Abstract

Hepatitis E virus (HEV) is a single-stranded, positive-sense RNA virus in the family Hepeviridae. Hepatitis E caused by HEV is a clinically important global disease. There are currently four well-characterized genotypes of HEV in mammalian species, although numerous novel strains of HEV likely belonging to either new genotypes or species have recently been identified from several other animal species. HEV genotypes 1 and 2 are limited to infection in humans, whereas genotypes 3 and 4 infect an expanding host range of animal species and are zoonotic to humans. Historical animal models include various species of nonhuman primates, which have been indispensable for the discovery of human HEV and for understanding its pathogenesis and course of infection. With the genetic identification and characterization of animal strains of HEV, a number of naturally occurring animal models such as swine, chicken, and rabbit have recently been developed for various aspects of HEV research, including vaccine trials, pathogenicity, cross-species infection, mechanism of virus replication, and molecular biology studies. Unfortunately, the current available animal models for HEV are still inadequate for certain aspects of HEV research. For instance, an animal model is still lacking to study the underlying mechanism of severe and fulminant hepatitis E during pregnancy. Also, an animal model that can mimic chronic HEV infection is critically needed to study the mechanism leading to chronicity in immunocompromised individuals. Genetic identification of additional novel animal strains of HEV may lead to the development of better naturally occurring animal models for HEV. This article reviews the current understanding of animal models of HEV infection in both natural and experimental infection settings and identifies key research needs and limitations.

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Key Words: hepatitis E virus (HEV); animal models; hepatitis E; cross-species infection; rabbit; swine; chicken

Introduction

Hepatitis E virus (HEV)

HEV is classified in the genus Hepeivirus of the family Hepeviridae (Meng et al. 2012) The mammalian HEV is comprised of four recognized genotypes and at least two putative genotypes that are distinct in geographic locale as well as host. Genotype 1 HEV causes the majority of outbreaks of hepatitis E in humans in Asia. Genotype 2 HEV includes one Mexican strain and multiple African strains and causes large outbreaks in humans. In addition to humans, genotype 3 HEV has been isolated from a wide variety of animal species, including wild and domestic swine (de Deus et al. 2008; Meng et al. 1997; Takahashi et al. 2004), deer (Takahashi et al. 2004), mongoose (Nakamura et al. 2006), rats (Lack et al. 2012), and rabbits (Cossaboom et al. 2011; Zhao et al. 2009), and is associated with zoonotic transmission causing sporadic, cluster, and chronic cases of hepatitis E in humans (Meng 2010). Genotype 4 HEV infects humans as well as wild and domestic swine with zoonotic transmission to humans causing sporadic cases of hepatitis E (Meng 2013) (Table 1).

In addition to the zoonotic genotypes 3 and 4 HEV strains mentioned above, genetically divergent strains of HEV have been also been identified from several other animal species, including chicken (Haqshenas et al. 2001; Marek et al. 2010; Payne et al. 1999), bat (Drexler et al. 2012), fish (Batts et al. 2011), rat (Johne et al. 2011), ferret (Raj et al. 2012), and wild boar (Takahashi et al. 2011) (Figure 1). Additionally, antibodies to HEV have been reportedly detected from horses (Saad et al. 2007) and ruminant species including cattle, sheep, and goats (Sanford et al. 2012a); however, the source of seropositivity in these species remains unknown (Meng 2013). The animal host range of HEV has expanded dramatically over the past decade from the initial identification of HEV in swine in 1997 (Meng et al. 1997) and chickens in 2001 (Haqshenas et al. 2001) to a multitude of animal species acting as both reservoir and host more recently. Further research will likely expand the host range of the virus to include additional novel strains from many more other animal species. Reclassification and nomenclature changes for HEV...
are eminent with the recent identification of several novel strains (Table 1).

Hepatitis E

HEV is transmitted via the fecal-oral route through contaminated food or water and typically causes an acute icteric disease known as hepatitis E (Meng 2010; Purcell 2001). The majority of patients experience an asymptomatic course of disease in which the virus is quickly cleared with no major complication (Meng 2010). Symptomatic patients experience a range of symptoms, including anorexia, jaundice, darkened urine coloration, hepatomegaly, myalgia, elevated alanine aminotransferase (ALT) levels in the blood, and occasionally abdominal pain, nausea, vomiting, and fever (Purcell 2001). Acute HEV infection in humans begins with a typical incubation period of 2 weeks to 2 months, a transient viremia period with viral shedding in the feces, a symptomatic phase lasting days to weeks, and jaundice apparent 2 to 3 weeks into the course of infection (Purcell 2001). The severity of HEV infection is considered dose dependent with alcohol use or other concurrent hepatic diseases as contributing factors (Purcell 2001). Immunocompromised individuals infected with HEV such as organ transplant recipients are at a high risk of developing chronic hepatitis E (Kamar et al. 2008; Kamar et al. 2013). Pregnancy-associated complications with concurrent HEV infection include death of both the mother and fetus, abortion, premature birth, and death of the baby shortly after birth with no known mechanism for the severe hepatitis E manifestation (Navaneethan et al. 2008). The mortality rate ranges from 0.5 to 4% in immune-competent individuals and concurrent pregnancy attributed to increases in mortality up to 25% (Aggarwal 2011).

Hepatitis E affects humans in both industrialized and developing countries worldwide. Industrialized countries experience sporadic and cluster cases of hepatitis E associated with ingestion of contaminated animal meats, shellfish, and contact with infected animals (Meng 2013; Teo 2010). Developing countries such as Bangladesh, Egypt, Mexico, China, and India and parts of Africa experience large waterborne outbreaks due to poor sanitation conditions and a hyperendemic status in the population (Teo 2010). Genotype 1 and 2 HEV strains are limited to the human population, whereas genotypes 3 and 4 strains are zoonotic and infect humans and other animals. Human to human transmission of HEV is considered rare; however, transmission through blood products by transfusion has been reported (Pavio et al. 2010).

