Review

Molecular Genetics of Aichivirus C (Porcine Kobuvirus) in China

Lei Chen, Ling Zhu, Zhi-Wen Xu*, Wan-Zhu Guo

Animal Biotechnology Center, College of Veterinary Medicine of Sichuan Agricultural University, 46# Xinkang Road, Yucheng District, Ya'an, 625014, Sichuan province of P.R. China

Abstract | *Aichivirus C* is a member of the *Kobuvirus* genus within the *Picornaviridae* family and is widely distributed in both healthy and diarrheic pigs from China. Due to the high detection rate in the severe diarrhea in China, *Aichivirus C* is thought to be a potential pathogeny of pig diarrhea. The review represented the discovery of *Aichivirus C* in China, and made a brief summary about molecular and epidemiology characterizations of Chinese *Aichivirus C* strains. Mutiple *Aichivirus C* strains and *Aichivirus C* variants are circulating in China. Recombination events were also observed in Chinese *Aichivirus C* strains. More further studies are needed to clarify the evolutionary features and pathogenicity of *Aichivirus C*.

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***Correspondence** | Zhi-Wen Xu, College of Veterinary Medicine of Sichuan Agricultural University, China; **E-mail** | wzguo@126.com
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Introduction

Members of the *Picornaviridae* family are small, non-enveloped viruses with a linear, single-stranded, and positive-sense RNA genome. This family includes important pathogens of both human and animals and is divided into 17 genera: *Enterovirus, Aphthovirus, Cardiovirus, Hepatovirus, Parechovirus, Erbovirus, Teschovirus, Avihepatovirus, Aquamavirus, Cosavirus, Dicipivirus, Megrivirus, Salivirus, Sapelovirus, Senecavirus, Tremovirus, and Kobuvirus*. The *Kobuvirus* genus contains three officially recognized species: *Aichivirus A* (human *Aichi virus*), *Aichivirus B* (*Bovine kobuvirus*), and *Aichivirus C* (*Porcine kobuvirus*) (ICTV). The RNA genomes of kobuviruses range from 8.2–8.4 kb, which include a 5'UTR, a leader (L) protein, three structural proteins (VP0, VP3, and VP1), seven non-structural proteins (2A, 2B, 2C, 3A, 3B and 3D), a 3'UTR and a poly (A) tail (Reuter et al., 2011). The Kobuvirus has a wide range of host specificity and kobuviruses have been detected in humans, cattle, pigs, sheep, wild boars, bats, dogs, cats, goats and rodents (Yamashita et al., 2003; 1991; Reuter et al., 2008; 2010; 2013; Kapoor et al., 2011; Li et al., 2010; Phan et al., 2011). The *Aichivirus A* was first isolated in Japan from faecal samples of a patient suffering from acute gastroenteritis in 1991 and the *Aichivirus B* was detected in faecal samples from clinically healthy cattle in 2003 in Japan (Yamashita et al., 2003; 1991). The *Aichivirus C* was first reported in faecal samples of pig collected from a Hungarian farm in 2007, and later in 2009. Yu et al. have reported the first detection of *Aichivirus C* in China (Reuter et al., 2008; Yu et al., 2009). Since then, *Aichivirus C* has been detected in Thailand, Spain, Japan, Korea, the United States, Brazil, the Netherlands, and recently in the Czech Republic (Yu et al., 2009; Barry et al., 2011; Ribeiro et al., 2013; Khamrin et al., 2009; Halaihel, et al., 2011; Khamrin et al., 2010; An et al., 2011; Verma et al., 2013; Dufkova et al., 2012).

Researchers from worldwide have done much work on *Aichivirus C* to clarify its epidemic characteristics, distribution, evolutionary features and genome sequences. Among all those currently published reports about *Aichivirus C*, most of which described the infection situations in China. Chinese scholars from different districts have carried out many approaches

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to determine the prevalence and molecular characterizations of *Aichivirus C* in China.

Discovery of *Aichivirus C* in China

When detecting calicivirus in porcine fecal specimens from healthy piglets by reverse transcription-PCR (RT-PCR), an unexpected nonspecific band was observed on agarose gel electrophoresis. Nucleotide sequence of this 1,185bp fragment was determined, and further molecular and phylogenetic analysis revealed that the porcine faecal specimen contained a novel species of Kobuvirus. This was the first detection of *Aichivirus C* in China (Yu et al., 2013).

