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Experimental infection of rabbit with swine-derived hepatitis E virus genotype 4

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ABSTRACT

Hepatitis E virus (HEV) is a zoonotic virus that is capable of causing cross-species infection. Rabbits can be experimentally infected with human- and swine-derived HEV-4 in the species *Orthohepevirus* A, and avian-derived HEV-3 in the species *Orthohepevirus* B suggesting rabbits can serve as an animal model for zoonotic HEV infection study. However, these studies show that the infectivity of swine HEV isolates in rabbits is not consistent. In this study, the animal study was conducted by the experimental infection of rabbit with a swine-derived HEV-4 isolated in China (designated CHN-SD-sHEV) for 28 weeks post-inoculation (wpi) in compassion to that infected with a rabbit-derived HEV-3 (designated HEV-SX-rHEV). Two rabbits were euthanized every 2 wpi for pathological examinations. The results showed that rabbits infected with CHN-SD-sHEV had the viremia and virus fecal shedding from 1 wpi to 22 wpi and seroconverted from 10 to 28 wpi. Meanwhile, elevated ALT levels were detected at 2 wpi. Moreover, virus replication was confirmed by the detection of both positive- and negative-strand HEV RNAs in the livers and spleens. Diarrhea and hepatocellular lesions were also observed in some animals. In contrast, rabbits experimentally infected with CHN-SX-rHEV exhibited earlier seroconversion, viremia and virus fecal shedding and hepatocellular lesions. Taken together, our data demonstrate that in comparison to the previously reported cases, the swine-derived HEV-4 isolated in China could cross-species infect rabbit accompanied with prolonged virus fecal shedding and liver lesions.

1. Introduction

Hepatitis E virus (HEV) is a quasi-enveloped, single-stranded positive-sense RNA virus which belongs to the family *Hepeviridae*, a family comprised of highly diverse viruses. Some HEV isolates can also cause cross-species infection (Smith et al., 2015). As the causative agent of hepatitis E, HEV causes self-limiting hepatitis with mortality ranging from 0.5 to 3% in the general population, but with mortality as high as 25% in pregnant women (Labrique et al., 2012). The discovery of HEV in swine in 1997 established HEV as a zoonotic disease (Meng, 2013). Since then, hepatitis E cases have been frequently reported in developed countries and endemic HEV has been shown to be present in many developing countries (Abravanel et al., 2017; Anheyer-Behmenburg et al., 2017; Doceul et al., 2016; Park et al., 2016).

The family *Hepeviridae* contains two genera: *Orthohepevirus* and *Piscihepevirus* (Smith et al., 2015). *Orthohepevirus* A HEV species has four major genotypes (1–4) in which HEV-1 and HEV-2 are only restricted to humans, while HEV-3, HEV-4, and HEV-7 are zoonotic (Doceul et al., 2016; Purcell and Emerson, 2008). So far, HEV-3 and HEV-4 have been isolated from pigs, while only HEV-3 isolates have been identified in rabbits (Kamar et al., 2012; Zhao et al., 2009). Sequence comparisons demonstrated that HEV-3 isolates from pigs and rabbits share high identities with human-derived HEV-3 suggesting the zoonotic potential of HEV-3 isolates (Liu et al., 2017). Moreover, under experimental conditions it was shown that swine HEV can cause cross-species infection in rabbits and vice versa, while rabbit and swine HEV isolates can both cross-infect rhesus monkeys (Cossaboom et al., 2012; Han et al., 2014; Liu et al., 2013; Meng et al., 1998b).

It is generally accepted that rabbits are the natural host of HEV-3ra (Zhao et al., 2009), and the pathogenicity of different rabbit HEV isolate in rabbits is various (Cheng et al., 2012; Han et al., 2014; Liu et al., 2014; Zhang et al., 2015). Moreover, it has been confirmed that rabbits

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can be experimentally infected with human- and swine-derived HEV-4 as well (Han et al., 2014; Ma et al., 2010); however, the up-to-date reports regarding to the infectivity of HEV-4 isolated from swine in rabbit are not consistent (Han et al., 2014; Liu et al., 2014; Zhang et al., 2015). Therefore, the goal of this study was to evaluate the infectivity of a HEV-4 isolated in China (designated CHN-SD-sHEV) in rabbits. The results showed that CHN-SD-sHEV infected rabbits exhibited prolonged viremia and virus fecal shedding and seroconversion. The virus replicated in the livers and spleens. Some animals showed diarrhea and hepatocellular lesions. These findings indicate that swine-derived HEV-4 isolated in China can cross-species infects rabbit accompanied with the prolonged infection and clinical characteristics.

2. Materials and methods

2.1. Virus and animals

The swine-derived HEV-4 (CHN-SD-sHEV, GenBank No. KF176351) was isolated from a bile sample collected from a 32-week-old pig in a slaughterhouse in China (Wang et al., 2014). The rabbit-derived HEV-3 (CHN-SX-rHEV, GenBank No. KX227751) was isolated from fecal samples collected from a specific pathogen free (SPF) rabbit vendor in China (Liu et al., 2017). The samples containing HEVs were diluted in phosphate-buffered saline (PBS) (pH 7.4) as a 10% (w/v) suspension. After subsequent filtering through 0.45-μm and 0.22-μm filters to clarify the suspensions, viral titers were determined by semi-quantitative nested reverse transcription PCR (RT-nPCR), as previously reported. Titers of swine and rabbit HEV stocks were adjusted to 10^4 genomic equivalents/ml (GE/ml) (Kasorndorkbua et al., 2002).

