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Detection and genetic characterization of porcine pegivirus from pigs in China

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Abstract

Porcine pegiviruses (PPgV) have been first discovered in serum samples from domestic pigs in Germany in 2016 and then in the USA in 2018. To date, there is no documentation with respect to the presence of PPgVs in domestic pigs in China. Herein, we attempted to determine the presence and prevalence of PPgV in China and its genetic characterization. In this study, 469 sera were tested and 34 (7.25%) were positive for PPgV. An ascending trend of the positive rate for PPgV was observed from suckling piglets (1.61%) to nursing piglets (1.85%), finishing pigs (6.56%), and sows (11.34%). The complete genome sequence of a representative strain of PPgV, PPgV_GDCH2017, and the complete E2 gene of 17 PPgV isolates discovered in this study was determined. Sequence analysis indicated that PPgV_GDCH2017 was highly related to other PPgVs with nucleotide and amino acid identities ranging from 87.3 to 97.4% and 94.6–99.3%, respectively, in the complete coding region. Phylogenetic analyses demonstrated that the PPgV_GDCH2017 discovered in this study was closely related to the PPgVs from the USA and clustered in the same genus with pegiviruses from other hosts. The topology of the phylogenetic tree based on the complete E2 gene was consistent with that based on the complete genome of PPgV. Further studies on pathogenicity and pathogenesis of PPgVs are needed.

Keywords Porcine pegivirus · Detection · Genetic characterization · Phylogenetic analysis

Pegivirus is a member in the *Pegivirus* genus, a recently approved genus in the family *Flaviviridae* [1]. Pegiviruses widely infect human populations [2]. Besides, pegivirus can infect a broad range of hosts, including primates [3, 4], bats [5], equines[6, 7], and rodents [8]. Porcine pegiviruses (PPgV) have been first discovered in serum samples from domestic pigs with a detection rate of 2.2% in Germany in 2016 [9] and then identified at a rate of 15.1% tested blood samples in pigs in the USA in 2018 [10]. The infections of pegiviruses might cause clinical diseases. For example, the equine pegivirus was reported to be associated with Theiler's disease [6]. Human pegivirus infection is predominantly asymptomatic, but some studies have shown an association with the occurrence of non-Hodgkin's lymphoma[11]. The pathogenicity of porcine pegiviruses is unclear, and no obvious clinical signs were observed in pigs attributable to PPgV infection [9, 10]. To date, there is no documentation with respect to the presence of PPgVs in domestic pigs in China. Hence, we attempted to determine the presence of PPgV and its genetic characterization.

A total of 469 blood samples from sows, suckling piglets, nursing piglets, and finishing pigs were collected in ten pig farms in Jiangxi and Guangdong province in China during October 2017 to January 2018 (Table 1). The total RNA of serum samples was extracted using RNAiso Plus (TaKaRa, Dalian, China) following the manufacturer's instructions. For PPgV identification, a nested reverse transcription-polymerase chain reaction (nested RT-PCR) was established with the primers (5′-outer primer: 5′-CCGTTCTATGGCCAC

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Table 1 Prevalence of porcine pegivirus in China

Province	District	Farm	Pig no., positive no., positive rate			
			Sow	Suckling piglet	Nursing pig	Finishing pig
Guangdong	Zhaoqing	A	24, 4, 16.67%	24, 0, 0	24, 0, 0	21, 2, 9.52%
Jiangxi	Ji'an	B	27, 7, 25.93%	13, 0, 0	5, 0, 0	5, 0, 0
	Ji'an	C	NS	NS	2, 0, 0	NS
	Yichun	D	30, 3, 10.00%	5, 0, 0	7, 0, 0	9, 0, 0
	Fuzhou	E	40, 3, 7.50%	20, 1, 5.00%	20, 0, 0	15, 1, 6.67%
	Ji'an	F	30, 0, 0	NS	NS	NS
	Ganzhou	G	30, 3, 10.00%	NS	30, 1, 3.33%	11, 1, 9.09%
	Ganzhou	H	17, 3, 17.64%	NS	NS	NS
	Ganzhou	I	NS	NS	20, 1, 5.00%	NS
	Ganzhou	J	40, 4, 10.00%	NS	NS	NS
		Total	238, 27, 11.34%	62, 1, 1.61%	108, 2, 1.85%	61, 4, 6.56%

NS no sample collected

AAGTT-3′, 3′-outer primer: 5′-TATAGGGGCACTCAC CGAAC-3′; 5′-inner primer 5′-CGCCATCTCATCTTC).

