Small Animal Models of Hepatitis E Virus Infection

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Novel hepeviruses have been recovered from many different animal species in recent years, increasing the diversity known to exist among the *Hepeviridae*, which now include two genera, *Piscihepevirus* and *Orthohepevirus*. Multiple viral genotypes in the *Orthohepevirus A* species are able to replicate and cause acute hepatitis E in humans, and thus represent an important public health problem in industrialized as well as developing countries. Although hepatitis E virus (HEV) infections typically result in acute and self-limited hepatitis, immunocompromised and transplant patients are vulnerable to prolonged infections and to chronic hepatitis. Cell culture systems have been established for several HEV strains and offer new opportunities for the study of HEV biology. Similarly, a variety of new small animal models have been developed, using either nonhuman hepeviruses in their cognate hosts as surrogates for human HEV, or human HEV infection of immunodeficient mice with chimeric livers engrafted with human hepatocytes. These new models provide several advantages over previous nonhuman primate models of hepatitis E infection and will facilitate studies of pathogenicity, cross-species infection, mechanisms of virus replication, and vaccine and antiviral agent development. This article reviews the current understanding of small animal models for HEV.

Hepatitis E virus (HEV) is classified in the genus *Hepevirus* of the family *Hepeviridae* (Meng et al. 2012; Kenney and Meng 2018). It is a small, round nonenveloped virus, 27–34 nm in diameter. The genome of genotype (gt)1 HEV was first molecularly cloned in 1990 (Reyes et al. 1990) and is a 7.2-kb, single-strand, positive-sense RNA molecule containing three discontinuous and partially overlapping open reading frames (ORFs) (Tam et al. 1991). The 5' end of the RNA terminates in an m⁷G cap structure, and the 3' terminus of the RNA is polyadenylated (Tam et al. 1991; Magden et al. 2001). ORF1 encodes a large polyprotein with several

putative functional domains, but it is unclear whether this polyprotein undergoes proteolytic processing resulting in separate mature non-structural proteins as in other positive-strand viruses. ORF2 encodes the major capsid protein, which self-assembles into virus-like particles (VLPs) when expressed by recombinant baculoviruses (Li et al. 2000, 2011). ORF3 encodes a phosphoprotein with multiple functions and is associated with virion release in particular (Nagashima et al. 2011).

HEV causes waterborne epidemic and sporadic acute hepatitis E in developing countries and sporadic, zoonotic infections in devel-

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oped countries (Emerson and Purcell 2003; Nelson et al. 2011, 2018; Dalton and Izopet 2018). HEVs isolated from humans are grouped into four major genotypes designated 1 to 4 (gt1 to gt4) on the basis of their nucleotide and deduced amino acid sequences (Liu et al. 2008). HEV gt1 and gt2, which infect only humans, are transmitted primarily by the fecal–oral route (Balayan et al. 1983). They infect ~20 million people every year and cause waterborne hepatitis outbreaks in regions of Asia, Africa, and Central America with low standards of sanitation. HEV gt1 and gt2 viruses cause ~70,000 deaths every year (Rein et al. 2012) with very high morbidity in pregnant women who have a mortality risk as high as 20% (Khuroo et al. 1981).

Human HEV gt3 and gt4 infections occur most commonly in developed countries of Europe, the Americas, and Asia. These infections are sporadic and very likely zoonotic in nature, transmitted to humans from species that include domesticated pigs, wild boar, and deer (Tei et al. 2003; Li et al. 2005; Meng 2010). Probable zoonotic transmission of an HEV gt7 virus from a camel also has been described recently (Lee et al. 2015). Because HEV infection is associated with viremia, HEV may also be transmitted by blood transfusion. Importantly, such autochthonous hepatitis E infections can persist in immunocompromised individuals, including transplant recipients as well as patients with hematologic malignancies or poorly controlled human immunodeficiency virus (HIV) infection (Kamar et al. 2013).

