Isolation and full-genome sequencing of porcine sapelovirus strain in piglets from Hainan province, China

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Short report

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Abstract

Background: Porcine sapelovirus (PSV) is a species of the genus Sapelovirus within the family Picornaviridae, which associated with facute diarrhoea, respiratory distress, reproductive failure, and severe neurological disorders in swine. The first isolate strian of PSV was reported in Hainan province, China in 2019.

Results: We report the isolation, genomic sequence of PSV isolated from pig diarrhea samples. The PSV strain was correctly identified by RT-PCR, IFA, WB assays, which appeared spherical with a diameter of approximately 25 nm by TEM. We named the strain PSV HaN01-CH2019, and its full genomes were 7,551 bp nucleotides in length. Phylogenetic analysis revealed that PSV HaN01-CH2019 and Chinese HuN01 strain are related in comparing with other reference strains.

Conclusions: We successfully isolated the first PSV strain in Hainan province and prepared polyclonal antibodies. It is evident that PSV infection has occurred in Hainan province, and therefore, active molecular and serological investigation is important to swine population. Moreover, veterinarians must pay attention to this diarrhoea and reinforce biosecurity measures to prevent PSV spread.

Background

Porcine sapelovirus (PSV) that previously designated porcine enterovirus 8 in the genus Enterovirus is positive-sense single stranded RNA viruses within a family of Picornavirales, which is classified into the genus Sapelovirus belonging to the family Picornaviridae[1]. So far, the genus Sapelovirus consists of porcine sapelovirus, avian sapelovirus, simian sapelovirus and unclassified sapelovirus[1–5]. The full genome of PSV is approximately 7.5 kb nucleotides in length and share a similar genomic organization compared to other picornaviruses, which consists of a 5’ terminal protein called VPg (Viral Protein genome-linked), a 5’ untransated region (UTR), a large open reading frame (ORF), a 3’ UTR and a poly(A) tail of variable length[6–9]. The single polyprotein encoded by a large ORF is subsequently cleaved into a leader (L) protein, four structural proteins (VP4, VP2, VP3 and VP1) and seven functional proteins (2A, 2B, 2C, 3A, 3B, 3C and 3D[7, 10, 11].

According to molecular and serological investigation revealed that PSV was highly prevalent in pigs around the world [5, 8, 12–14]. PSV is a major pathogen worldwide, but there are few studies on the pathogenicity of PSV[5, 13, 15]. PSV has been reported to be associated with a wide spectrum of symptoms ranging from asymptomatic infection to clinical signs (including diarrhoea, polioencephalomyelitis, pneumonia, and reproductive disorders), and its infection in swine is most frequently asymptomatic and mainly transmits by a faecal-oral route[15, 16].

Here, we describe the isolation of PSV from swine fecal samples from diarrhoea and biological characterization of PSV isolates. The PSV HaN01-CH2019 strain was characterized using an Immunofluorescence Assay (IFA) and Western Blot assay with a polyclonal antibody specific for VP1 of

PSV capsid protein, RT-PCR assay with primer pair specific for the PSV 5'UTR region and transmission electron microscopy[17].

Methods

Fecal samples from a swine farm in Hainan province with diarrhea in May 2019. To determine etiology, fecal samples from diarrhoea were detected using TGEV, PEDV, PDCoV, PRV-A, PKV and PSV specific primers[17, 18]. Total RNA of the specimens was extracted using the TRIzol reagent (Invitrogen, Grand Island, NY, USA) and 0.2 μg of total RNA was reverse transcribed using a First Strand cDNA Synthesis Kit (Vazyme, China) according to the manufacturer's instructions. PCR amplification was performed using various virus-specific primers (listed in Additional file 1: Table S1). Virus isolation and purification were carried out in a PK-15 cells culture system from the PSV-positive specimens and labeled PSV HaN01-CH2019. Rabbit anti-PSV VP1 polyclonal antibody was prepared in our lab using recombinant PSV VP1 protein. Identification of PSV HaN01-CH2019 using immunofluorescence assay (IFA), Western blot, and plaque assay was carried out as described previously[19]. Complete genome sequencing was performed using 7 pairs of overlapping primers (Additional file 1: Table S1) based on the published genomic sequences of the PSV V13 (GenBank ID: NC_003987), csh (GenBank ID: HQ875059), JD2011 (GenBank ID: KF539414.1), QT2013 (GenBank ID: KJ463384.1), JXXY-a2 (GenBank ID: MH626634.1), JXXY-C (GenBank ID: MH626635.1), USA/IA33375/2015 (GenBank ID: KX574284.1), ISU-SHIC (GenBank ID: KX810827.1), IVRI/PSV/SPF/C-6/2015 (GenBank ID: KY053835.1), KS055217 (GenBank ID: KJ821021.1), KS05151 (GenBank ID: KJ821020.1), HuN4 (GenBank ID: KX354743.1), HuN3 (GenBank ID: KX354742.1), HuN2 (GenBank ID: KX354741.1), HuN1 (GenBank ID: KX354740.1), KS04105 (GenBank ID: KX354740.1), and YC2011 strains (GenBank ID: JX286666). The phylogenetic tree was constructed by the neighbor joining method, with 1000 bootstrap replicates, using MEGA7.0 software.

