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Short communication

Hepatitis E virus was not detected in feces and milk of cows in Hebei province of China: No evidence for HEV prevalence in cows

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ABSTRACT

Hepatitis E virus (HEV) is an important human pathogen, with pigs and other species serving as natural animal reservoirs. Recently, the report of detection of genotype 4 HEV in dairy milk with high positive rate in Yunnan province of China has attracted extensive attention. To assess the zoonotic risk of cows as HEV reservoir and transmission of HEV through dairy milk, 467 fecal samples of cows, 276 fresh milk samples, and 140 retail milk samples were collected across Hebei Province, China, from March 2017 to May 2018, and detected for HEV RNA. Fecal samples of rabbit or pig were also collected for HEV detection from farms of mixed farming with cows or farms neighboring cow farms. HEV RNA was not detected in any cow feces or in any milk samples, but 9.3% feces of pigs and 18.9% feces of rabbits were positive for HEV RNA. In addition, all of the dairy milk samples undergone HEV antigen and anti-HEV antibody detections, but none was positive. Phylogenetic analysis showed that all of the HEV isolates from pigs belonged to genotype 4 and those from rabbits were genotype 3-rabbit HEV. The results indicate that, currently in Hebei province of China, HEV is not apparently prevalent in cows and hence there is no zoonotic transmission risk through dairy milk towards humans, albeit the genotype 4 and 3 (rabbit) HEV are prevalent in pigs and rabbits respectively.

1. Introduction

Hepatitis E virus (HEV) is a major cause of acute viral hepatitis in many developing countries, and sporadic hepatitis E cases have been reported increasingly in many industrialized countries. HEV is a single-stranded, positive-sense RNA virus belonging to the family *Hepeviridae*. *Hepeviridae* family consists of two genera, *Orthohepevirus* and *Piscihepevirus*. *Orthohepevirus* genus has four viral species, designated *Orthohepevirus* A–D (Smith et al., 2014). All variants of HEV known to infect humans belong to the species *Orthohepevirus* A (Purdy et al., 2017). HEV strains within the species *Orthohepevirus* A are tentatively grouped into eight genotypes: genotypes 1 and 2 strains were exclusively identified from humans and associated with waterborne epidemics in developing countries; genotypes 3 and 4 strains were isolated from both humans and some animal species, and are responsible for sporadic cases in developed countries; whereas genotypes 5 and 6 have only been isolated from wild boar, and genotypes 7 and 8 have been isolated from camels (Purdy et al., 2017). Thus far, genotypes 3 and 4 HEV strains are recognized as zoonotic pathogens, which can be transmitted from animal reservoirs to humans.

A cluster of evidences documented sporadic cases of hepatitis E acquired via closely contacting with animals and consumption of undercooked meat (Pavio et al., 2015). Zoonotic transmission of genotypes 3 and 4 HEV raises many concerns with the increasing reports of local acquired sporadic hepatitis E cases in developed countries. In the past two decades, waterborne outbreak caused by genotypes 1 and 2 HEV almost disappeared in China, and currently most hepatitis E cases are sporadic, caused by genotype 4 HEV (Geng and Wang, 2016). Although the geographical distribution and host range of genotypes 3 and 4 HEV are different to some extent, pigs are the primary natural reservoir of both genotypes (Meng, 2013; Pavio et al., 2015). Genotype 3 strains of HEV have also been genetically identified from a number of other animal species, including wild boars (de Deus et al., 2008), deer (Takahashi et al., 2004), mongooses (Nakamura et al., 2006) and goats (Di Martino et al., 2016). Genotype 3 HEV also includes some unassigned strains, such as rabbit HEV provisionally assigned as subtype

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Abbreviations: HEV, Hepatitis E virus; ORF, open reading frame; RT-nested PCR, reverse transcription nested polymerase chain reaction; GE/mL, genome equivalents per milliliter; HEV-Ag, HEV antigen; Anti-HEV-Ab, Anti-HEV antibody; ELISA, enzyme linked immunosorbent assay

