Phylogenetic and pathogenicity analysis of a novel lineage of caprine parainfluenza virus type 3

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Abstract

Caprine parainfluenza virus type 3 (CPIV3) was first identified in goats named JS2013 in China. In 2019, a sheep herd broke a disease with respiratory disease in Hebei province, China. In order to confirm the pathogen of the disease, the nasal swabs, stool swabs and blood samples were collected from the sheep. Virus isolation was performed on MDBK cells and identification was conducted by RT-PCR. The complete genome of the isolate was sequenced and phylogenetic analyzed. In order to evaluate the pathogenicity of the virus, five seronegative sheep were experimental infected with the virus suspension. The phylogenetic analyses based on the complete genome and the M gene indicated that the isolate strain was distinguished distinct from previously reported CPIV3 lineage of JS2013. The virus-inoculated sheep displayed the syndrome with depression, cough, and fever. Virus shedding were detected by RT-PCR from nasal swabs. All infected showed virus shedding during 2 - 21dpi and viremia could be detected in serum samples. Gross pathological assessment of sheep in infected group showed gross lesion in the lungs. Histopathological observation results indicated that lungs had mild to moderate interstitial pneumonia , with thickened alveolar walls, decreased alveolar space, and increased amounts of inflammatory cells infiltration. This is the first report of pathogenicity of the novel lineage of sheep-derived CPIV3. The results would be helpful for further studies on the prevention and control strategies for CPIV3 infections in goat and sheep.

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ABSTRACT

Caprine parainfluenza virus type 3 (CPIV3) was first identified in goats named JS2013 in China. In 2019, a sheep herd broke a disease with respiratory disease in Hebei province, China. In order to confirm the pathogen of the disease, the nasal swabs, stool swabs and blood samples were collected from the sheep. Virus isolation was performed on MDBK cells and identification was conducted by RT-PCR. The complete genome of the isolate was sequenced and phylogenetic analyzed. In order to evaluate the pathogenicity of the virus, five seronegative sheep were experimental infected with the virus suspension. The phylogenetic analyses based on the complete genome and the M gene indicated that the isolate strain was distinguished distinct from previously reported CPIV3 lineage of JS2013. The virus-inoculated sheep displayed the syndrome with depression, cough, and fever. Virus shedding were detected by RT-PCR from nasal swabs. All infected showed virus shedding during 2 - 21dpi and viremia could be detected in serum samples. Gross pathological assessment of sheep in infected group showed gross lesion in the lungs. Histopathological observation results indicated that lungs had mild to moderate interstitial pneumonia , with thickened alveolar walls, decreased alveolar space, and increased amounts of inflammatory cells infiltration. This is the first report of pathogenicity of the novel lineage of sheep-derived CPIV3. The results would be helpful for further studies on the prevention and control strategies for CPIV3 infections in goat and sheep.

KEYWORDS

caprine parainfluenza virus type 3, sheep, pathogenicity, phylogenetic, respiratory disease

1 | INTRODUCTION

Parainfluenza virus type 3 (PIV3) is one of the most important viral respiratory pathogens for humans and many species of animals (Silvina S Maidana, 2012). The virus belongs to the genus respirovirus with respiratory syncytial virus (RSV), human parainfluenza virus (HPIV), and canine distemper virus (CDV), bovine parainfluenza virus type 3 (BPIV3), caprine parainfluenza virus type 3 (CPIV3) in the family Paramyxoviridae. The members of this virus family are enveloped and have genomes consisting of a single segment of negative-sense RNA, which usually have a length of 15kb (Selim, Çomakli, Selçuk, & Pathogens, 2019; Walker, P. J. et al., 2019). The genome encodes six structural proteins including the nucleocapsid (N), phosphoprotein (P), matrix (M), fusion (F), hemagglutinin-neuraminidase (HN), and large (L) proteins. Three accessory proteins are encoded by the P gene, including the C, V, and D proteins (Ohkura et al., 2015).