The complete nucleotide sequence of *Aichivirus C* strain Y-1-CHINA/2008 isolated from China was released by Yu et al. 2009. The isolate Y-1-CHI was 88.62%, 58.66%, and 48.86% identical to those of *Aichivirus C* (strain S-1-HUN), *Aichivirus B* (strain U-1), and *Aichivirus A* (strain A846/88), respectively. Phylogenetic tree revealed that Y-1-CHI and S-1-HUN belong to the same species, *Aichivirus C* (Yu et al., 2011).

Epidemiology of *Aichivirus C* in China

The *Aichivirus C* is widely distributed in China, in both apparently healthy and diarrheic pigs. Up to now, 28/34 (82.4%) first-level administrative subdivision districts have been reported the existence of *Aichivirus C* in porcine intestine and faecal samples (Fig. 1) (Yu et al., 2013; Wang et al., 2012; Chen et al., 2012; Shi et al., 2013; Zhang et al., 2013; Fan et al., 2013; Zhang et al., 2013; Tang et al., 2012; Xiang et al., 2013; Chen et al., 2013).

In different regions, the prevalence rate of *Aichivirus C* ranged from 11.2 to 100% (Table 1) (Yu et al., 2013; Wang et al., 2012; Chen et al., 2012; Shi et al., 2013; Zhang et al., 2013; Fan et al., 2013; Zhang et al., 2013; Tang et al., 2012; Xiang et al., 2013; Chen