In addition to human HEV, novel HEV-like viruses have been identified in a wide variety of animal species, including wild boars, monkeys, rabbits, rats, minks, moose, ferrets, red foxes, camels, kestrel, little egrets, chickens, bats, and cutthroat trout (Haqshenas et al. 2001; Zhao et al. 2009; Johne et al. 2010a; Batts et al. 2011; Drexler et al. 2012; Raj et al. 2012; Yamamoto et al. 2012; Bodewes et al. 2013; Krog et al. 2013; Lin et al. 2014; Takahashi et al. 2014; Woo et al. 2014; Reuter et al. 2016). The family *Hepeviridae* is comprised of two genera, *Orthohepevirus* and *Piscihepevirus* (Smith et al. 2014; see also Smith and Simmonds 2018), in a classification scheme recently approved by the International Com-

mittee on Taxonomy of Viruses (ICTV) (see ictvonline.org/virusTaxonomy.asp). The *Orthohepevirus* genus includes four species: *Orthohepevirus A*, which includes isolates from humans, monkeys, pigs, wild boar, deer, mongoose, rabbits, and camels; *Orthohepevirus B*, which includes isolates from chickens; *Orthohepevirus C*, which includes isolates from rats, greater bandicoots, Asian musk shrews, ferrets, and mink; and *Orthohepevirus D*, which includes isolates from bats. The species assignments of isolates from moose, kestrel, and little egrets remain uncertain. Cutthroat trout virus is classified within the genus *Piscihepevirus*.

Human, camel, rabbit, ferret, and rat HEV have been propagated in various cell lines, including human hepatocellular carcinoma cells (PLC/PRF/5 or HepG2), human lung cancer cells (A549), and several nonhuman mammalian cell lines (Tanaka et al. 2007; Shukla et al. 2011; Jirintai et al. 2012, 2014; Li et al. 2016c, d). Such cell culture systems are useful for understanding mechanisms of replication, cell entry, and virion morphogenesis. However, they do not allow investigation of the molecular basis underlying differences in the pathogenicity of HEV genotypes and the ability of gt3 viruses to cause persistent infection.

Further progress in defining mechanisms of liver disease and immune control in humans with acute and chronic HEV infections will depend on the availability of suitable animal models. Development of approaches to prevent or treat severe acute infection, especially in the setting of pregnancy as well as chronic infections, would also be aided by an animal model that recapitulates features of human infection. Chimpanzees are susceptible to HEV and have been used to characterize virological and immunological aspects of HEV infection (Meng et al. 1998; Yu et al. 2010; Purcell et al. 2013; Lanford et al. 2018). However, these animals are no longer available for invasive biomedical research. Other nonhuman primates (particularly macaque species) and swine also have been used to model acute (Balayan et al. 1983, 1990; Halbur et al. 2001; Li et al. 2004, 2016d; Meng 2010; Purcell et al. 2013) and more recently persistent HEV infection in the setting of immune suppression

(Cao et al. 2017; Gardinali et al. 2017). Cynomolgus and rhesus monkeys are susceptible to gt1–4 and gt7 HEV infection and were useful in evaluating the response to HEV vaccine candidates as detailed in Lanford et al. (2018). Swine also can be readily infected with gt3 and gt4 HEV, with mild or no disease, and are a likely reservoir for gt3 and gt4 HEV (Halbur et al. 2001). These nonhuman primate and swine models are potentially very useful, but they are expensive and cumbersome for studies of HEV pathogenesis. Moreover, they do not always recapitulate clinical aspects of hepatitis E, showing minimal elevations in serum liver enzymes and only moderate pathological liver lesions (Halbur et al. 2001; Meng 2010; Cullen and Lemon 2018). In this review, we focus on HEV infection models using small animals, including rabbits, ferrets, rats, tree shrews, Mongolian gerbils, and mice with chimeric human livers.

RABBITS (*Oryctolagus* sp.)