Animal Models of Human HEV

Several animal species serve as useful models for human HEV because of their susceptibility to infection by human HEV strains (Table 2). Cynomolgus and rhesus monkeys are susceptible to genotypes 1 to 4 HEV and serve as the primary model for genotypes 1 and 2 human HEV strains (Meng 2010; Purcell and Emerson 2001). Swine serve as a reservoir for genotypes 3 and 4 human HEV and a natural animal host for HEV infection (Halbur et al. 2001). Despite production of efficient virus replication, both the nonhuman primate and swine models have limitations in reproducing clinical aspects of hepatitis, with minimal elevations in serum levels of liver enzymes and moderate pathological liver lesions present (Halbur et al. 2001; Meng 2010). Rabbit HEV recently identified in China (Zhao et al. 2009), the United States (Cossaboom et al. 2011), and France (Izopet et al. 2012) likely serves as a useful model of genotype 3 human HEV infection with successful transmission of the virus to cynomolgus monkeys (Liu et al. 2013) and pigs (Cossaboom et al. 2012), thereby demonstrating the potential for cross-species infection (Table 2).

Historical Animal Models for Human Hepatitis E

In 1983, an experimental transmission study to a human volunteer as well as cynomolgus monkeys identified virus-like-particles (VLPs) in stool by immune electron microscopy that became what is now HEV (Balayan et al. 1983). After ingestion of the stool samples collected from Afghan patients with signs consistent with viral hepatitis, the human volunteer

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<th>Animals with naturally occurring HEV infections</th>
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developed clinical symptoms consistent with acute viral hepatitis with a non-A, non-B hepatitis etiology, and VLPs were identified in the volunteer’s stool samples (Balayan et al. 1983). In addition, cynomolgus macaques inoculated with fecal samples containing the VLPs responded similarly with elevations of serum liver enzymes, histopathologic liver lesions, excretion of VLPs in feces, and antibody responses with confirmed reaction to the VLPs (Bradley et al. 1987). Antiviral antibodies were confirmed in serum samples obtained from patients involved in outbreaks of non-A, non-B, acute hepatitis in various other countries, confirming an association between the identified virus and human infections (Khuroo 2011; Purcell and Emerson 2001).

Nonhuman primates such as rhesus macaque and chimpanzee have served as important animal models for HEV. Following the initial infection of cynomolgus monkeys with fecal samples containing VLPs, various species of nonhuman primates were utilized in attempts to better characterize the then-unknown virus (Balayan et al. 1983; Bradley et al. 1987). Macaques developed clinical signs consistent with acute viral hepatitis, occasionally excreted the VLPs in feces, and developed antiviral antibodies (Arankalle et al. 1995;
Chimpanzees and tamarins were inconsistently infected in these initial attempts (Arankalle et al. 1988; Bradley et al. 1987; McCaustland et al. 2000). Subsequent attempts to infect other nonhuman primate species have yielded mixed results, with tamarins occasionally developing infection, but chimpanzees (Arankalle et al. 1988), pig-tailed macaques (Tsarev et al. 1993b), vervets (Tsarev et al. 1993b), owl monkeys (Ticehurst et al. 1992), squirrel monkeys (Tsarev et al. 1993b), and patas monkeys were all susceptible through experimental infection. Rhesus and cynomolgus monkeys both in captivity and wild caught had serological evidence of natural exposure to HEV, and the seroprevalence was age dependent, with the majority positive for anti-HEV antibodies at 1 year and older for both species (Arankalle et al. 1994; Purcell and Emerson 2001; Tsarev et al. 1993a). Based on the transmission studies, chimpanzees (Arankalle et al. 1988), rhesus monkeys (Arankalle et al. 1995), and cynomolgus monkeys (Bradley et al. 1988; Tsarev et al. 1993a) were the most susceptible to both human strains of HEV (genotypes 1–4) and animal strains of HEV (genotypes 3 and 4) and are considered suitable models for HEV studies (Purcell and Emerson 2001) (Table 2). However, the restricted procedures, limited animal resources, and ethical concerns have limited the widespread use of these historical nonhuman primate models in HEV research today.

Naturally Occurring HEV Infections in Animals

Known Animal Strains of HEV

The ever-expanding host range for HEV currently includes a variety of animal species that serves as both reservoirs for human infections as well as hosts of genetically diverse but related viruses (Table 1). The most well-characterized animal strains of HEV include genotypes 3 and 4 swine HEV from domestic and wild pigs (Meng et al. 1997) and avian HEV genotypes 1 to 3 (Haqshenas et al. 2001; Huang et al. 2002). Rabbit HEV has recently been identified and genetically characterized as a genotype 3 from rabbits in China (Zhao et al. 2009), the United States (Cossaboom et al. 2011), and France (Izopet et al. 2012). Rats (Johne et al. 2011) and ferrets (Raj et al. 2012) each carry HEV-related strains genetically distinct from other mammalian and avian HEV and cluster together as a new putative genus proposed as Orthohepevirus (Meng 2013). Cutthroat trout virus (Batts et al. 2011) resembles mammalian HEV in its genomic organization despite low nucleotide sequence identity and likely represents a putative genus proposed as Piscihepevirus (Meng 2013). Genotype 3 HEV has also been isolated from wild mongooses in Japan (Nakamura et al. 2006). Recently, a novel phylogenetic clade of HEV obtained from Western
African, Central American, and European bat species was identified, although evidence for transmission from bats to humans was lacking (Drexler et al. 2012). Deer have been implicated in foodborne transmission of genotype 3 HEV to humans and share high nucleotide sequence identity to genotype 3 wild boar HEV in Japan (Takahashi et al. 2004) (Figure 1). The genetic identification of these diverse animal strains of HEV provided opportunities for developing new and useful naturally occurring animal models for HEV in the future.

