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Prevalence of hepatitis E virus and porcine caliciviruses in pig farms of Guizhou province, China

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ABSTRACT

Background: Hepatitis E virus (HEV) and caliciviruses are enteric pathogens of humans and animals, and pigs have been considered an important reservoir of these viruses.

Objectives: The aim of this study is to determine the infection rates of HEV and caliciviruses (sapovirus [SaV] and norovirus [NoV]) in different age groups of pigs in Guizhou province, China, and characterize the strains that are prevalent in this mountainous area.

Materials and Methods: A total of 209 stool samples from healthy pigs of different ages were collected from 6 pig farms in Guizhou province from May to June 2009 and tested for HEV, SaV, and NoV by reverse-transcription polymerase chain reaction (RT-PCR).

Results: The overall prevalence of porcine HEV and porcine SaV was 6.7% (15/209) and 1.0% (2/209), respectively. No NoV was detected. The prevalence rates of porcine HEV infection were 15.4% in piglets aged < 1 mon (4/26), 6.8% in piglets aged between 1 and 4 mon (3/44), 12.5% in piglets aged ≈ 4 mon (6/48), and 1.1% in sows aged between 6 and 14 mon (2/91). Porcine SaV was detected only in piglets (7.7%, 2/26). All 10 HEV isolates belonged to genotype 4, clustering with a human HEV strain (AF103940) from an adjacent province.

Conclusions: This is the first report on the existence of porcine SaV in swine in Guizhou province, China. The clustering of the porcine HEV isolates with a human strain suggests cross-species transmission between swine and humans in this area.

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► Implication for health policy/practice/research/medical education:

HEV and porcine SaV infection existed in swine in Guizhou province, China. Health policy makers should be aware of the potential dangers of these viruses and thinking the best way for prevention. We recommend all researchers in laboratory fields as well as health policy makers to read this interesting article.

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1. Background

Hepatitis E virus (HEV) is a member of the genus Hepevirus and has a 7.2 kb positive-sense RNA genome that contains 3 open reading frames (ORFs) (1). Based on a sequence analysis, HEV strains have 4 major genotypes

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(genotype 1-4). HEV is considered to be a zoonotic agent, and researchers have suggested that swine is a principal reservoir of HEV that infects humans (2-6). The family Caliciviridae is divided into 4 genera: Norovirus (NoV), Sapovirus (SaV), Lagovirus, and Vesivirus. NoV and SaV are common causes of gastroenteritis in humans and have been detected in several animal species—SaV in swine and mink and NoV in swine, cattle, mouse, lion, and dog (7). Porcine calicivirus infections have been reported in industrialized and developing countries (8, 9).

Porcine SaV and porcine NoV are considered zoonotic agents due to the genetic and antigenic similarities between porcine and human strains and the occurrence of recombination (10-15). Porcine HEV and porcine caliciviruses are transmitted primarily through the fecal-oral route and are excreted in feces. Some reports have shown that genotype 4 HEV is transmitted freely between swine and humans in eastern and southern China; however, other studies have generated contrasting results for other areas of China (6, 16, 17). We have shown that porcine caliciviruses exist on pig farms in eastern China and that porcine SaV causes diarrhea outbreaks in piglets. The infection rates for porcine SaV and NoV in this area are 0.9% and 0.2%, respectively. The prevalence of HEV in stool samples of Chinese swine is 9.6% to 26.1%, and the overall seroprevalence in humans is 43% in rural communities of southern China (16-18). However, no coinfection of HEV and caliciviruses has been observed in humans or animals.

2. Objectives

Little is known about the genetic diversity of the porcine HEV and caliciviruses that circulate in southwestern China, particularly in Guizhou province, a developing mountainous area. Therefore, the aim of this study was to determine the infection rates of HEV and caliciviruses in various age groups of pigs and characterize the strains that are prevalent in this area.