Forty-two 6-week-old SPF New Zealand White rabbits were purchased from the SPF animal center of Xi'an Jiaotong University. Prior to inoculation, all rabbits were confirmed negative for anti-HEV antibodies and HEV RNA, as determined by an enzyme-linked immunosorbent assay (ELISA) and RT-nPCR, respectively. The animal experimental protocol was approved by the Committee of Laboratory Animal Welfare and Ethics, Northwest A&F University.

2.2. Inoculation of rabbits with swine- and rabbit-derived HEV isolates

Rabbits were divided randomly into three groups (14/group). Group 1 to group 3 rabbits were inoculated intravenously with CHN-SD-sHEV, CHN-SX-rHEV infectious stock (3×10^4 GE), or PBS, respectively. Four rabbits randomly selected from each group were kept for 28 wpi, and two rabbits of each group were euthanized at 2, 4, 6, 8, and 10 wpi.

2.3. Sample collection

Blood and fecal samples from each rabbit were collected prior to inoculation and were collected weekly after virus challenge at 1 to 28 weeks post-inoculation (wpi). Blood plasma samples from each rabbit were tested for alanine aminotransferase (ALT) levels. Serum samples were tested separately for antibodies to swine and rabbit HEVs using indirect ELISA. Serum and fecal samples were also tested for HEVs RNA by RT-nPCR and real-time RT-PCR, respectively. Bile, liver, and spleen samples during necropsy were collected and stored at -80 ℃. Liver and spleen tissue samples were homogenized in 10% (w/v) sterile phosphate buffered saline (PBS) yielding suspensions from each group that were used for detection of both positive- and negative-strand HEVs RNA by RT-nPCR.

2.4. RT-nPCR, RT-qPCR, and DNA sequencing of HEVs RNA

Swine HEV RNA isolated from serum, fecal, bile, liver, and spleen samples from CHN-SD-sHEV-inoculated rabbits were tested according to the method described by Huang et al. (Huang et al., 2002). Rabbit HEV RNAs in samples from CHN-SX-rHEV inoculated rabbits were detected according to the method described by Geng et al. (Geng et al., 2011). In addition, RNA of swine and rabbit HEVs present in serum samples were also quantified by RT-qPCR methods as previously described (Jothikumar et al., 2006).

Positive PCR products showing the presence of swine, and rabbit HEVs RNA were sequenced using an ABI 3730 Genetic Analyzer (JinSiTe Biotech Co., Nanjing, China). Multiple alignments among swine, and rabbit HEV strains sequences were carried out using Lasergene MegAlign software (DNASTAR Inc., Madison, WI, USA).

2.5. Detection of anti-HEVs antibodies and ALT concentrations

Anti-swine and anti-rabbit HEVs IgG antibodies were separately detected in serum samples using indirect ELISAs as previously described (Liu et al., 2017; Wang et al., 2014). Briefly, purified truncated CHN-SD-sHEV and CHN-SX-rHEV capsid proteins expressed in *Escherichia coli* were used as the coating antigens for detecting anti-sHEV and anti-rHEV antibodies, respectively, in the indirect ELISAs. After the coated plates were blocked and washed, serum samples (100 μL/well) were added into each well and incubated for one hour at room temperature (RT). After three washes, horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch,West Grove, PA, USA) diluted 1:4000 (100 μL/well) was added to the wells and incubated for one hour. After washing three times, 3,3′,5,5′-tetramethylbenzidine (TMB) was added to each well and the plates were incubated in the dark for 15 min at RT. The colorimetric reaction was stopped by adding 3 M H_2SO_4 (50 μL/well) and optical density (OD) values were read at 450 nm using an automated microplate reader (Bio-Rad, USA). Each serum sample was tested in duplicate wells. The OD values of the pre-inoculation samples were used to determine cutoff values.

ALT concentrations in plasma samples from rabbits were measured using standard methods on a SmartSpec 3000 spectrophotometer. Rabbits were considered positive for hepatitis if ALT levels exceeded pre-challenge ALT levels by more than two-fold (Ma et al., 2010).

2.6. Evaluation of pathological and histopathological changes in liver tissues

Gross pathological lesions from livers of rabbits in each group were evaluated during necropsies and were also recorded as digital pictures. Liver samples were also fixed in 10% neutral buffered formalin and processed for routine histological examination. Histopathological liver lesions were scored according to lesion severity based on standard scoring systems, with scores ranging from 0 to 4 (0, no lesions; 1, < 5 foci; 2, 5 to 8 foci; 3, 9 to 15 foci; 4, > 15 foci) (Billam et al., 2005).

2.7. Statistical analysis

Histopathological lesions were scored by counting lesion foci. Categorical (dichotomous) variables were analyzed by logistic regression using either the LOGISTIC or the GENMOD procedure in SAS version 8.02 (SAS Institute, Inc., Cary, NC, USA). Counts of lesion foci were modeled as either Poisson or negative binomially distributed variables by use of the GENMOD procedure in SAS.

3. Results

3.1. Clinical presentation of HEV-infected rabbits

Clinical signs such as decreased feed consumption, diarrhea, or mortality were not observed in any rabbits in the negative control group for the duration of the study. However, two rabbits (No.1 and 12) exhibited diarrhea in the CHN-SD-sHEV-challenged group from 3 to 4 wpi (data not shown). For the CHN-SX-rHEV group, four rabbits (No.16, 18, 22 and 24) exhibited diarrhea from 2 to 3 wpi.