**Animal Species with Only Serological Evidence of HEV Infection**

The presence of anti-HEV antibodies in serum indicates the exposure to and potential infection by HEV. Ruminant species, including goats (Sanford et al. 2012a), cattle, and sheep, have been reported as seropositive to anti-HEV antibodies without definitive genetic identification of HEV (Meng 2013). Horses were reportedly a potential reservoir of HEV by the presence of anti-HEV antibodies and viral RNA; however, the close relatedness of virus from horses to human HEV strains in Cairo (97–100% nucleotide sequence identity) raises questions about the authenticity of these sequences as true virus from horses (Saad et al. 2007). Anti-HEV antibodies have also been reportedly detected in both dogs and cats with no detection of HEV-related sequences (Pavio et al. 2010). Definitive genetic identification of the sources for anti-HEV seropositivity in these animal species will discover new animal strains of HEV, thus leading to the potential development of additional naturally occurring animal models for HEV in the future.

**Naturally Occurring Animal Models for Human Hepatitis E**

**Swine Model**

*Discovery and Prevalence of Swine HEV in Pigs*

Discovered from pigs in the United States in 1997, swine HEV became the first known animal strain of HEV (Meng et al. 1997) and has since been identified in domestic and wild swine worldwide. Swine HEV was initially discovered via identification of anti-HEV seropositive adult pigs followed by a prospective study on piglets from a herd in Illinois that led to the recovery of a novel virus (Meng et al. 1997). The novel virus was successfully transmitted to specific-pathogen-free (SPF) pigs, and the same virus was recovered from the experimentally infected SPF pigs, thereby satisfying Koch’s postulates (Meng 2010).

Swine serve as a major reservoir for zoonotic genotypes 3 and 4 HEV (Meng 2010). Serological and molecular prevalence studies of swine HEV yield widely variable results in both domestic and wild swine species in essentially all swine-producing countries. In the United States, the majority of pigs develop seropositivity to HEV by 3 months of age with dispersion of the virus in most herds (Meng et al. 1997). Swine HEV infection is highly prevalent in both domestic and wild swine irrespective of the human population (Meng et al. 1999). In Canada, HEV RNA was detected in 98.04% (50/51) of fecal samples and 49.02% of plasma samples (Leblanc et al. 2007), while the overall anti-HEV antibody prevalence in pigs was 88.8% in Quebec, 80.1% in Ontario, and 25% in Prince Edward Island (Yoo et al. 2001). In a nationwide study of 3925 pigs of 1 to 6 months of age in Japan, 93% (109/117) of farms and 57% of swine were positive for immunoglobulin (Ig)G anti-HEV, and 84% of pigs were seropositive by 6 months of age (Takahashi and Okamoto 2014). Likewise, descriptive studies on HEV prevalence in wild boars in Japan vary from 4.5 to 34.3% in anti-HEV seropositivity and 1.1 to 13.3% in HEV RNA detection (Takahashi and Okamoto 2014). In The Netherlands, the highest prevalence of swine HEV RNA at 53% (51/97) was found on farms housing pigs 5 to 27 weeks of age (Rutjes et al. 2009), whereas 98% (204/210) of swine herds in Spain were seropositive since 1985, indicating the long-standing enzootic infection in swine herds in Europe (Casas et al. 2009b). Swine HEV is equally dispersed in developing and industrialized countries worldwide.

**Pathogenesis and Course of Infection of Swine HEV in the Pig Model**

Following the subclinical course of HEV infection, swine develop only mild microscopic lesions in the liver and associated lymph nodes (Meng et al. 1997). Microscopic lesions include mild to moderate multifocal and periportal lymphoplasmacytic hepatitis and mild focal hepatocellular necrosis (Halbur et al. 2001). A prospective study of four piglets naturally infected by swine HEV identified no apparent gross lesions during necropsy in 19 different tissues, including liver, kidney, spleen, small and large intestines, tonsil, and pancreas, but characteristic microscopic lesions of hepatitis and lymphoplasmacytic enteritis in all, as well as multifocal lymphoplasmacytic interstitial nephritis in three of the four (Meng et al. 1997). Under experimental conditions, pigs infected with swine HEV developed no clinical abnormalities but were consistent in microscopic liver lesions as the naturally infected pigs, and HEV RNA was detectable in feces, liver tissues, and bile (Halbur et al. 2001). Pathological lesions in wild boars have not been investigated; however, the similarity between domestic and wild swine strains leads to speculation that clinical and pathologic effects are likely similar.

Domestic pigs are typically infected by HEV at 2 to 4 months of age with a transient viremia lasting 1 to 2 weeks and fecal viral shedding lasting 3 to 7 weeks (de Deus et al. 2008). By 18 weeks of age, as much as 86% of pigs are naturally infected (Leblanc et al. 2007). The source of infection is thought to be virus shed in large amounts in feces with transmission via fecal-oral route most common (Meng et al. 2010).
Naïve pigs acquire and spread the virus through direct contact between pigs as well as fecally contaminated feed and water sources in their environment. Following the waning of maternal antibodies around 8 weeks of age, piglets become infected by swine HEV and seroconvert first with IgM anti-HEV antibodies that peak in conjunction with peak fecal viral shedding, followed by seroconversion of IgG anti-HEV antibodies peaking at 4 months of age with subsequent clearance of the virus from the feces (de Deus et al. 2008; Leblanc et al. 2007). In contact-infected piglets, HEV RNA was detected in feces by 7 days postinfection, with the infectious period estimated as approximately 49 days (Bouwknegt et al. 2009).