3. Materials and Methods

A total of 209 stool samples from healthy pigs of various ages were collected randomly from 6 middle- or large-scale pig farms (200-2000 sows each) in Guizhou province from May to June 2009. Fresh stool samples were collected and prepared immediately as 10% (w/v) suspensions in PBS (0.01 M phosphate, pH 7.2-7.4, 0.15 M NaCl, 0.1% DEPC). RNA was extracted from 200 μ l of 10% fecal suspension with TRIzol (Invitrogen, USA) per the manufacturer's instructions after low-speed centrifugation. RNA pellets were dissolved in 25 μ l of RNase-free water, and reverse-transcription was performed immediately. Two sets of primers were used to detect human and porcine caliciviruses, as reported (9). Universal HEV primers were used to detect all 4 HEV genotypes as described (19). Reverse-transcription was performed in a 10- μ l reaction containing 2 μ l 5 \times RT buffer,

Table 1. Frequency of HEV and SaV by RT-PCR in stool samples in pigs of different age

Age (mo)	Samples tested (No.)	Positive samples for HEV [No. (%)]	Positive samples for SaV [No. (%)]
Sow (6-14)	91	1 (1.1%)	0 (0.0%)
Finisher (4)	48	6 (12.5%)	0 (0.0%)
Nursery pig (1-2)	44	3 (6.8%)	0 (0.0%)
Piglet (<1)	26	4 (15.4%)	2 (7.7%)
Total	209	14 (6.7%)	1.0%

0.5 μ l (200 units) AMV reverse transcriptase (TaKaRa, Japan), 1 μ l (25 mM) primer, and 0.5 μ g extracted RNA at 42°C for 1 hr. The parameters for the PCR have been reported (2, 20).

The PCR products were analyzed on a 1.5% agarose gel, stained with ethidium bromide (0.5 μ g/ml), and visualized under UV light (Figure 1). The expected DNA bands were purified with the AxyPrep DNA Gel Extraction Kit (Axygen, USA) and cloned into the pMD18-T vector (TaKaRa, Japan). The inserts were sequenced on a DNA analyzer (Applied Biosystems 3730; Invitrogen, USA). To avoid contamination, negative and positive controls were added from RT-PCR step to nucleotide sequencing. After multiple alignment with CLUSTAL W (version 1.4), MEGA, version 4.0 was used to construct phylogenetic trees of the HEVs and SaVs by bootstrap analysis (1000 repeats).

4. Results

Porcine HEV and porcine SaV were detected in 83% (5/6) and 33% (2/6) of the farms in this study, respectively. No NoV was detected. Moreover, there was no pig that was coinfecte

d with HEV and SaV. All specific PCR bands were TA-cloned and sequenced. Viral strains with identical nucleotide sequences were considered a unique strain

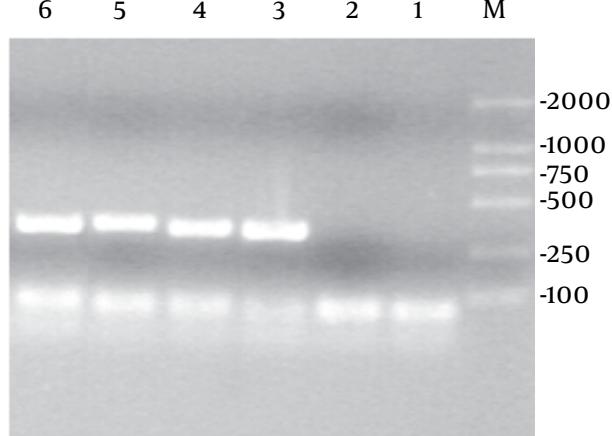
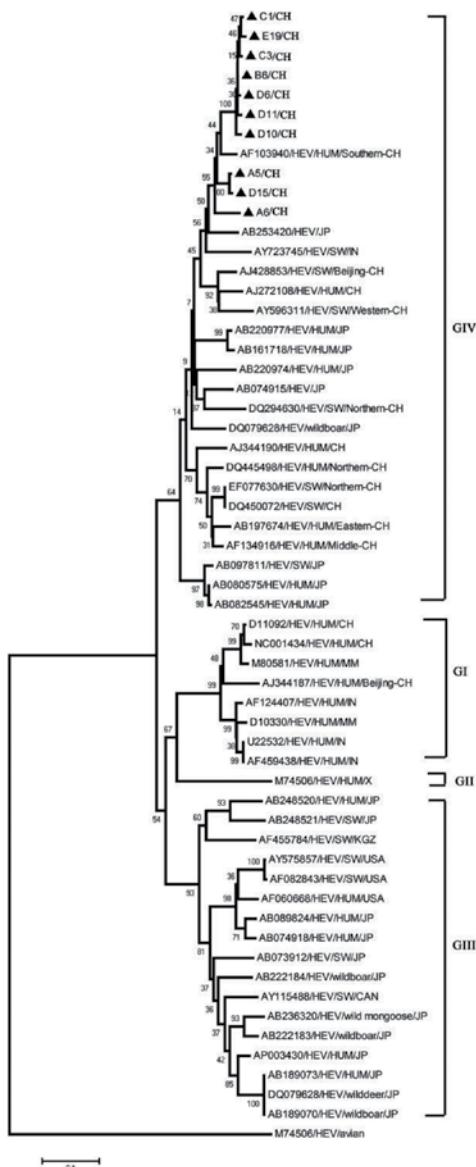