TGTCA-3′, and 3′-inner primer: (5′-CGTCAATTGTTG GTGTGAGG-3′) designed based on the conserved region of the genome sequence of PPgVs available in GenBank (Accession No. KU351669). Amplicons were confirmed by direct sequencing. For the determination of the full-length genome sequence of PPgV, 12 pairs of primers covering the full genome of PPgV and two sets of primers for rapid amplification cDNA ends (5′- and 3′-ends) were designed (Table S1). The complete E2 gene of PPgV was amplified by the primer pair of 2F and 2R as shown in Table S1. The sequences of each fragment hitting PPgV during BLAST search were assembled and annotated by importing into SeqMan in DNAStar *Lasergene* v 7.10 (DNAStar, Inc., Madison, WI, USA). The viral structural proteins and non-structural proteins (NS) were predicted based on the amino-terminal portion of the polyprotein by cellular signal peptidases or signal peptide peptidase as reported previously [1, 8]. Potential N-linked glycosylation sites of structural proteins were analyzed by the online software of NetNGlyc 1.0 Server. The predicted amino acid (aa) sequences of the proteins were put into Pfam database (http://pfam.xfam.org) for homology analysis with other pegiviruses and/or members in the Family *Flaviviridae* [12]. Afterwards, phylogenetic trees were generated by neighbor-joining method with a Bootstrap value of 1000 replicates using MEGA 6 program [13].

For PPgV identification, a size of 250-bp sequence was obtained from serum samples, which shared a 99% nucleotide (nt) identity to PPgV when searched against GenBank with BLAST. Of 469 serum samples tested, 34 were positive for PPgV, an average positive rate of 7.25%, which indicated that the frequency of PPgV in pigs in China was higher than that (2.2%) in pigs in Germany but lower than that (15.1%) in pigs in USA [9, 10]. The infection rates were variable among these farms tested (Table 1). Yang et al. have found that PPgV could infect all ages of pigs [10]. Similar situations were noted in this study. An ascending trend of the positive rate for PPgV was observed from suckling piglets (1.61%) to nursing piglets (1.85%), finishing pigs (6.56%), and sows (11.34%). As shown in Table 1, the sows had the highest PPgV infection rate than that of the other three growing stages of pigs.

The complete genome sequence of PPgV_GDCH2017 (accession no. MG874672) determined in this study was 9756 nt in length excluding the poly A tail. Like the other members of the family *Flaviviridae*, it contained a large ORF encoding a 2972-aa putative multifunctional polyprotein. The predict 5′- and 3′-UTR was 613 nt and 224 nt in length, respectively. Based on the cleavage sites for cellular signal peptidases and viral proteases, which was just similar to that of the members of other strains of pegivirus, the polyprotein of PPgV_GDCH2017 was organized as NH2-envelope (E) 1-E2-protein X-nonstructural (NS) 2-NS3-NS4A-NS4B-NS5A-NS5B-COOH (Fig. 1A). In accordance with bat pegivirus (BPgV) [1] and equine pegivirus (EPgV) [7], PPgV polyprotein had four potential signalase cleavage sites at residues 22, 192, 537, and 753, respectively. PPgV encoded several short proteins containing multiply basic aa at the N'-terminus, followed by two envelope glycoproteins E1 and E2, which was homologous to GBV-C envelope (Accession No. in pfam: PF12786). The next 216 aa showed no homology to PF12786, but was predicted to be the X protein represented in BPgV and EPgV. N-linked glycosylation analysis indicated three structural proteins E1, E2, and X contained 1, 6, and 3 predicted glycosylation sites, respectively. NS2 (PF01538) was located to immediate downstream of putative X protein, and the NS2-NS3 cleavage site was relatively conserved among the identified pegiviruses (Fig. 1a). NS3 harbored motifs common to viral helicase in the family *Flaviviridae* (peptidase S29, PF02907; DEAD domain, PF07652). Meanwhile, the