Result

We successfully isolated PSV HaN01-CH2019 strain from piglets with diarrhoea, which was negative for PKV, TGEV, PEDV, PDCoV or PRV-A, but positive for PSV(Fig. 1). As shown in Fig. 2a, PSV HaN01-CH2019 strain induced typical cytopathic effects characterized by rounding and shrinkage and degeneration of PK-15 cells at 18 h post-infection after 4 passages. The viral titer of fourth passage PSV HaN01-CH2019 strain was 7.2×10^8 PFU/mL. The plaque morphology was distinct and plaque size was approximately 1.5–2.5 mm in PK cells (Fig. 2b). To confirm the isolation of PSV, immunofluorescence assay and Western blot analysis were conducted using home-made rabbit polyclonal anti-PSV VP1 antibody. As shown in Fig. 2c, cells infected by the isolate reacted with the specific rabbit polyclonal antibody against PSV VP1 protein by IFA. Meanwhile, Western blot analysis showed approximately 36 kilodalton (kDa) band in cells infected with the isolate (Fig. 2d, lane 2) but not in the mock cells (Fig. 2d, lane 1). TEM assay revealed icosahedral, nonenveloped viral particles with a diameter of approximately 25 nm(Fig. 2e). These results indicated that PSV strain HaN01-CH2019 was successfully isolated.

The complete genome of PSV HaN01-CH2019 strain has been amplified, sequenced and deposited in GenBank (accession number: MT080999). The strain consists of 7,551 bp nucleotides (nt) in length, including a 491-nt 5'UTR, an open reading frame (ORF) encoding a 2,324 amino acid polyprotein and a 88-nt 3' UTR contains a partial poly(A) sequence. Analysis of the whole genome sequence of PSV HaN01-CH2019 strain indicated that it shares high nucleotide identities (83.7 ~ 87.4%) with all previous PSV genomes in the GenBank (Fig. 3a), and the polyprotein shares 92.9 ~ 95.2% amino acid sequence identity (Fig. 3b). Two phylogenetic tree results were constructed based on a complete genome sequence or ploy protein amino acid sequence and demonstrated that the PSV HaN01-CH2019 strain is closely related to PSV-HuN01 strain.

Discussion

PSV infection in piglets can induce diarrhoea and intestinal pathologys. Currently, the pathogenicity of PSV has been poorly studied[3]. In this study, a Chinese PSV strain isolated from Hainan province was reported for the first time. The PSV strain was identified by RT-PCR, IFA, WB and TEM assays. By comparing the homology of the whole genome sequence with the polyprotein amino acid sequence inferred in this study, it was found that PSV HaN01-CH2019 and the Chinese strain HuN01 were related to other reference strains.. Notably, epidemiological investigation revealed that the PSV-positive rates and co-infection of PSV/PEDV in diarrhea samples were much higher than in asymptomatic samples, which indicating that PSV may contribute collectively to enteric disease of pigs along with other porcine pathogens[5, 20]. Although PSV is an ancient virus, regard to its pathogenic and immunobiology has been poorly understood[21]. Consequently, investigating pathogenesis and epidemiological features of PSV is crucial to facilitate the development of antiviral strategies and offer effective control measures against PSV infection.