Table 1
Seroconversion, viremia and fecal shedding of rabbits during the course of the study[a].

Virus Inocula	No. of seroconversion/viremia/fecal shedding/total no. tested at indicated wpi										
	0	1	2	3	4	5	6	7	8	9	10
CH-SD-sHEV	0/0/0/14	0/11/13/14	0/11/12/14	0/8/10/12	0/6/7/12	0/5/6/10	0/3/5/10	0/4/5/8	0/4/4/8	0/2/3/6	1/2/3/6
CHN-SX-rHEV	0/0/0/14	0/12/14/14	5/10/13/14	9/6/10/12	10/6/9/12	8/4/7/10	9/5/7/10	8/4/5/8	8/4/6/8	5/3/5/6	5/2/4/6
PBS	0/0/0/14	0/0/0/14	0/0/0/14	0/0/0/12	0/0/0/12	0/0/0/10	0/0/0/10	0/0/0/8	0/0/0/8	0/0/0/6	0/0/0/6

[a] The number of rabbits showed seroconversion, viremia, and fecal shedding from 0 to 10 wpi, and the data from 11 to 28 wpi were shown in Fig.1.

3.2. Seroconversion for anti-swine and anti-rabbit HEVs antibodies

Prior to inoculation, all rabbits were seronegative for both antigens used. All rabbits in negative control group were seronegative for anti-HEV antibody throughout the study (Table 1 and Fig. 1C). Seroconversion to anti-swine HEV IgG antibodies in the CHN-SD-sHEV challenged rabbits was assessed separately at 10 wpi (Table 1). However, in the CHN-SX-rHEV-challenged group, anti-rabbit HEV IgG antibodies were first observed at 2 wpi (Table 1).

For four rabbits from each group were monitored until 28 wpi, 2/4 and 1/4 rabbits in CHN-SD-sHEV and CHN-SX-rHEV infected groups, respectively, remained seropositive at 28 wpi (Fig. 1A, B), while no animals in PBS inoculated control group were seropositive throughout the study (Fig. 1C).

3.3. Detection of HEVs RNA in serum, feces, liver, spleen, and bile samples

Prior to inoculation, serum and fecal samples from all rabbits were negative for sHEV-4 and HEV-3ra RNA (Table 1). Detection of HEVs RNA from different samples is a common indicator of HEV infection. In the negative control group, serum, feces, liver, spleen, and bile samples from the rabbits were all negative for HEVs RNA throughout the study (Tables 1 and 2). However, HEVs RNA was detected at variable levels in serum, fecal, bile, liver, and spleen samples among the two other groups (Tables 1 and 2). Swine HEV RNA could be detected in feces and sera as early as 1 wpi, as was also observed for the CHN-SX-rHEV group (Fig. 1A, B and Table 1). Meanwhile, detection of HEV RNA in fecal samples was also observed for up to 22 wpi in CHN-SD-sHEV-challenged rabbits (Fig. 1A). For the CHN-SX-rHEV group, detection of HEV RNA from fecal samples lasted for up to 28 weeks in two rabbits (Fig. 1B), which was detectable for a longer duration of time than for the CHN-SD-sHEV group.

Conversely, for liver, spleen, and bile samples collected from necropsied rabbits at 2, 4, 6, 8, and 10 wpi, positive results for detection of HEVs RNA were observed (Table 2). In the CHN-SD-sHEV group, from 2 to 8 wpi there was at least one rabbit positive for swine HEV RNA in liver, spleen, and bile samples (Table 2). However, at least one rabbit was positive for rabbit HEV RNA in these samples at within the time frame of 2 to 10 wpi in the CHN-SX-rHEV group (Table 2).

3.4. Detection of negative-stranded HEV RNA in liver and spleen samples

As a single-stranded positive-sense RNA virus, negative-stranded viral RNA is produced as the essential intermediate stage of HEV replication. Therefore, detection of negative-stranded HEV RNA from tissues is a necessary indicator for HEV replication. To analyze replication of swine and rabbit HEVs in inoculated rabbit, testing to detect negative-strand RNA was conducted for liver and spleen samples obtained from necropsied rabbits. As shown in Table 2, no rabbits were positive for negative-strand HEV RNA in the negative control group. In contrast, in the CHN-SD-sHEV-challenged group, liver samples from necropsied rabbits were positive for negative-strand swine HEV RNA from 2 to 8 wpi, while spleen samples were only positive at 4 and 6 wpi. Furthermore, negative-strand HEV RNA was detected in both liver and spleen samples at 2 to 8 wpi in the CHN-SX-rHEV group.

3.5. Quantitation of HEV RNA in serum samples

Besides detection of HEV RNA in serum samples by RT-nPCR, viral concentrations were also quantitatively evaluated by RT-qPCR. In the CHN-SD-sHEV group, the highest viral copy numbers in the serum samples of the four rabbits were $10^{4.1}$ to $10^{5.2}$ and the duration times of viral RNA detection in sera of two rabbits lasted for approximately 10 weeks (Fig. 2A). However, in the CHN-SX-rHEV group, the peak viral copy numbers in sera from the four rabbits were from $10^{6.2}$-$10^{6.7}$, which was higher than values for the CHN-SD-sHEV group (Fig. 2B), with virus detected in three rabbits in the group at 21 to 28 weeks (Fig. 2B). In the negative control group, the peak numbers of viral copies in all four rabbits were from $10^{1.2}$-$10^{1.5}$ (Fig. 2C).