Genotypes 3 and 4 HEV infections carry a subclinical course in both naturally and experimentally infected swine with no observable clinical disease or elevation in liver enzymes (Halbur et al. 2001). Experimentally, swine are readily infected via intravenous inoculation; however, oral route of inoculation is inefficient (Meng 2011).

Wild boars are assumed to be a natural reservoir for HEV as well because of the recovery of nearly genetically identical strains of HEV in deer cohabiting forestry land in Japan (Takahashi et al. 2004). As a model for human HEV infections, swine efficiently produce infection with genotypes 3 and 4 HEV and act as the main reservoir for foodborne and zoonotic HEV transmission to humans. Therefore, this naturally occurring swine model is very useful for the study of various aspects of HEV replication, pathogenesis, and cross-species infection (Meng 2010) (Table 2). The major drawback of the naturally occurring swine HEV model is that it does not reproduce a hepatic disease with overt clinical signs, thus limiting its usefulness in pathogenicity studies.

### Chicken Model

**Discovery and Prevalence of Avian HEV in Chickens**

Avian HEV was identified in the United States in 2001 from chickens with Hepatitis-Splenomegaly syndrome (HSS) (Haqshenas et al. 2001). Big Liver and Spleen Disease Virus presented similarly in chickens in Australia with approximately 80% nucleotide sequence identity to avian HEV (Marek et al. 2010; Payne et al. 1999). These two syndromes (HSS and BLS) are now known to be caused by variants of the same virus within the avian HEV clade. Avian HEV currently consists of at least three genotypes (1–3) throughout the world and shares approximately 60% nucleotide sequence identity with human HEV strains (Marek et al. 2010) but is not known to infect humans.

Avian HEV infection in chickens affects approximately 71% of chicken flocks and 30% of individuals overall within the United States (Huang et al. 2002). Avian HEV transmits most likely via fecal-oral route and spreads easily between and within chicken flocks (Meng et al. 2008). The infection in chickens is age dependent, affecting 17% of chickens <18 weeks of age, but 36% of adults were positive for anti-HEV antibodies (Huang et al. 2002).

**Pathogenesis and Course of Infection of Avian HEV in the Chicken Model**

Following avian HEV infection, few birds show clinical signs prior to death. Postmortem evaluations reveal regressive ovaries, serosanguinous abdominal fluid, enlarged, hemorrhagic, and necrotic livers, and enlarged spleens (Meng et al. 2008). Microscopic evaluations identify inflammatory cellular infiltrations within the liver parenchyma (Billam et al. 2005). Likewise, experimentally infected birds consistently present with lymphocytic periphlebitis and phlebitis in the liver at microscopic evaluation with enlarged and hemorrhagic livers in approximately 25% of the infected birds at gross evaluation (Meng et al. 2008). Avian HEV has been shown to successfully cross species barriers and infect turkeys (Sun et al. 2004); however, attempts to experimentally infect rhesus macaques (Huang et al. 2004) and mice were unsuccessful (Meng et al. 2008).

Avian HEV genotypes 1 to 3 correspond with mortality rates in chickens ranging from 0.3 to 1.0% of the overall flock and a high level of subclinical infection. Typical clinical signs include egg drop, hepato-splenomegaly, and acute death of birds. In flocks displaying signs of avian HEV infection, as much as 20% of hens present egg drop, significantly reducing production (Meng et al. 2008). In an age-dependent fashion, broiler breeders and laying hens of 30 to 72 weeks of age display the highest level of mortality (Huang et al. 2002). As a model for HEV infection in humans, avian HEV genotypes 1 to 3 are far more limited in their host range, but this naturally occurring chicken model offers a unique hepatic disease model (HSS) that can be used to study at least some aspects of human hepatitis E disease (Table 2).

### Rabbit Model

**Discovery and Prevalence of Rabbit HEV in Rabbits**

Farmed rabbits from the Gansu Province in China tested positive for anti-HEV antibodies, and full-length genomic sequences of HEV were determined and found to be most closely related to the zoonotic genotype 3 HEV (Zhao et al. 2009). Subsequently, studies on farmed and wild rabbits in the United States (Cossaboom et al. 2011) and France (Izopet et al. 2012) confirmed the presence of rabbit HEV related to genotype 3.

Anti-HEV antibody presence in rabbits is highly prevalent, with 57%, 54.6%, and 34.6% of farmed rabbits in the Gansu province of China, Beijing, and Virginia, USA testing positive, respectively (Cossaboom et al. 2011; Zhao et al. 2009). The detection of rabbit HEV RNA in fecal and serum samples also indicates a widespread infection of the virus, with 7.5%, 7.0%, and 15.9% of respective farmed rabbits positive (Cossaboom et al. 2011; Zhao et al. 2009). A study in France identified a similar proportion of farmed rabbits positive for HEV RNA at 7.0%, whereas 23.0% of wild rabbits were also positive (Izopet et al. 2012).
Pathogenesis and Course of Infection of Rabbit HEV in the Rabbit Model

The rabbit likely acts as a reservoir for HEV, because the rabbit HEV belongs to the zoonotic genotype 3 that infects humans. The close genetic and antigenic relationship to other mammalian HEV strains indicates the potential of rabbit HEV infection in rabbits to serve as a useful, naturally occurring animal model for human HEV study. Experimentally, rabbits are susceptible to infection by human HEV genotype 4, and rabbit HEV has been successfully transmitted to pigs (Cossaboom et al. 2012) and cynomolgus macaques (Liu et al. 2013). Anti-HEV antisera from rat, swine, human, and chicken strains of HEV cross-react with the rabbit HEV capsid protein (Cossaboom et al. 2012). Experimental infection studies in rabbits indicate the ability to produce local hepatocellular necrosis on microscopic evaluation; however, rabbits respond subclinically to experimental infection with HEV with little to no overt signs of disease. Following experimental infection, rabbits shed virus in their feces, seroconvert, and show elevations in serum alanine aminotransferase levels, indicating acute liver damage (Ma et al. 2010). More in-depth studies on pathogenicity and cross-species infections are warranted to further characterize the usefulness of rabbit HEV as a naturally occurring model for human HEV.

