Characterization of a Porcine Sapelovirus Strain Isolated in China

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Research

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Abstract

Background: Porcine sapelovirus (PSV) infections have been associated with a wide spectrum of symptoms ranging from asymptomatic infection to clinical signs including diarrhea, pneumonia, reproductive disorders, and polioencephalomyelitis. Although PSV is an important pathogen due to its wide distribution and high prevalence rate, researches on PSV have been relatively few so far.

Methods: In this study, we isolated a PSV strain, SHCM2019, from swine faecal specimens from PK15 cells. To investigate the molecular characteristics and pathogenicity of the PSV strain, the genomic sequence of the SHCM2019 strain was analysed, and the clinical manifestations and pathological changes exhibited by inoculated neonatal piglets were observed.

Results: Sequencing results showed that the full-length genome of the SHCM2019 strain was 7567 nucleotides in length excluding the 27-nucleotide poly(A) tail. Phylogenetic analyses demonstrated that the virus isolate belongs to PSV and was classified into the China cluster. RNA recombination analysis indicated that there may be a recombination breakpoint upstream of the 3D region in PSVs. Pathogenicity research demonstrated that the virus isolate could cause diarrhoea and pneumonia in piglets.

Conclusion: This study presented the isolation of a recombinant PSV strain, SHCM2019 and demonstrated the isolate was pathogenic. Our results may provide a reference for future research investigating of the pathogenic mechanism and evolutionary characteristics of PSV.

Introduction

The genus *Sapelovirus*, belonging to the family *Picornaviridae*, consists of three species: *Avian sapelovirus*, *Sapelovirus A* (porcine sapelovirus), and *Sapelovirus B* (simian sapelovirus) [1]. Porcine sapelovirus (PSV) is a non-enveloped, positive-sense, single-stranded RNA virus, which was previously known as porcine enterovirus 8 (PEV-8) in the genus *Enterovirus* [2]. The genome of PSV measures approximately 7500 bp in length and consists of a 5' untranslated region (UTR), intermediate coding region, 3' untranslated region (UTR) and poly(A) tail. The intermediate coding region contains only a large open reading frame (ORF) that encodes a polyprotein precursor about 2,330 amino acids in length, which is subsequently cleaved by the self-coding protease into 12 mature functional proteins. The structure of the polyprotein is consistent with the L-4-3-4 structure of other picornaviruses, in which a leading peptide L leads to four structural polypeptides (VP4, VP2, VP3, and VP1) and seven nonstructural polypeptides (2A, 2B, 2C, 3A, 3B, 3C, and 3D) [3–5].

Since PSV was first reported in the UK in 1958 [6], it has been identified in many countries, including Canada, Japan, Australia, Brazil, Spain and Korea, and China and other countries worldwide [5, 7–12]. PSV infection rates in pigs have been investigated globally, with prevalence rates ranging from 7.1% in India [13] to 71.0% in Hungary [14]. PSV infections have been associated with a wide spectrum of symptoms ranging from asymptomatic infection to acute fatal encephalomyelitis, reproductive disorders, diarrhoea and pneumonia [12, 15–17]. Prodelalova reported that PSV infection rates were higher in

asymptomatic pigs than in diarrhoeal pigs [15]. However, Bin Zhang demonstrated a higher prevalence of PSV in diarrhoeal pigs by using metagenomic analysis of pig faeces [18]. In addition, Deok-Song Kim demonstrated that PSV can cause intestinal lesions and colonize the villous epithelial cells in the small intestine after PSV infection [19]. Another important characteristic of PSV infection is that infected pigs may also be infected with porcine parvovirus, swine fever virus, porcine reproductive and respiratory disorder syndrome virus, enterovirus and other infections, leading to clinical atypical symptoms in sick pigs [20–22]. Latent infections and mixed infections of PSV often cause the symptoms of PSV to be covered up and overlooked [15], enabling the spread of PSV infection, which poses a considerable threat to the pig industry.

In this study, a PSV strain was isolated using PK15 cells. The strain is closely related to Chinese isolates, and there may be a recombination break point in the 3D region. Animal regression experiments demonstrated that the isolated strain was pathogenic to piglets. The study laid a foundation for the research on the pathogenic mechanism of PSV.