The PIV3 infections were found in a wide variety of mammals including cattle, humans, non-human primates, rhinoceros (Fischer-Tenhagen et al.,2000), pigs (Qiao, Janke, & Elankumaran, 2010), dogs, dolphin (Qiao et al., 2010), sheep (Mao et al., 2019), goats (Li,W. et al., 2016), bison (Zarnke & Erickson, 1990), guinea pigs (Hao, Wang, Mao, Yang, & Jiang, 2019), black and white rhinoceros (Fischer-Tenhagen, Hamblin, Quandt, & FroLich, 2000), moose (Thorsen & Henderson, 1971) chamois (Luzzago, Ebranati, Lavazza, Besozzi, & Lauzi, 2020), bighorn sheep (Parks, Post, Thorne, & Nash, 1972), camels (Eisa, Karrar, & Abdel Rahim, 1979), and water buffaloes (Silvina S Maidana, 2012). BPIV3 has demonstrated strong hazard to both adult and young cattle, which was identified one of the viral respiratory agents caused bovine respiratory disease syndromes (BRDC) (Ren et al., 2015). The cross-species infections have been reported, including BPIV3 in sheep, HPIV3 in guinea pigs and Atlantic bottlenose dolphins (Ohsawa et al., 1998; Kirsten et al., 2015).

The first CPIV3 strain, JS2013 was isolated from goats (Li, W. et al., 2014). To date, the CPIV3 strains isolated from sheep and goats were classified as a similar clade with the JS2013 strain (Mao et al., 2019). Moreover, the pathogenesis of CPIV3 infection in goats and the guinea pigs were conducted and reported. However, no other lineage of CPIV3 was detected in sheep and goat herds in the world. In this study, a caprine parainfluenza virus strain was isolated from sheep suffered severe respiratory disease with high morbidity and varied mortality in Hebei province of China. The phylogenetic analysis and pathogenicity indicated that the virus is distinct from previously reported CPIV3 strains.

2 | MATERIALS AND METHODS

2.1 | Samples and virus isolation

The nasal swabs, stool swabs and blood samples were collected from the sheep showed respiratory clinical signs with nasal discharge and cough in Hebei province, China in 2019. The nasal swab samples were quickly transferred to the laboratory and filtered through 0.22 μm membrane (Millipore). The filtered supernatants were inoculated to a monolayer of Madin–Darby bovine kidney (MDBK) cells cultured in DMEM (GIBCO, USA) supplemented with 2% fetal bovine serum, 100U/ml penicillin and 100ug/ml streptomycin at 37, 5% CO_2 incubator. The cytopathic effect (CPE) was observed for 5 days. After three passages, the cell cultures were harvested and freezing-thawing three times. The supernatant of cultures was collected and stored at -80 for RT-PCR assay.

2.2 | RT-PCR assay

Total RNA was extracted using PrimeScrip TM II 1st Strand cDNA Synthesis Kit (TaKaRa, Dalian, China) and the cDNA was subject to amplification using Premix Taq (TaKaRa, Dalian, China). A CPIV3 specific primer pair of fusion protein gene (the forward primer 5'-CATTGAATTCATACTCAGCAC-3' and reverse primer 5'-AGATTGTCGCATTT(AG)CCTC-3' was used for the reactions of amplification (Lyon et al., 1997). The reaction was using a 25 ul reaction volume and optimized to 9.5 ul of RNase-free water, 15.5ul of reaction mix (1ul of Mix 20 uM each of primers forward 0.5 ul and reverse 0.5 ul, 12.5 ul of Premix Taq, 2 ul of template RNA. The reaction was carried out using a Veriti 96-Well thermal cycler (Thermofisher) with the following temperature profile: 95°C for 5 min, followed by 35 cycles of 30 s at 95°C, 30 s at 50°C and 60 s,then with a final extension at 72 for 10 min. The PCR product were detected by electrophoresis through a 1.5% agarose gel and visualized under UV light.