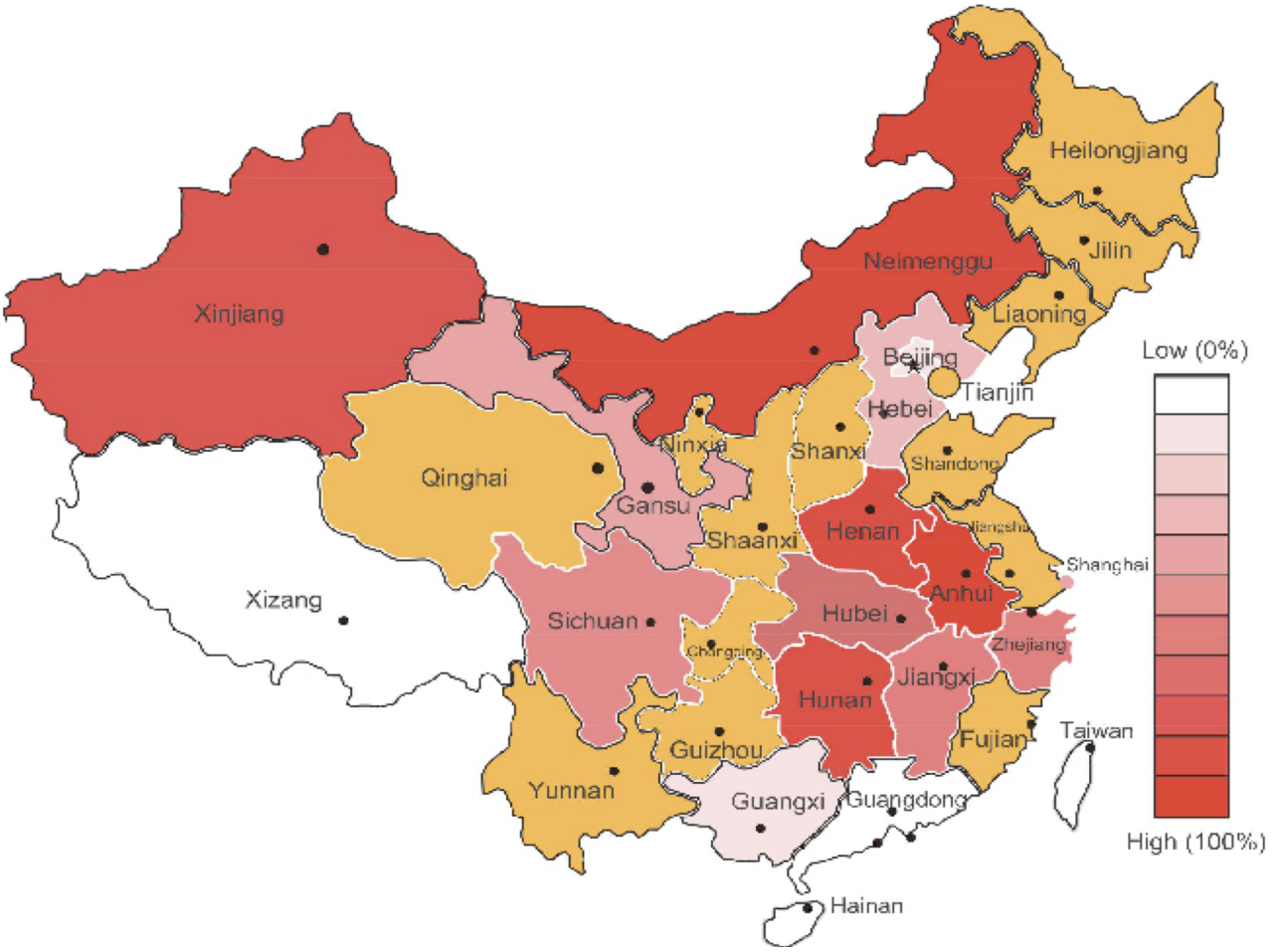

Figure 1: *Distribution of Aichivirus C in China . Different shades of red colour indicate various-total infection rates of Aichivirus C in porcine faeces and intestine samples. Light red to dark red colours are corresponding to 0% to 100% Aichivirus C infection rates. For districts where no literature has been reported, the presence of Aichivirus C is indicated by no colour. Districts in orange indicate Aichivirus C exists in this region, but currently the prevalence rate is unclear.*

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Table 1: *Prevalence rate of Aichivirus C in pigs from different regions of China*

Districts	Infection Rates	Faeces Consistency (Diarrheal or Healthy)
Beijing	11.2% (133/1190)	Healthy
Shanghai Total: 38.8% (45/116)	61.2% (30/49)	Diarrheal
	22.4% (15/67)	Healthy
Hebei	30.12% (97/322)	Healthy
Zhejiang	60% (3/5)	Diarrheal
Anhui	100% (8/8)	Diarrheal
Jiangxi	59.7% (37/62)	Diarrheal
Henan	91.7% (11/12)	Diarrheal
Hubei Total: 66.3% (271/409)	76.8% (262/341)	Diarrheal
	13.2% (9/68)	Healthy
Hunan	88.1% (37/42)	Diarrheal
Sichuan Total: 53.4% (87/163)	64.3% (72/112)	Diarrheal
	29.4% (15/51)	Healthy
Gansu	40.1% (57/142)	Diarrheal
Guangxi	11.8% (2/11)	Diarrheal
Neimenggu	96.2% (2/26)	Unknown
Xinjiang	84.2% (16/19)	Unknown

et al., 2013). Total infection rate of *Aichivirus C* was extremely high in a few provinces: 100% (8/8) in Anhui, 91.7% (11/12) in Henan, 88.1% (37/42) in Hunan, 96.2% (25/26) in Neimenggu, 84.2% (16/19) in Xinjiang. In addition, statistical analysis has concluded that *Aichivirus C* infection was more susceptible to young animals and pigs with diarrhoea (Chen et al., 2013; Wang et al., 2011).

The *Aichivirus C* has been reported in pig serum in Hungary and Brazil (Barry et al., 2011; Reuter et al., 2010). In China, pig serum from Gansu (33.3%, 2 out of 6) and also from Hunan, Hubei and Henan (total infection rate of those there provinces: 6.5%, 6 out of 92) have been tested for infection with *Aichivirus C*. Furthermore, recently, the *Aichivirus C* was detected in sow colostrum samples by Xiang et al. 2013 from IVDC (China Institute of Drug Control): 66.7% (2/3) in Beijing, 80% (4/5) in Xinjiang, and 84.2% (16/19) in Neimenggu (Xiang et a., 2013).