Materials And Methods

Cells and clinical samples

PK-15 cells were grown in Dulbecco's modified Eagle's medium (DMEM, HyClone, USA) containing 10% heat-inactivated foetal bovine serum (FBS; Invitrogen, Australia) and 1% antibiotics (10,000 units/mL penicillin and 10,000 µg/mL streptomycin) and cultured at 37 °C with 5% CO₂. The faecal specimens were collected from diarrheic piglets from a pig farm in Shanghai, China, in May 2019. Subsequently, these faecal samples were used for PCR analysis to detect a panel of potential pathogens including porcine sapelovirus (PSV), porcine kobuvirus (PKV), porcine astrovirus (PAstV), porcine deltacoronavirus (PDCoV), porcine torovirus (PToV), porcine teschovirus (PTV), porcine sapovirus (PSaV), porcine epidemic diarrhoea virus (PEDV), transmissible gastroenteritis virus (TGEV), porcine rotavirus (PoRV) and bovine viral diarrhoea virus (BVDV).

Virus isolation

PK-15 cells were inoculated with faecal specimens that were positive for PSV RNA but negative for PEDV, TGEV, PKV, PAstV, PDCoV, PToV, PTV, PSaV, PoRV, and BVDV RNA. In brief, the positive faecal samples were suspended in sterile phosphate-buffered saline (PBS) containing 1,000 units/mL penicillin and 1,000 µg/mL streptomycin and centrifuged at 3,500 × g for 10 min. The supernatants were filtered through 0.22-µm-pore membrane filters, followed by inoculation PK15 cells for 1 h at 37 °C. Next, the inoculum was discarded, and the cells were washed three times with sterile PBS. Subsequently, DMEM supplemented with 0.5 µg /mL TPCK-trypsin (Gibco™, USA) was added, and cell culture was continued at 37 °C with 5% CO₂, with daily observation for cytopathic effect (CPE). When more than 80% of cells showed CPE, the supernatant and cells were harvested, frozen and thawed three times and stored at -80 °C for the next virus passage.

RT-PCR

Total RNA was extracted from the culture supernatant of PSV inoculation using TRIzol Reagent (Takara, China) according to the manufacturer's instructions. The cDNAs were synthesized in a final volume of 20 μ L containing: 10 μ L RNA, 4 μ L 5 $\times$ RT buffer, 1 μ L dNTPs (10 mM), 2 μ L random primer (25 μ M), 0.5 μ L Moloney murine leukaemia virus (M-MLV) reverse transcriptase (TaKaRa, China), 0.5 μ L RNase inhibitor (TaKaRa, China), and 2 μ L diethylpyrocarbonate (DEPC)-treated water. The reaction was incubated at 42 °C for 1 h followed by incubation at 70 °C for 15 min. cDNA was used for PCR with the following primers, PSV-F: 5'-GATGTGGCGCATGCTCTT-3', PSV-R: 5'-TGCTGCCTCCTGTGTTGTTAT-3'. Amplified products were separated by electrophoresis in a 1.5% agarose gel and purified using a DNA gel extraction kit (Tiangen, China). The purified PCR products were subsequently cloned into the PEASY-Blunt Zero vector (TransGen-Biotech, China) and sequenced (Biosune, China). Sequences were analysed using the BLAST search program (<http://blast.ncbi.nlm.nih.gov/>).

Transmission electron microscopy (TEM)

PK-15 cells infected with the mentioned strain and showing over 80% CPE were frozen and thawed, three times and centrifuged at 2,000 $\times$ g for 30 min to remove the cell debris. Crude virus was pelleted from the clarified supernatant by ultracentrifugation at 9,8900 $\times$ g at 4 °C for 3 h in a TYPE-70 Ti rotor (Beckman, USA). The resulting pellet was resuspended in a small amount of PBS and was subsequently layered onto a 20–60% (w/v) discontinuous iodixanol solution (OptiPrep™ Density Gradient Medium, Sigma-Aldrich, USA) by centrifugation at 124,400 $\times$ g at 4 °C for 3 h in a SW-55 Ti rotor (Beckman, USA). The virus band at the interface was collected and placed onto a formvar grid (Electron Microscopy Sciences, USA) for 5 min, and excess liquid was subsequently removed by filter paper. The samples were observed using a transmission electron microscope (Hitachi, Japan) for the morphological identification of the virus.