Figure 1. PCR products for HEV and SaV. Lane 1 to lane 6: negative control for HEV, negative control for SaV, positive control for HEV, HEV-positive sample, positive control for SaV, SaV-positive sample

Figure 2. Neighbor-joining tree of the phylogenetic relationship between HEV strains based on a 304-nt fragment of ORF2. Bootstrap values, expressed as percentages of 1000 replications, are given at the branch point. The 10 newly identified HEV strains



(GenBank accession numbers: HQ292706-15 for A5/CH-E19/CH) are marked by filled triangles.

in the phylogenetic analysis, yielding 10 strains with distinct sequences of HEV and 2 unique strains of SaV. The overall prevalence of porcine HEV and porcine SaV was 6.7% (15/209) and 1.0% (2/209), respectively. The prevalence rates of porcine HEV infection were 15.4% in piglets aged <1 mon (4/26), 6.8% in piglets aged between 1 and 4 mon (3/44), 12.5% in piglets aged \approx 4 mon (6/48), and 1.1% in sows aged between 6 and 14 mon (2/91). The prevalence of HEV in piglets and finisher pigs aged \approx 4 mon was significantly higher than in other age groups ($P < 0.01$). Porcine SaV was detected only in piglets (7.7%,

2/26) (Table 1).

By phylogenetic analysis, all 10 HEV strains belonged to genotype 4 (Figure 2). They shared 90% to 99% nucleotide homology and 92% to 94% homology with a human HEV strain (AF103940), which was isolated from the Guangxi Zhuang Autonomous Region, which neighbors Guizhou province (21). The 2 porcine SaVs belonged to SaV GIII and shared 96% nucleotide homology (Figure 3). They clustered with a Brazilian strain (FJ876073), sharing 91% nucleotide homology with it. Yet, they shared a maximum of 84% nucleotide identity with the strain (FJ374683) from eastern China. Notably, they shared 84% nucleotide homology with a human SaV from China (AF439862).

5. Discussion

HEV causes outbreaks in developing countries and sporadic hepatitis in industrialized countries (22, 23). Swine is considered a principal reservoir of the HEV that infects humans in regions of China (6). HEV RNA has been detected in swine stool samples in over 16 provinces and regions of China. In this study, 14 of 209 (6.7%) fecal samples were positive for HEV RNA. Some studies have observed that growing pigs have the highest HEV shedding rates, whereas we noted that piglets had the highest rate. The climate, presence of a river, and water supply and management can affect the prevalence of HEV (24). Therefore, we posited that our results were attributed to the specific climate, location, and sanitary conditions of the farms in our study. The lower overall prevalence of HEV might be due to the sows' previous exposure to HEV and the resulting acquired immunity and lower infection rate.