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3ra, as its genotyping is still under consideration (Doceul et al., 2016; Zhao et al., 2009). Genotype 4 HEV isolates have been restricted to humans and pigs until recently, as genotype 4 sequences were isolated from cows. The first report on genotype 4 HEV detected in dairy cows was from Xinjiang, China (Hu and Ma, 2010). HEV strains have also been found in yak in Qinghai, China (Xu et al., 2014). However, in most cases, HEV RNA could not be detected in the investigated cattle or cow, even in populations of cattle or cow with a very high positive rate of HEV antibodies (Dong et al., 2011; Geng et al., 2010; Prpić et al., 2015). Hence, whether cow or cattle could be a reservoir of HEV remains uncertain.

Recently, Huang et al. reported that genotype 4 HEV was highly prevalent in dairy cows in Yunnan province of China, and the infectious virus is excreted into milk (Huang et al., 2016). As fresh milk is widely consumed, the safety of milk stands as an important problem of public health. The report by Huang et al. (2016) raised many concerns regarding the risks of HEV transmission through dairy milk. The aim of this study is to determine whether HEV strains are circulating among cows in other areas of China, and to assess the risk of HEV transmission through dairy milk.

2. Material and methods

2.1. Sample collection and processing

From March 2017 to May 2018, feces and milk samples of dairy cows were collected from dairy cow farms in six prefectures across Hebei Province in northern China (Fig. 1). In each area, judgement sampling method was used: the majority of the farms are small and medium-scale domestic farms with poor sanitation, among which several are mixed farms, where cows are kept together with other animals; Most samples are collected from cows aged from 1 to 3 years, and some fecal samples from calves before milk producing. Most of the cows are Holstein cow, and the rest are Jersey cow. If the farm was a mixed farm or there were pig or rabbit farms near the cow farms, feces of pigs and rabbits were collected (Table 1). In addition, 140 retail fresh milk samples without disinfection treatment were purchased from street retailers in Baoding city during January to May 2018.

Twenty percent (wt/vol) fecal suspensions were prepared by using sterile phosphate buffered saline (PBS; pH 7.4) containing 2% bovine serum albumin (BSA) and centrifugated at 4000 g for 20 min, and the supernatants were separated and recentrifuged at 10,000 g for 5 min to get the clearer supernatants. The milk samples were centrifuged at 4000g for 5 min, and the liquid phase between the sediment and the creamy top was harvested. The processed fecal and milk samples stored at −70 °C until further use.

Table 1
Sources of the collected fecal and dairy milk samples.

Geographic locations	Cows			Pigs		Rabbits	
	Farms	Feces	Milk	Farms	Feces	Farms	Feces
Baoding	2	50	125	2	17	1	10
Cangzhou	3	32	67			1	10
Hengshui	4	69		2	15	2	19
Shijiazhuang	2	55	34	1	13	2	14
Tangshan	5	86	60	1	2		
Zhangjiakou	37	175		3	17		
Total	53	467	276	9	64	6	53

2.2. HEV RNA extraction and RT-nested PCR

Viral RNAs were extracted from the prepared fecal and milk samples using EasyPure Viral DNA/RNA Kit (TransGen Biotech, Beijing, China) according to the manufacturer's instructions as previously described (Zhao et al., 2015). A 200 μL volume of each sample was used for RNA extraction and the RNA was recovered in 40 μL elution buffer. Two sets of HEV universal primers were used to amplify RNA from conserved regions of the viral genome. One set was used to amplify a 332 bp segment of ORF1 (Johne et al., 2010), and the other set to amplify a 348 bp segment of ORF2 region (Geng et al., 2013b) (Table 2). The procedures of RT-nested PCR were same as previously described (Geng et al., 2013b). Positive control samples of genotype 4 (swine HEV) and genotype 3 (rabbit HEV) were included.