Rabbit (*Oryctolagus* sp.) HEV was first detected in farmed rabbits from China. Analysis of the full-length genomic sequences showed that rabbit HEV strains are most closely related to the gt3 HEV, which causes zoonotic infections in humans (Zhao et al. 2009). Since then, many rabbit HEV strains have been detected in farmed as well as wild and specific pathogen-free (SPF) rabbits in the United States, China, Korea, Germany, Italy, the Netherlands, and France (Cossaboom et al. 2011; Burt et al. 2016; Di Bartolo et al. 2016; Ahn et al. 2017; Hammerschmidt et al. 2017; Liu et al. 2017), suggesting that HEV infection is common in rabbits and that they are a natural host for this virus. Rabbit HEV replicated in PLC/PRF/5 and A549 cell lines as efficiently as gt3 HEV (Jirintai et al. 2012). Of 919 HEV-infected patients studied in France during 2015–2016, five were infected with virus closely related to rabbit HEV, suggesting that rabbit HEV strains may be transmitted to humans and cause occasional zoonotic infection (Abravanel et al. 2017). Rabbit HEV has been successfully transmitted to pigs (Cossaboom et al. 2012) and cynomolgus macaques (Liu et al. 2013), confirming the po-

tential for cross-species transmission (Liu et al. 2017).

Experimental infection studies indicate that rabbits shed virus in feces, develop antibodies to HEV, and show elevations in serum alanine aminotransferase (ALT) activity indicative of acute liver damage when infected with HEV (Ma et al. 2010). Intriguingly, experimental infection of pregnant rabbits with rabbit HEV resulted in high mortality as well as vertical transmission of virus, with local hepatocellular necrosis evident in microscopic evaluation of the liver (Xia et al. 2015). In contrast, nonpregnant rabbits experimentally infected with rabbit HEV demonstrated little to no overt signs of disease.

The close genetic and antigenic relatedness of rabbit HEV to other mammalian HEV strains suggests that infected rabbits may serve as a useful, naturally occurring animal model for the study of HEV pathogenesis. However, rabbits do not appear to be uniformly susceptible to infection with HEV strains that infect humans. In one study, gt1 HEV was not infectious for rabbits, and although rabbits inoculated with gt3 HEV seroconverted they demonstrated neither viremia nor fecal viral shedding (Cheng et al. 2012). Similarly, intravenous and oral inoculation of gt4 HEV resulted in infection in only some rabbits. Another report confirmed that only two of nine rabbits inoculated with gt4 HEV became infected, and that gt1 HEV was incapable of eliciting any markers of HEV infection in rabbits (Ma et al. 2010). Our preliminary experimental results also suggest that rabbits are not susceptible to gt7 HEV infection.

In summary, although rabbits cannot be reliably infected with human HEV strains, rabbits infected with rabbit HEV may provide an appropriate parallel animal model of HEV infection, especially in the medically important setting of pregnancy for which no other animal model of enhanced maternal disease currently exists.

FERRETS (*Mustela putorius*)

Ferrets (*M. putorius*), which have long been used as an animal model of influenza virus infection, may also be valuable for the study of

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HEV infection. HEV was first detected in ferrets from the Netherlands in 2012 (Raj et al. 2012), but since then many other ferret HEV strains have been identified in ferrets used as laboratory animals or kept as pets in the United States and Japan (Raj et al. 2012; Yang et al. 2013; Li et al. 2015b). Ferret HEV is distinct from human HEV and is widespread among ferrets in Europe, the Americas, and Asia.

The ferret HEV genome contains the three ORFs (1–3) present in the human HEV genome. ORF1 encodes a nonstructural protein of 1596

amino acids (aa), ORF2 encodes a capsid protein of 654 aa, and ORF3 encodes a phosphoprotein of 108 aa (Raj et al. 2012). A putative fourth ORF (ORF4) overlapping with ORF1 was also identified in the ferret HEV genome but is of unknown function. Ferret HEV is rather distantly related to human HEV strains and shares the highest nucleotide sequence identity (72.3%) with rat HEV (Fig. 1). Nucleotide sequence identity with human gt1–gt4 HEV, rabbit and avian HEVs ranges from 54.5% to 60.5% (Raj et al. 2012). However, antigenic analyses demonstrate