Additional research should be performed to determine any differences in the characteristics of infection in this mountainous area. Evidence is accumulating that HEV is zoonotic and that pigs are considered a major reservoir of human infection. By phylogenetic analysis of its partial or entire genome, genotype 4 HEV is transmitted freely between humans and swine in eastern China and some provinces of southern China (6, 25). Nevertheless, our analysis of HEV infection in central China revealed no evidence of cross-species transmission between humans and swine (24). In our study, all 10 HEV isolates belonged to genotype 4, clustering with a human strain (AF103940) from an adjacent province contains both Guizhou and Guangxi Zhuang Autonomous Region, which suggests that cross-species transmission between swine and human occurred in this area.

Although genotype 3 HEV was detected in provinces of China, including Shanghai, Henan, Anhui, and Zhejiang, genotype 4 is the principal strain in China (24). All 10 stains belonged to genotype 4, and no genotype 3 strains were detected. Genotype 3 strains are more sensitive than genotype 4 to strict sanitation procedures, such as sterilization of antibiotic-laden environments and the adoption of an all-in/all-out management system. The genotype 3 strains might have been new 'immigrants'

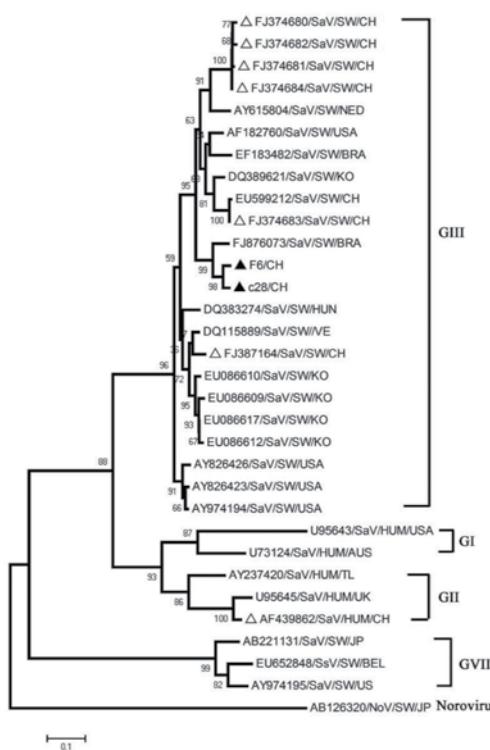


Figure 3. Neighbor-joining tree of the phylogenetic relationship between SaV strains based on a 309-nt fragment of the RNA-dependent RNA polymerase gene. Bootstrap values, expressed as percentages of 1000 replications, are given at the branch points. Genbank accession numbers for the reference strains are marked at each branch points. The 2 newly identified SaV strains (Genbank accession numbers: HQ292717 for C28/CH; HQ292716 for F6/CH) are marked by filled triangles, and other Chinese SaV strains are marked by unfilled triangles.

to China that are unable to survive such unfavorable conditions, resulting in decreased infection compared with better adapted, 'native' HEV genotype 4 strains (25). These hypotheses explain why genotype 4 strains are more prevalent and are emerging as the chief strains in China.

Porcine calicivirus infections have been reported in industrialized and developing countries. SaV and NoV are common in animals, and some of them are genetically closely related to human strains (12, 26). Moreover, more potential recombinant strains of SaV and NoV have been reported recently (12, 27, 28). The detection of SaV and NoV in many countries and various species implicates a potential zoonotic risk of cross-species infections. We reported that porcine SaV and NoV infection exists in pigs in eastern China (9, 29). Two porcine SaV strains were observed in the current study, both of which were isolated from piglets. The overall prevalence rate for SaV was 1.0%, which is similar to that in eastern China (0.9%) but significantly lower than in Brazil (30.1%), Venezuela (17.6%), and the USA (62%) (9, 27-32). NoV infection is seasonal, peaking in winter (from October to April), most notably in February and March (33). Not finding NoV infection in our study might be partially due to this epidemic characteristic of NoV.

Or phylogenetic analysis indicated that the 2 porcine SaV isolates clustered with a Brazilian isolate, suggesting that they have a common genetic origin with the Brazilian strain rather than with Chinese isolates. Moreover, porcine SaVs exist in not only eastern China but also southwestern China, although the infection rate there is low. The relationship between human calicivirus and porcine calicivirus in China is unknown due to the limited number of available sequences, necessitating further research.

