tool for studying HEV infection. Non-human primates (NHPs) and pigs are the main animal models used for studying HEV infection, but ethical and financial concerns restrict the use of NHPs in research. Therefore, new small animal models have been developed which help more progress in HEV research. In this review, we give updates on the animal models used for studying HEV infection, focusing on the applicability of each model in studying different HEV infections, cross-species infection, virus-host interaction, evaluation of anti-HEV therapies and testing potential HEV vaccines.

1. Introduction

Hepatitis E virus (HEV) infection is an emerging pathogen in industrialized countries. HEV became endemic not only in developing countries but also in developed countries (Webb and Dalton, 2019). HEV is the most common cause of acute viral hepatitis resulting in 20 million infections and 70,000 deaths annually (Debing et al., 2016b; Rein et al., 2012).

HEV belongs to the *Hepeviridae* family which is divided into two genera: the *Orthohepevirus* and the *Piscihepevirus* that encompasses the cutthroat trout virus. The *Orthohepevirus* includes four species: *Orthohepevirus A, B, C and D,* the *Orthohepevirus A* species includes 8 genotypes (gt) (Smith et al., 2016, 2015). Genotypes HEV-1 and HEV-2 infect humans only and they are common in developing countries. While HEV-3 and HEV-4 are zoonotic and pigs, wild boars, and rabbits are the main reservoirs for these isolates (Kenney, 2019; Meng et al., 1997; Schlosser et al., 2014; Zhao et al., 2009). HEV-5 and HEV-6 were isolated from wild boars in Japan, they are highly divergent from HEV-3 and HEV-4 strains, and these isolates are not infectious to humans (Smith et al., 2015; Takahashi et al., 2011). HEV-7 and HEV-8 were isolated from camels (Woo et al., 2016, 2014).

The *Orthohepevirus B* includes avian HEV strains, the *Orthohepevirus C* includes isolates from rat, greater bandicoot, Asian musk shrew, ferret and mink; and the *Orthohepevirus D* includes isolates from the bat (Smith et al., 2016, 2015).

HEV causes acute, chronic, and extrahepatic manifestations. Acute HEV infection is mostly self-limited, especially in immunocompetent patients (Debing et al., 2016b). Chronic HEV infection, defined by the presence of HEV RNA more than 3 months in the patient samples, is developed in immunocompromised patients such as HIV-infected patients, organ transplants and patients with hematological disorders (Kamar et al., 2008; Riveiro-Barciela et al., 2014; Scotto et al., 2015; von Felden et al., 2019). The renal and nervous systems are the main targets for HEV replication outside the liver (Kamar et al., 2011, 2012; Zhou et al., 2017). Pegylated interferon-alpha and ribavirin are used for the treatment of HEV infection (Kamar et al., 2010, 2014).

HEV is a small icosahedral single-stranded, positive-sense RNA virus, including four overlapping open reading frames (ORF1, ORF2, ORF3 and ORF4) with a 5′capping and a 3′-poly A-tail. ORF1 encodes enzymes required for HEV replication, such as RNA dependent RNA polymerase, helicase, cysteine protease and methyltransferase. ORF2 encodes the structural capsid protein, and ORF3 encodes a small phosphoprotein that plays a role in virion morphogenesis (Kenney and Meng, 2019a). ORF4 protein is mostly conserved among HEV-1 isolates

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and it is induced under the effect of host endoplasmic reticulum stress. ORF4 interacts with multiple viral and host proteins and assembles a protein complex that stimulates viral RNA dependent RNA polymerase (Nair et al., 2016).

The study of HEV had been hampered for long period of time due to the lack of an efficient cell cultures that support the replication of all HEV isolates. Although certain viral strains of HEV-3 and HEV-4 have been adapted to grow efficiently *in vitro*, HEV-1 viruses poorly replicate in the cell culture system (Nguyen et al., 2014; Shukla et al., 2011; Tanaka et al., 2007, 2009). Several animal models have been developed to study HEV infection (Meng, 2003; Meng et al., 1998; Purcell and Emerson, 2001). These *in vivo* models are the best tools for studying the outcomes of HEV infection, virus-host interaction, evaluation of anti-HEV viral therapies and testing potential HEV vaccines. In this review, we give updates on the animal models used for studying HEV infection, highlighting the advantages and limitations of each model.

1.1. Non-human primates

Non-human primates (NHPs), such as chimpanzees, rhesus macaques, cynomolgus monkey, pig-tailed macaques, vervets, owl monkeys, African green monkeys, squirrel monkeys and tamarins have shown susceptibility to different HEV isolates (Krawczynski et al., 2011; Li et al., 2006; Purcell and Emerson, 2001; Purcell et al., 2013).

Experimental infection of NHPs (chimpanzee, rhesus macaques, and cynomolgus monkey) with HEV-1 and HEV-3 preparations lead to the development of acute hepatitis similar to the course of acute HEV infection in humans. Elevation of liver enzymes such as alanine transaminases (ALT) and aspartate transaminases (AST), viremia, seroconversion and excretion of infectious particles in stool were recorded in the infected animals (Choi et al., 2018; Purcell et al., 2013; Tsarev et al., 1994). The infection of NHPs with fecal preparations containing human derived HEV-1 (SAR-55 strain) was done either by the intravenous or oral route; the intravenous route was more successful and required less inoculum dose (Tsarev et al., 1994). Chimpanzees were more susceptible to HEV-1 infection than rhesus macaques and cynomolgus monkeys, while the elevation in liver enzymes was higher in cynomolgus monkeys and rhesus macaques than chimpanzees (Purcell et al., 2013). The clinical course of HEV-4 infection in rhesus macaques was variable; both acute and chronic infections were reported in the infected animals (Huang et al., 2016b, 2008). Chronic HEV infection was established in rhesus macaques inoculated with swine derived HEV-4 (KM01 strain) and in 3 out of 4 of cynomolgus monkeys treated with the immunosuppressant and challenged with Brazilian swine derived HEV-3 (Gardinali et al., 2017; Huang et al., 2016b). Extrahepatic manifestations of HEV infection were observed in the kidney and CNS of HEV infected NHPs (Geng et al., 2016; Huang et al., 2016b; Zhou et al., 2017). NHPs were used in reverse genetics system to produce infectious HEV from infectious cDNA. Recently, Li et al reported that cynomolgus monkeys were susceptible to HEV-5 and HEV-7 produced *in vitro* by a reverse genetic system suggesting a possibility of zoonotic transmission for these isolates (Li et al., 2019, 2016c).