3.6. Gross and microscopic lesions

Throughout the study, no gross hepatic lesions were observed in livers from any necropsied rabbits in any group. The data on microscopic lesions in the liver are summarized in Table 3. However, slight to severe microscopic lesions were observed as an indicator of hepatitis in liver tissue samples, which included lymphocytic venous periphlebitis, balloon-like lesions, and hepatocellular necrosis. Lymphocytic venous periphlebitis was observed in liver sections of 8/14 rabbits in the CHN-SD-sHEV group, all (14/14) rabbits in the CHN-SX-rHEV group, and only one rabbit in the negative control group (Fig. 3B). In addition, balloon-like hepatocellular lesions were observed in the liver sections of 6/14 rabbits (No.3, 4, 7, 9, 10 and 13) in the CHN-SD-sHEV group and of 10/14 rabbits (No.15, 17, 19, 20, 22, 23, 24, 26, 27 and 28) in the CHN-SX-rHEV group (Fig. 3E, F). Moreover, hepatocellular necrosis and focally intense lymphocytic venous periphlebitis were observed in liver sections of 5/14 animals in the CHN-SD-sHEV group (Fig. 3C). Furthermore, for the CHN-SX-rHEV group, liver sections of 8/14 rabbits demonstrated extensive hepatocellular necrosis (Fig. 3D). Taken together, the histological presentation of liver lesions ranged from moderate to severe in the CHN-SD-sHEV and CHN-SX-rHEV groups (Fig. 3, Table 3).

3.7. ALT evaluation in serum samples

During the entire study, all four rabbits from each group were monitored weekly for the ALT levels in serum samples. In the CHN-SD-sHEV group, a peak ALT level of 106–123 U/L was observed in three rabbits at 2 wpi (Fig. 4A). By contrast, in the CHN-SX-rHEV group, all four rabbits showed increased ALT levels (125–148 U/L) in serum samples at 1 wpi (Fig. 4B). No major ALT level changes were observed in the negative control group (Fig. 4C).

4. Discussion

To date, several animal models for HEV *in vivo* studies have been established (Cheng et al., 2012; Cordoba et al., 2012; Guo et al., 2007; Krawczynski et al., 2011). Among all of these models, non-human primates such as cynomolgus and rhesus monkeys and SPF pigs have been predominantly used for HEV pathogenicity studies (Cordoba et al., 2012; Krawczynski et al., 2011). However, the limited availability and