Immunofluorescence assay (IFA)

To characterize the PSV strains, IFA was performed with a polyclonal antibody specific for the PSV-VP1 protein. Briefly, PK-15 cells in 6-well plates were mock infected or infected with the SHCM2019 strain, incubated for 24 h, fixed in 4% paraformaldehyde for 30 min at 4 °C, and subsequently permeabilized with 0.3% Triton X-100 (Biodee, China) for 10 min at room temperature (RT). After the cells were washed three times with PBS, they were blocked with 5% bovine serum albumin (BSA; Sigma, USA) at RT for 1 h. Mouse anti-PSV VP1 protein PcAbs (prepared in our laboratory) and Alexa Fluor™ 488-conjugated rabbit anti-mouse IgG (Invitrogen, USA) were used as primary and secondary antibodies, respectively. The cell nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI; Beyotime, China) for 5 min at RT. After the cells were washed with PBS, the stained cells were observed with a fluorescence microscope (Carl Zeiss, Germany).

Sequence and phylogenetic analysis

Third-generation viral RNA was extracted from PK-15 cells infected with the virus isolate using TRIzol Reagent (Takara, China). Reverse transcription and PCR were performed with Moloney murine leukaemia virus (M-MLV) reverse transcriptase (Takara, China) and TransStart FastPfu DNA polymerase, respectively. The PCR products were cloned using the pEASY-Blunt Cloning Kit (TransGen-Biotech, China), and the resulting plasmids were sent to Biosune (Shanghai, China) for sequencing. The 5'-UTR and 3'-UTR sequences of the isolated strain were obtained using a 5'-3' RACE kit (Takara, China) according to the manufacturer's instructions. The sequences fragments were assembled with DNASTar v. 7.1. The complete genome sequences were deposited into NCBI GenBank (<https://www.ncbi.nlm.nih.gov/>) under accession number MN685785. All of the complete sequences of the polyprotein gene and reference sequences obtained from GenBank were utilized in sequence alignments and phylogenetic analyses. Phylogenetic trees were constructed using the neighbour-joining method in MEGA v. 7.0, with bootstrap values being calculated for each node from 1,000 replicates. Recombination analysis was performed using SimPlot software v. 3.5.1 and Recombination Detection Program (RDP) v. 4.10.

Experimental infection of piglets

To assess the pathogenicity of the isolated SHCM2019 strain in piglets, four neonatal piglets from one sow were used. Before inoculation, we collected faecal samples from all piglets to ensure that none of the piglets was infected with PSV. After the piglets were observed for 7 days (to ensure that none was symptomatic), they were assigned at random to two groups (two pigs per group). Each piglet in group 1 (L1 and L2) was given 5 mL PSV-SHCM ($10^{7.41}$ TCID₅₀/mL) by the oral route, and piglets in group 2 (C1 and C2) received 5 mL DMEM orally as a negative control. After inoculation, all piglets were monitored each day for overt clinical signs. Faecal samples from each pig were collected for viral RNA detection on days 0, 1, 2, 3, 4, 5 and 6 post-inoculation (PI), and serum samples were collected for seroconversion tests on days 0, 1, 3, and 6 PI. All piglets were killed by bleeding at 6 dpi, and one portion of the tissue samples (intestine, lung, and brain) was fixed with 4% paraformaldehyde solution and sent to the company to make case slices; another portion of the tissue samples was collected for viral RNA detection. Viral shedding from faeces or tissues was tested by real-time PCR.

Ethics statement

All animal experiments were performed under the guidance of the Institutional Animal Care and Use Committee at the Center for Disease Control and Prevention (CDC) and the Laboratory Animal Care International accredited facility.

Results

Virus isolation and identification

PSV-positive faecal samples were utilized for virus isolation in PK15 cells. After six generations of blind transmission, the clarified supernatant of each generation was detected by RT-PCR and sequenced, and the sequencing results showed that the PSV may be initially isolated (Fig. 1A). Distinct CPE was observed

first in PK15 cells in the third blind passage. Compared with uninoculated PK-15 cells, PSV-inoculated PK-15 cells were characterized by rounding, shrinking and detachment of the cells after 30–36 hpi, and at 70 h, the cells detached from the surface of the cultivation flask (Fig. 1B).

To determine the morphological characteristics of this virus, PSV particles from the SHCM2019 strain in PK-15 cell culture media were imaged through transmission electron microscopy (TEM). TEM of a negatively stained sample revealed icosahedral, three-dimensional symmetrical, nonenveloped viral particles with a diameter of approximately 25–30 nm (Fig. 1C). No other virus-like particles were observed. Furthermore, virus propagation of the fifth passage of the SHCM2019 strain and cell nuclei was confirmed by detecting PSV antigens with IFA using PSV VP1-specific PABs and DAPI (Fig. 1D). Distinct green signals were observed in the PSV SHCM2019 strain infected PK-15 cells. These results demonstrated that isolated virus was PSV.