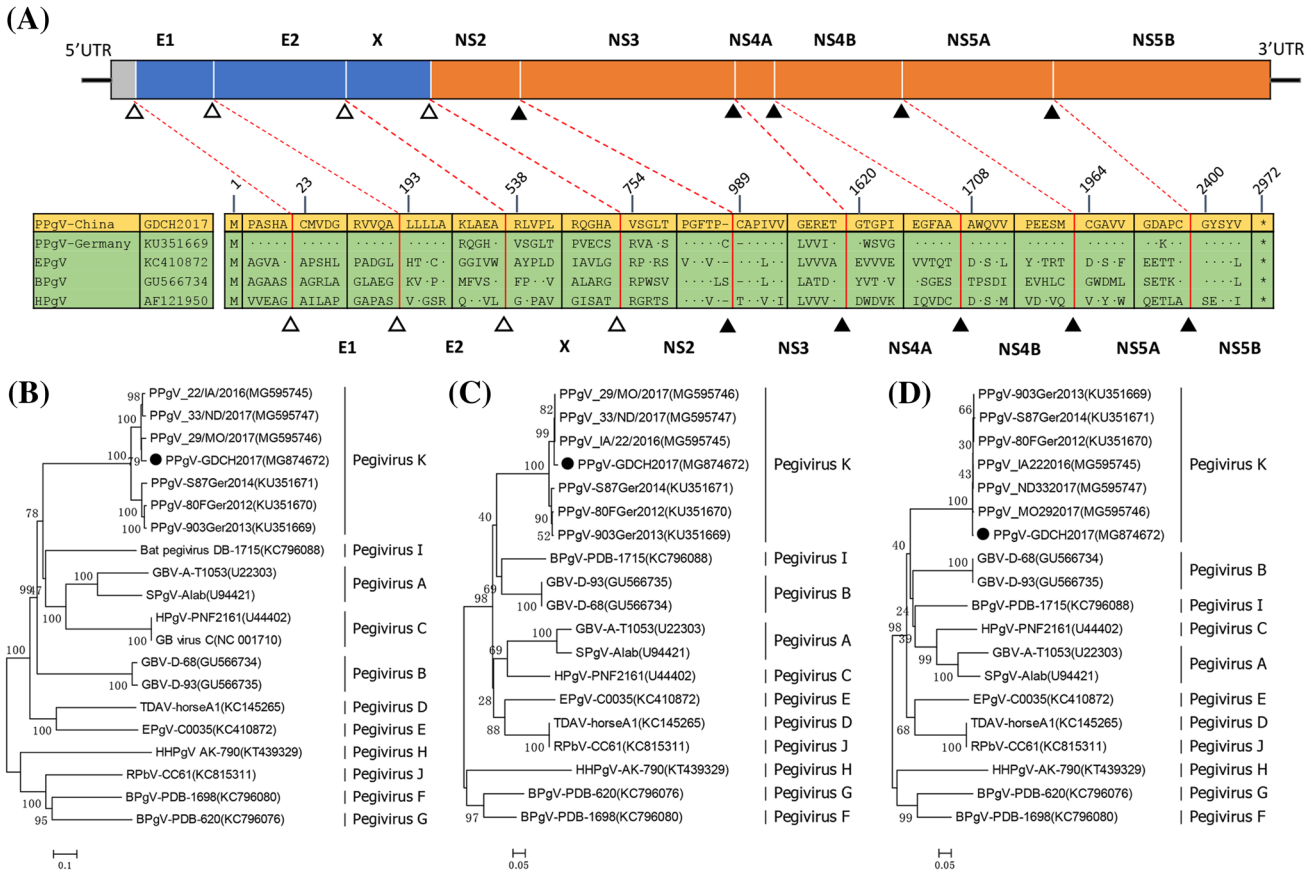

Fig. 1 Genome diagram and the predicted signalase cleavage sites of pegiviruses (**a**) and phylogenetic trees of pegiviruses based on the nucleotide of the complete coding region (**b**), and amino acid sequences of RdRp (**c**) and helicase (**d**). Vertical arrows in part A indicate possible N-linked glycosylation sites (N×[S/T]); black triangles, predicted signalase cleavage sites; and triangles proposed cleavage sites of autocatalytic S2–NS3 proteinase

conserved residues compatible with other *Flaviviruses* and *Hepaciviruses* were presented in NS4A (PF01006), NS4B (PF01001), NS5A (zinc finger domain PF08300; domain 1b, PF08301), and NS5B (RdRp, PF00998).

Sequence homology analysis indicated that PPgV_GDCH2017 was highly related to other PPgVs with nt and aa identities ranging from 87.3 to 97.4% and 94.6–99.3%, respectively, in the complete coding region. This Chinese PPgV strain shared 95.3–97.4% nt identities with US PPgV strains, and 87.3–89.7% nt identities with German PPgV strains. Phylogenetic analyses of the complete coding region of pegiviruses (pegivirus A–K) also showed that the Chinese PPgV strain was clustered in a branch with three US PPgV strains (Fig. 1b). Phylogenetic trees based on the amino acid sequences of RNA-dependent RNA polymerase (RdRp) and helicase, coded by NS5B and NS3, were topologically similar with that on the complete coding region (Fig. 1c, d).

