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Short communication

Detection of three novel atypical porcine pestivirus strains in newborn piglets with congenital tremor in southern China

Xin Zhang[a,1], Rui Dai[a,1], Qunhui Li[b], Qingfeng Zhou[b], Yangyang Luo[b], Limiao Lin[b], Yingzuo Bi[a], Feng Chen[a,*]

[a] College of Animal Science, South China Agricultural University & Guangdong Provincial Key Lab of Agro-Animal Genomics and Molecular Breeding, Guangzhou 510642, PR China
[b] Guangdong Wen's Foodstuffs Group Co. Ltd., Yunfu 527439, PR China

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ABSTRACT

Atypical porcine pestivirus (APPV) have been discovered in swine herds from three provinces in China, suggesting a wide distribution in China. This study reports the occurrence of three novel APPV strains in China. They were detected from newborn piglets with clinical signs of congenital tremors (CT) in Guangdong Province, China. The complete genomic sequences of three novel APPV strains exhibited only 80.5%–84.1% nucleotide sequences homology with other APPV reference sequences available in GenBank. Phylogenetic analysis showed that these novel APPV strains formed independent branch from the American, German, Netherlandish, Australian and other Chinese strains. These results will help us better understand the epidemiology and genetic characteristics of APPV in China.

1. Introduction

Congenital tremor (CT) commonly occurred in newborn piglets, and characterized by tremors of the head and limbs that vary in severity. Furthermore, the tremor was associated with variable degrees of hypomyelination in the brain and spinal cord (de Groof et al., 2016). Subsequent studies suggested that atypical porcine pestivirus(APPV) can cause CT in piglets (Arruda et al., 2016; de Groof et al., 2016; Schwarz et al., 2017). After the first report in the United States (Hause et al., 2015), APPVs were also detected in many countries, including Spain, Germany, the Netherlands, Brazil, Austria and China (Beer et al., 2017; de Groof et al., 2016; Mosena et al., 2018; Munoz-Gonzalez et al., 2017; Schwarz et al., 2017; Shen et al., 2018; Zhang et al., 2017). Therefore, APPV infection has become a worldwide distribution. Here, three novel APPV strains from newborn piglets with CT in China were characterized.

2. Materials and methods

Thirty tissue samples including brains, lungs, spleens and lymph nodes of suspected APPV infected newborn piglets exhibiting clinical signs of CT were collected from three separate swine herds in southern China, Guangdong Provinces from November 2017 to March 2018. The morbidity of CT in newborn piglets from three herds is 5.9%, 6.0% and 6.3%, respectively. The CT-affected piglets showed a severe lateral shaking and were often incapable of sucking milk, which led to elevated preweaning death rates. None were more than 3 days old and all samples were collected from different animals. All tissue samples were routinely processed and stored at −80 °C for subsequent analysis. Ten pairs of primer (Table 1) targeting various fragments of nearly full-length gene for RT-PCR were designed with Primer software (Molecular Biology Insights Inc., Cascade, CO, USA) based on the available APPV genome sequences in GenBank database. The total RNA was extracted from these tissue samples using the AxyPrep Viral DNA/RNA Miniprep Kit (Axygen Biotechnology, Hangzhou, China) following manufacturer's instructions. RT-PCR amplification for APPV full-length genomes sequences were performed using ten primers. The PCR products were purified by a Gel Band Purification Kit (Omega Bio-Tek, USA) and then cloned into a pMD-19 T vector (TaKaRa Bio Company, USA) using the In-fusion PCR Cloning Kit (TaKaRa Bio Company, USA). The positive clones were sequenced on an ABI 3730 capillary DNA-sequencing instrument. Then, the APPV GD-MH01–2018 GD-DH01–2018 and GD-BZ01–2018 strains genome sequences were assembled in Lasergene SeqMan Program (DNASTAR, USA). Phylogenetic trees were

* Corresponding author at: College of Animal Science, South China Agricultural University, 483 Wushan Road, Tianhe District, Guangzhou 510642, PR China.
E-mail address: fengch@scau.edu.cn (F. Chen).
[1] These authors contributed equally to this work.

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Table 1
Primers targeting various fragments of nearly full-length APPV gene for RT-PCR.