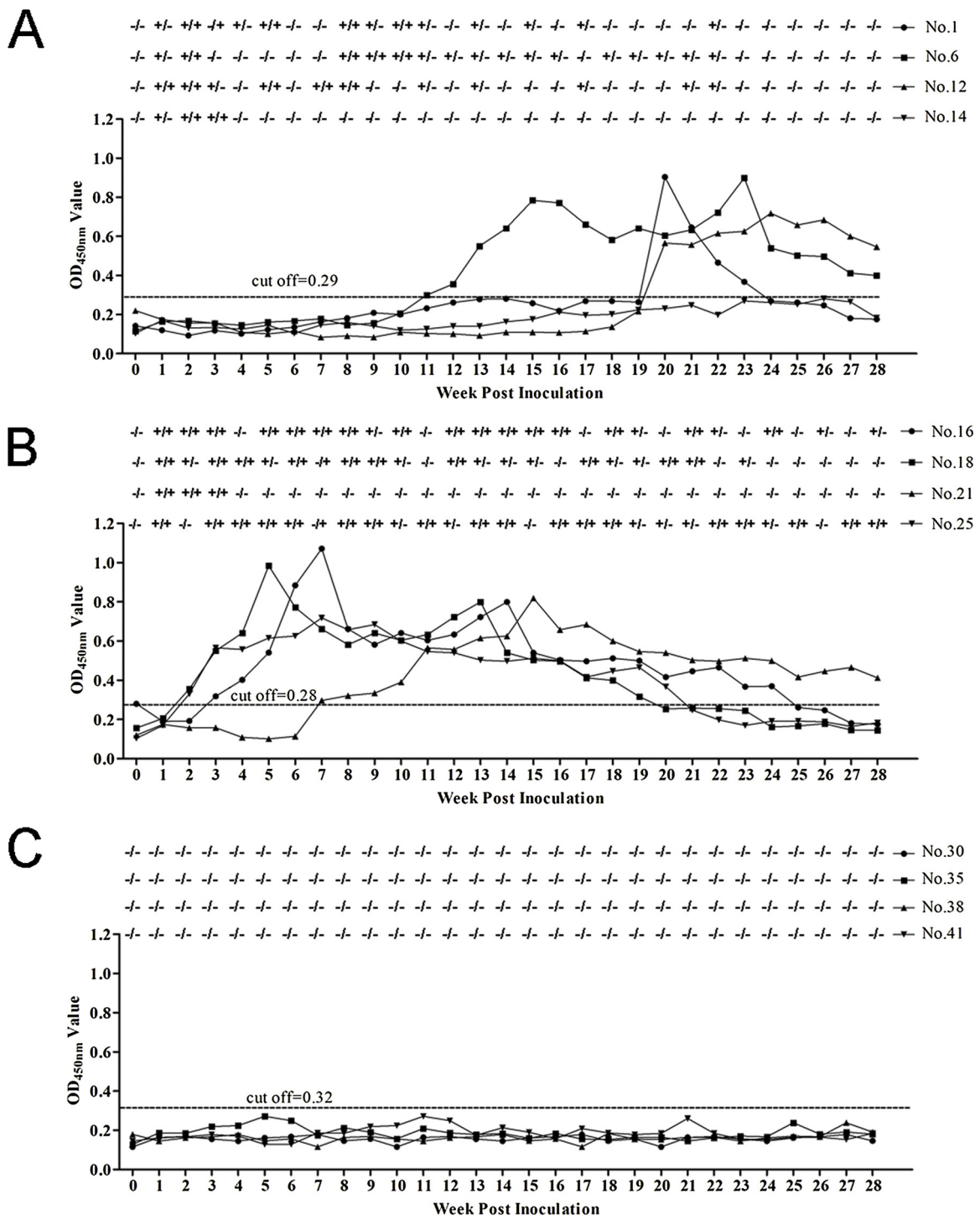

Fig. 1. Time course of seroconversion, viremia, and fecal virus shedding in SPF rabbits inoculated with distinct HEV isolates originating from various species. Rabbits inoculated with CHN-SD-sHEV(A) and CHN-SX-rHEV (B), respectively; Rabbits inoculated with PBS buffer (C) as a negative control. HEV RNA was detected by RT-nPCR. Presence and absence of HEV RNA in feces or serum are indicated by "+" and "-", respectively. Serum and fecal samples were collected at pre-inoculation and various wpi.

high cost of primates and swine, along with the difficulties of handling them, have severely restricted use of such animal models for large scale studies. Since the discovery of rabbit as an HEV host, many studies were conducted to determine if rabbit is also susceptible to swine HEV under experimental conditions (Liu et al., 2014; Wu et al., 2017; Zhang et al., 2015). Unfortunately, it has been documented that rabbits inoculated with swine HEV developed lower levels of viremia and fecal virus shedding (Liu et al., 2014; Wu et al., 2017), suggesting an active but weak replication of swine HEV in rabbit. In contrast to these previous reported results, our study showed that 13 of 14 rabbits inoculated with CHN-SD-sHEV developed fecal virus shedding at 1 wpi and that from 3 of 4 remaining rabbits lasted for 22 wpi (Fig.1A). These results suggest that CHN-SD-sHEV isolate can cause prolonged infection of rabbits with pathological damage of the tissues, which may be explained by the possibilities that this swine-derived HEV-4 isolate has higher virulence since it has different genomic organizations (Cheng et al., 2012), and

Table 2
Detection of HEV RNAs in samples from rabbits necropsied at different wpi[a].

Virus Inocula	Sample	No. of positive strain RNA (no. of negative strain RNA)/ total no. of rabbits tested at different wpi					
		2	4	6	8	10	28
CH-SD-sHEV	Serum	1/2	2/2	2/2	1/2	1/2	0/4
	Faces	2/2	2/2	2/2	1/2	1/2	0/4
	Liver	1(1)/2	2(2)/2	2(2)/2	1(1)/2	0(0)/2	0(0)/4
	Spleen	1(0)/2	2(1)/2	2(2)/2	1(0)/2	0(0)/2	0(0)/4
	Bile	2/2	2/2	1/2	1/2	0/2	0/4
CHN-SX-rHEV	Serum	2/2	2/2	1/2	1/2	1/2	1/4
	Faces	2/2	2/2	2/2	2/2	1/2	2/4
	Liver	2(1)/2	2(2)/2	2(2)/2	1(1)/2	1(0)/2	0(0)/4
	Spleen	2(1)/2	2(2)/2	2(2)/2	1(1)/2	1(0)/2	0(0)/4
	Bile	2/2	2/2	2/2	1/2	0/2	0/4
PBS	Serum	0/2	0/2	0/2	0/2	0/2	0/4
	Faces	0/2	0/2	0/2	0/2	0/2	0/4
	Liver	0(0)/2	0(0)/2	0(0)/2	0(0)/2	0(0)/2	0(0)/4
	Spleen	0(0)/2	0(0)/2	0(0)/2	0(0)/2	0(0)/2	0(0)/4
	Bile	0/2	0/2	0/2	0/2	0/2	0/4

[a] Each of two rabbits was necropsied at 2, 4, 6, 8 and 10 wpi. The remaining four rabbits were necropsied at 28 wpi.

higher infectious dosage (Meng et al., 1998a). In addition, two rabbits (No.1 and 12) exhibited diarrhea in early infection followed with seroconversion, viremia, virus shedding, and liver lesion and six rabbits (No.3, 4, 7, 9, 10 and 13) had hepatocellular ballooning lesions of livers. However, the correlation of diarrhea with other clinical exhibitions needs to be confirmed.

In present study, two different HEV capsid proteins used as coating antigens for detecting anti-sHEV and anti-rHEV antibodies in the indirect ELISAs share > 93% amino acid identity and the antibodies to one antigen does cross-react with another. It has been reported that HEV infection via vein inoculation is evidenced first by virus fecal shedding, followed by viremia and liver enzyme elevation and finally by seroconversion (Tsarev et al., 1992), which was consistent with our finding that seroconversion was observed in some rabbits, but disappeared at later times (Fig. 1).