When compared with pegiviruses from other hosts, the nt (aa) identities of PPgV_GDCH2017 ranged from 53.9 to 61.8% (46.7–55.8%), 42.8–59.6% (41.1–53.7%), 60.1–60.6% (52.9–55.7%), 43.3% (41.9%), and 46.9–50.4% (46.7–51.1%) in the complete coding region of human pegivirus (HPgV), bat pegivirus (BPgV), new world primate pegivirus (SPgV), rodent pegivirus (RPgV) and EPgV, respectively (Table S2). We also analyzed the phylogenetic relationships of PPgVs, pegiviruses from other hosts (HPgV, BPgV, SPgV, RPgV, and EPgV), and viruses in other three genera (*Flavivirus, Hepacivirus*, and *Pestivirus*) in the family *Flaviviridae* (Fig. S1A). All of the pegiviruses formed a separate cluster which was distant from the members from genera *Flavivirus, Hepacivirus*, and *Pestivirus*. Phylogenetic trees on the amino acid sequences of RdRp and helicase, coded by NS5B and NS3, were topologically similar with that on complete coding region (Fig. S1B). Analyses of conserved amino acid sequences of RdRp and helicase were introduced to elucidate the evolutionary relationships between PPgV and other members in the family *Flaviviridae* based on the methodology guided by Koonin et al. [16, 17]. Similar to the previous studies [1], eight motifs were identified in RdRp based on the enzymic function of amino acid sequence. Of the amino acid sequences in the eight motifs, 15 amino acids were found conserved in all members in four genera in the

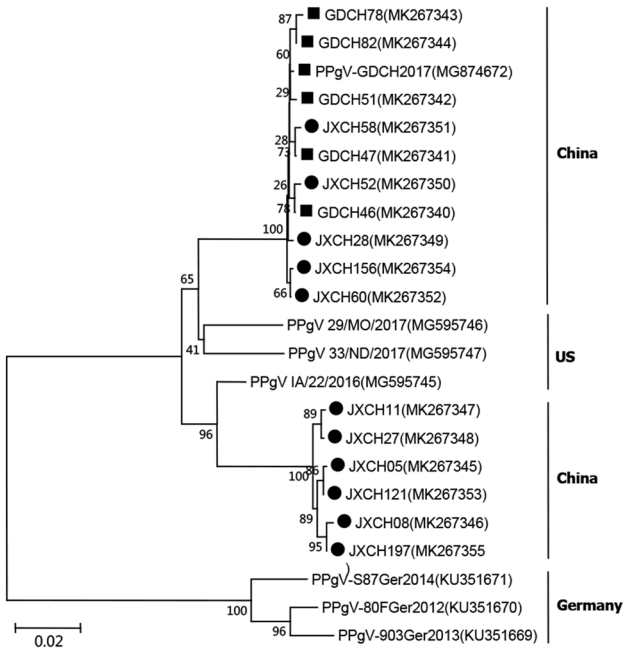

Fig. 2 Neighbor-joining phylogenetic tree of full-length E2 gene sequences of PPgV strains from China compared with global strains. Circle and square denote Chinese strains detected from Jiangxi and Guangdong province, respectively

family *Flaviviridae* (Fig. S2). The helicase contains seven motifs, and 19 amino acids were found conserved in the members in the family *Flaviviridae* (Fig. S3). To investigate the genetic diversity of PPgVs, the complete E2 gene of 17 strains of PPgV isolated from Guangdong and Jiangxi province, China, was determined and characterized. Similarity of E2 gene among Chinese PPgV isolates ranged from 92.1 to 99.9% at the nt level, and 96.8–100% at the aa level, respectively. The 17 Chinese strains shared 92.7–96.0% nt identity and 95.1–99.1% aa identity with US isolates and had a lower homology with German strains, ranging from 83.0 to 85.1% nt and 93.1–95.4% aa identity (Table S3). A phylogenetic analysis indicated that these PPgVs were grouped with US isolates and distinct from three German isolates (S87Ger2014, 80FGer2012, and 903Ger2013), which formed a separate group of PPgV (Fig. 2).

In summary, we for the first time identified PPgV in pigs in China, and the positive rate of PPgV detected in this study was higher than that determined in Germany, but lower than that reported in the USA. Phylogenetic analyses demonstrated that the PPgV_GDCH2017 discovered in this study was closely related to the US PPgV strains and clustered in the same phylogenetic branch with other PPgVs, and pegiviruses from other hosts in the same genus. The findings from this study provide a useful start point for the research on PPgV in China and increase our knowledge in pegivirus virology. Further studies on pathogenicity and pathogenesis of PPgVs are needed.

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Author Contributions DL, DPS, YY, and KL performed the experiments, analyzed the data, and wrote the manuscript. FFZ and WFY participated in conducting the experiments; DYH, KL, and QW were responsible for samples collection; ZD and LYW participated in data analyses. DPS and YXT designed and supervised the experiments. All authors read and approved the final manuscript.

Compliance with Ethical Standards

Conflict of interest The authors declare that there are no conflicts of interests.

Research involving human participants and/or animals All procedures involving animals were in accordance with ethical standards.

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