Primer	Sequence (5′-3′)	Target
APPV-1-fw	TAACCAGGCCTCTAGTACCACA	1–22
APPV-1-rev	CACCACAGGATAGCTCCGAT	2328–2347
APPV-2-fw	GTGGCCTACACAGATTTGGAT	2282–2302
APPV-2-rev	GCCAGTTAATGTCAGTGCTGT	3488–3508
APPV-3-fw	CTTAGGTTAGTCGGAGGTAGTG	3281–3302
APPV-3-rev	GTCAATGGTGAGGTCTCCA	4202–4220
APPV-4-fw	TGCAAAGAAGAATGACTC	3919–3936
APPV-4-rev	AACACCATTGAGTACCTG	4939–4956
APPV-5-fw	GGGTGGTCYGGAYTACCRATAA	4737–4758
APPV-5-rev	CTTTCYAGGATTTGCTTCTC	6018–6037
APPV-6-fw	GTKGAAGTGAGAATGYTGGAGAG	5958–5980
APPV-6-rev	CCTTCCAGCAGTGAGAGGATTA	7335–7356
APPV-7-fw	ATGGAATCAGCAGTGGTGGT	7129–7148
APPV-7-rev	TTCCGATGGTGGCATTCATAA	8458–8478
APPV-8-fw	TATCCCTTAGGTCGTGCGC	8083–8101
APPV-8-rev	TCAGAGTAGGTTACTTCCTCGTATGTG	9653–9679
APPV-9-fw	ATGGACAAAYATGAAAATGAGCAAGTC	9564–9590
APPV-9-rev	ACTCCTATCCCTGGRTCATA	11,028–11,047
APPV-10-fw	TATCCCGGATGAACTACAGCATGACT	10,879–10,904
APPV-10-rev	GGATGCATGCATGCCTGCAGGTCG	11,468–11,491

Primer design based on the consensus sequence of available APPV genome sequences in GenBank database (KY475593.1, KX95076.1, KX950762.1, KY612413.1, KY652092.1, MH102210.1, KY475592.1, KY624591.1, KR011347.1, MF167290.1, KU041639.1).

constructed based on the available complete genome in GenBank with MEGA 7.0 software using neighbor-joining analysis with 1000 bootstrap replicates to calculate pairwise distances for providing confidences to the clustering.

3. Results and discussion

Atypical porcine pestivirus is a novel and genetically very distinct pestivirus associated with congenital tremors which make ambulation challenging, and subsequently, piglets have difficulty nursing. Up to date, the prevalence of APPV-infected CT in newborn piglets in China have been reported by multiple articles, and APPV genomes have been identified in swine herds from Guangdong, Guangxi and Guizhou provinces of China (Shen et al., 2018; Yuan et al., 2017; Zhang et al., 2018; Zhang et al., 2017). Some commercial pig farm in China occurred CT with up to 50% of morbidity during epidemically period (Shen et al., 2018). We came to know that clinical signs of CT were long-term presence in these swine herds in that time period, by consulting with the veterinarians in those three pig farms. Hence, APPV prevalence may cause extensive economic losses in China.

Table 2
Nucleotide sequence homologies of complete genomic sequences based on GD-MH01–2018 GD-DH01–2018 and GD-BZ01–2018 strains and reference sequences available in GenBank.

	GD-MH01–2018	GD-DH01–2018	GD-BZ01–2018
KY475593.1_China_GD-SD_2016	80.8	82.3	83.9
KX950762.1_China_GD2	80.8	83.0	84.1
KX950761.1_China_GD1	80.8	83.0	84.0
KY612413.1_China_GD3	80.8	82.9	84.0
KX929062.1_Netherlands_NL1	82.0	82.0	82.5
KX778724.1_Austria_AUT-2016_C	81.7	82.0	82.3
KU194229.1_USA_ISDVDL2014016573	81.5	81.6	82.1
MG792803.1_China_JX-JM01–2018A01	81.2	81.4	82.1
LT594521.1_Germany_APPV_GER_01	82.1	82.2	82.3
KY652092.1_China_Porcine_pestivirus_1	81.3	81.5	82.2
MH102210.1_China_GX04/2017	81.0	81.2	81.9
KY475592.1_China_GZ01_2016	81.3	81.5	82.2
KY624591.1_China_GD	81.3	81.7	82.1
KR011347.1_USA_000515	81.5	81.5	82.1
MF167291.1_Germany_Ger-NRW_L277	81.9	82.1	82.5
MF167290.1_Germany_Ger-NRW_CT-59	82.0	82.1	82.5
KU041639.1_Germany_Bavaria_S5/9	81.6	81.9	82.0
MF167292.1_Germany_CN-CQ_11/39	80.5	80.6	81.3
GD-MH01–2018	–	97.5	95.3
GD-DH01–2018	–	–	94.0
GD-BZ01–2018	–	–	–