Figure 1. Phylogenetic tree based on complete genomic sequences of hepeviruses recovered from fish, humans, monkey, swine, wild boar, deer, rabbit, mongoose, camel, ferret, rat, moose, kestrel, little egret, and avian sources. Sources of hepatitis E virus (HEV) strains follow GenBank accession numbers. Sequence alignment was performed with ClustalW. *Orthohepevirus* species assignments are indicated on the right (see also Smith and Simmonds 2018).

that ferret HEVs are cross-reactive with gt1, gt3, gt4, and rat HEVs. As might be expected from their greater nucleotide sequence identity, the rat and ferret HEVs showed stronger antigenic cross-reactivity to each other than either did to human HEV. However, antibody directed against ferret HEV does not neutralize gt3 HEV, suggesting that these two HEVs belong to different serotypes (Yang et al. 2013).

The level of nucleotide sequence identity (full genome) between ferret HEV (classified within the species *Orthohepevirus C*) and human HEV gt1–gt4 (*Orthohepevirus A*) is <55%, and there is as yet no evidence for the zoonotic potential of ferret HEV. An infection experiment indicated that laboratory rats (Wistar), nude rats (Long Evans run/run), and cynomolgus macaques are also not susceptible to ferret HEV, suggesting that the capacity of ferret HEV to replicate in other commonly used experimental animals is very limited (Li et al. 2015c). However, a cell culture system has been established for ferret HEV using the human hepatocellular carcinoma cell line, PLC/PRF/5; infectious virus particles recovered from cell culture supernatants were shown to be associated with membranes (Li et al. 2016c).

Like other HEVs, ferret HEV can be transmitted by the fecal–oral route (Li et al. 2016c). Three patterns of infection in ferrets have been observed, including subclinical infection, acute hepatitis as measured by transaminase eleva-

tions, and persistent infection. In one study, the ALT was increased in 65.8% (25 of 38) of naturally infected ferrets testing positive for ferret HEV RNA; ALT levels were higher than 2000 IU/L in some ferrets (Li et al. 2016a). Histologic changes induced in the liver by ferret HEV infection have yet to be characterized (Li et al. 2016a, 2017). ALT elevations have also been documented after experimental transmission of the virus to ferrets, suggesting that liver disease is caused solely by ferret HEV and not a coinfecting virus (Fig. 2) (Li et al. 2016a).

Importantly, we recently identified persistent HEV infection in six of 63 ferrets imported into Japan from a ferret farm in the United States (Li et al. 2016a). Animals were viremic through at least 130 days of follow-up. It is notable that this persistent infection developed naturally in ferrets not treated with immune suppressive drugs. Naturally occurring persistent gt3 HEV infection has also been described in a captive Japanese snow monkey (*Macaca fuscata*) (Yamamoto et al. 2012) that served as a source of virus for other macaques in the colony. Why some ferrets and monkeys develop prolonged or persistent infections in the absence of immune-suppressive therapy remains unclear. Ferrets may be particularly useful in efforts to define factors leading to persistence and to identify protective immune responses to HEV. Such studies may provide a better understanding of persistent HEV infections occurring in solid-

Figure 2. Experimental infection of ferrets with ferret hepatitis E virus (HEV). Animals were inoculated orally with virus recovered from the supernatant fluids of infected PLC/PRF/5 cells. (From Li et al. 2016a; adapted, with permission, from Elsevier © 2016.)

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organ transplant recipients who have received immunosuppressive drugs, and in patients with other immunosuppressive conditions, including HIV infection and hematological malignancy (Kamar et al. 2008; Dalton et al. 2009; le Coutre et al. 2009; Ollier et al. 2009). It will be also interesting to determine whether persistent HEV infection is associated with chronic hepatitis and triggers the onset of hepatic cirrhosis or even hepatocellular carcinoma.