NHPs were used to study the virulence of HEV isolates; HEV-3 was less virulent than HEV-1 and HEV-2 as determined by measurement of the peak of ALT level; the highest peak of ALT was caused by HEV-2, followed by HEV-1, while HEV-3 caused a slight elevation in ALT level (Purcell et al., 2013). In addition, NHPs are suitable candidates for studying cross-species transmission; they are susceptible to swine derived HEV-3 (Meng et al., 1998), rabbit derived HEV-3 (Liu et al., 2013), cow derived HEV-4 (Huang et al., 2016a) and camel derived HEV-7 (Li et al., 2016c), but not to ferret derived HEV-C2 (Li et al., 2015b), rat derived HEV-C1 or avian HEV strains (Huang et al., 2004). A recent study showed the capability of HEV-8, isolated from Bactrian Camel in China, to infect cynomolgus macaques; Chronic hepatitis, systemic infection, and renal pathology were observed in the infected monkeys (Wang et al., 2019).

The pathogenesis of HEV infection was studied in NHPs; chimpanzees and rhesus macaques were used to study the hepatic immune response against HEV infection. Most of the differentially expressed genes in HEV-infected chimpanzees were also affected in HCV-infected chimpanzees but at lower magnitudes. In HEV-1 infected chimpanzees, the number of upregulated genes peaked during the first week of viremia and returned to baseline with the cessation of viremia such as interferon stimulating genes and chemokines (Yu et al., 2010). The expression profiles of host immune response genes against HEV gt3 and gt1 infections were evaluated in rhesus macaques. In HEV-1 infected macaques, the hepatic immune response-related genes were downregulated in early viremia and the expression of these genes was upregulated during the peak of viremia when the animal became seropositive. While the expression levels of these genes were upregulated in early viremia and during the peak of viremia in HEV-3 infected animals. The expression levels of these genes were reduced or normalized after clearance of both infections (Choi et al., 2018). In addition, the pathogenesis of HEV infection during pregnancy was tested in rhesus macaques, but this trial was not successful since the virus did not transmit to the offspring (Tsarev et al., 1995).

NHPs are excellent models to evaluate the efficacy of potential HEV vaccines (Purcell et al., 2003; Zhang et al., 2015, 2002).

Although NHPs have many applications in HEV research, ethical (EU, 2010; Reardon, 2019) and financial concerns are the main limitations of this model.

1.2. Pigs

HEV was isolated for the first time from pigs in 1997 in the United States (Meng et al., 1997). Pigs are reservoirs for HEV-3 and HEV-4 (Kenney and Meng, 2019b; Meng et al., 1998, 1997). Pigs have been experimentally infected with swine derived HEV 3 and human derived HEV-3; fecal excretion of the virus was observed after 1 week of infection and the animals seroconverted to anti-HEV IgG at week 2 of infection (Meng et al., 1998). Also, pigs have been infected with human derived HEV-4 (strain TW6196E); fecal virus shedding and viremia were detected variably from week 1 to week 8 post infection, and seroconversion occurred by week 4 post infection (Feagins et al., 2008). In addition, the extrahepatic replication of HEV in the small intestine, colon, spleen, and kidney was also recorded in this model (Williams et al., 2001). Infected pigs only develop a subclinical infection with mild-to-moderate histopathologic lesions of hepatitis and no evidence for the elevation of liver transaminases limiting the use of this model for studying HEV pathogenicity (Meng, 2003). Similar to NHPs, the inoculation of pigs with swine derived HEV-3 *via* the intravenous route was more efficient than the oral route (Kasorndorkbua et al., 2004). Pigs were used to study chronic HEV infection and assess immune correlates during chronicity (Cao et al., 2017). Pigs were used in the cross-species transmission studies; infection of pigs with human derived HEV-3, human derived HEV-4, rabbit derived HEV-3 and wild boar derived HEV-3 strains was successfully established (Cossaboom et al., 2012; Meng, 2003; Schlosser et al., 2014). However, pigs are not susceptible to experimental infection with human derived HEV-1, human derived HEV-2 (Meng, 2003) and rat derived HEV-C1 (Cossaboom et al., 2012).

The pathogenesis of swine derived HEV-3 was studied in the swine model. HEV modulates several cellular pathways involved in cholesterol and lipid metabolism or cell survival (Rogee et al., 2015). Pigs were used also in vaccine studies (Sanford et al., 2012).

However, a limited presentation of clinical disease and the inability to study human derived HEV-1 and HEV-2 infection are the drawbacks of pigs as an animal model for HEV.

1.3. Rabbits

In 2009, HEV was first isolated from farmed rabbits in China (Zhao

et al., 2009). Rabbit HEV strains are closely related to human HEV-3. Rabbit HEV strains were isolated from humans confirming zoonotic origin (Abravanel et al., 2017). Rabbits have been used to study HEV infection and pathogenesis caused by the rabbit derived HEV-3, but not other HEV isolates. Infection of rabbits with different rabbit derived HEV-3 strains led to the development of a typical course of acute HEV infection; shedding of the virus in feces, viremia, elevation of liver transaminases, seroconversion and histopathological changes in the liver were recorded in HEV infected rabbits (Cheng et al., 2012; Ma et al., 2010). Chronic HEV infection was developed in the rabbit model after challenging with the rabbit derived HEV-3 (CH-BJ-rb14 strain) (Wang et al., 2017). Besides, extrahepatic replication of HEV was observed in the salivary gland, tonsil, spleen, thymus gland, lymph node, heart, lung, intestine, and kidney of HEV infected rabbits (Wang et al., 2017; Wu et al., 2017). Wu et al. reported the detection of HEV RNA in the saliva of some rabbits infected with swine derived HEV-4 raising the possibility of HEV transmission by droplet (Wu et al., 2017).

Rabbits are susceptible to rabbit derived HEV-3 strains (Cheng et al., 2012; Ma et al., 2010), human derived HEV-4 (Cheng et al., 2012), swine derived HEV-4 (Liu et al., 2019), and wild boar derived HEV-3 (Schlosser et al., 2018). However, the rabbit model is not susceptible to infection by human derived HEV-1, human derived HEV-3 and camel derived HEV-7 (Cheng et al., 2012; Li and Wakita, 2019; Ma et al., 2010). Similar to NHPs and pigs, the intravenous inoculation seems to be more effective than oral gavages in the establishment of HEV infection in the rabbit model (Cheng et al., 2012).

The impact of HEV infection on pregnancy was studied in rabbits and vertical transmission of HEV from pregnant rabbits to the fetus was recorded. (Xia et al., 2015). Rabbits were used for testing the antiviral therapies (Gong et al., 2018), and evaluating the efficacy of HEV vaccines (Cheng et al., 2012; Liu et al., 2014; Schlosser et al., 2018)

The main limitation of this model is the inability to study HEV infections caused by human derived HEV-1 and human derived HEV-3 since rabbits are not susceptible to infection by these isolates.