Full genome sequence and phylogenetic analyses

The complete genome sequence of the SHCM2019 strain has been determined to be 7,567 nucleotides, which is predicted to contain only a single, 6996-nt ORF flanked by a 466-nt 5' UTR and 106-nt 3' UTR. The single ORF encodes a 2331-aa viral polyprotein, which shares the same organization of L-4-3-4 and similar predicted cleavage sites as those previously found in other sapeloviruses [23]. However, the length of the polyprotein gene has been observed to be different in PSV strains and ranges from 6969 nt to 6999 nt, and it is relatively conserved in Chinese PSVs, exhibiting a length of 6996 nt, except for the strain with 3 nucleotides inserted in HuN4. A previous study observed that the 5' terminal nucleotide residues of PSV were UU [5], but the two 5' terminal residues were AC for the SHCM2019 strain, similar to the Chinese csh and English V13 strains, which indicating that the sequence was incomplete.

The phylogenetic tree was constructed by the neighbour-joining method based on the complete polyprotein gene of the SHCM2019 strain and other representative picornaviruses from the NCBI database. The phylogenetic tree of the polyprotein gene indicated that the PSV/SHCM2019 clustered together with the other PSVs, which further confirmed that the isolated strain was PSV. Obviously, PSVs had genetic diversity, and the Chinese PSV strains were closely related to the Korean PSV strains (Fig. 2). In addition, the SHCM2019 strains showed high nucleotide (84.7–94.0%) and deduced amino acid (92.9–98.3%) identities with the other PSV strains, but showed relatively low nucleotide and polyprotein sequence identities with the avian and simian sapelovirus strains.

To determine potential recombination events within the polyprotein genome sequence of the SHCM2019 strain that was identified, we performed recombination analysis using SimPlot v. 3.5.1 software and RDP v. 4.10. Standard similarity plot analysis was performed using the SimPlot program and the PSV/SHCM2019 sequences were used as separate queries. Next, we performed a bootscanning analysis to identify potential breakpoints in the recombinants. The SHCM2019 strain showed a high degree of nucleotide sequence similarity with HeB04 in the VP1,VP2 and VP3 regions, whereas the 3D regions were highly similar to those of ISU-SHIC, which suggests that there may also be a restructuring break point in 3D (Fig. 3A,B).

Experimental infection, necropsy, and histopathological examination

To determine the virulence of the PSV/SHCM2019 strain, two eight-day-old, healthy piglets were inoculated with PSV/SHCM2019. Inappetence and lethargy appeared in the inoculated pigs (group 1) at 2 days PI, and watery diarrhoea occurred at 5 days PI compared to two mock-inoculated piglets (Fig. 4D). Necropsy results showed that the intestinal tube of PSV-infected pigs was thin and translucent, the intestinal contents were pulpy or watery, and there was obvious congestion in the lungs compared to uninfected pigs (Fig. 4E,F). The piglets in the control group had no obvious clinical symptoms, the anus was clean, the necropsy indicated that the intestines were thick and elastic, and their contents were normal (Fig. 4A-C). Diseased tissues were also examined histopathologically. Histopathological results showed that the villi of the small intestine were slightly atrophic, and submucosal oedema was obvious (Fig. 5C). The lung was observed to be damaged by thickened alveolar walls, serous exudation and inflammatory cell infiltration in the interalveolar septa (Fig. 5D). There was no significant change in the control group (Fig. 5A,B).

To explore the excretion and distribution of PSV, RNA detection was conducted on faecal samples collected daily and tissue samples obtained after necropsy of piglets. The results showed that there were high RNA copy numbers in faecal samples at 3–6 dpi (Fig. 6A). In addition, PSV was determined to be widely distributed in lung and intestinal tissues, but was not detected at high level in lymph and brain tissues. However, PSV wasn't detected in the heart, liver, or kidney (Fig. 6C,D). The results of enzyme-linked immunosorbent assay (ELISA) showed that the IgG antibody levels of PSV-infected pigs were significantly higher than those of uninfected pigs, and antibody levels increased significantly after 6 days of inoculation (Fig. 6B). These results confirmed the pathogenicity of PSV/SHCM2019 to piglets.

Discussion

At present, most studies on PSV conducted domestically and abroad have been epidemiological investigations, and few studies have examined pathogenicity and mechanism of this virus. In this report, we describe the isolation of a PSV strain, SHCM2019, from pig farms in Shanghai. The strain was identified as PSV by RT-PCR, IFA and TEM assays, and its pathogenicity in piglets was confirmed.