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Conflict of interest

None declared.

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References

1. Panda SK, Thakral D, Rehman S. Hepatitis E virus. *Rev Med Virol*. 2007;17(3):151-80.
2. Huang FF, Haqshenas G, Guenette DK, Halbur PG, Schommer SK, Pierson FW, et al. Detection by reverse transcription-PCR and genetic characterization of field isolates of swine hepatitis E virus from pigs in different geographic regions of the United States. *J Clin Microbiol*. 2002;40(4):1326-32.
3. Ijaz S, Arnold E, Banks M, Bendall RP, Cramp ME, Cunningham R, et al. Non-Travel-Associated Hepatitis E in England and Wales: Demographic, Clinical, and Molecular Epidemiological Characteristics. *J Infect Dis*. 2005;192(7):1166-72.
4. Lu L, Li C, Hagedorn CH. Phylogenetic analysis of global hepatitis E virus sequences: genetic diversity, subtypes and zoonosis. *Rev Med Virol*. 2006;16(1):5-36.
5. Takahashi M, Nishizawa T, Okamoto H. Identification of a genotype III swine hepatitis E virus that was isolated from a Japanese pig born in 1990 and that is most closely related to Japanese isolates of human hepatitis E virus. *J Clin Microbiol*. 2003;41(3):1342-3.
6. Zheng Y, Ge S, Zhang J, Guo Q, Ng MH, Wang F, et al. Swine as a Principal Reservoir of Hepatitis E Virus That Infects Humans in Eastern China. *J Infect Dis*. 2006;193(12):1643-9.
7. Scipioni A, Mauroy A, Vinjé J, Thiry E. Animal noroviruses. *Vet J*. 2008;178(1):32-45.
8. Green KY, Ando T, Balayan MS, Berke T, Clarke IN, Estes MK, et al. Taxonomy of the Caliciviruses. *J Infect Dis*. 2000;181(Suppl 2):S322-S30.
9. Shen Q, Zhang W, Yang S, Chen Y, Ning H, Shan T, et al. Molecular detection and prevalence of porcine caliciviruses in eastern China from 2008 to 2009. *Arch Virol*. 2009;154(10):1625-30.
10. Bull RA, Tanaka MM, White PA. Norovirus recombination. *J Gen Virol*. 2007;88(Pt 12):3347-59.
11. Martella V, Decaro N, Lorusso E, Radogna A, Moschidou P, Amorisco F, et al. Genetic heterogeneity and recombination in canine noroviruses. *J Virol*. 2009;83(21):11391-6.
12. Martella V, Lorusso E, Banyai K, Decaro N, Corrente M, Elia G, et al. Identification of a porcine calicivirus related genetically to human sapoviruses. *J Clin Microbiol*. 2008;46(6):1907-13.