To validate the RT-nested PCR and exclude a possible inhibitory effect of feces and milk of the cow on RNA detection, 10 μL of 10^4 GE/mL (genome equivalents per milliliter) in phosphate-buffered saline (PBS) of genotypes 4 and 3 HEV supernatants prepared from swine and rabbit feces spike into 1 mL of the fecal supernatants or milk samples of cows, and then the prepared samples were conducted RNA isolation and RT-nested PCR. The spiked swine HEV of genotype 4 or rabbit HEV could be constantly detected out of cow fecal supernatant and milk

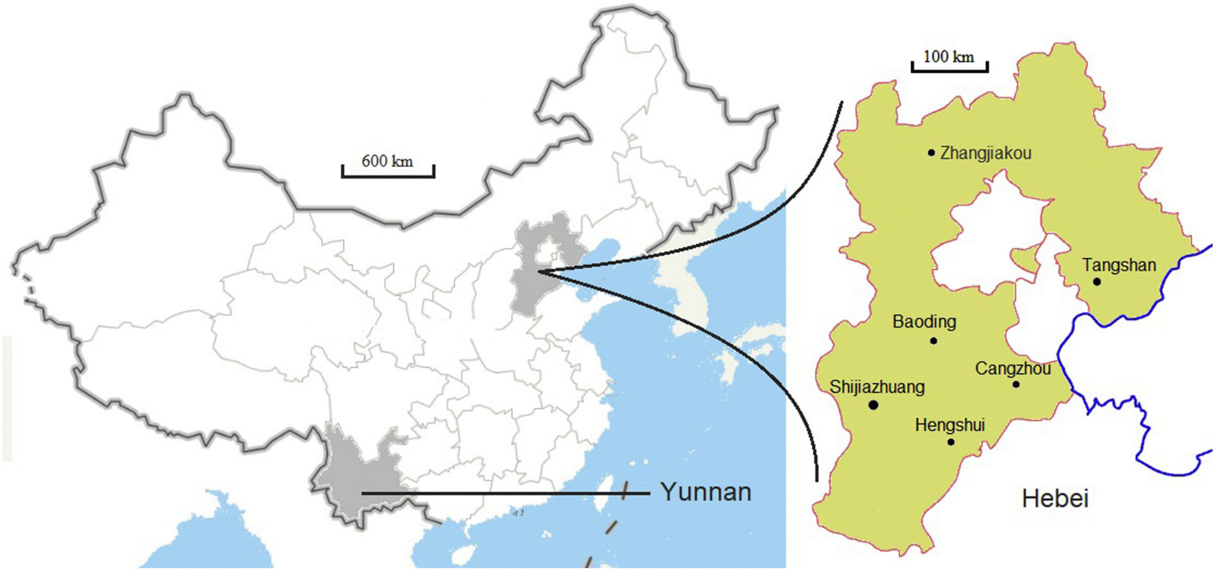

Fig. 1. Geographical origin of cow samples from Hebei province, China. The areas from which the samples were collected are marked by black dots. HEV in the milk has been reported in Yunnan Province, China (Huang et al., 2016).

Table 2
RT-Nested PCR primers for HEV RNA amplification.

Amplified region	Primer designation	Primer's sequences (5′-3′)	Product length(bp)
ORF1	HEV-cs	TCGCGCATCACMTTYTTCCARAA	470
	HEV-cas	GCCATGTTCCAGACDGTRTTCCA	
	HEV-csn	TGTGCTCTGTTTGGCCCNTGGTTYMG	332
	HEV-casn	CCAGGCTCACCRGARTGYTTCTTCCA	
ORF2	SEBO1	AAYTATGCWCAGTACCGGGTTG	730
	SEEO1	CCCTTATCCTGCTGAGCATTCTC	
	SEBI1	GTYATGYTYTGCATACATGGCT	348
	SEEI1	AGCCGACGAAATYAATTCTGTC	

D = A, G or T; M = A or C; N = A, C, G or T; R = A or G; Y = C or T.

respectively. Thus, the sensitivity of the assay for fecal supernatant and dairy milk is at least 100 GE/mL.