1.4. Human liver chimeric mice (humanized mice)

They are immunodeficient mice (uPA$^{+/+}$-SCID, uPA$^{+/+}$/NOG and FRG mice) suffering from a severe, chronic liver disease, *via* toxic transgene or knockout of essential protein, that allows replacement of the diseased murine hepatocytes by the transplanted functional primary human hepatocytes (Foquet et al., 2017; Meuleman et al., 2005). Human liver chimeric mice were reported as animal models for human hepatotropic pathogens such as hepatitis B virus, hepatitis C virus, hepatitis D virus and *Plasmodium falciparum* (Dorner et al., 2011; Lutgehetmann et al., 2012; Morosan et al., 2006). Recently humanized mice were reported as an elegant model for studying HEV infection. Humanized mice were successfully infected with HEV-1 and HEV-3 derived from various sources; patient samples, chimpanzees' fecal preparation, and cell culture derived virus. Viremia, fecal virus shedding and HEV antigens (Ags) (ORF2, ORF3) were persistently detected in the infected mice, while liver enzymes were not elevated in the infected mice (Allweiss et al., 2016; Sayed et al., 2017a, b; Sayed et al., 2019; van de Garde et al., 2016). HEV RNA and HEV ORF2 Ag could be detected in the stool and plasma of infected mice up to 4 months post infection suggesting that this model is a suitable candidate for studying chronic HEV infection (Sayed et al., 2017a, b; Sayed et al., 2019). HEV RNA was also detected in the liver and spleen of the infected mice, while the brain and kidney of the infected mice were negative for HEV RNA. These findings are probably because these organs were not humanized in origin, indicating that this model is not suitable for studying extrahepatic manifestations (Sayed and Meuleman, 2017). In addition, humanized mice were used to study the virulence of HEV genotypes; the viral load was lower in HEV-3 infected mice than HEV-1 infected mice regardless of the dose of inoculum (Sayed et al., 2017a). The infection of humanized mice with human derived HEV-1 and human derived HEV-3 preparations was successfully established *via* intravenous and intrasplenic injection, while intraperitoneal and oral inoculation (single or multiple gavages) failed. Cohousing of HEV-1 infected mice with naïve mice lead to transmission of the infection to the naïve mice, while cohousing of HEV-3 infected mice with naïve mice did not establish infection in the naïve ones (Allweiss et al., 2016; Sayed et al., 2017a, b; Sayed et al., 2019).

Up to date the cross-species infection has not been studied in depth in this model. The infection of humanized mice using chimpanzee or rhesus macaques fecal preparations containing human derived HEV-1 (SAR-55 strain) was proven indicating that this model could be a suitable candidate for studying cross-species transmission (Sayed et al., 2017a, b; Sayed et al., 2019; van de Garde et al., 2016, 2017)

Moreover, humanized mice were used to study the virus-host interaction, especially the hepatic immune response against HEV infection. Since the adaptive immune system is lacking in humanized mice, this model is a unique tool to study the impact of HEV infection on host innate immune response (Sayed, 2019; Sayed et al., 2017a; van de Garde et al., 2017). In addition, humanized mice were used for the evaluation of novel anti-HEV therapies such as ribavirin, Interferon α and silvesterol (Sayed et al., 2017a, 2019; Todt et al., 2018; van de Garde et al., 2016).

Although humanized mice are a promising tool for future HEV research, some limitations are linked to this model such as the lack of an adaptive immune response, the lack of clinical presentation of acute HEV infection, the inability to use this model for studying extrahepatic manifestations and the high cost of this model.

1.5. Mice (non-humanized)

Mice are a questionable model for HEV infection. It was reported that C57BL/6 mice were not susceptible to human derived HEV-1, swine derived HEV-3 and wild boar-derived HEV-4 (Li et al., 2008). Also, all trials to infect C57BL/6 mouse strains (wild-type, IFNAR$^{-/-}$, CD4$^{-/-}$, CD8$^{-/-}$) and BALB/c nude mice with wild boar derived HEV-3 failed (Schlosser et al., 2018). Similarly, non-transplanted immunodeficient mice (uPA$^{+/+}$/ SCID, FRG background) were not susceptible to human derived HEV-1 and HEV-3 infections (Sayed et al., 2017a; Sayed and Meuleman, 2017; Sayed et al., 2017b). On the other hand, others reported that Balb/c nude mice could be infected by HEV-4 isolated from a swine stool in China. Detection of HEV Ags and HEV RNA in liver, spleen, kidney, jejunum, ileum, and colon were recorded in the infected mice. In addition, liver enzymes were elevated and anti-HEV IgG was generated in these mice (Huang et al., 2009). Balb/c mice were used in cross-species transmission studies; the mice were infected with swine derived HEV-4 and rabbit derived HEV-3 isolates (Huang et al., 2009; Sun et al., 2018). Extrahepatic replication was recorded in the brain of Balb/c nude mice infected with HEV-4 (Zhou et al., 2017). More recently, Balb/c mice were used in reverse molecular genetics to produce HEV-4 from infectious cDNA (Yu et al., 2018) and to study the pathogenesis of HEV-4 during pregnancy (Yang et al., 2019a,b).

The discrepancy in the previously mentioned data is still unclear, but it could be attributed to the difference in the viral isolate, source of the virus and/or the species tropism. Mice were not susceptible to wild boar derived HEV-3, swine derived HEV-3 (DQ079632), wild boar derived HEV-4 (DQ079628), human derived HEV-1 and human derived HEV-3, while they were susceptible to swine derived HEV-4. Importantly, only one HEV isolate (KM01) is infectious to non-humanized mice. Further studies should be done to confirm the susceptibility of mice to other HEV-4 isolates.

Because of the discrepancy of data, the use of mice as a candidate for HEV infection is still questionable.