For HEV RNA detection in rabbits of CHN-SX-rHEV and CHN-SD-sHEV groups, two different RT-nPCR methods (Geng et al., 2011; Huang et al., 2002) were used for fecal shedding and viremia appearance (Fig.1), and one RT-qPCR method (Jothikumar et al., 2006) was used for virus RNA quantity in serum (Fig.2). In the CHN-SX-rHEV group, the peak viral copy numbers ($10^{6.2}$-$10^{6.7}$) in sera from the four rabbits was higher than the CHN-SD-sHEV groups' ($10^{4.1}$-$10^{5.2}$) (Fig. 2). This result suggested that the infectivity of HEV-3ra was higher than sHEV-4 in

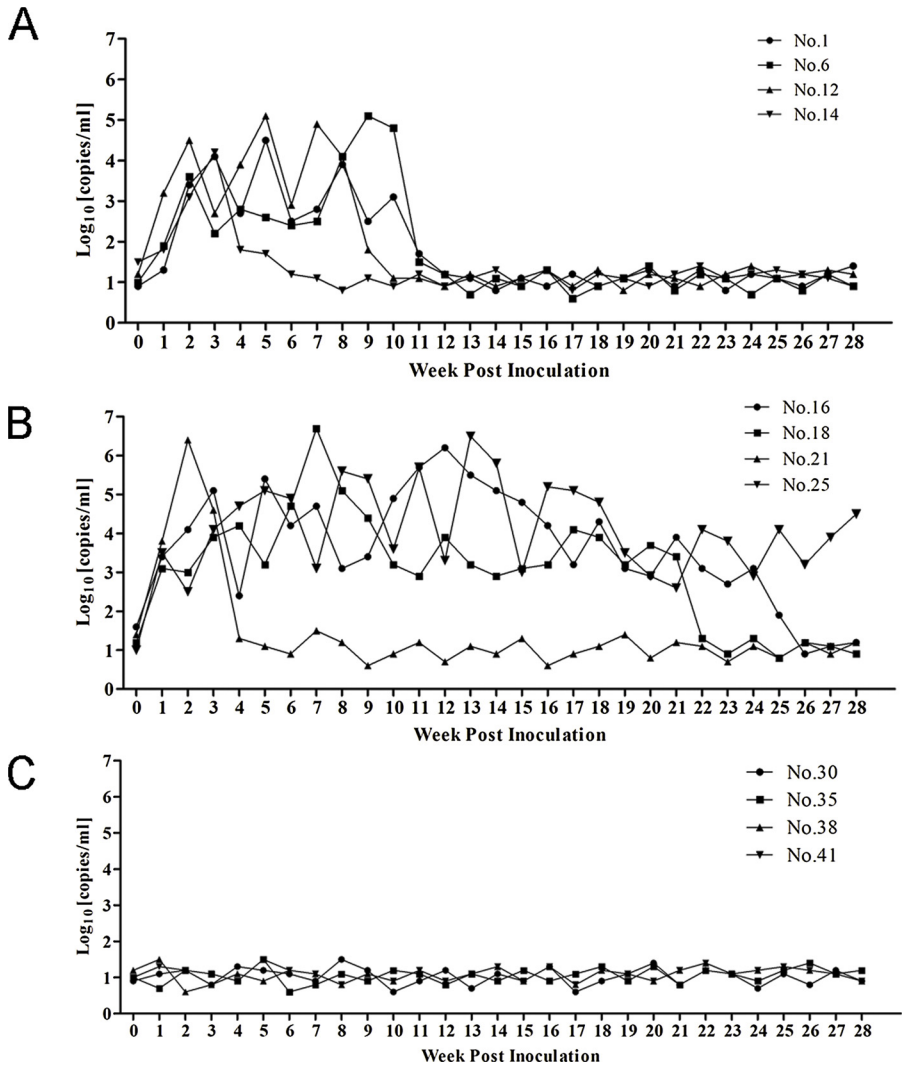

Fig. 2. Dynamics of RT-qPCR quantified viral loads in serum samples of rabbits inoculated with HEV isolated from various host species. Rabbits inoculated with CHN-SD-sHEV (A), CHN-SX-rHEV (B) and PBS buffer (C), respectively. Serum samples were collected at pre-inoculation and various wpi. Y axis represents $\log_{10}$ [copies/ml] of HEV RNA in serum.

Table 3
Number of rabbits with microscopic liver lesions[a].

Virus Inocula	No. of rabbits with lesions (mean score) at indicated wpi					
	2	4	6	8	10	28
CH-SD-sHEV	2(2)	2(3.5)	2(3)	2(2)	2(2)	4(1)
CHN-SX-rHEV	2(3.5)	2(4)	2(3)	2(2.5)	2(2)	4(1.5)
PBS	0(0)	1(1)	2(1.5)	1(1)	1(1)	1(1)

[a] Each of two rabbits was necropsied at 2, 4, 6, 8 and 10 wpi. The remaining four rabbits were necropsied at 28 wpi. The liver lesions include lymphoplasmacytic, periphlebitis, phlebitis, and necrotic lesions.

rabbit. Meanwhile, there is an oscillation of the RNA detection in blood and feces during the observation period in both inoculated groups (Fig. 1). One possible explanation is that the sensitivity of the RT-nPCR assays we used affected the results. Sine liver, bile, and spleen were main tissues for HEV distribution (Wu et al., 2017), we only tested HEV RNAs in these tissues (Table 2). In addition, the fact that CHN-SD-sHEV was only detected in serum and feces, but not in the bile, liver, and spleen at 10 wpi (Table 2) may be because HEV directly entered liver