To further characterize this strain, we sequenced the complete genome of the SHCM2019 strain. The full-length genome of the strain was determined to 7,567 nucleotides, consisting of a 5' UTR of 465 nucleotides, a 3' UTR of 106 nucleotides, and a single open reading frame (ORF) of 6,996 nucleotides that encodes a protein precursor of 2,331 amino acids. BLAST results showed that the nucleotide similarity between the isolates and PSV-YC2011 was as high as 90%. The antigenic epitome analysis of VP1 showed that the SHCM2019 strain was not significantly different from other PSV strains, and the slow evolution of PSV strain could help to better control the disease.

Phylogenetic analysis based on the polyprotein gene showed that the PSV isolates from China had genetic diversity, and were closely related to the South Korean strain, but were distantly related to the PSV isolates from Europe and America, suggesting that the transmission of PSV may be significantly affected by the region. At the same time, we constructed an evolutionary tree of the VP1 gene (see additional file 1), but the topological structure and branching of the evolutionary tree were slightly different from the protein coding region, and were not completely consistent with the previously reported evolutionary tree of VP1 [24], which indicated that PSV could not be divided into genotypes at present. The VP1 protein, located in the outermost layer of PSV virions, is the main protein that enteroviruses induce the body to produce neutralizing antibodies and maintain viral infection [25]. At present, many picornaviruses are genotyped by VP1 sequence alignment [26].

The driving forces of viral evolution include gene mutation, natural selection, gene drift, and viral gene recombination. Mutation and recombination are two important mechanisms governing virus evolution. The results of recombination analysis showed that there was recombination between PSV isolates, and the recombination sites may be located in the 3D region, which was not consistent with the findings of previous studies [24, 27–29]. The 3D protein is a virus-specific RNA polymerase [3], and its ability to synthesize is, in turn, an important factor affecting recombination. In view of the diversity of recombination sites reported to date [24, 27–29], we must accelerate the process of research on the molecular mechanism of PSV.

The PSV infection rate was high in both domestic and wild pigs [9], but in most cases, the pigs infected with PSV did not exhibit clinical symptoms [8, 9, 14, 20], which suggests that PSV is not consistently pathogenic. As a result, when there is mass diarrhea in a swine herd, most researchers mainly focus on porcine circovirus type 2 (PCV-2) and porcine epidemic diarrhoea virus (PEDV), while PSV, which can cause similar symptoms, does not attract similar attention [30, 31]. In this study, the SHCM2019 strain was found to replicate in the intestinal tracts of piglets and cause diarrhoea and a degree of pneumonia in pigs, suggesting that the SHCM2019 strain is pathogenic. PCV-2 infection itself is asymptomatic, and infected pigs occasionally develop severe postweaning multisystem depletion syndrome under viral coinfection or immune stimulation [32]. Similarly, PSV may also require currently unidentified factors to show full pathogenicity in pigs, which warrant further investigation.

Conclusion

In this study, a swine sapelovirus strain was isolated and named SHCM2019. The isolated strain was confirmed as PSV by RT-PCR, IFA and TEM assays. The molecular characteristics and pathogenicity of the SHCM2019 strain were studied. Phylogenetic analyses showed that the virus isolate belongs to PSV and was classified into the China cluster. Recombination analysis indicated that there may be a recombination breakpoint upstream of the 3D region in PSVs. Pathogenicity research demonstrated that the SHCM2019 strain is pathogenic. To better assess the adverse effects of PSV on pig populations, we need to further explore the epidemiological characteristics and pathogenic mechanisms of PSV.

Abbreviations

PSV

Porcine sapelovirus; PEV-8: Porcine enterovirus 8; UTR: Untranslated region; ORF: Open reading frame; DMEM: Dulbecco's modified Eagle's medium; FBS: Foetal bovine serum; PKV: Porcine kobuvirus; PAsV: Porcine astrovirus; PDCoV: Porcine deltacoronavirus; PToV: Porcine torovirus; PTV: Porcine teschovirus; PSaV: Porcine sapovirus; PEDV: Porcine epidemic diarrhoea virus; TGEV: Transmissible gastroenteritis virus; PoRV: Porcine rotavirus; BVDV: Bovine viral diarrhoea virus; PCV-2: porcine circovirus type 2; CPE: Cytopathic effect; PBS: Phosphate-buffered saline; M-MLV: Moloney murine leukaemia virus; DEPC: Diethylpyrocarbonate; TEM: Transmission electron microscopy; IFA: Immunofluorescence assay; RT: Room temperature; BSA: Bovine serum albumin; DAPI: Diamidino-phenylindole; RDP: Recombination Detection Program; ELISA: enzyme-linked immunosorbent assay.