1.6. Rats

Rats were reported as potential models for HEV infection. The

infection of Wistar rats with human derived HEV-1, swine derived HEV-3 and wild boar-derived HEV-4 was not successful (Li et al., 2013) suggesting that rats are not an appropriate animal model for studying human derived HEV strains or other HEV isolates. The infection of Wistar rats with rat derived HEV-C1 lead to a transient excretion of the virus in rat stool, and seroconversion, but the liver enzymes were not elevated in the infected rats (Li et al., 2013). HEV particles recovered from rat stool were infectious and established the infection in the naïve rat (Li et al., 2013). Nude (athymic) rats infected with rat derived HEV-C1 developed a persistent infection with the excretion of massive quantities of the virus. Rat HEV RNA was detectable in the liver and intestine, but not in heart, lung, kidney, salivary gland or muscle indicating that rats are not a suitable animal model for studying extrahepatic manifestations (Li et al., 2013). Rats are not good candidates for studying the cross-species infection since rats are neither susceptible to infection with HEV-3, derived from pig stool or cell culture supernatant, nor to ferret derived HEV-C2 (Debing et al., 2016a; Li et al., 2015b). On the other hand, two out of eight Wistar rats inoculated intravenously with wild boar derived HEV-3 developed anti-HEV antibodies and HEV RNA was detectable in the stool of one animal. Notably, dexamethasone treatment in these animals did not enhance the susceptibility to wild boar-derived HEV-3 infection (Schlosser et al., 2018). Although experimental infection of rat with swine derived HEV-3 did not lead to active infection, HEV-3 was isolated from the spleen and intestine of Norway rats captured around a pig farm in Japan, probably rats moved through the pig pens and they got infected with HEV-3 by contact with pigs' contaminants (Kanai et al., 2012). Rats were used in the reverse genetics system to develop rat derived HEV-C1 from infectious cDNA clones (Debing et al., 2016a). Although rats are not a suitable model for studying human derived HEV isolates, they were used for screening of anti HEV therapy; ribavirin effectively inhibited rat derived HEV (LA-B350 strains) replication in athymic nude rats (Debing et al., 2016a).

The clinical presentation of HEV infection is limited in the rat model. Also, rats could not be infected with human derived HEV isolates or other HEV animal isolates and these are the main limitations of this model.

1.7. Ferrets

Ferret derived HEV isolates (HEV-C2) are different than human derived HEV isolates, but they are close to rat derived HEV-C1. Natural HEV infection in ferrets exhibits three patterns: sub-clinical infection, acute hepatitis, and persistent infection (Li et al., 2016a). Experimental inoculation of ferrets with cell culture supernatant containing ferret derived HEV-C2 *via* oral route led to the development of acute hepatitis. Detection of HEV RNA in the stool and sera, seroconversion and a significant elevation of liver enzymes indicated liver damage in the inoculated animals suggesting that ferrets are potential candidates for studying the virus pathogenesis and immunological response against HEV infection (Li et al., 2016b). Up to our knowledge, cross-species studies have not been evaluated in the ferret model. Further studies should be done to verify the susceptibility of this model to other HEV isolates. But since the ferret derived HEV-C2 isolates are divergent from human derived HEV isolates, ferrets might not be suitable candidates for studying human HEV strains, evaluation of anti-HEV therapies or potential vaccines.

1.8. Mongolian gerbils

The Mongolian gerbils (*Meriones unguiculatus*) were reported as promising models for HEV infection. Mongolian gerbils challenged intraperitoneal with swine derived HEV-4 developed an active infection characterized by viremia, fecal viral shedding, elevation of liver transaminases, and seroconversion to anti-HEV IgG. In addition, HEV was consistently detectable in liver, and occasionally in other organs such as kidney, spleen and the small intestine of the infected gerbils (Li et al., 2009; Yang et al., 2015). Importantly, Mongolian gerbils were successfully infected with a human derived HEV-1 strain isolated from acute HEV infected patient; excretion of the virus in the feces, the moderate elevation of liver transaminases and histopathological changes in the liver, spleen, and kidney were observed in the infected gerbils (Hong et al., 2015). The numbers of mast cells were relatively high mainly in the liver and small intestine of the infected gerbils suggesting that mast cells play a role in HEV pathogenesis (Liu et al., 2018). Mongolian gerbils were used to study extrahepatic manifestations of HEV, especially the neurological disorders associated with HEV infection.(Shi et al., 2016). In addition, HEV caused a disruption in the blood-testis barrier of the infected gerbils and induced germ cell apoptosis (Soomro et al., 2017). Since Mongolian gerbils were successfully infected with swine derived HEV-4 and human derived HEV-1, they could be a potential candidate for cross-species transmission studies. However, up to our knowledge, there is no report on the susceptibility of Mongolian gerbils to HEV strains isolated from other animals such as rabbit derived HEV-3, rat derived HEV-C1, …etc. Also, there is not any report about the susceptibility of Mongolian gerbil to other human derived HEV isolates such as HEV-2, HEV-3 and HEV-7.

1.9. Chickens

In 2001, the avian HEV strain was identified in chickens with hepatitis-splenomegaly syndrome (HSS). Phylogenetic analyses revealed that avian HEV is genetically related to, but distinct from, other known HEV strains (Haqshenas et al., 2001). Chicken can be infected with avian HEV *via* intravenous or oronasal route; the virus is shed in feces and HEV RNA could be detected in bile, serum, and liver samples. In addition, liver lesions characteristic of HSS has been observed, making it a homologous animal model system to study HEV pathogenesis and replication (Billam et al., 2005). Moreover, extrahepatic replication of avian HEV in the gastrointestinal tract was recorded in the infected chicken (Billam et al., 2008). Chickens were used in reverse molecular genetics to produce avian HEV from infectious cDNA, the produced viruses were infectious to naïve chicken (Huang et al., 2005). In addition, the chicken model was used to study the impact of the hypervariable region (HVR) on the efficiency of HEV replication. Avian HEV mutant strain with a complete deletion of HVR region was injected intrahepatic in chickens. The chickens remained seronegative and HEV RNA was not detectable in the feces nor the plasma of the chicken indicating that HVR is involved in the efficiency of HEV RNA replication (Pudupakam et al., 2011). A recent study reported a novel avian HEV strain as a causative agent for hepatic rupture hemorrhage syndrome (HRHS) that is characterized by rapid viremia, chronic fecal shedding, detection of the virus in the liver and spleen and 40% loss of the infected chickens (Su et al., 2018). Vertical transmission of HEV was not complete in the chicken model, suggesting that it is not an ideal model for studying HEV pathogenesis during pregnancy (Guo et al., 2007a). The chicken model was used in vaccine studies; chickens immunized with avian HEV capsid protein induced protection against avian HEV infection (Guo et al., 2007b).

Chickens are not a suitable model for studying human derived HEV isolates, because the clinical course in chickens is different from that typically found in humans. In addition, chicken is not an ideal model for cross-species transmission.

1.10. Tree shrews (Tupaia belangeri chinensis)

Yu et al. reported that HEV infection was successfully established in three tree shrews inoculated intravenously with swine derived HEV-4 ; Seroconversion, detection of HEV RNA in the serum, stool, liver, bile, spleen, and kidney tissue, detection of HEV capsid protein in the liver, spleen, and kidneys and the development of acute liver lesions were signs of active infection. In addition, HEV infection was transmitted to naive tree shrews cohoused with HEV infected animals (Yu et al., 2016)

Limited data is known about the use of tree shrews as a model for HEV infection since only one report is available.