Molecular genetics of *Aichivirus C* in China

There are total 11 *Aichivirus C* complete genome sequences are available in GenBank from China. The complete RNA genomes of *Aichivirus C*, WUH1, Y-1-CHI, SH-W-CHN, K-11/2012/CH and K-4/2012/CH strains, were similar to the sequence of *Aichivirus C* prototype strain S-1-HUN, which consist of 8210 nucleotides (nt) in length excluding the poly(A) tail. The genome sequence of S-1-HUN shared 89.2%, 88.6%, 89%, 86% and 89% identity at the nucleotide level to WUH1, Y-1-CHI, SH-W-CHN, K-11/2012/CH and K-4/2012/CH, respectively (Yu et al., 2011; Wang et al., 2012; Fan et al., 2013; Reuter et al., 2010; Lin et al., 2012). Interestingly, compared to the standard *Aichivirus C* strain S-1-HUN, there were six *Aichivirus C* variants in-China. The CH/HNXX-4/2012 had a 30-amino-acid deletion in the 2B protein and a threonine amino acid insertion in the VP1 gene (Cao et al., 2012). The genome sequence of CH/HZ is 8101 nt (excluding the poly-A tail); an adenine insertion at position 124, an 18-nt deletion in the 5'UTR, and a 90-nt deletion in the 2B coding region were also observed (Shi et al., 2013). The RNA genomes of GS-1/2012/CH and GS-2/2012/CH consist 8121 nt, excluding the poly (A) tail, and they both have a 90-nucleotide deletion in the 2B protein and a single nucleotide insertion in the 3'UTR. Besides, possible 3C/3D cleavage site of GS-1/2012/CH and GS-2/2012/CH is Q/C, while it is Q/S in S-1-HUN genome sequence (Fan et al., 2013). The *Aichivirus C* isolates XX and swK-oV CH441 have genomes of 8147 nt and 8149 nt, respectively, which were shorter than the S-1-HUN (8210 nt).

Partial sequences of *Aichivirus C* 3D gene and VP1

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gene from China were also largely characterized. The 3D RNA-dependent RNA polymerase gene is a relatively conserved region and has been used to determine phylogenetic relationships among kobuviruses (Reuter et al., 2011). The VP1 region is the most exposed and immunodominant portion of the capsid protein, and is the most variable structural protein among all kobuviruses (Reuter et al., 2011; Yamashita et al., 2003). Characterizations of the 3D protein gene and VP1 gene play an important role in the molecular epidemiology and genetic evolution of *Aichivirus C*. Phylogenetic trees based on the partial 3D or VP1 sequences revealed that there were multiple lineages of *Aichivirus C* circulating in China. In addition, Chen et al. 2013 have recently reported co-infection of multiple *Aichivirus C* strains in a single pig, and significant recombination breakpoints observed in the co-infection strains demonstrated that multiple *Aichivirus C* strains in the same pig could have arisen from recombination events (Chen et al., 3013). Wang et al. 2012 indicated that early recombination events might have facilitated the genome of SH-W-CHN (Wang et al., 2012). Significant recombination signals were also detected in GS-1/2012/CH [27]. Amino acid mutations were observed in both structural proteins and non-structural proteins. Mutations and recombination events may have contributed to the genetic diversity of *Aichivirus C* and act as a driving force for the evolution of virus genomes. However, such finding requires further investigations.

Discussion and Summary

Since the end of 2010, massive outbreaks of diarrhoea have occurred in suckling piglets in China; however, the etiological agent has yet to be determined. Affected pigs exhibited signs of watery diarrhoea, dehydration, and vomiting with morbidity ranging 80–100% and mortality between 50–90%. High detection rate of *Aichivirus C* in those porcine diarrhoea samples made researchers suspect that *Aichivirus C* may be a causative agent of the diarrhoea or has atleast contributed to aggravate the disease.

Failure of culturing *Aichivirus C* in vitro has hindered the research about its pathogenicity. Considering *Aichivirus C* is also widely existent in healthy swinery, most people probably would ignore its importance. Less intentions hase been paid to these asymptomatic infections, providing suitable conditions for evolution and persisting of *Aichivirus C* in pig hosts. Recombination events and variants are common in Chinese *Aichivirus C* group. Recombination and mutation possibly could convert *Aichivirus C* to a potential causative agent. We could not exclude the possibility that *Aichivirus C* variants generated through recombination or other evolutionary forces are associated with the large-scale outbreak of severe diarrhoea in suckling piglets in China. Further characterization and epidemiological studies are required to determine the exact role of *Aichivirus C* variants in swine disease. We hope data about *Aichivirus C* from China would facilitate its evolutionary researches and pathogenicity study progress in near future.

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