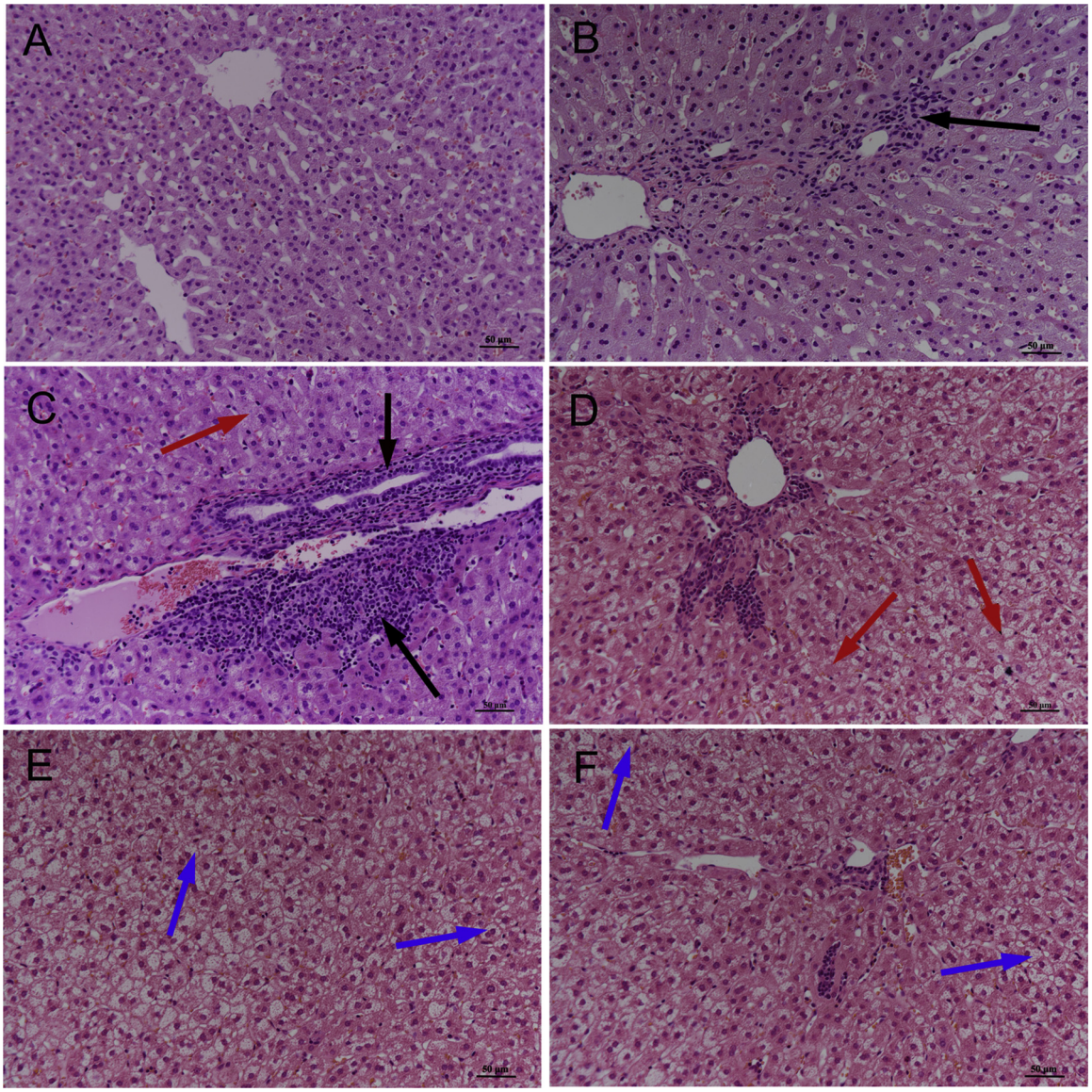

Fig. 3. Various characteristics of microscopic lesions in livers from rabbits inoculated with HEV isolated from various host species. (A) Liver sections from rabbits showing no visible pathological signs of HEV infection in the negative control group (No. 35); (B) Local lymphocytic venous periphlebitis (arrow) in the CHN-SD-sHEV group (No. 8); (C) Local hepatocellular necrosis (red color arrow) and focally intense lymphocytic venous periphlebitis (black color arrow) in the CHN-SD-sHEV group (No. 4); (D) Extensive hepatocellular necrosis (arrow) in the CHN-SX-rHEV group (No. 20); (E) Liver sections from swine HEV-inoculated rabbits (No. 9) showing local balloon-like hepatocellular lesion (arrow); (F) Liver sections from the CHN-SX-rHEV inoculated rabbit (No. 19) showing extensive balloon-like hepatocellular lesion (arrow); Tissues were stained with hematoxylin and eosin (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