Other Potential Naturally Occurring Animal Models of Hepatitis E

Rat Model

A rat strain of HEV was identified in Hamburg, Germany in 2009 from Norway rats collected in sewers (Johne et al. 2011). The strain shared 59.9% and 49.9% nucleotide sequence identity with human and avian HEV strains, respectively, indicating a putative new mammalian genotype. Studies from Japan, China, Indonesia, the United States, and Germany also indicate the presence of a rat strain of HEV based upon the presence of anti-HEV antibodies, and in addition to Germany, rat HEV has been genetically identified from rats in a number of other countries, including the United States (Purcell et al. 2011) and Japan. In the United States, a variable prevalence of anti-HEV antibodies in rats of the genus Rattus exists, ranging from 44% to 90% in different states. In addition to the rat HEV (Purcell et al. 2011), a genotype 3 HEV RNA has been reportedly detected in wild rats in the United States (Lack et al. 2012), although independent confirmation of this report is still lacking.

Naturally infected rats had no overt signs of illness related to HEV infection. In experimental studies on laboratory rats infected with rat HEV, seroconversion and fecal shedding of virus were detected; however, no clinical signs were apparent (Li et al. 2013). Histopathologic evaluation of hepatic tissues from the infected laboratory rats identified mild portal inflammation, parenchymal foci of necrosis, and aggregates of lymphocytes and Kupffer cells within the lobules, indicating evidence of mild hepatitis consistent with acute HEV infection (Purcell et al. 2011). As a potential model for human HEV infection, inoculation of rats with mammalian HEV genotypes 1, 2, and 4 failed to produce an efficient infection, and rat HEV failed to infect rhesus monkeys (Purcell et al. 2011) (Table 2). In a separate report, Wistar rats were not susceptible to experimental infection by genotypes 1, 3, or 4 HEV, and rat HEV elicited evidence of infection as expected (Li et al. 2013). Additionally, both swine HEV and avian HEV also failed to elicit a productive infection in rats, further demonstrating the limited utility of rats as a naturally occurring model or an experimental model of human HEV infection (Krawczynski et al. 2011; Purcell et al. 2011).

Ferret Model

A ferret strain of HEV was genetically identified in the Netherlands in 2010. Phylogenetic analysis revealed its clustering with the rat HEV (Raj et al. 2012). Nucleotide sequence identity with known genotypes 1 to 4, rabbit, and avian strains of HEV ranged from 54.5% to 60.5%, with the highest sequence identity to rat HEV at 72.3% (Raj et al. 2012). Little is known about the ferret HEV, and the current knowledge relies on one set of samples obtained from household pets with no known illness (Raj et al. 2012). Whether ferret HEV can serve as a useful model for HEV is unclear; however, given the close genetic relatedness of the ferret HEV to rat HEV, the usefulness of ferret HEV as a naturally occurring animal model for HEV is likely limited.

Application of Naturally Occurring Animal Models for HEV Studies

Vaccine Studies

The mouse models have been used for identification of immunogenic properties of HEV antigen and preliminary immunization trials in HEV vaccine development (Table 3). However, the mice species were nonpermissive for HEV infection and were unable to be used for HEV challenge studies (Krawczynski et al. 2011). Therefore, the mouse models are mainly used for preliminary assessment of HEV vaccine antigen immunogenicity studies. HEV DNA vaccine constructs, purified VLPs, and recombinant subunit capsid protein all lead to the development of immune responses in the mouse model (Krawczynski et al. 2011).

Rhesus and cynomolgus monkeys were utilized in the vaccine preclinical and challenge studies to identify potential candidate vaccines that elicit protective immunity against known HEV genotypes (Krawczynski et al. 2011). The HEV capsid-based recombinant vaccine candidates elicit protective immune responses. A recombinant vaccine proved to be efficacious in phase II clinical trials in young men, with 95% protection against HEV genotype 1 in Nepal (Shrestha et al. 2007). Another recombinant vaccine proved to be efficacious in phase II and III clinical trials in the general
population (ages 16–65 years) and in pregnant women for human HEV genotypes 1 and 4 with 100% protection (Zhu et al. 2010). Efficacy was achieved with both two- and three-dose regimens with no serious adverse events and minimal side effects (Shrestha et al. 2007; Zhu et al. 2010). Rhesus monkeys immunized with the candidate vaccines were subsequently challenged by intravenous inoculation of human HEV genotypes 1, 2, and 3 for evaluation of protective immunity (Krawczynski et al. 2011). In both cases, the macaques were protected against homologous and heterologous challenge by HEV strains (Krawczynski et al. 2011). The large-scale clinical trial of a capsid-based recombinant vaccine involving 11,165 individuals (Zhu et al. 2010) has led to the approval of the first commercial HEV vaccine in China (Proffiitt 2012).