RATS (*Rattus* sp.) AND NUDE RATS

Rat HEV was first identified in 2010 in feces of wild rats (*Rattus* sp.) from Germany (Johne et al. 2010a). Since then, the virus has been detected in animals from several countries in Europe and Asia and from the United States (Johne et al. 2014). The host species are mainly rat species (*Rattus norvegicus*, *Rattus rattus*, among other), but viruses very closely related to rat HEV have also been found in the greater bandicoot (*Bandicota indica*) and the Asian musk shrew (*Suncus murinus*) (Guan et al. 2013; Li et al. 2013a,b). Phylogenetic analyses indicate that rat HEV is most closely related to ferret HEV (Fig. 1) (Raj et al. 2012), and both viruses have been classified with the *Orthohepevirus C* species (Smith et al. 2014). Like the ferret HEV genome, the rat HEV genome also contains four ORFs, including ORFs 1–3, which encode a nonstructural polyprotein, the capsid protein, and a small phosphoprotein, respectively, and which are present in all HEV-related viruses. ORF4 overlaps with the 5' region of ORF1, and as in the ferret virus is of unknown function (Johne et al. 2010b). Initial studies failed to show replication of rat HEV in rat-derived cell lines (Johne et al. 2010a). However, rat HEV has been propagated successfully in the human hepatoma cell lines PLC/PRF/5, HuH-7, and HepG2 (Jirintai et al. 2014).

Analyses of the amount of rat HEV-RNA present in different tissues of infected wild rats suggest that the virus is hepatotropic (Johne et al. 2010a). This was confirmed in laboratory rats intravenously inoculated with tissue homogenates containing rat HEV (Guan et al. 2013). Immunodeficient nude rats inoculated with rat HEV developed persistent infections

with long-term shedding of large quantities of virus (Guan et al. 2013). In contrast, experimentally infected Wistar rats showed transient virus shedding and developed antirat HEV-specific antibodies. Hepatitis was not observed after experimental infections of rats (Purcell et al. 2011; Guan et al. 2013). These results suggest that rat HEV might provide an infection model but is unlikely to be helpful for studies of pathogenesis.

Because nude rats were found to be highly susceptible to rat HEV infection, with prolonged shedding of high titers of rat HEV, we used these animals to develop a reverse molecular genetics system for the virus (Li et al. 2015a). Rat HEV was recovered from nude rats without any changes in its genome. The rat HEV-infected nude rats showed persistent infection that could be useful as an animal model for antiviral agent screening. In fact, ribavirin effectively inhibited replication of the LA-B350 strain of rat HEV in athymic nude rats, confirming the suitability of the rat model for antiviral studies (Debing et al. 2016).

The zoonotic potential of rat HEV is not clear at present. Efforts to experimentally infect rhesus monkeys and pigs with rat HEV did not result in evidence of virus replication (Purcell et al. 2011; Cossaboom et al. 2012). In addition, rats and even nude rats were not susceptible to experimental infection by HEV gt1–4 (Guan et al. 2013; Li et al. 2016b), suggesting that rats are not suitable for studying human HEV.

MONGOLIAN GERBILS (*Meriones unguiculatus*)

Several reports suggest the possibility of using Mongolian gerbils (*M. unguiculatus*) as an animal model of HEV infection. In one study, 14 Mongolian gerbils were successfully infected after intraperitoneal inoculation of gt4 HEV recovered from swine (Li et al. 2009). Viremia and fecal virus shedding persisted for nearly 4 weeks; virus was also detected within the liver as well as the kidneys, spleen, and small intestine. Histopathological changes included multifocal, lymphohistiocytic infiltrates, portal tracts, or irregular distribution throughout the parenchyma. HEV antigens were detected in the liver and

intestine. In another study, gt1 HEV-infected gerbils showed histopathological changes within the liver, spleen, and kidney (Hong et al. 2015). HEV RNA was detected in fecal samples from 7 to 42 days after inoculation, but ALT levels were only moderately increased. However, in both of these studies, HEV-specific antibodies could not be detected in HEV-infected animals. Further studies will be needed to confirm whether Mongolian gerbils are truly susceptible to human HEV infection.