Declarations

Abbreviations

PSV, porcine sapelovirus; PKV, porcine kobuvirus; TGEV, transmissible gastroenteritis virus; PEDV porcine epidemic diarrhoea virus; PDCoV porcine deltacoronavirus; PRV-A, porcine rotavirus A; ORF, an opern reading frame; UTR, untranslated region; aa, amino acids; PCR, Polymerase Chain Reaction; NJ, Neighbor-joining (NJ); PFU, Plaque forming unit; nt, nucleotides;

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The fecal samples from piglets with diarrhoea were kindly provided by a pig farmer in Hainan province (China) and stored in -80 °C. All primers used in this study were designed using Primer Premier 5.0 software. Data analyses were performed by the neighbor-joining (NJ) method with 1000 bootstrap r

eplicates using MEGA 7.0 software.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

HZ, XL and JX performed the experiments. HZ wrote the manuscript. HZ, XL and HC analyzed the data. PQ and XL designed the experiments. All authors reviewed the manuscript. All authors read and approved the final manuscript.

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References

1. Tseng CH, Tsai HJ. Sequence analysis of a duck picornavirus isolate indicates that it together with porcine enterovirus type 8 and simian picornavirus type 2 should be assigned to a new picornavirus genus. Virus Res. 2007;129(2):104-14.
2. Zhang W, Kataoka M, Doan HY, Ami Y, Suzaki Y, Takeda N, et al. Characterization of a Novel Simian Sapelovirus Isolated from a Cynomolgus Monkey using PLC/PRF/5 Cells. Sci Rep. 2019;9(1):20221.
3. Kim DS, Kang MI, Son KY, Bak GY, Park JG, Hosmillo M, et al. Pathogenesis of Korean SapelovirusA in piglets and chicks. J Gen Virol. 2016;97(10):2566-74.
4. Krumbholz A, Dauber M, Henke A, Birch-Hirschfeld E, Knowles NJ, Stelzner A, et al. Sequencing of porcine enterovirus groups II and III reveals unique features of both virus groups. J Virol. 2002;76(11):5813-21.
5. Li Y, Du L, Jin T, Cheng Y, Zhang X, Jiao S, et al. Characterization and epidemiological survey of porcine sapelovirus in China. Vet Microbiol. 2019;232:13-21.
6. Lan D, Ji W, Yang S, Cui L, Yang Z, Yuan C, et al. Isolation and characterization of the first Chinese porcine sapelovirus strain. Arch Virol. 2011;156(9):1567-74.
7. Son KY, Kim DS, Kwon J, Choi JS, Kang MI, Belsham GJ, et al. Full-length genomic analysis of Korean porcine Sapelovirus strains. PLoS One. 2014;9(9):e107860.
8. Tassoni L, Zamperin G, Monne I, Beato MS. Nearly Complete Genome Sequence of a Sapelovirus A Strain Identified in Swine in Italy. Microbiol Resour Announc. 2019;8(29).
9. Hales LM, Knowles NJ, Reddy PS, Xu L, Hay C, Hallenbeck PL. Complete genome sequence analysis of Seneca Valley virus-001, a novel oncolytic picornavirus. J Gen Virol. 2008;89(Pt 5):1265-75.
10. Bai H, Liu J, Fang L, Kataoka M, Takeda N, Wakita T, et al. Characterization of porcine sapelovirus isolated from Japanese swine with PLC/PRF/5 cells. Transbound Emerg Dis. 2018;65(3):727-34.
11. Chen J, Chen F, Zhou Q, Li W, Song Y, Pan Y, et al. Complete genome sequence of a novel porcine Sapelovirus strain YC2011 isolated from piglets with diarrhea. J Virol. 2012;86(19):10898.
12. Hanke D, Pohlmann A, Sauter-Louis C, Hoper D, Stadler J, Ritzmann M, et al. Porcine Epidemic Diarrhea in Europe: In-Detail Analyses of Disease Dynamics and Molecular Epidemiology. Viruses. 2017;9(7).
13. Arruda PH, Arruda BL, Schwartz KJ, Vannucci F, Resende T, Rovira A, et al. Detection of a novel sapelovirus in central nervous tissue of pigs with polioencephalomyelitis in the USA. Transbound Emerg Dis. 2017;64(2):311-5.