Pigs have been used as a model for HEV vaccine trials and assessment of cross-protective potentials of recombinant HEV antigens, which is essential for the development of vaccines that protect against the zoonotic genotypes 3 and 4 strains of HEV. Pigs vaccinated with truncated recombinant capsid antigens derived from three different animal strains of HEV specifically induce strong anti-HEV IgG responses, and these responses are partially cross-protective against a genotype 3 mammalian HEV (Sanford et al. 2012b). In addition, prior infection of pigs with a genotype 3 swine HEV induces protective immunity enabling resistance against challenge by heterologous and homologous strains of genotypes 3 and 4 HEV (Sanford et al. 2011), further demonstrating that swine are a good naturally occurring animal model for HEV vaccine research.

Chicken has also been used as a model for HEV vaccine studies. Immunization of chickens with avian HEV capsid protein induces protective immunity against avian HEV challenge (Guo et al. 2007), thus confirming the role of HEV capsid protein in eliciting protective immunity. The identification of B-cell epitopes within the avian HEV capsid protein that are unique to avian, swine, or human strains of HEV is useful for future development of diagnostic immunoassays as well as vaccine design (Guo et al. 2006). In chickens immunized with keyhole limpet hemocyanin-conjugated capsid peptides, protection against avian HEV challenge was not achieved. In contrast, in chickens immunized with recombinant avian HEV capsid antigen, complete protective immunity against avian HEV challenge was obtained, indicating that the immunodominant epitopes in avian HEV capsid are not protective (Guo et al. 2008). Rabbits may also be useful for HEV vaccine challenge and efficacy trials with further characterization of the course of infection and disease (Cheng et al. 2012).

### Pathogenesis Studies

Historically, primates served as the main animal model for HEV pathogenicity studies; however, due to ethical concerns, availability of animals, restrictions on their use, and difficulty in assessing clinical relevance because primates are not the natural host for HEV, additional naturally occurring animal models have recently been used for pathogenicity studies (Billam et al. 2005; Purcell and Emerson 2001) (Table 3). Rhesus (Arankalle et al. 1995) and cynomolgus monkeys (Aggarwal et al. 2001; Bradley et al. 1987; Tsarev et al. 1993a) have been widely used to study HEV infection and pathogenesis; however, differences in liver enzyme elevations, virus excretion, serologic, and histopathologic results between species and in relation to a human host exist. The chimpanzee model has also been useful in analyzing the course of human infections and pathogenicity with genotype 1 and 2 human HEV, and host gene responses to HEV, although the use of chimpanzees in HEV study is currently less frequent and more restricted (Arankalle et al. 1988; McCaustland et al. 2000). The mechanisms leading to a chronic course of HEV infection in immunocompromised individuals as well as elevated mortality rates in pregnant women of up to 25% are largely unknown due to the inability to identify an appropriate animal model (Meng 2013). Inoculation of pregnant rhesus monkeys with genotype 1 HEV failed to identify a difference with nonpregnant monkeys and was

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**Table 3 Applications of available animal models including naturally occurring animal models for various aspects of HEV studies**

<table>
<thead>
<tr>
<th>Animal species</th>
<th>Application</th>
</tr>
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<tbody>
<tr>
<td>Chimpanzee</td>
<td>Pathogenesis, molecular biology and virus replication, cross-species infection</td>
</tr>
<tr>
<td>Rhesus monkey</td>
<td>Vaccine, pathogenesis, molecular biology and virus replication, cross-species infection</td>
</tr>
<tr>
<td>Cynomolgus monkey</td>
<td>Vaccine, pathogenesis, molecular biology and virus replication, cross-species infection</td>
</tr>
<tr>
<td>Owl monkey</td>
<td>Cross-species infection</td>
</tr>
<tr>
<td>Rodents</td>
<td>Vaccine, cross-species infection</td>
</tr>
<tr>
<td>Swine</td>
<td>Vaccine, pathogenesis, molecular biology and virus replication, cross-species infection</td>
</tr>
<tr>
<td>Chicken</td>
<td>Vaccine, pathogenesis, molecular biology and virus replication, cross-species infection</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Vaccine, pathogenesis, cross species infection</td>
</tr>
</tbody>
</table>
unsuccessful in reproducing the elevated mortality rates seen in pregnant women or the development of the reported severe and fulminant hepatitis E (Tsarev et al. 1995).

With the discovery of swine HEV, domestic swine became a potential model for HEV pathogenicity study, although such studies are limited due to the fact that swine infected by swine or human HEV develop only subclinical infection with mild-to-moderate pathologic lesions of hepatitis (Meng 2003; Meng et al. 1997). As a natural reservoir for genotypes 3 and 4 HEV, pigs serve as a homologous model system to evaluate transmission routes and the epidemiology of infection. Experimental infections of pigs with HEV are often limited by the inability to produce a natural course of infection via the natural oral route of inoculation. Even using high titers of infectious HEV stocks, many experiments have failed to initiate a productive infection via the oral route in swine (Kasomdorkbua et al. 2004). In a controlled contact exposure setting, it was shown that first, second, and third generation-infected pigs produced a force of infection Ro at 8.8, indicating that HEV was able to spread among the contacts randomly; however, the true field conditions (pig farms) could not be assessed (Bouwkneet et al. 2009). Oral inoculation of bile containing infectious HEV lead to a produce infection in pigs characterized by the detection of HEV RNA in the bile, although the infection was less efficient than the intravenous route of inoculation and the sentinel animals did not show viremia, seroconversion to anti-HEV IgG, or the presence of HEV RNA in the feces by 64 days postinfection and at the time of necropsy (Casas et al. 2009a). In addition, the intravenous route of inoculation of swine HEV and human HEV in the pig model produced characteristic pathological liver lesions, although clinical sign of hepatitis were lacking (Halbur et al. 2001). To assess the enhanced pathogenic effect of HEV infection during pregnancy observed in humans, pregnant gilts were inoculated with swine HEV (Kasomdorkbua et al. 2003). Whereas the gilts developed active HEV infection, the offspring remained seronegative, and no clinical disease was noted (Kasomdorkbua et al. 2003), further confirming the results from the pregnant monkey study. Therefore, the naturally occurring swine model is limited for HEV pathogenicity studies, although this model has been useful for various other aspects of HEV research (Bouwkneet et al. 2009; Casas et al. 2009a; Feagins et al. 2008; Huang et al. 2005).