TREE SHREWS (*Tupaia belangeri chinensis*)

Tree shrews (*T. belangeri chinensis*) are small Euarchonta mammals native to the tropical forests of Southeast Asia. Because they are closely related to primates, tree shrews have been studied in the past as potential animal models of hepatitis B virus (HBV) and hepatitis C virus (HCV) infection. A recent study suggests that tree shrews can be infected with human gt4 HEV RNA, resulting in detection of HEV RNA in the serum, stool, liver, bile, spleen, and kidney tissue (Yu et al. 2016). HEV capsid protein was detected in the liver, spleen, and kidneys. The histological examination of the liver and serum ALT elevations were indicative of mild liver injury. Both immunoglobulin (Ig)G and IgM antibodies were detected in experimentally infected animals (Yu et al. 2016). Interestingly, uninoculated animals cohoused with experimentally infected animals also developed evidence of infection. The potential of tree shrews to serve as a model of human HEV infection merits further study.

MURINE XENOGRRAFT MODELS OF HEV INFECTION

Infections of rats and ferrets with their cognate *Orthohepevirus C* viruses provide interesting opportunities for studying general features of HEV infection, but these small animal species are not permissive for replication of the *Orthohepevirus A* viruses that cause disease in humans. A murine model of hepatitis E would be particularly valuable given recent advances in murine genetics and the abundance of tools

and reagents available to study infections in mice. However, hepeviruses have not been isolated from mice, and C57BL/6 mice have been confirmed experimentally to be nonsusceptible to human HEV infection (Li et al. 2008). Humanized mice with chimeric human livers have contributed significantly to our understanding of pathobiology, virus–host interactions, and antiviral therapy for hepatitis B, C, and D. As described in Hirai-Yuki et al. (2018), Alb-uPA/SCID beige mice engrafted with human hepatocytes (Mercer et al. 2001) also support replication of human hepatitis A virus (HAV). It is not surprising, therefore, that human liver chimeric mice are susceptible to HEV infection.

Allweiss et al. (2016) described the first human liver chimeric mouse model of HEV infection. uPA/SCID/beige (USB) mice repopulated with primary human hepatocytes were susceptible to HEV infection. Chimeric mice infected with a gt1 HEV showed a rapid increase in viremia and large quantities of virus in serum, liver, bile, and feces for >25 weeks. In contrast, viremia in gt3 HEV-infected mice developed more slowly and resulted in lower amounts of virus in all analyzed tissues compared with the gt1 HEV-infected animals. HEV-infected human hepatocytes could be visualized in these mice using HEV ORF2- and ORF3-specific antibodies and in situ hybridization probes complementary to HEV RNA (Allweiss et al. 2016). Humanized uPA-NOG (uPA^{+/+}NOD/Shi-*scid*/IL2Rγ^{-/-}) and FRG mice are also susceptible to gt1 or gt3 HEV infection (van de Garde et al. 2016; Sayed et al. 2017).

Thus, human liver chimeric mice represent an attractive small animal model for the study of HEV infection and replication as well as the evaluation of novel antiviral and preventive strategies. As an example, it was recently shown that interferon α can rapidly clear HEV infection in humanized mice with chimeric livers (van de Garde et al. 2017), and that ribavirin can suppress virus replication (Allweiss et al. 2016). Moreover, such mice have been used to establish the infectious nature of HEV in human plasma, showing that it has the potential to cause transfusion-associated HEV infection (Sayed et al. 2017). These animals do lack a

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functional immune system, however, and so studies of protective host immune responses may be limited to in vivo studies of virus neutralization by passively administered antibodies.