2. Animal models used for studying chronic HEV Infection

HEV infection can be developed to chronicity especially in case of suppression of the immune system such as HIV infection, leukemia or organ transplantation (Kamar et al., 2015, 2008). Chronic HEV infection is a risk factor for the development of HEV cirrhosis or extrahepatic manifestations (Kamar et al., 2011; Peron, 2016). It is difficult to study the pathogenesis of chronic HEV infection using *in vitro* cell culture system; therefore, animal models are suitable candidates for this purpose. Chronic HEV infection was established in rhesus macaques inoculated with swine derived HEV-4 (KM01 strain). HEV RNA was consistently detected in the serum and stool of infected macaques up to 272–650 days post inoculation, slight elevation of liver transaminases and very weak and transient humoral immune response were recorded in the chronically infected macaques (Huang et al., 2016b). Similarly, chronic HEV infection was reported in 3 out of 4 of cynomolgus monkeys treated with the immunosuppressant and challenged with Brazilian swine derived HEV-3 strain; persistent viremia and fecal shedding lasted for 160 days, but the infected monkeys did not develop fibrosis or cirrhosis (Gardinali et al., 2017).

Similarly, pigs were used to study chronic HEV infection. Chronic HEV infection was developed in pigs treated with immunosuppressive drugs; 8 out of 10 of immunosuppressed HEV-infected pigs continued fecal virus shedding up to 22 weeks post-infection. The levels of the liver enzyme such as γ-glutamyl transferase and fecal virus shedding were higher in immunocompromised HEV-infected pigs than mock treated HEV infected pigs. In HEV infected immunocompromised pigs, the serum levels of Th1 cytokines IL-2 and IL-12, Th2 cytokines IL-4 and IL-10 and IFN-γ-specific CD4 + T-cell responses were reduced during the acute phase of infection, while TNF-α-specific CD8 + T-cell responses increased during the chronic phase of infection (Cao et al., 2017).

In addition, chronic HEV infection, characterized by infiltration of inflammatory cells in the portal area of the liver, venous dilation, and some degree of fibrosis, was developed in the rabbit model after challenging with the rabbit derived HEV-3 (CH-BJ-rb14 strain). Prolonged viremia and fecal shedding of viral particles similar to chronic HEV infection in humans were observed in the infected animals (Wang et al., 2017).

Also, humanized mice were used to study chronic HEV infection. Viremia and fecal excretion of the infectious viral particles were persisted up to 16 weeks post-infection. In addition, HEV ORF2 Ag was continuously detectable in the mice samples (plasma and stool) (Allweiss et al., 2016; Sayed et al., 2019). The viral load was higher in the mouse stool than mouse plasma at the same time point, while HEV ORF2 Ag level was higher in the mouse plasma than the mouse stool suggesting that a large amount of ORF2 Ag present in the plasma is attributed to non-infectious particles; a similar conclusion was made from the analysis of HEV infected human sera (Montpellier et al., 2018; Sayed et al., 2019).

3. Animal models used for studying the pathogenesis of HEV during pregnancy

HEV virus causes severe outcomes during pregnancy and the mortality rate can reach up 25–30 %.in the women during their third trimester (Patra et al., 2007). Complications of HEV during pregnancy depend on several factors such as virus genotypes, viral load, immune status, and hormonal factors (Navaneethan et al., 2008). HEV-1 virus causes most of the complications associated with HEV infection during pregnancy such as abortion, premature delivery, death of a live-born baby soon after birth or fetal and/or maternal mortality. While the course of HEV-3 during pregnancy is a mild to moderate, spontaneously self-limited (Navaneethan et al., 2008; Pischke et al., 2017). The pathogenesis of HEV during pregnancy was studied *in vitro*. Knegendorf et al. showed that HEV is capable of completing the full viral life cycle in placental-derived JEG-3 cells (Knegendorf et al., 2018). Similarly, HEV could replicate in organ cultures of maternal fetus interface including the decidua basalis and fetal placenta as well as placental stromal cells causing alterations in the tissue secretome and severe tissue injury (Gouilly et al., 2018). Animal models were also used to study HEV pathogenesis during pregnancy and the possibility of vertical transmission of HEV from mother to offspring. HEV infection was successfully established in pregnant rabbits. Two out of six infected pregnant rabbits miscarried and three of the remaining pregnant rabbits died. The infected pregnant rabbits developed acute HEV infection. HEV Ag and HEV RNA were detected in the placental tissue of the infected pregnant rabbits. In addition, HEV RNA was detected in the stool of one surviving offspring and the newborns seroconverted at 3 months of age indicating the vertical transmission of HEV from pregnant rabbits to the fetus (Xia et al., 2015).

Balb/c mice were used to study the pathogenesis of HEV during pregnancy. Mice were infected with swine derived HEV-4 in various stages (early, middle and late) of pregnancy. Miscarriages (87.5%) occurred in mice infected during the middle stage of pregnancy. HEV RNA and HEV-Ag were detected in various tissues of the infected mice as well as the fetal liver. Th1 biased immune status was the cause of fetus loss in the aborted mice (Yang et al., 2019a,b)

Besides, the pathogenesis of HEV infection during pregnancy was tested in rhesus macaques. The typical severe clinical course normally observed during pregnancy, such as fulminant hepatitis and the severe outcome was not observed in the pregnant rhesus monkeys infected with HEV-1 and the infection did not transmit to their offspring indicating that NHPs are not the ideal model for studying HEV infection during pregnancy (Tsarev et al., 1995). In the chicken model, infectious avian HEV was detected in chicken egg-white samples, but the vertical transmission of HEV was not complete in this model, suggesting that it is not an ideal model for studying HEV pathogenesis during pregnancy (Guo et al., 2007a).

4. Animal models used for studying HEV extrahepatic manifestations

Extrahepatic manifestations have been reported in association with HEV infection such as neurological disorders, glomerulonephritis, cryoglobulinemia, acute pancreatitis, thrombocytopenia, and hemolytic anemia. The mechanisms of extrahepatic manifestations are still unclear, but they may be caused by either direct HEV replication in these tissues, or indirectly by various immune-mediated mechanisms (Pischke et al., 2017; Wedemeyer et al., 2012). Some extrahepatic manifestations are genotype-specific; acute pancreatitis cases are mainly caused by HEV-1 viruses, while renal manifestations are caused by genotype 3 HEV. Limited data is available for the frequency or the percentage of extrahepatic manifestations. Two studies in France and UK showed that neurological manifestations are not common in HEV infected patients as they have been reported in 5.5%–7.5% of the patients (Kamar et al., 2011; Woolson et al., 2014). HEV infection, mainly HEV-3 isolates, was detected in 5–10% of patients diagnosed with Guillain-Barré syndrome and in 11% of patients with neuralgic amyotrophy (Pischke et al., 2017). Kamar et al. reported the link between HEV infection and renal impairment. Out of 51 HEV infected patients, 8 patients developed glomerulonephritis without any other possible causes (Kamar et al., 2012). Few case reports have been reported for other extrahepatic manifestations such as thyroiditis, myocarditis, and hematological disorders (Pischke et al., 2017).