Avian HEV infection in chickens leads to HSS, including egg drop, regressive ovaries, and serosanguinous abdominal fluid (Meng et al. 2008). The presence of liver gross abnormalities in the chicken model shares similarities with the course of HEV infection and disease in humans, allowing a better characterization of HEV pathogenesis in this naturally occurring chicken model (Billam et al. 2005). Additionally, the chicken model affords the opportunity to mimic the natural route of HEV infection with oronasal delivery of virus inocula (Billam et al. 2005). Although the route of virus inoculation affects the timing of seroconversion (i.e. develops earlier than oronasal), the patterns of seroconversion, viremia, and development of clinical and pathologic lesions are similar to those seen in HEV infection in humans (Billam et al. 2005). In a comparative pathogenicity study utilizing a strain of avian HEV (HEV-VA) strain obtained from a clinically healthy chicken and the prototype avian HEV strain recovered from a diseased chicken, no significant differences were seen in pathogenicity, indicating that other unknown factors may also be involved in the HEV pathogenesis (Billam et al. 2009). In another study, avian HEV-VA was capable of inducing histopathologic liver lesions despite failing to elicit a clinical disease in the chicken model (Kwon et al. 2011). With chickens being a naturally occurring animal model mimicking certain aspects of the human disease, identification of viral and host factors that determine pathogenicity in chickens would serve as a baseline for identifying these same factors in HEV infection in humans. A major drawback for this model is that chickens are not susceptible to infection by mammalian HEV strains.

Rabbits inoculated with human HEV genotypes 1 and 4 failed to produce clinical disease, elevated liver enzymes, or significant histopathologic lesions as seen in rabbits inoculated with rabbit HEV (Ma et al. 2010). Genotype 4 human HEV was capable of infecting only 2 of 9 rabbits, thus confirming the ability for cross-species infection but at an inefficient level (Ma et al. 2010). Genotype 1 human HEV was incapable of eliciting any markers of HEV infection in rabbits, and thus the usefulness of the rabbit model for HEV pathogenicity study remains to be determined.

**Molecular Biology and Virus Replication Studies**

Experimental infections of rhesus and cynomolgus monkeys have been widely used for infectivity and replication studies through intravenous and intrahepatic inoculations (Aggarwal et al. 2001; Krawczynski et al. 2011; Tsarev et al. 1993a) (Table 3). Hepatic expression of HEV antigens indicating viral replication in conjunction with the detection of HEV RNA in bile and feces were identified in rhesus macaques prior to the appearance of gross lesions within the liver parenchyma (Krawczynski et al. 2011). Chimpanzees and rhesus macaques have been successfully used to determine the infectivity of infectious cDNA clones of HEV via intrahepatic inoculation of RNA transcripts synthesized from cloned cDNA genome of HEV (Emerson et al. 2001). The nonhuman primate model has been indispensable in studying HEV replication, especially during the early days after the virus discovery.

The naturally occurring swine model has played important roles in understanding the molecular mechanism of HEV replication. By using the swine model, a genotype 3 swine HEV infectious clone was established without the need of an in vitro cell culture system (Huang et al. 2005; Krawczynski et al. 2011). Intrahepatic inoculation of pigs with capped RNA transcripts from HEV infectious clones provided a unique means to study the effect of in vitro genetic manipulation of HEV genome on virus replication and pathogenicity (Huang et al. 2005). The identification of an attenuated
mutant HEV (pSHEV-1) led to the characterization of specific amino acid residues (F51L, T59A, and S390L) in the capsid protein that are important for virus attenuation in the swine model (Cordoba et al. 2011). Both the T59A and S390L mutations drastically lowered viral RNA loads in intestinal contents, bile, and liver and shortened the duration of fecal viral shedding (Cordoba et al. 2011).

The hypervariable region (HVR) in ORF1 of HEV varies considerably between different HEV genotypes and among HEV strains. By using the swine model, it was demonstrated that the HVR of HEV is dispensable for HEV infectivity, although a near-complete deletion of the HVR attenuated the virus (Pudpakam et al. 2009). By using the chicken model, the impact of complete HVR deletion on virus infectivity was further tested using an avian HEV mutant with a complete HVR deletion. Although the HVR deletion mutant was still replication competent in LMH chicken cells in vitro, the complete HVR-deletion mutant resulted in a loss of avian HEV infectivity in the chicken model (Pudpakam et al. 2011).

The small ORF3 protein of HEV is multifunctional and involved in virus replication in vivo (Huang et al. 2007; Kenney et al. 2012). Using a homologous naturally occurring pig model, the authentic initiation site for HEV ORF3 translation was identified as the third in-frame AUG codon in the junction region (Huang et al. 2007). A mutant virus with a mutation in the third in-frame AUG completely abolished the virus infectivity in the pig model, whereas mutations in the first and second in-frame AUG codons in the junction region did not affect the virus infectivity in pigs (Huang et al. 2007). Furthermore, by utilizing the naturally occurring chicken model, it was demonstrated that the PSAP motif in the ORF3 of avian HEV is involved in particle release from the cell and viral fecal shedding (Kenney et al. 2012). Taken together, in the absence of an efficient cell culture system for HEV, these naturally occurring swine and chicken models are important for studying the molecular mechanisms of HEV replication.