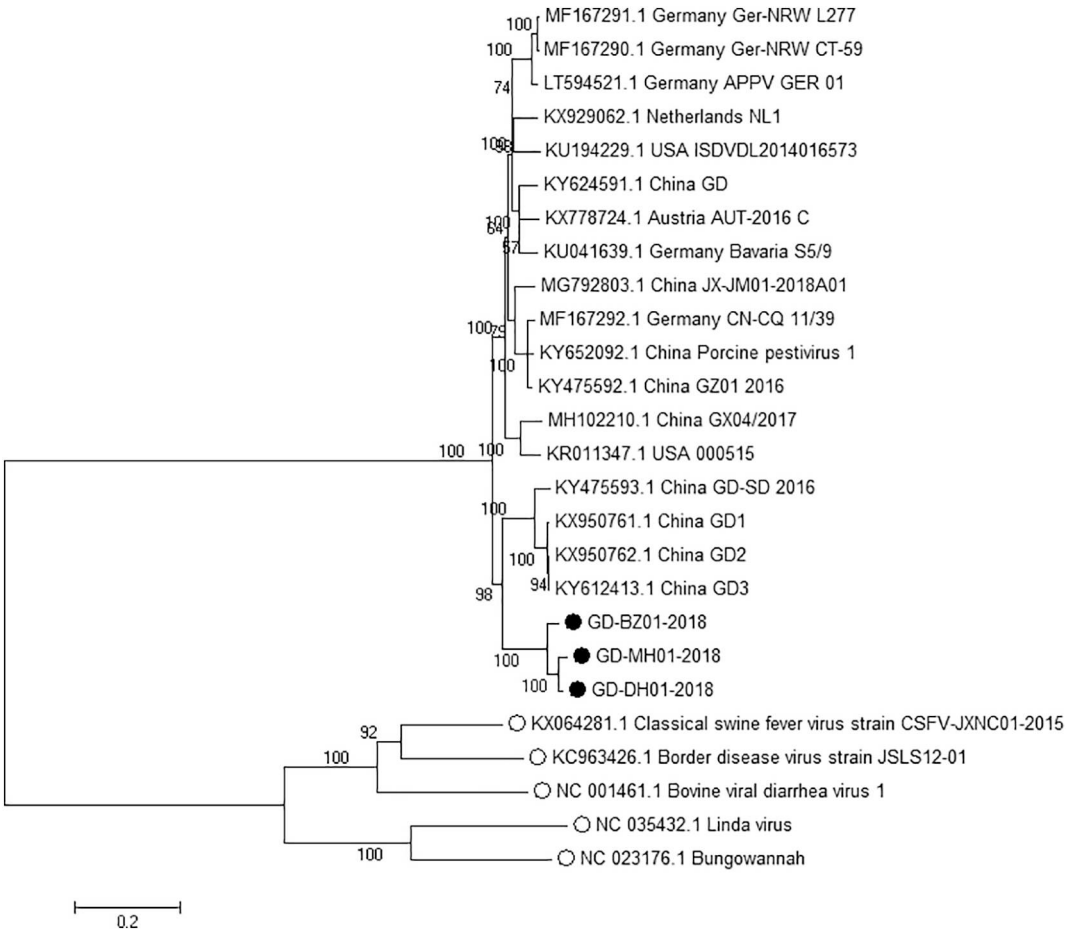

Fig. 1. Phylogenetic reconstruction based on polyprotein sequence of pestivirus species. ● indicates the GD-MH01–2018 GD-DH01–2018 and GD-BZ01–2018 strains described in this study.

In this study, three novel atypical porcine pestivirus strains, GD-MH01–2018 GD-DH01–2018 and GD-BZ01–2018, were identified from three separate swine herds in China. The nearly complete genome sequences of three novel APPV strains were determined by reverse transcription-PCR (RT-PCR) using ten pairs of primers and deposited in GenBank under the accession no. MH493894 to MH493896. The genome of GD-MH01–2018 GD-DH01–2018 and GD-BZ01–2018 include 11,491 nucleotides in length and encode a polyprotein consisted of the 3636 amino acids, which is consistent with other APPV strains (Hause et al., 2015). To examine the molecular characteristics of these three novel APPV genomes, nucleotide sequence identity analysis was performed using representative APPV sequences. Sequence analysis showed that the ORF sequences of these three novel APPV strains were found to share 94.0%–97.5% nucleotide sequence identities with each other. However, they shared only 80.5% –84.1% nucleotide sequence homologies with those of the virus strains from Germany, the USA, the Netherlands Austria and China (Table 2). These results indicated that the three sequences obtained in this study were distant to other APPV reference sequences available in GenBank. Therefore, APPV has a high variability in China.