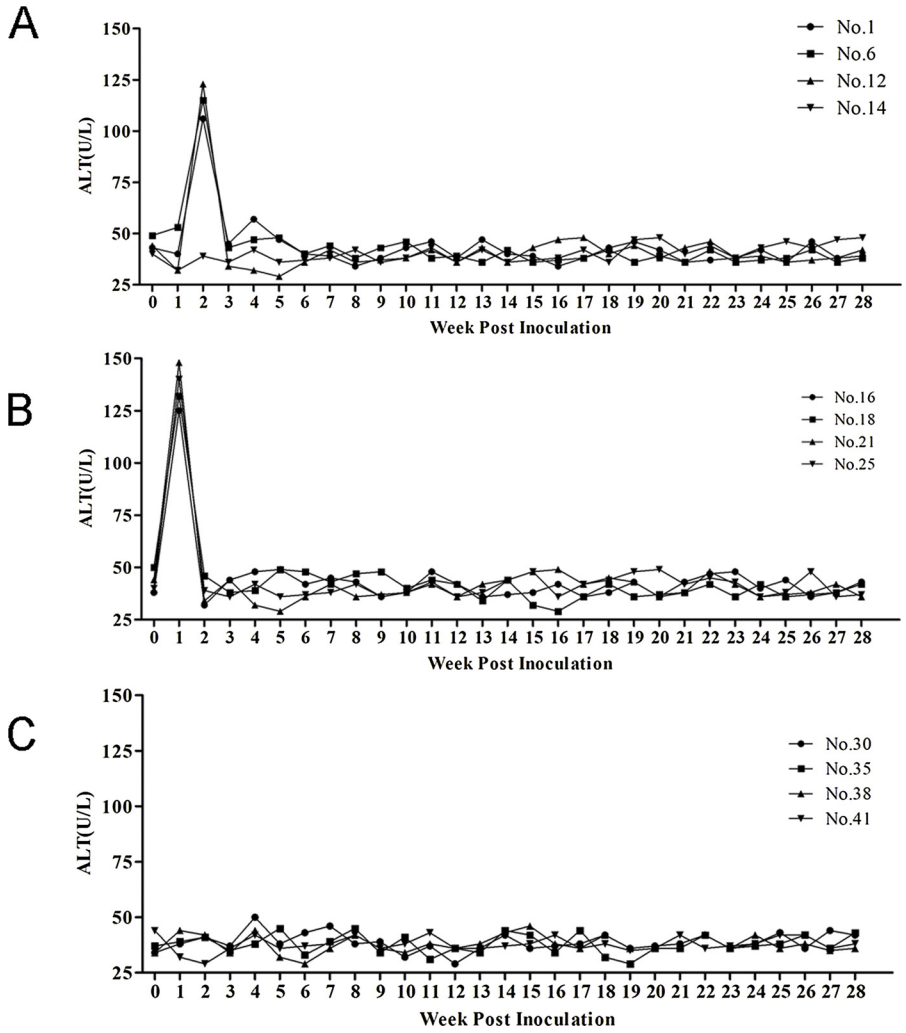

Fig. 4. Levels of ALT liver enzyme in sera of rabbits inoculated with HEV isolates from various host species. Rabbits inoculated with CHN-SD-sHEV (A), CHN-SX-rHEV (B) and PBS buffer (C), respectively.

through blood circulation and reached to gastrointestinal tract passing bile and then reenters blood circulation.

Since the discovery of HEV zoonosis, both domestic and wild animals have been identified as natural reservoirs for zoonotic HEV strains. Recently, a rabbit HEV strain originating in France matched a closely related human HEV isolate (from a French patient) bolstering support for possible zoonotic transmission of a genotype 3 rabbit HEV to a human (Izopet et al., 2012). As further evidence, zoonosis of rabbit HEV was subsequently confirmed by experimental infection of cynomolgus macaques by rabbit HEV (Liu et al., 2013). Recently, rabbits have also been identified as a potential zoonotic reservoir for human hepatitis E (Caruso et al., 2015), although few studies have investigated pathogenicity of rabbit HEV isolates in their natural hosts (Cheng et al., 2012; Han et al., 2014). Previous reports indicated that rabbit HEV usually caused subclinical infection, with variable rabbit infectivity observed in rabbits in different reports. Similar to the previous report (Han et al., 2014), CHN-SX-rHEV infected rabbits showed the persistent fecal virus shedding which was lasted at 28wpi. However, the elevated serum ALT level was only detected at 1wpi (Fig. 1).

It is not surprising that rabbit-derived HEV-3 infection of rabbits have longer seroconversion, viremia, and virus fecal shedding than swine-derived HEV-4 infected rabbits (Fig.1) which is similar with the previously report (Han et al., 2014) and although the same viral doses were used, the overall course of disease was various which is consistent with that reported previously (Tsarev et al., 1993). It is speculated that HEV cross-species infection generally needs to adapt to the new host before it can replicate more efficiently (Cossaboom et al., 2012). However, our study showed that CHN-SD-sHEV and CHN-SX-rHEV infected rabbits exhibited the viremia and virus shedding as early at 1 wpi (Fig. 1 and Table 1). In addition, since both inoculated viruses from bile and faces are all non-enveloped HEV (Nagashima et al., 2017), it is not likely that the different pathogenicity of CHN-SD-sHEV and CHN-SX-rHEV infected rabbits is resulted from the origins of the viruses.

In summary, recent identification of animal HEV strains has raised public health concerns regarding zoonosis and food safety (Dalton et al., 2014; Garbuglia et al., 2015; Guillois et al., 2016; Lapa et al., 2015). In the present study, we demonstrate that the swine-derived HEV-4 isolated in China could cross-species infect rabbit accompanied with prolonged virus fecal shedding and liver lesions and provide new insights for understanding swine-derived HEV-4 pathogenicity in rabbits and zoonosis among diverse hosts.

Conflict of interest statement

The authors declare that they have no competing interests.

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