14. Kumari S, Ray PK, Singh R, Desingu PA, Varshney R, Saikumar G. Pathological and molecular investigation of porcine sapelovirus infection in naturally affected Indian pigs. Microb Pathog. 2019;127:320-5.
15. Schock A, Gurrala R, Fuller H, Foyle L, Dauber M, Martelli F, et al. Investigation into an outbreak of encephalomyelitis caused by a neuroinvasive porcine sapelovirus in the United Kingdom. Vet Microbiol. 2014;172(3-4):381-9.
16. Abe M, Ito N, Sakai K, Kaku Y, Oba M, Nishimura M, et al. A novel sapelovirus-like virus isolation from wild boar. Virus Genes. 2011;43(2):243-8.
17. Palmquist JM, Munir S, Taku A, Kapur V, Goyal SM. Detection of porcine teschovirus and enterovirus type II by reverse transcription-polymerase chain reaction. J Vet Diagn Invest. 2002;14(6):476-80.
18. Ding G, Fu Y, Li B, Chen J, Wang J, Yin B, et al. Development of a multiplex RT-PCR for the detection of major diarrhoeal viruses in pig herds in China. Transbound Emerg Dis. 2019.
19. Fan W, Zhang D, Qian P, Qian S, Wu M, Chen H, et al. Swine TRIM21 restricts FMDV infection via an intracellular neutralization mechanism. Antiviral Res. 2016;127:32-40.
20. Jiang C, He H, Zhang C, Zhang X, Han J, Zhang H, et al. One-step triplex reverse-transcription PCR detection of porcine epidemic diarrhea virus, porcine sapelovirus, and porcine sapovirus. J Vet Diagn Invest. 2019;31(6):909-12.
21. Sutton KM, Lahmers KK, Harris SP, Wijesena HR, Mote BE, Kachman SD, et al. Detection of atypical porcine pestivirus genome in newborn piglets affected by congenital tremor and high preweaning mortality1. J Anim Sci. 2019;97(10):4093-100.