To determine the relationship between the three sequences obtained in this study and previously published APPV strains, phylogenetic analysis was performed. Phylogenetic analysis based on the ORF sequences indicated these novel APPV strains formed independent branch from the American, German, Netherlandish, Australian and other Chinese strains (Fig. 1). The E2 protein is immunodominant and possesses neutralization epitopes and consequently is the pestivirus protein that exhibits the greatest amount of diversity (Ridpath and Bolin, 1997). Therefore, a phylogenetic tree for the full-length E2 gene of APPV was also constructed to address the evolutionary relationship for different APPV strains. Similar to the complete sequences, the full-length E2 gene of these novel APPV strains maintained a high genetic distance with other APPV isolates available in GenBank (Fig. 2). These results indicated the high genetic diversity of APPV, not being able to cluster this virus according to the geographic region.

In summary, this study reports three novel atypical porcine pestivirus strains were detected in newborn piglets with congenital tremor in Guangdong province, China. The sequences obtained in this study were distant to the American, German, Netherlandish, Australian and other Chinese strains. These data demonstrated APPV exhibited the high level of genetic variability when comparing available APPV sequences. Therefore, the genome information of this study will help in analyses of the epidemiology and evolutionary characteristics of APPV in swine herds in China.

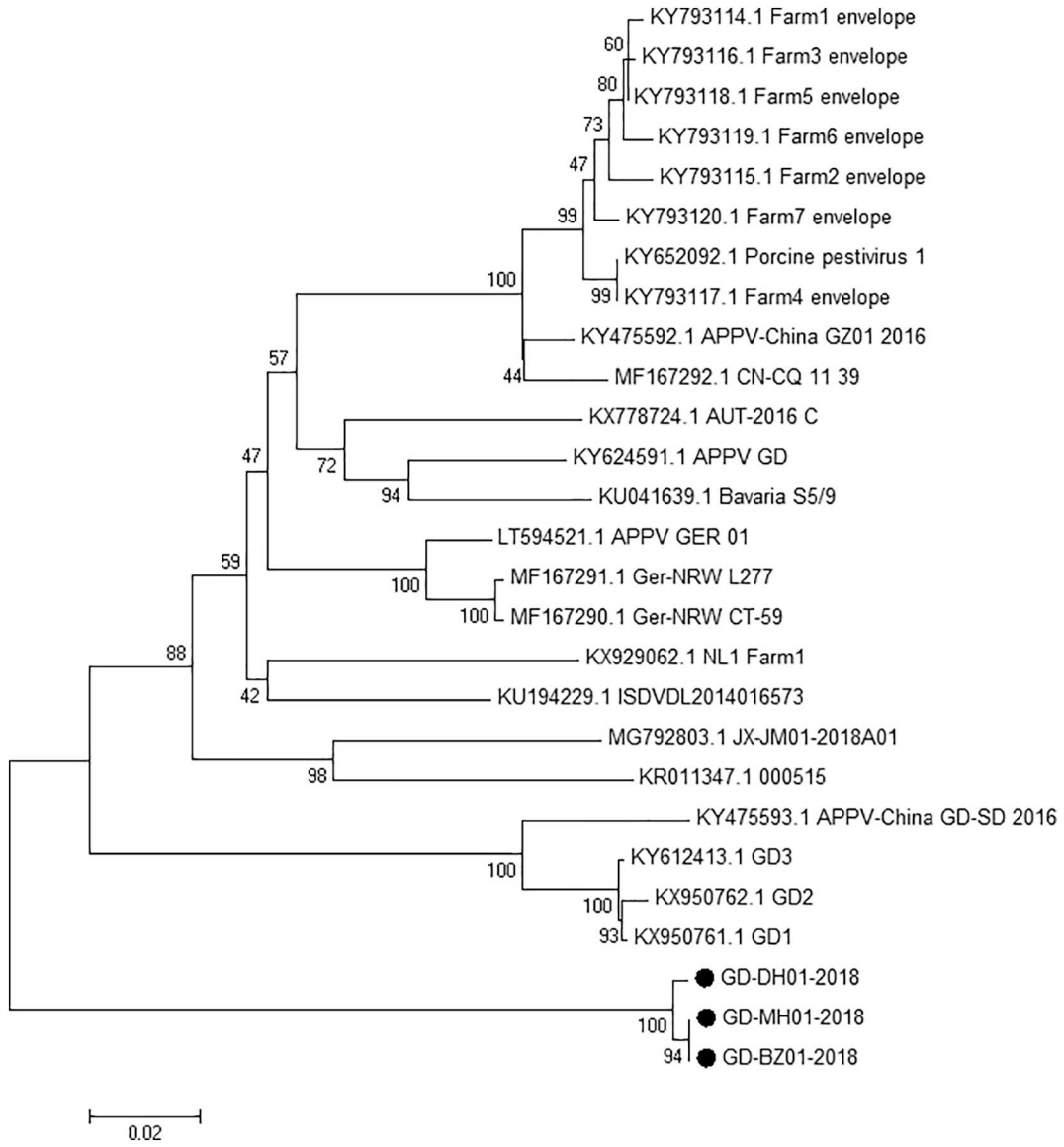

Fig. 2. Phylogenetic tree of E2. ● indicates the E2 genes of GD-MH01–2018 GD-DH01–2018 and GD-BZ01–2018 strains described in this study.