Extrahepatic replication of HEV and the excretion of HEV in urine were studies in NHPs. Two cynomolgus monkeys were inoculated with human derived fecal preparation containing HEV-4 and HEV-1 viruses. The animal became infected and they excreted HEV RNA and HEV Ag in their urine. The ratios of HEV-Ag to RNA in the urine samples were

significantly higher than those in the corresponding serum and fecal samples. Importantly, the excreted viral particles in the urine were infectious as shown by the development of acute HEV infection in naïve monkey challenged with the urine of HEV infected monkey. HEV Ag was observed in the liver and kidney sections of the infected monkeys. In addition, several pathological changes were observed in the kidney, including tubular protein casts and interstitial infiltration of inflammatory cells, while the parameters of routine urinalysis remained within the normal ranges in HEV infected monkeys (Geng et al., 2016).

Also, rhesus macaques were used to study the neurologic manifestation associated with HEV infection. Two monkeys were challenged with swine derived HEV-4 (KM01 strain), HEV RNA was detected in the serum, liver and brain tissues of the monkey and HEV ORF2 protein was expressed in the granule layer of cerebellum. Similarly, HEV ORF2 protein and HEV RNA were detected in the brain of Balb/c nude mice infected with the same viral isolate (swine derived HEV-4) (Zhou et al., 2017).

Mongolian gerbils were used to study extrahepatic manifestations of HEV, especially the neurological disorders associated with HEV infection. HEV-4 virus crossed the blood-brain barrier, replicated in the brain and spinal cord producing various pathological changes such as perineural invasion, microglia nodule, myelin degeneration, neuron necrosis and lymphocyte infiltration (Shi et al., 2016).

Also, extrahepatic replication of HEV and kidney injury were recorded in the rabbit model challenged with rabbit derived HEV-3 (CH-BJ-rb14 strain). Positive and negative-stranded HEV RNA and HEV ORF3 protein were detected in the kidneys, indicating virus replication in the tissue. In addition, infiltration of lymphocytes and plasma cells in the renal interstitium and obvious protein casts in the cavities of renal tubules suggesting the kidney injury. HEV RNA was detectable in the urine of the infected rabbits (Wang et al., 2017).

In the pig model, negative-strand HEV RNA was detected in the small intestines, lymph nodes, colons, and liver of the pigs infected with swine derived HEV-3 and human derived HEV-3, indicating extrahepatic replication of HEV in these tissues (Williams et al., 2001).

5. Animal reservoirs and transmission

Pigs are an animal reservoir for HEV-3 and HEV-4 (Meng, 2003; Meng et al., 1997). In natural HEV infected pigs, fecal viral shedding, seroconversion to anti-HEV IgG were recorded, while no observable clinical disease was observed in the animals (Meng, 2003; Meng et al., 1998, 1997). Pigs become infected with HEV through the ingestion of feces-contaminated feed or water or direct contact with an infected animal (Meng, 2011). Several studies showed that persons exposed to pigs such as pig farmers, slaughterers, butchers, and swine veterinarians have a higher prevalence of anti-HEV- antibodies than non-exposed persons suggesting zoonotic HEV transmission through contact with the infected pigs (Meng, 2003). HEV replicates in the liver, colon, duodenum, lung, spleen, muscle and lymph node of the infected pigs (Williams et al., 2001). The presence of HEV RNA in muscle or other pig organs entering the food chain such as liver and sausages increases the risk of zoonotic HEV transmission through the ingestion of the contaminated pork products (Meng, 2011; Pavio et al., 2014). One study showed that 7 out of 13 patients developed acute HEV infection after eating raw figatellu (pig liver common in France), and HEV-3 RNA was recovered from figatelli purchased in supermarkets (Colson et al., 2010).

Rabbits are a natural reservoir for HEV. HEV was detected in farmed and wild rabbits globally (Ahn et al., 2017; Cossaboom et al., 2011; Di Bartolo et al., 2016; Hammerschmidt et al., 2017; Zhao et al., 2009). HEV RNA was detected in the plasma, bile, liver, and stool of the natural HEV infected rabbits. Also, anti-HEV antibodies were detected in infected animals (Izopet et al., 2012). Rabbit HEV strains are closely related to human HEV strains. A 93-nucleotide insertion in the X domain of the ORF1 of the human strain and all the rabbit HEV strains circulating in France was found suggesting that HEV infected rabbits could be a source of HEV infection to humans (Izopet et al., 2012). Abravanel et al. showed that 5 out of 919 HEV-infected patients in France were infected with a rabbit HEV strain and none of these patients had direct contact with rabbits, suggesting foodborne or waterborne infections (Abravanel et al., 2017). Also, pet rabbits were reported to be infected with HEV (Caruso et al., 2015; Di Bartolo et al., 2016). This could increase the risk of zoonotic HEV transmission to humans.

In 2010, rat HEV was first detected in the feces of wild rats (*Rattus norvegicus*) in Germany (Johne et al., 2010). After that, several reports have shown the detection of rat HEV-C1 in different rat species (*Rattus norvegicus, Rattus rattus*) globally suggesting that rats are a natural reservoir for rat HEV-C1 strains(Li and Wakita, 2019; Murphy et al., 2019; Purcell et al., 2011). The nucleotide sequence identity of rat derived HEV-C1 to human HEV strains is approximately 60%, while the rat derived HEV-C1 is closely related to ferret derived HEV-C2 (Kenney and Meng, 2019b; Li and Wakita, 2019; Raj et al., 2012). The genome of rat derived HEV-C1 strain contains four ORFs, ORF1-3 as those present in other HEV isolates and ORF4 is of unknown function (Johne et al., 2010; Li and Wakita, 2019). Experimental infection of pigs with rat derived HEV-C1 did not lead to active infection (Cossaboom et al., 2012), while HEV-3 was isolated from the spleen and intestine of Norway rats captured around a pig farm in Japan (Kanai et al., 2012). Recently, two cases in Hongkong and Canada were diagnosed with rat derived HEV. The first case was a liver transplant immunocompromised recipient who developed persistent hepatitis caused by rat HEV-C1 isolate. HEV-C RNA was detectable in feces, blood, saliva and liver samples and HEV-C Ag was detectable in the liver. Although this patient had preexisting anti-HEV antibodies, they were not protective against HEV-C infection (Sridhar et al., 2018). The second case was immunocompetent who developed severe acute hepatitis caused by a novel strain of rat HEV-C1 that showed 85% homology with a European HEV rat strain (acc.MF480317)(Andonov et al., 2019).