2.3. Detection of anti-HEV antibody and antigen

All of the reprocessed milk samples were detected for HEV-antigen (HEV-Ag) and anti-HEV antibody (anti-HEV-Ab) respectively by using enzyme linked immunosorbent assay (ELISA) kits of Wantai Biopharmaceutical Inc., Beijing, China, according to the manufacturer's instructions. The HEV-Ag detection kit is based on an indirect sandwich ELISA, in which a combination of three monoclonal antibodies against the HEV ORF2 protein were coated on the solid phase to capture antigen and goat polyclonal anti-ORF2 antibodies were used for detection (Zhang et al., 2006). The anti-HEV-Ab detection kit is based on a direct-sandwich ELISA, in which both coated and detecting enzyme antigens were HEV ORF2 protein, and thus a cluster of immunoglobulins IgG, IgM and IgA of anti-HEV could be captured and detected.

To validate the ELISA and exclude a possible inhibitory effect of the dairy milk on ELISA detection, a stocked serum sample from an acute hepatitis E patient was diluted serially in phosphate-buffered saline (PBS) or in milk samples followed by ELISA for HEV-Ag and anti-HEV-Ab. The values of S/CO of the serial dilutions in PBS and in milk were not significantly different. Thus, the ELISA kits for HEV-Ag and HEV-Ab detection are capable of detecting dairy milk.

2.4. DNA sequencing and phylogenetic analysis

The amplified products were purified and bidirectionally sequenced with the internal PCR primers by Sangon Biotech Co., Ltd. (Shanghai, China). The acquired sequences in ORF1 and ORF2 regions were aligned against the corresponding regions of the published sequences available in the GenBank respectively, using Clustal Omega. Phylogenetic analysis was performed with a neighbor-joining algorithm (Molecular Evolutionary Genetics Analysis, MEGA7.0).

3. Results

3.1. HEV RNA was detected in feces of rabbits and pigs, but not in feces and milks of cows

All fecal samples collected from cows, pigs and rabbits, and dairy milk samples were processed for RT-nested PCR to amplify HEV RNA of ORF1 and ORF2 regions respectively.

Of the total of 467 fecal samples of cows and 416 diary milk samples, no sample was positive for HEV RNA by using any protocols to amplify the fragment in ORF1 or ORF2 region. Of the 64 pig feces, 5 samples were positive for both ORF1 and ORF2 fragments, one sample was only positive for ORF2 fragment. In total, 6 swine samples from 4 farms were positive for HEV RNA, the average positive rate of HEV was 9.3% (6/64) among pigs and 44.4% (4/9) among the pig farms. Of the 53 rabbit feces, 10 samples were positive for both ORF1 and ORF2 fragments, showing a positive rate of 18.9% (10/53); HEV RNA positive samples were found in all of the 6 rabbit farms (100%).

3.2. Neither HEV-Ag nor anti-HEV-Ab was detected in dairy milk

All of the 276 dairy milk samples collected from cow farms and 140 dairy milk samples collected from retail vendors underwent HEV-Ag and anti-HEV-Ab detection. None of the milk samples were positive for HEV-Ag or anti-HEV-Ab.

3.3. The isolates from pigs and rabbits belonged to HEV genotypes 4 and 3 respectively

All of the PCR products from the 6 positive swine fecal samples and 10 positive rabbit fecal samples were sequenced, while two rabbit ORF1 fragments with weak bands were failed to get clear sequences. Thus, among all of the HEV isolates of this study, 5 sequences of swine and 10 sequences of rabbits in ORF2 region, and 6 sequences of swine and 8 sequences of rabbits in ORF1 region were identified and have been deposited in GenBank with the accession numbers from MH607617 to MH607645.

By comparing, aligning and conducting phylogenetic analysis with known HEV sequences from GenBank, for the 348 bp sequences of the ORF2 capsid region, all 5 sequences from swine clustered within genotype 4, and the 10 sequences from rabbits clustered within rabbit HEV, a sub-cluster of genotype 3 in the phylogenetic tree (Fig. 2A). The same result was obtained by phylogenetic analysis of the 332 bp sequences of ORF1, all 6 sequences from pigs clustered within genotype 4, and all 8 sequences from rabbits clustered within rabbit HEV (Fig. 2B).