Figures

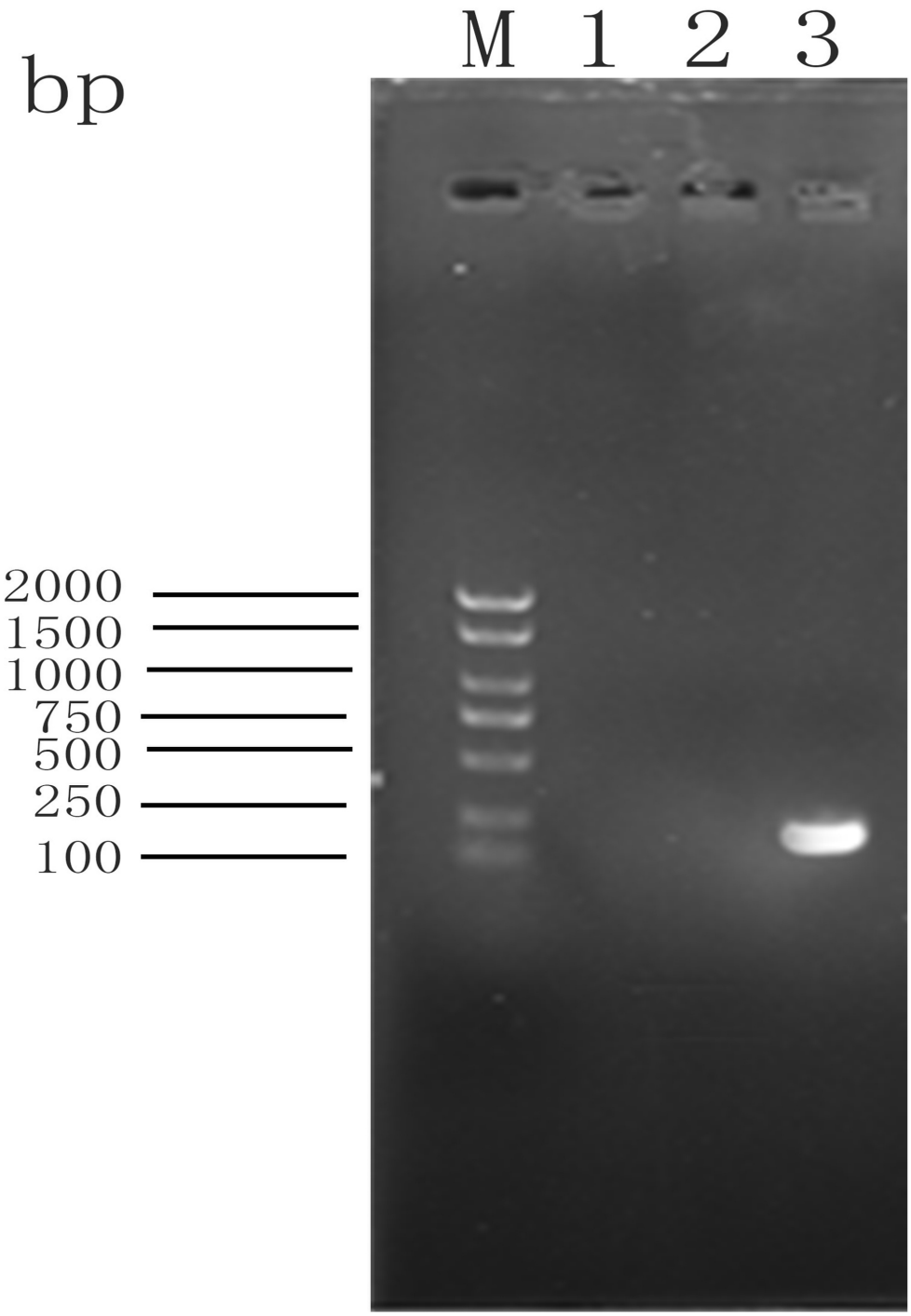

Figure 1

PCR detection of porcine sapelovirus from swine fecal samples from diarrhoea. PCR amplification identified the causative agent in the fecal samples from diarrhoea using specific primers. Lane 1 is for negative control. Lane 2 is for PK-15 cells. Lane 3 is for PSV (183 bp). Lane M is DNA marker (from top to bottom is 2000-1500-1000-750-500-250-100 bp).