The zoonotic potential of rat HEV is still unclear. The infection could be transmitted to humans by ingestion of food contaminated by rat feces during the food supply or processing. Contaminated blood products with rat HEV-C1 could be another possible cause of the infection. Further studies should be done to verify these points.

A ferret HEV-C2 strain was first identified in the Netherlands in 2010 (Raj et al., 2012). After that, ferret HEV RNA was detected in 7.1% of pet ferrets in Japan (Li et al., 2015a). Ferret derived HEV-C2 was also detected from laboratory ferrets imported from a ferret farm in the U.S (Li et al., 2017). Ferret derived HEV-C2 is closely related to rat derived HEV-C1, but it is divergent from human derived HEV-1- HEV-4 and other HEV isolates. Nucleotide sequence identity with human derived HEV-1- HEV-4, rabbit derived HEV-3, and avian HEVs ranges from 54.5% to 60.5%, while nucleotide sequence identity with rat derived HEV-C1 is (72.3%)(Li and Wakita, 2019). Like the genome of rat derived HEV-C1, the genome of ferret derived HEV-C2 contains four ORFs; ORFs (1–3) present in the human HEV genome. A putative fourth ORF (ORF4) overlapping with ORF1 was also identified in the ferret HEV genome but is of unknown function (Li and Wakita, 2019; Raj et al., 2012). Up to now, there is no evidence for the zoonotic potential of ferret derived HEV-C2.

Recently, HEV RNA and anti-HEV antibodies were detected in NHPs present naturally in zoos and farms suggesting that these animals could be a reservoir for HEV (Caballero-Gomez et al., 2019; Dogadov et al., 2019; Yang et al., 2019a,b).

Wild boars are another animal reservoir for HEV. HEV-3, HEV-4, HEV-5, and HEV-6 were isolated from the wild boars (Sato et al., 2011; Schlosser et al., 2014; Takahashi et al., 2011). Several studies have shown that pig and wild boar derived HEV isolates sharing 90–98% homology (Nishizawa et al., 2005; Schielke et al., 2009). Wild boars naturally infected with HEV-3 developed chronic HEV infection; fecal viral shedding and viremia were observed for more than five months.

Table 1
The susceptibility of HEV animal model to human derived HEV and other HEV isolates.

Animal	Figure	Cross-Species infection	Susceptibility to Human HEV	Susceptibility to other HEV isolates
Non-human primates		Yes	HEV 1-4 HEV-7	• camel derived HEV-7 and HEV-8 • swine derived HEV-3 and HEV-4 • rabbit derived HEV-3 • cow derived HEV-4
Pig		Yes	HEV-3 and HEV-4	• Swine derived HEV-3 • Rabbit derived HEV-3 • Wild boar derived HEV-3
Rabbit		Yes	HEV-4	• Rabbit derived HEV-3 • Swine derived HEV-4 • Wild boar derived HEV-3
Humanized Mice		Should be[a]	HEV-1 and HEV-3	NT
Mice non humanized		Yes[b]	No	Swine derived HEV-4 Rabbit derived HEV-3
Rat		No	No	Rat derived HEV-C1
Ferret		No	No	Ferret derived HEV-C2
Mongolian gerbil		Yes	HEV-1	Swine derived HEV-4
Chicken		No	No	Avian HEV

[a]Infection was proven using the fecal preparation of NHPs, but in-depth cross species infection has not been done yet.
[b]NT: Not tested.
[c]Discrepancy data about this model.

Although the infected animal developed anti-HEV antibodies, the immune system could not clear the virus. HEV infection was transmitted to domestic pigs cohoused with the HEV infected wild boar (Schlosser et al., 2014, 2015). Several studies have shown a higher HEV seroprevalence in the hunters of wild boars that general population indicating a zoonotic transmission through contact with the animals (Schielke et al., 2015; Toyoda et al., 2008). In addition, HEV RNA was detectable in some food products derived from wild boars such as liver, meat, and sausages (Pavio et al., 2017). Several HEV-related hepatitis cases were linked to the ingestion of wild boar products suggested a foodborne transmission to humans (Li et al., 2005; Masuda et al., 2005, 2000).

HEV RNA and anti-HEV antibodies were also detected in deer (Anheyer-Behmenburg et al., 2017; Medrano et al., 2012; Thiry et al., 2017). HEV isolates found in deer belong to HEV-3 (Pavio et al., 2017; Thiry et al., 2017). HEV RNA was also detectable in the liver and meat products of the deer (Pavio et al., 2017). Four HEV infected cases were reported in Japan and linked to the consumption of meat from Sika deer suggesting a zoonotic transmission through the ingestion of contaminated meat products (Tei et al., 2003). However, a recent study in Germany has shown that the HEV loads found in livers and muscle of deer were consistently lower than wild boar suggesting that deer are not a true reservoir of HEV but they become infected accidentally by sharing the same habitat as wild boar (Anheyer-Behmenburg et al., 2017).

HEV was detected in 37% of cows present in mixed farms with pigs in China, the isolated viruses from cows belonged to HEV-4 subtypes 4 h, the same isolate detected in the pigs and in humans in the same region. Importantly, HEV infected cows excreted infectious HEV particles in their milk that represent a possible zoonotic transmission through the ingestion of contaminated milk products (Huang et al., 2016a).

Anti-HEV antibodies, but not HEV RNA, have been detected in different animal species such as sheep, buffalo, horses, cats, and dogs suggesting that these animal species have been exposed to HEV or a closely related agent, but they are not true reservoirs for HEV (Doceul et al., 2016).

6. Animal models used for evaluation of antiviral therapies and vaccines

Ribavirin is used in the treatment of chronic HEV, but mutant viruses with reduced sensitivity to ribavirin have recently been described (Debing et al., 2014; Todt et al., 2016). Also, ribavirin is contraindicated during pregnancy due to its teratogenicity effect, so novel antiviral therapies are highly needed (Debing and Neyts, 2014). Animal models can be used for the evaluation of potential anti-HEV therapies and HEV vaccines. Rabbits were used for testing antiviral therapies such as ribavirin and Babao Dan (BD) which is a traditional medicine widely used for the treatment of chronic hepatitis in China. BD was effective in the prevention and treatment of HEV infection in rabbits challenged with rabbit derived HEV-3. BD caused a significant reduction in fecal virus shedding, fecal virus titers and protecting the liver tissue from pathological injury. Ribavirin treatment was more effective in rapidly clearing HEV than BD (Gong et al., 2018). Also, rabbits have been used to evaluate the efficacy of HEV vaccines. Rabbits immunized with HEV 239 vaccine, HEV 179p, HEV-3 recombinant capsid protein or rat capsid protein showed protection against homologous and heterologous HEV challenge. High anti-HEV titers and no sign of active infection were observed in the vaccinated rabbits (Cheng et al., 2012; Liu et al., 2014; Schlosser et al., 2018).