4. Discussion

To determine whether HEV is prevalent in cows and to assess whether the milk is contaminated with HEV, we collected feces and milk samples from dairy cows of different ages in different regions of Hebei Province for HEV detection. Since the study of Huang et al. (2016) noted a rural area in Yunnan with mixed farming systems to have a high prevalence of genotype 4 HEV in cows, we focused on collecting samples from a few small-scale mixed feeding farms with poor sanitary conditions. Two RT-nested PCR protocols were used respectively for each sample to ensure the detection HEV RNA. One protocol used consensus primers to amplify ORF2 region was able to detect HEV genotypes 1, 3 and 4 (Geng et al., 2013b); the other protocol developed initially for rat HEV detecting was capable of detecting a more broad-spectrum of HEV types (Johne et al., 2010). However, no HEV-specific sequence could be amplified from the samples of cows despite repeated RT-PCR testing. In addition to the RT-nested PCR assays, we also tried to detect HEV-Ag and anti-HEV-Ab in milk samples, but no sample was positive. Thus, there are no signs of an epidemic of genotype 3 or 4 HEV infection in dairy cows in Hebei Province.

Studies have shown that genotype 4 HEV is the predominant zoonotic genotype in China (Liu et al., 2012; Geng and Wang, 2016). In this study, HEV RNA was detected in the fecal samples of both pigs and rabbits; all the strains from pigs belong to genotype 4, whereas the strains from rabbits are clustered around rabbits HEV. Hence, while genotypes 4 and 3 (rabbit) HEV are circulating among pigs and rabbits respectively in this area as previously known (Geng et al., 2013a), we found no evidence for cross-species transmission of genotypes 4 and 3 HEV from pigs and rabbits to cows even in mixed farming farms. We also found no cross-species transmission of genotype 4 and rabbit HEV between pigs and rabbits. Overall, the genotype 4 and rabbit HEV still remain to exhibit apparent host restriction in this region.

Our results are apparently different with the results obtained in Yunnan province by Huang et al. (2016), who reported that 37.1% of investigated cows were actively infected with genotype 4 HEV and 100% of the infected cows secret HEV in their milk. Following the study