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References

Arruda, B.L., Arruda, P.H., Magstadt, D.R., Schwartz, K.J., Dohlman, T., Schleining, J.A., Patterson, A.R., Visek, C.A., Victoria, J.G., 2016. Identification of a Divergent Lineage Porcine Pestivirus in Nursing Piglets with Congenital Tremors and Reproduction of Disease following Experimental Inoculation. PLoS One 11, e0150104.

Beer, M., Wernike, K., Drager, C., Hoper, D., Pohlmann, A., Bergermann, C., Schroder, C., Klinkhammer, S., Blome, S., Hoffmann, B., 2017. High Prevalence of Highly Variable Atypical Porcine Pestiviruses Found in Germany. Transbound. Emerg. Dis. 64, e22–e26.

de Groof, A., Deijs, M., Guelen, L., van Grinsven, L., van Os-Galdos, L., Vogels, W., Derks, C., Cruijsen, T., Geurts, V., Vrijenhoek, M., Suijskens, J., van Doorn, P., van Leengoed, L., Schrier, C., van der Hoek, L., 2016. Atypical Porcine Pestivirus: a possible Cause of Congenital Tremor Type A-II in Newborn Piglets. Viruses 8.

Hause, B.M., Collin, E.A., Peddireddi, L., Yuan, F., Chen, Z., Hesse, R.A., Gauger, P.C., Clement, T., Fang, Y., Anderson, G., 2015. Discovery of a novel putative atypical porcine pestivirus in pigs in the USA. The Journal of general virology 96, 2994–2998.

Mosena, A.C.S., Weber, M.N., da Cruz, R.A.S., Cibulski, S.P., da Silva, M.S., Puhl, D.E., Hammerschmitt, M.E., Takeuti, K.L., Driemeier, D., de Barcellos, D., Canal, C.W., 2018. Presence of atypical porcine pestivirus (APPV) in Brazilian pigs. Transbound. Emerg. Dis. 65, 22–26.

Munoz-Gonzalez, S., Canturri, A., Perez-Simo, M., Bohorquez, J.A., Rosell, R., Cabezon, O., Segales, J., Domingo, M., Ganges, L., 2017. First report of the novel atypical porcine pestivirus in Spain and a retrospective study. Transbound. Emerg. Dis. 64, 1645–1649.

Ridpath, J.F., Bolin, S.R., 1997. Comparison of the complete genomic sequence of the border disease virus, BD31, to other pestiviruses. Virus Res. 50, 237–243.

Schwarz, L., Riedel, C., Hogler, S., Sinn, L.J., Voglmayr, T., Wochtl, B., Dinhopl, N., Rebel-Bauder, B., Weissenbock, H., Ladinig, A., Rumenapf, T., Lamp, B., 2017. Congenital infection with atypical porcine pestivirus (APPV) is associated with disease and viral persistence. Vet. Res. 48 (1).

Shen, H., Liu, X., Zhang, P., Wang, L., Liu, Y., Zhang, L., Liang, P., Song, C., 2018. Identification and characterization of atypical porcine pestivirus genomes in newborn piglets with congenital tremor in China. J. Vet. Sci. 19, 468–471.

Yuan, J., Han, Z., Li, J., Huang, Y., Yang, J., Ding, H., Zhang, J., Zhu, M., Zhang, Y., Liao, J., Zhao, M., Chen, J., 2017. Atypical Porcine Pestivirus as a Novel Type of Pestivirus in Pigs in China. Front. Microbiol. 8, 862.

Zhang, K., Wu, K., Liu, J., Ge, S., Xiao, Y., Shang, Y., Ning, Z., 2017. Identification of atypical porcine pestivirus infection in swine herds in China. Transbound. Emerg. Dis. 64, 1020–1023.

Zhang, H., Wen, W., Hao, G., Hu, Y., Chen, H., Qian, P., Li, X., 2018. Phylogenetic and genomic characterization of a novel atypical porcine pestivirus in China. Transbound. Emerg. Dis. 65, e202–e204.