Cross-Species HEV Infection Studies

Rhesus monkeys are widely used in HEV cross-species infection studies because of the ability to be infected by all four genotypes of human HEV and development of virologic, pathologic, and serologic characteristics consistent with HEV infection (Krawczynski et al. 2011) (Table 3). Two rhesus monkeys and one chimpanzee were successfully infected with a genotype 3 swine HEV, resulting in acute viral hepatitis, seroconversion to anti-HEV antibodies, fecal virus shedding, viremia, and slight elevations in ALT, thus serving as experimental surrogates for human HEV infections (Meng et al. 1998). Rhesus macaques were also successfully infected with an Indian strain of genotype 4 swine HEV as evidenced by viremia and seroconversion to anti-HEV antibodies (Arankalle et al. 2006). In addition, cynomolgus monkeys were readily infected with rabbit HEV with the development of viremia, elevated liver enzymes, presence of fecal virus shedding, and seroconversion to HEV antibodies indicating that, similar to other genotype 3 HEV strains, the rabbit HEV may likely infect humans (Liu et al. 2013).

The swine model has been used to study the cross-species infection and susceptibility of human HEV. SPF pigs are readily infected by the genotype 3 and 4 strains of human HEV (Cordoba et al. 2012; Feagins et al. 2008; Meng 2003). In infected pigs, seroconversion occurred by 28 days postinoculation, and fecal viral shedding and viremia occurred by 7 to 56 days postinoculation, indicating that swine serve as an excellent model for human HEV infection (Feagins et al. 2008). By using the swine model, it was demonstrated that intergenotypic chimeric HEVs with the genotype 4 human HEV capsid gene cloned in the backbone of genotype 3 swine HEV are infectious in pigs, furthering confirming the zoonotic nature of genotypes 3 and 4 HEV (Feagins et al. 2011). Swine were also shown to be susceptible to infection by rabbit HEV but resistant to infection with the rat HEV (Cossaboom et al. 2012). The pig model will be important in identifying the genetic elements in the virus genome that determine the cross-species HEV infection between humans and swine.

Under experimental conditions, avian HEV from chickens has been demonstrated to cross species barriers and infect turkeys (Sun et al. 2004); however, attempts to infect rhesus monkeys with avian HEV were unsuccessful (Huang et al. 2004), suggesting that avian HEV has a limited host range and is not zoonotic. Turkeys inoculated with avian HEV seroconverted by 4 to 6 weeks postinfection, viremia was detected by 2 to 6 weeks, and a control negative turkey became infected by direct contact (Sun et al. 2004), indicating that the infection is readily transmissible in a new host.

Rodents including Balb/c nude mice (Huang et al. 2009), C57BL/6 mice (Li et al. 2008), Wistar rats (Li et al. 2013), and Mongolian gerbils (Li et al. 2009) may potentially serve as animal models for some aspects of HEV study; however, few transmission studies in these species resulted in productive infections, and some of these reports have not yet been independently confirmed.

Rabbits serve as a natural host for genotype 3 HEV and are susceptible experimentally to infection by human HEV genotype 4 (Liu et al. 2013). Experimental rabbit HEV inoculations in rabbits yield viremia with fecal virus shedding, seroconversion, and mild hepatic lesions consistent with HEV infection (Ma et al. 2010). The rabbit strain of HEV was shown capable of infecting swine and rhesus monkeys, indicating the ability of rabbit HEV to infect across species barrier (Cossaboom et al. 2012). The susceptibility to cross-species infection of rabbits by human HEV genotypes 3 and 4 indicated the potential use of rabbits as an alternate model for human HEV.

Future Perspectives

Despite recent advances in the genetic identification of novel animal strains of HEV, characterization of the course of infection and disease in a variety of animal hosts, the underlying molecular mechanisms of HEV replication, pathogenesis, and
cross-species infection remain largely unknown, in part due to the lack of an efficient cell culture and a practical animal model for HEV. Several naturally occurring animal models such as swine, chicken, and rabbit have recently been developed and shown to be useful for various aspects of HEV studies. However, a reproducible hepatic disease animal model for the study of human HEV pathogenicity is still lacking. For example, the observed severe and fulminant hepatitis E in pregnant women could not be reproduced in pregnant pigs or pregnant rhesus monkeys. Such an animal model will be critical in identifying the underlying mechanisms of fulminant hepatitis E during pregnancy. Currently, chronic and persistent HEV infection is an emerging and significant clinical problem in immunocompromised individuals such as organ transplant recipients. Unfortunately, a useful animal model that can mimic chronic HEV infection is still lacking and thus hindering our understanding of the mechanism for progression into chronicity and the progress of developing effective antivirals against chronic hepatitis E. Clearly, identifying additional animal models that can more adequately mimic the course of HEV infection and outcomes of disease in humans are important for future HEV research. The expanding host range of HEV offers the opportunities to identify potential new animal strains of HEV that could lead to the development of better naturally occurring animal model(s) for HEV. Therefore, genetic identification and characterization of additional novel animal strains of HEV are warranted, and development of an efficient cell culture to propagate different strains of HEV in vitro will be the key for future development of a cost-effective, modified live-attenuated vaccine against HEV.

Acknowledgments

The author’s research on HEV is supported by grants from the National Institutes of Health (R01AI074667 and R01AI050611). D.M.Y. is supported by a training grant from the National Institutes of Health (T32OD010430–06). We thank Dr. Dianjun Cao for his expert help in the construction of the phylogenetic tree. This article encompasses a comprehensive literature review that is available in the PubMed database with a focus on naturally-occurring animal models for human HEV infection. Due to the narrow scope of the topic and space constraints, many important HEV articles may be unintentionally excluded from this review. We have attempted to include the most recent and relevant publications in order to provide the reader with up to date information on this specific topic.

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