Also, humanized mice were used for the evaluation of anti-HEV therapies such as ribavirin, Interferon α and silvesterol. Humanized mice treated with ribavirin for 2–6 weeks showed a reduction in the viral load and HEV ORF2 Ag level in the plasma, stool and liver of the treated mice (Allweiss et al., 2016; Sayed et al., 2019). Similarly, treatment of humanized mice with IFN-α injections lead to clearance of HEV RNA from mouse stool (van de Garde et al., 2017). Also, humanized mice were used to evaluate silvestrol, a natural compound isolated from the plant *Aglaia foveolate*. Humanized mice treated with silvestrol showed a significant reduction in fecal viral load, especially during the first few days of treatment (Todt et al., 2018).

NHPs were also used to evaluate the efficacy of potential HEV vaccines. In one study, the HEV vaccine containing a recombinant HEV capsid antigen was highly immunogenic and efficacious in preventing HEV infection in rhesus macaques challenged with different isolates of

Table 2
Applications of HEV animal models.

Animal	HEV infection			Application of the model in HEV research			
	Acute	Chronic	Extrahepatic Manifestation	Pregnancy study	Evaluation of anti-HEV therapy	Evaluation of vaccine	Virus-host interaction
NHPs	Yes	Yes	Yes	Failed	?[a]	Yes	Yes
Pigs	Yes	Yes	Yes;	?[a]	Yes	Yes	Yes
Rabbits	Yes	Yes	Yes	Yes	Yes	Yes	?[a]
Human liver chimeric mice	No	Yes	No[b]	?[a]	Yes	NA	Yes
Mice (non- humanized)	Yes[c]	?[C]	Yes[c]	Yes[c]	?[a]	?[a]	?[a]
Rats	Yes[d]	Yes In nude rat	No	?[a]	Yes	?[a]	?[a]
Ferrets	Yes	Yes	?[a]	?[a]	No	No	No
Mongolian gerbil	Yes	No	Yes	?[a]	?[a]	?[a]	?[a]
Chicken	Yes	?[a]	Yes	No	?[a]	Yes	?[a]
Tree shrews	Yes	?[a]	?[a]	?[a]	?[a]	?[a]	?[a]

?[a]unknown, up to our knowledge.
[b]no extrahepatic manifestations were recorded with HEV gt1 and gt3 strains, other isolates have not been tested yet in this model.
[C]Discrepancy data about this model.
[d]No elevation in liver enzymes, and a transient excretion of the virus in the rat stool.
NA: not applicable.

Table 3
Advantage, limitations and cost of animal models used in studying HEV infection.

Animal	Advantages	Limitations	Cost[a]
Non-human primates	- Used in cross-species infection. - Susceptible to human derived HEV 1-4 - Studying immune response against HEV infection. - Evaluation of HEV vaccine. - Clinical presentations (acute, chronic and extrahepatic manifestations) could be studied.	- Ethical concerns - High Cost - Not suitable for studying HEV pathogenesis during pregnancy. - Difficult in operation.	≥ 500-1000$
Pig	- Used in cross-species infection. - Susceptible to human derived HEV3-4. - Studying immune response against HEV infection. - Used in vaccine studies.	- Limited clinical presentations - Not susceptible to human derived HEV1 and HEV-2	Around 100$
Rabbit	- Used in cross-species infection - Clinical presentations (acute, chronic and extrahepatic manifestations) could be studied. - Evaluation of HEV vaccine and antiviral therapies - Studying HEV pathogenesis during pregnancy.	- Not susceptible to human derived HEV1 and HEV-3	Around 100-150$
Humanized Mice	- Replication of HEV inside human hepatocytes Evaluation of antiviral therapies. - Studying innate immune response against HEV infection.	- lack clinical presentation of acute HEV infection - lack adaptive immune response - High cost. - No extrahepatic manifestation.	Around 150-500$
Mice non humanized	- Used to study HEV pathogenesis during pregnancy. - Study neurologic manifestation associated with HEV infection.	- Questionable model due to discrepancy of data. - Only one isolate (swine derived HEV-4 (KM01) could infect	Around 10$
Rat	- Used to study rat derived HEV-C1 which is recently isolated from human cases. - Could be used for evaluation of anti-HEV therapy.	- Not susceptible to study human derived HEV isolates - Not suitable for cross species studies	Around 20-40$
Ferret	- Study the pathogenesis of ferret derived HEV-C2	- Limited data about this model.	Around 65- 250$
Mongolian gerbil	- Potential candidate for cross-species studies - Study the pathogenesis of human derived HEV-1 - Study neurologic manifestations associated with HEV infection.	- Limited data about this model.	Around 90-105$
Chicken	- Could be used in vaccine studies. - Study the pathogenesis of avian HEV	- Not susceptible to study human derived HEV isolates.	Around 10-25$

[a] The prices mentioned in the table are approximate prices since they differ from one place to another. Also, these prices represent direct cost, other indirect costs including technician fees, animal preparation, food and supplements, cages, animal medication and vaccination, feed jar,…etc are not included.

HEV-1 and HEV-3 (Purcell et al., 2003). In another study, macaques vaccinated with HEV 239 vaccine (Hecolin) developed anti-HEV antibodies and were protected against challenge with homologous and heterologous HEV isolates (Zhang et al., 2015, 2002).

Pigs were used in vaccine studies. Prior infection of pigs with swine derived HEV-3 provided a protection for the pigs and prevented developing viremia and fecal virus shedding upon challenge with homologous swine derived HEV-3 and heterologous human derived HEV-4 (Sanford et al., 2011). Also, truncated recombinant capsid antigens of swine derived HEV-3, rat derived HEV-C1 and avian HEV strains could induce strong immune responses in pigs and partially cross-protect against HEV-3 (Meng strain) infection (Sanford et al., 2012).

7. Summary and conclusion

Animal models are useful tools for studying HEV infection. The models above-mentioned are widely used in HEV research, but major limitations are still present in each model. Each model has susceptibility to different HEV isolates, summarized in Table 1. Still, limited data are available for the susceptibility of the new small animal models to

various HEV isolates. Animal models allow the study of various aspects of HEV infection including infection outcome, cross-species infection, viral pathogenesis, and host immune response against HEV. With the emergence of cell culture adapted strains, there are many aspects of HEV infection that are best studied utilizing *in vitro* methodology. However, there is not an ideal model that can be utilized in all previous aspects, since each model has its applications, advantages and limitations. Summarized in Table 2 and Table 3.

Author contributions

I.M.S., A.A.E and M.A.E wrote the manuscript.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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