13. Muller B, Klemm U, Mas Marques A, Schreier E. Genetic diversity and recombination of murine noroviruses in immunocompromised mice. *Arch Virol*. 2007;152(9):1709-19.
14. Wang QH, Han MG, Cheetham S, Souza M, Funk JA, Saif LJ. Porcine noroviruses related to human noroviruses. *Emerg Infect Dis*. 2005;11(12):1874-81.
15. Wang QH, Han MG, Funk JA, Bowman G, Janies DA, Saif LJ. Genetic diversity and recombination of porcine sapoviruses. *J Clin Microbiol*. 2005;43(12):5963-72.
16. Li RC, Ge SX, Li YP, Zheng YJ, Nong Y, Guo QS, et al. Seroprevalence of hepatitis E virus infection, rural southern People's Republic of China. *Emerg Infect Dis*. 2006;12(11):1682-8.
17. Zhang W, Yang S, Ren L, Shen Q, Cui L, Fan K, et al. Hepatitis E virus infection in central China reveals no evidence of cross-species transmission between human and swine in this area. *PLoS One*. 2009;4(12):e8156.
18. Jinshan, Jirintai, Manglai D, Takahashi M, Nagashima S, Okamoto H. Molecular and serological survey of hepatitis E virus infection among domestic pigs in Inner Mongolia, China. *Arch Virol*. 2010;155(8):1217-26.
19. Cooper K, Huang FF, Batista L, Rayo CD, Bezanilla JC, Toth TE, et al. Identification of genotype 3 hepatitis E virus (HEV) in serum and fecal samples from pigs in Thailand and Mexico, where genotype 1 and 2 HEV strains are prevalent in the respective human populations. *J Clin Microbiol*. 2005;43(4):1684-8.
20. Jiang X, Huang PW, Zhong WM, Farkas T, Cubitt DW, Matson DO. Design and evaluation of a primer pair that detects both Norwalk- and Sapporo-like caliciviruses by RT-PCR. *J Virol Methods*. 1999;83(1-2):145-54.
21. Li K, Zhuang H, Zhu W. Partial nucleotide sequencing of hepatitis E viruses detected in sera of patients with hepatitis E from 14 cities in China. *Chin Med J (Engl)*. 2002;115(7):1058-63.
22. Erker JC, Desai SM, Schlauder GG, Dawson GJ, Mushahwar IK. A hepatitis E virus variant from the United States: molecular characterization and transmission in cynomolgus macaques. *J Gen Virol*. 1999;80 (Pt 3):681-90.
23. Velazquez O, Stetler HC, Avila C, Ornelas G, Alvarez C, Hadler SC, et al. Epidemic transmission of enterically transmitted non-A, non-B hepatitis in Mexico, 1986-1987. *JAMA*. 1990;263(24):3281-5.
24. Zhang W, Shen Q, Mou J, Yang ZB, Yuan CL, Cui L, et al. Cross-species infection of hepatitis E virus in a zoo-like location, including birds. *Epidemiol Infect*. 2008;136(8):1020-6.
25. Li L, Zhu Y, Fu H, Wei X, Wang L, Liang J, et al. Full-genome nucleotide sequence and analysis of a Chinese swine hepatitis E virus isolate of genotype 4 identified in the Guangxi Zhuang autonomous region: evidence of zoonotic risk from swine to human in South China. *Liver Int*. 2009;29(8):1230-40.
26. Guo M, Chang KO, Hardy ME, Zhang Q, Parwani AV, Saif LJ. Molecular characterization of a porcine enteric calicivirus genetically related to Sapporo-like human caliciviruses. *J Virol*. 1999;73(11):9625-31.
27. Nayak MK, Chatterjee D, Nataraju SM, Pativada M, Mitra U, Chatterjee MK, et al. A new variant of Norovirus GII.4/2007 and inter-genotype recombinant strains of NVGII causing acute watery diarrhoea among children in Kolkata, India. *J Clin Virol*. 2009;45(3):223-9.
28. Oka T, Miyashita K, Katayama K, Wakita T, Takeda N. Distinct genotype and antigenicity among genogroup II sapoviruses. *Microbiol Immunol*. 2009;53(7):417-20.
29. Zhang W, Shen Q, Hua X, Cui L, Liu J, Yang S. The first Chinese porcine sapovirus strain that contributed to an outbreak of gastroenteritis in piglets. *J Virol*. 2008;82(16):8239-40.
30. Barry AF, Alfieri AF, Alfieri AA. High genetic diversity in RdRp gene of Brazilian porcine sapovirus strains. *Vet Microbiol*. 2008;131(1-2):185-91.
31. Martinez MA, Alcala AC, Carruyo G, Botero L, Liprandi F, Ludert JE. Molecular detection of porcine enteric caliciviruses in Venezuelan farms. *Vet Microbiol*. 2006;116(1-3):77-84.
32. Wang QH, Souza M, Funk JA, Zhang W, Saif LJ. Prevalence of noroviruses and sapoviruses in swine of various ages determined by reverse transcription-PCR and microwell hybridization assays. *J Clin Microbiol*. 2006;44(6):2057-62.
33. Verhoef L, Depoortere E, Boxman I, Duizer E, van Duynhoven Y, Harris J, et al. Emergence of new norovirus variants on spring cruise ships and prediction of winter epidemics. *Emerg Infect Dis*. 2008;14(2):238-43.