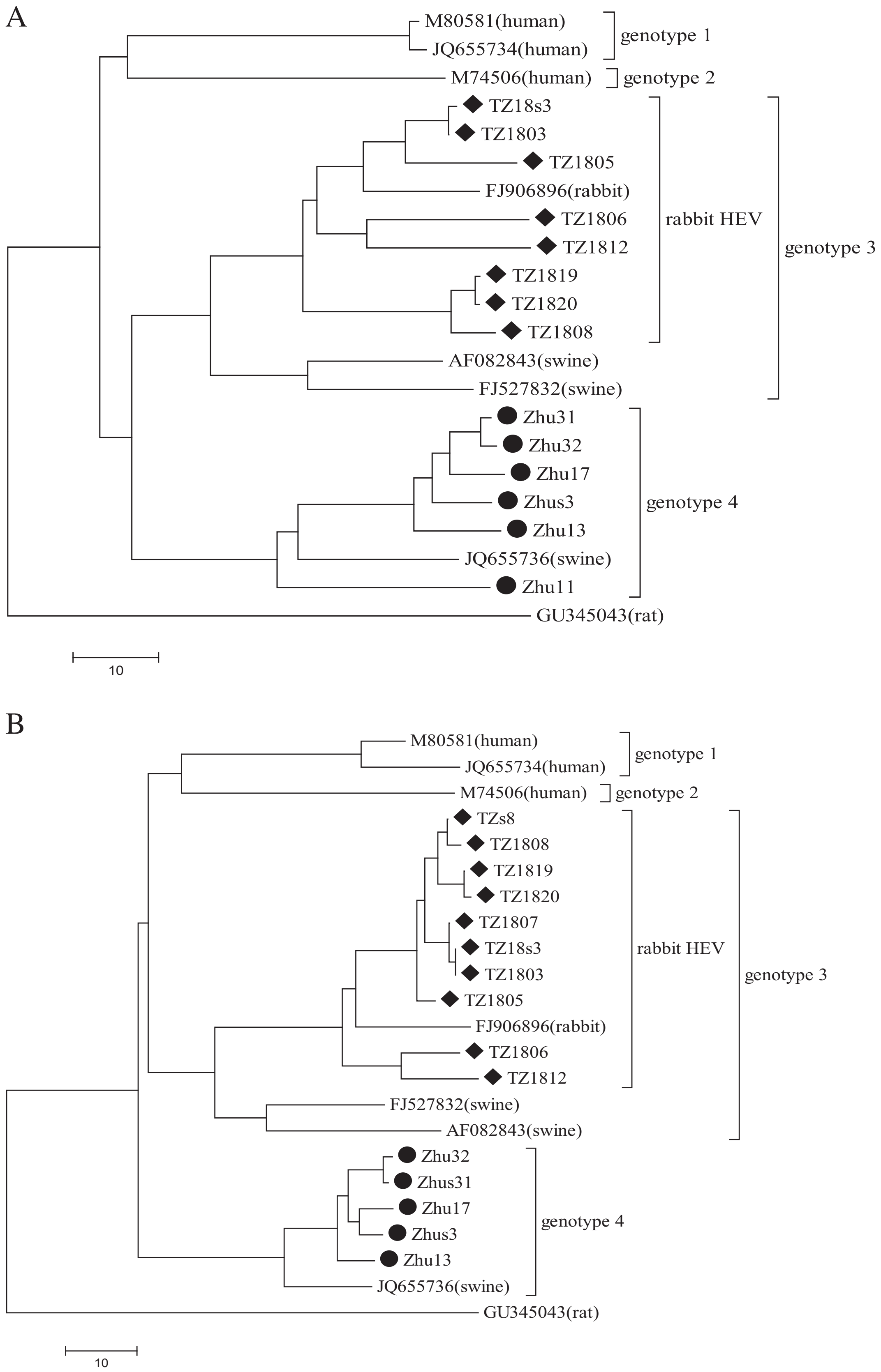

Fig. 2. Phylogenetic trees of the HEV strains from pigs and rabbits. Fig. 2A based on partial ORF1 sequences (332 nt) and Fig. 2B based on partial ORF2 sequences (348 nt) of HEV strains. The trees were constructed using the neighbor-joining method with MEGA 7.0. A scale bar representing the numbers of character state changes is proportional to the genetic distance. Filled circles and diamonds indicate strains detected from pigs and rabbits respectively in the current study.

of Huang et al. (2016), Baechlein and Becher (2017) have tried to determine the presence of HEV RNA in 400 milk samples collected in 2008 from dairy farms in Germany by using RT-PCR assay, but no sample was positive. Vercouter et al. (2018) also failed to find the presence of HEV in cow fecal and dairy milk samples collected in mixed and non-mixed farms in Flanders, Belgium, despite the circulation of genotype 3 HEV among pig farms in the same region. In addition to the current study, we have investigated the prevalence of HEV in domestic animals (pigs, goats, and cows) in 26 provinces including Yunnan in China, and genotype 4 HEV was isolated only from the serum samples of pig, but not from that of cattle and cows (Geng et al., 2010). These consistent results suggest that both genotypes 4 and 3 HEV infections are not common in cows.

We are unable to explain the discrepancy between our results and the results of Huang et al. (2016). The conditions of small mixed domestic farming pattern of cows and genotype 4 HEV as the predominant genotype in pigs are similar between Yunnan and Hebei provinces, thus farming differences of cows or genotype-dependent differences solely could not be the critical risk factors for HEV transmission among different species in Yunnan province as previously speculated (Baechlein and Becher, 2017; Vercouter et al., 2018). We also do not think that the discrepancy in results was due to the lower sensitivity of RT-nested PCR used in our study compared to real-time PCR used in the study of Huang et al. (2016), since Huang et al. obtained HEV sequences and we found HEV RNA in pigs and rabbits using RT-nested PCR. Considering the long distance of about 2500 km from Yunnan to Hebei province (Fig. 1), it is possible that HEV trans-species transmission to cows in a restricted region in Yunnan province is due to the combination of geographical environment, subtropical rain forest climate, and mixed animal feeding with poor sanitation.