CHICKEN MODEL OF AVIAN HEV

A novel HEV strain was identified in 2001 in chickens in the United States with hepatitis-splenomegaly syndrome (HSS) (Haqshenas

et al. 2001). This virus is now classified within the *Orthohepevirus B* species (Fig. 1). There are at least three genotypes (gt1–gt3) of avian HEV distributed throughout the world, together sharing ~60% nucleotide sequence identity with human HEV (Marek et al. 2010). Infections in chickens are often subclinical and result in mortality rates ranging from 0.3% to 1.0%. Infection results from ingestion of the virus, and leads to subcapsular hemorrhages, focal lymphocytic

Table 1. Comparative features of hepatitis E virus (HEV) infection in macaques, swine, and small animal models of HEV infection

Species	Known HEV susceptibility	Infection outcome		Severe hepatitis in pregnancy	Suitability for analysis of immunity	
		Acute resolution	Persistence		Humoral	Cellular ^a
Macaque ^b	<i>Orthohepevirus A</i> gt1, gt2, gt3, gt4, gt7	Yes	With immune suppression ^c	No ^d	Yes	Strong
Swine	<i>Orthohepevirus A</i> gt3, gt4	Yes	With immune suppression	No	Yes	Strong
Rabbit	<i>Orthohepevirus A</i> rabbit HEV (gt3), gt4	Yes	Unknown	Yes	Yes	Poor
Ferret	Ferret HEV (<i>Orthohepevirus C</i>)	Yes	Naturally occurring (frequency unknown)	Unknown	Yes	Intermediate
Rat	Rat HEV (<i>Orthohepevirus C</i>)	Yes	Unknown	Unknown	Yes	Intermediate
Chicken	Chicken HEV (<i>Orthohepevirus B</i>)	Yes	Unknown	N/A ^e	Yes	Poor
Mongolian gerbil	<i>Orthohepevirus A</i> gt1, gt4	Yes	Unknown	Unknown	Yes	Poor
Tree shrew	<i>Orthohepevirus A</i> gt4	Yes	Unknown	Unknown	Yes	Poor
Chimeric mouse (humanized liver)	<i>Orthohepevirus A</i> gt1, gt2, gt3, gt4	No	Yes	N/A	Yes	No

^aSuitability for studies of adaptive cellular immunity was classified as follows: “strong” when immunogenetic characterization of the species, availability of antibodies for detailed analysis of T-cell phenotype and function, and advanced reagents like major histocompatibility complex (MHC) tetramers have been generated; “intermediate” when there are gaps in immunogenetic characterization and/or the array of available reagents for characterization of T cells; and “poor” when immunogenetics or reagents availability are limited.

^bCynomolgus and rhesus macaques used in most studies (see Lanford et al. 2018 for details).

^cAs noted in the text (Yamamoto et al. 2012), naturally occurring persistent infection has been described in one captive Japanese macaque.

^dRhesus macaques infected with HEV gtl during pregnancy had no evidence of enhanced disease (Arankalle et al. 1993; Tsarev et al. 1995).

^eN/A, Not applicable.

periphlebitis, and hepatic phlebitis (Billam et al. 2005). Rhesus macaques were not susceptible to avian HEV infection when challenged experimentally, suggesting that avian HEV is probably not transmissible to primates (Huang et al. 2004). Similarly, chickens are not susceptible to human HEV infection. The usefulness of chickens as an animal model for human HEV is likely limited, but this naturally occurring chicken model offers a unique hepatic disease model (i.e., HSS) that might be used to study at least some aspects of human hepatitis E disease (Yugo et al. 2014).

CONCLUDING REMARKS

Novel hepeviruses have been identified in a variety of species, including small and readily available animals that are amenable to study in laboratory settings. Genomic characterization of these viruses has been important to sort out taxonomic relationships to HEV genotypes that infect humans, and to identify genetic features that might contribute to pathogenesis. At the same time, studies of virus replication, pathogenesis, and the nature of protective immune responses in these animals are somewhat preliminary. Further development of small animal models is nonetheless an imperative if mechanisms of progressive liver disease in chronic HEV infection and fulminant hepatitis E in pregnant women are to be understood. The ideal small animal model of HEV pathogenesis has yet to be identified (Table 1), but will ideally recapitulate the course of acute and chronic HEV infection in humans, and be amenable to studies of how innate and adaptive immunity might contribute to liver disease or provide protection from persistence.

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