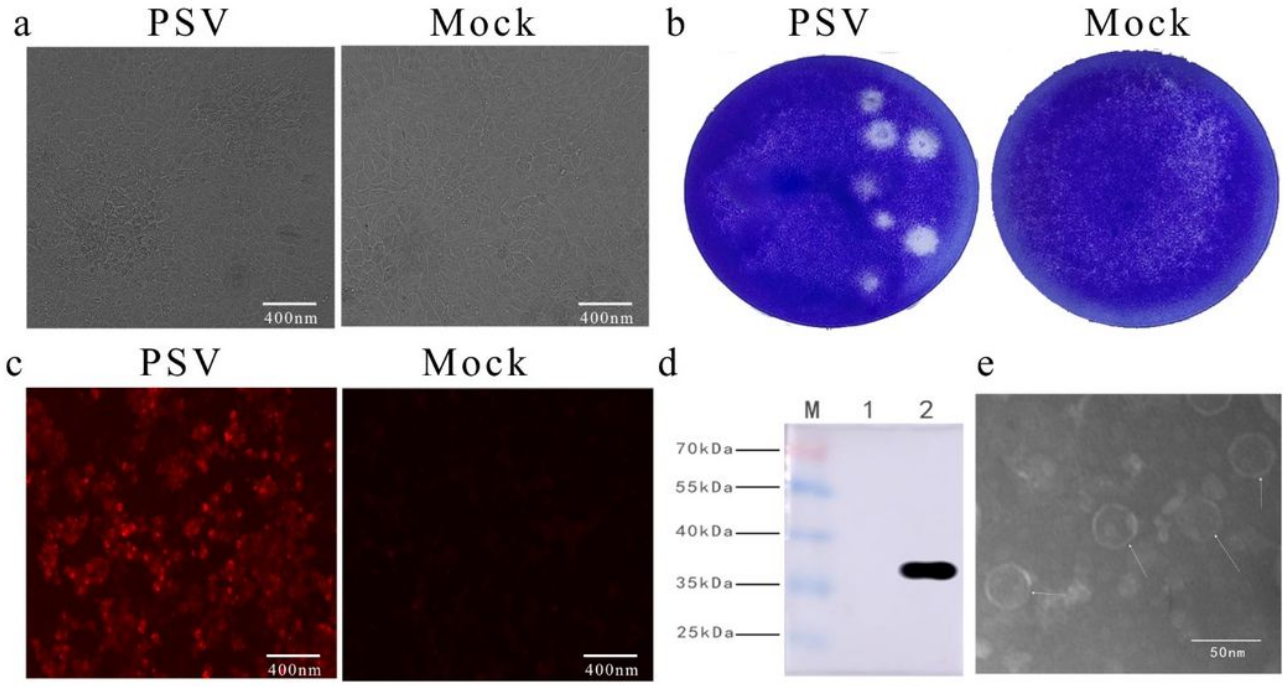

Figure 2

Identification of PSV HaN01-CH2019 strain. a The cytopathic effect of PK-15 cells infected with PSV HaN01-CH2019 strain at 18 h post-infection. b Plaque morphology inPK-15 cells cells infected with fourth-passage PSV HaN01-CH2019 strain at 18 h post-infection. c Immunofluorescence assay (IFA) of PK-15 cells infected with PSV HaN01-CH2019 strain at 18 h post-infection. Cells were stained with primary antibody using home-made rabbit anti-PSV VP1 polyclonal antibodies. d Western blot analysis of PK-15 cells infected with PSV HaN01-CH2019 strain at 12 h post-infection. e Electron micrograph (EM) of cultured PSV HaN01-CH2019 strain. Virus pelleted by ultracentrifugation was stained with 1% phosphotungstic acid and sprayed onto coated EM grids.

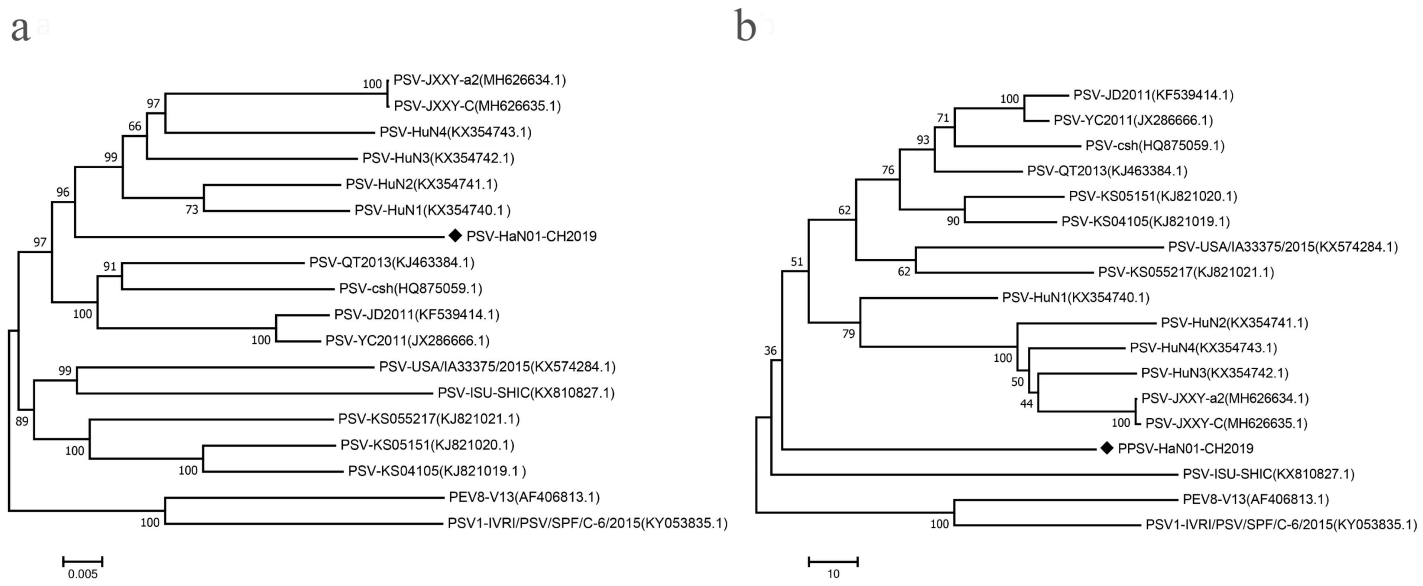

Figure 3

Phylogenetic analysis of PSV complete nucleotides and amino acid sequences. Phylogenetic trees were constructed using the neighbor-joining method, with 1000 bootstrap replicates, using MEGA7.0 software. The newly isolated PSV HB-CH-2016 strain is marked □. The number on every 100 branches indicates bootstrap values. a Phylogenetic analyses of PSV based on full-genome nucleotides. b Phylogenetic analyses of PSV based on full-polyprotein amino acids. Reference sequences retrieved from the GenBank are indicated by years of isolation, origins and accession numbers

Supplementary Files

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