Declarations

Acknowledgements

Not applicable.

Author Contributions

HLL conceived and designed this study; NNL and BQL conducted experiments; JT, JHC and YS contributed their technical knowledge; NNL and HLL wrote this paper; BQL and TJ were involved in interpreting the results and critically reading the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Data supporting the conclusions of this article are presented in the manuscript.

Ethics approval and consent to participate

All animal experiments were performed under the guidance of the Institutional Animal Care and Use Committee at the Center for Disease Control and Prevention (CDC) and the Laboratory Animal Care International accredited facility.

Consent to publication

Not applicable.

Competing interests

All authors declared that they have no competing interests.

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Figures

Figure 1

Isolation and identification of PSV. (A) RT-PCR detection of cell culture medium obtained through continuous passage. The first six passages of viruses yielded positive bands of ~624 bp in RT-PCR analysis with primers specific to PSV. (B) Cytopathic effects in PK-15 cells inoculated with PSV-positive samples. After three blind passages, PK-15 cells began to shrink and shed at 30 h, and were widely detached from the surface of the cultivation flask at 70 h. (C) Electron micrograph (EM) of cultured PSV strain SHCM2019. Virus pelleted by ultracentrifugation was stained with uranyl acetate and sprayed onto coated EM grid. (D) Immunofluorescence analysis of the PSV infected PK-15 cells by laser confocal microscopy. Immunofluorescence assay with DAPI confirmed that PSV strain SHCM2019 could be recognized by the anti-PSV polyclonal antibody.

Figure 2

Phylogenetic analysis of the polyprotein nucleotide sequence for PSV/SHCM2019. A phylogenetic tree was constructed by the neighbour-joining method in the MEGA7.0 software. The numbers at the branches are bootstrap values (%) following 1000 replicates. A dot has been added to the front of the PSV strain isolated in this study.

Figure 3

Identification of the recombination events based on the polyprotein gene of the potential recombinants. Similarity analysis of the polyprotein gene of potential recombinants using the SimPlot program with a 200 bp window size, 20 bp step size and 100 bootstrap replicates. The cut-off value in the bootstrapping test (>70%) is indicated by a dotted line, and the arrows indicate putative recombination breakpoints. (A) SHCM2019 represents the query sequence, and HeB04 (red line) and ISU-SHIC (blue line) represent the potential parental strains. (B) Genome origination and recombination-breakpoint identification in SHCM2019. The red area indicates the region of origin from ISU-SHIC, the blue area indicates the region of origin from HeB04, and the white area indicates an unknown origin.

Figure 4

Clinical symptoms and autopsy results of animals. (1) Clinical features around the anus in animals. The piglets in healthy control group were clean and had no attachments around the anus (A). The piglets in PSV-infected group developed watery diarrhoea with residues around the anus (D). (2) Autopsy results of animal intestines. The intestinal tract of piglets in healthy control group were thick and elastic (B). In PSV-infected group, the intestinal tube thinned and its contents were pulpy (E). (3) Autopsy results of animal lungs. The lung performance of piglets in healthy control group was normal (C). Local bleeding points appeared in the lungs of piglets in PSV-infected group (F).

Figure 5

Histopathological examination. (1) Examination of intestine. The intestinal villi of piglets in healthy controls (A). The intestinal villi of pigs infected with PSV were atrophic with obvious oedema of lamina propria and submucosa (C). (2) Examination of lungs. Lung pathology of piglets in healthy controls (B). The alveolar septa of pigs infected with PSV were thickened and had pale serous fluid exudation and many red blood cells in the alveolar septa(D).

Figure 6

Pathogenicity of the PSV/SHCM2019 strain in neonatal piglets. (A) Viral RNA shedding in faecal swabs after virus and DMEM inoculation. High levels of viral RNAs were shed in faecal samples of PSV-infected pigs (L1 and L2) at 3–6 dpi, and faecal swabs from DMEM-inoculated piglets (C1 and C2) were PSV viral RNA-negative. (B) IgG antibody levels were measured in infected and control pigs, and antibody levels increased significantly after 6 days of inoculation. (C-D) Viral RNAs were detected in some alimentary tract organs. PSV can be detected in duodenum, jejunum, ileum, caecum, colon, rectum, lung, brain and intestinal lymph, but not in the heart, liver and kidney.

Supplementary Files

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