At present, the molecular mechanism of host restriction versus cross species transmission of HEV is not clear. If cross-species transmission of HEV genotype 4 among domesticated animals was to only occur in certain areas, the mechanism and affecting factors are worthy of further investigation. We also believe whether HEV can be secreted into milk need to be further clarified with more evidence, since it involves major issues, such as mother to child transmission via breast-feeding and food safety of HEV through consumption of milk or milk products.

In summary, by using consensus primers to amplify two fragments located in ORF1 and ORF2 regions respectively, we detected no HEV RNA in the feces of cows and dairy milk samples collected from Hebei Province in northern China, despite finding high positive rate of genotype 4 HEV in pigs and genotype 3 HEV in rabbits as usual. Thus far, there is no evidence that HEV is prevalent among cattle and consequently no evidence for zoonotic infection of genotype 3 or 4 HEV through dairy milk in Hebei province.

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Conflicts of interest

None.

References

Baechlein, C., Becher, P., 2017. No evidence for zoonotic hepatitis E virus infection through dairy milk in Germany. Hepatology 65 (1), 394–395.

de Deus, N., Peralta, B., Pina, S., Allepuz, A., Mateu, E., Vidal, D., Ruiz-Fons, F., Martín, M., Gortázar, C., Segalés, J., 2008. Epidemiological study of hepatitis E virus infection in European wild boars (Sus scrofa) in Spain. Vet. Microbiol. 129 (1–2), 163–170.

Di Martino, B., Di Profio, F., Melegari, I., Sarchese, V., Robetto, S., Marsilio, F., Martella, V., 2016. Detection of hepatitis E virus (HEV) in goats. Virus Res. 225, 69–72.

Doceul, V., Bagdassarian, E., Demange, A., Pavio, N., 2016. Zoonotic hepatitis E virus: classification, animal reservoirs and transmission routes. Viruses 8 (10), e270.

Dong, C., Meng, J., Dai, X., Liang, J.H., Feagins, A.R., Meng, X.J., Belfiore, N.M., Bradford, C., Corn, J.L., Cray, C., Glass, G.E., Gordon, M.L., Hesse, R.A., Montgomery, D.L., Nicholson, W.L., Pilny, A.A., Ramamoorthy, S., Shaver, D.D., Drobeniuc, J., Purdy, M.A., Fields, H.A., Kamili, S., Teo, C.G., 2011. Restricted enzooticity of hepatitis E virus genotypes 1 to 4 in the United States. J. Clin. Microbiol. 49 (12), 4164–4172.

Geng, Y., Wang, Y., 2016. Epidemiology of Hepatitis E. Adv. Exp. Med. Biol. 948, 39–59.

Geng, Y., Wang, C., Zhao, C., Yu, X., Harrison, T.J., Tian, K., Wang, Y., 2010. Serological prevalence of hepatitis E virus in domestic animals and diversity of genotype 4 hepatitis E virus in China. Vector Borne Zoonotic Dis. 10 (8) (765–700).

Geng, Y., Zhang, H., Li, J., Huang, W., Harrison, T.J., Zhao, C., Zhou, Y., Lian, H., Wang, Y., 2013a. Comparison of hepatitis E virus genotypes from rabbits and pigs in the same geographic area: no evidence of natural cross-species transmission between the two animals. Infect. Genet. Evol. 13, 304–309.

Geng, Y., Zhao, C., Fan, J., Harrison, T.J., Zhang, H., Lian, H., Geng, K., Wang, Y., 2013b. Genotype analysis of hepatitis E virus from sporadic hepatitis E cases in northern China. Infect. Genet. Evol. 20, 413–417.

Hu, G.D., Ma, X., 2010. Detection and sequences analysis of bovine hepatitis E virus RNA in Xinjiang Autonomous Region. Bing Du Xue Bao 26 (1), 27–32.

Huang, F., Li, Y., Yu, W., Jing, S., Wang, J., Long, F., He, Z., Yang, C., Bi, Y., Cao, W., Liu, C., Hua, X., Pan, Q., 2016. Excretion of infectious hepatitis E virus into milk in cows imposes high risks of zoonosis. Hepatology 64 (2), 350–359.

Johne, R., Plenge-Bönig, A., Hess, M., Ulrich, R.G., Reetz, J., Schielke, A., 2010. Detection of a novel hepatitis E-like virus in faeces of wild rats using a nested broad-spectrum RT-PCR. J. Gen. Virol. 91 (Pt 3), 750–758.

Liu, P., Li, L., Wang, L., Bu, Q., Fu, H., Han, J., Zhu, Y., Lu, F., Zhuang, H., 2012. Phylogenetic analysis of 626 hepatitis E virus (HEV) isolates from humans and animals in China (1986–2011) showing genotype diversity and zoonotic transmission. Infect. Genet. Evol. 12 (2), 428–434.

Meng, X.J., 2013. Zoonotic and foodborne transmission of hepatitis E virus. Semin. Liver Dis. 33 (1), 41–49.

Nakamura, M., Takahashi, K., Taira, K., Taira, M., Ohno, A., Sakugawa, H., Arai, M., Mishiro, S., 2006. Hepatitis E virus infection in wild mongooses of Okinawa, Japan: demonstration of anti-HEV antibodies and a full-genome nucleotide sequence. Hepatol. Res. 34 (3), 137–140.

Pavio, N., Meng, X.J., Doceul, V., 2015. Zoonotic origin of hepatitis E. Curr. Opin. Virol. 10, 34–41.

Prpić, J., Černi, S., Škorić, D., Keros, T., Brnić, D., Cvetnić, Ž., Jemeršić, L., 2015. Distribution and molecular characterization of hepatitis E virus in domestic animals and wildlife in Croatia. Food. Environ. Virol. 7 (3), 195–205.

Purdy, M.A., Harrison, T.J., Jameel, S., Meng, X.J., Okamoto, H., Van der Poel, W.H.M., Smith, D.B., Ictv Report Consortium, 2017. ICTV virus taxonomy profile: Hepeviridae. J. Gen. Virol. 98 (11), 2645–2646.

Smith, D.B., Simmonds, P., International Committee on Taxonomy of Viruses Hepeviridae Study Group, Jameel, S., Emerson, S.U., Harrison, T.J., Meng, X.J., Okamoto, H., Van der Poel, W.H., Purdy, M.A., 2014. Consensus proposals for classification of the family Hepeviridae. J. Gen. Virol. 95 (Pt 10), 2223–2232.

Takahashi, K., Kitajima, N., Abe, N., Mishiro, S., 2004. Complete or near-complete nucleotide sequences of hepatitis E virus genome recovered from a wild boar, a deer, and four patients who ate the deer. Virology 300 (2), 501–505.

Vercouter, A.S., Sayed, I.M., Lipkens, Z., De Bleecker, K., De Vliegher, S., Colman, R., Koppelman, M., Supré, K., Meuleman, P., 2018. Absence of zoonotic hepatitis E virus infection in Flemish dairy cows. Int. J. Food Microbiol. 281, 54–59.

Xu, F., Pan, Y., Baloch, A.R., Tian, L., Wang, M., Na, W., Ding, L., Zeng, Q., 2014. Hepatitis E virus genotype 4 in yak, northwestern China. Emerg. Infect. Dis. 20 (12), 2182–2184.

Zhang, F., Li, X., Li, Z., Harrison, T.J., Chong, H., Qiao, S., Huang, W., Zhang, H., Zhuang, H., Wang, Y., 2006. Detection of HEV antigen as a novel marker for the diagnosis of hepatitis E. J. Med. Virol. 78 (11), 1441–1448.

Zhao, C., Ma, Z., Harrison, T.J., Feng, R., Zhang, C., Qiao, Z., Fan, J., Ma, H., Li, M., Song, A., Wang, Y., 2009. A novel genotype of hepatitis E virus prevalent among farmed rabbits in China. J. Med. Virol. 81 (8), 1371–1379.

Zhao, C., Geng, Y., Harrison, T.J., Huang, W., Song, A., Wang, Y., 2015. Evaluation of an antigen-capture EIA for the diagnosis of hepatitis E virus infection. J. Viral Hepat. 22, 957–963.