2.3 | Sequencing and Phylogenetic analysis

Total RNA was extracted and sequence analyses were performed directly on the RT-PCR product on the NovaSeq 6000 (Illumina). The library was prepared using Nextera XT reagents (Illumina). The representative PIV3 genomic sequences in GenBank were used for genetic and phylogenetic analyses. Multiple sequence alignments of viral genome and the M gene were carried out using Clustal W in the MegAlign program (DNAStar software), together with reference sequences of the identified CPIV3, BPIV3 and HPIV3 strains. Molecular phylogenetic trees were constructed using the Neighbour-Joining method with the MEGA7 software (Kumar, Stecher, & Tamura, 2016).

2.4 | Experimental infection

Ten 3-month old sheep were obtained from a farm in Inner Mongolia, China. All animals were negative to CPIV3 and apparently healthy, presenting no signs of depression, cough or other health disorders. The selected animals were transported to an animal facility in Inner Mongolia. All animal experiments were approved by the Institutional Animal Ethics Committee of Inner Mongolia University (Approval No. IMU-MO-2020-012).

Five sheep were intranasally infected with 4ml of CPIV3 TX01 strain containing HA titer at 2^5 and$10^{7.8}TCID_{50}$/ml and other five sheep were inoculated with the cell culture medium to serve as the controls. The temperature was taken at the same time for 21 days. Clinical assessment was observed daily from day 1 to day 21. Clinical signs including depression, cough, asthma, and other respiratory disease were recorded and scored using a scale of 0 – 3 (0 = absence; 1 = mild; 2 = moderate; 3 = severe).

The nasal swab specimens were collected at 1-day prior to challenge through 21 days post-challenge. The serum sample was collected at 0, 4, 7, 14, 21day. The samples were used for the determination of virus shedding and the viremia.

2.5 | Pathological and histopathological examination

The gross lesions in the lungs, trachea, lymph nodes and other tissues of sheep were observed and examined at necropsy. The extent of gross lesions was evaluated and expressed as - (normal), + (mild), ++ (moderate) and +++ (severe). The tissue samples of liver, spleen, lung, heart, kidney, intestine and mesenteric lymph node were collected and fixed in 10% buffered formalin for histopathologic analysis.

3 | RESULTS

The nasal swabs, stool swabs and blood samples were detected by RT-PCR, and the amplified PCR product was sized by electrophoresis as 400bp (data not shown). The PCR production was cloned and sequenced. Blast research showed the sequence was related to the CPIV3 strain JS2013 (KT215610) with identity 97.5%. The results showed that the isolate was CPIV3 and named TX01. The MDBK cells inoculated with nasal swab samples developed obvious cytopathic effects (CPE) as cells appeared aggregated and formed net-like in the monolayers after three passages. The complete genome sequence of the CPIV3 TX01 strain was submitted to GenBank with the accession number of MT756864. Pairwise genomic alignment revealed that the complete genome identity of the CPIV3 TX01 strain was varied from 74.2% - 75.4% with BPIV3, and 72.6% - 73.0% with the HPIV3 respectively. The genome sequence of the CPIV3 TX01 strain has 97.76% identity, which is the highest identity with the reference CPIV3 strain (GenBank accession number MF683167). (Figure 1a). The phylogenetic analyses of the M gene showed that TX01 strains closely relate with CPIV3 but form a separate branch of its own (Figure 1b). Additionally, high similarities of the nucleotide and amino acid sequences of the CPIV3 TX01 strain with reference strains of AHQJ2015-1, GS2007-2, JS2013, JSHA2016, and JSHA2014-1 in the coding regions of N, M, fusion protein, Hemagglutinin/neuraminidase(HN)and Large protein(L)were also be analyzed (Table 1).

The virus-inoculated sheep displayed the syndrome with depression, cough, and fever. The rectal temperatures raised from 2 - 12 dpi in the challenge group. The date was shown as the mean +- S.D (Figure 2) . The infected group showed virus shedding during 2–21 dpi and viremia could be detected in serum samples. The lungs of virus-inoculated sheep showed mild to moderate diffuse purple consolidation (Figure 3). The histopathology analysis showed that alveolar walls were obviously thickened, the blood vessels were dilated and congested, the fibrous histiocytosis and lots of inflammatory cells were infiltrated. No lesions were observed in the control group (Figure 4).

4 | DISCUSSION

Respiratory diseases have been causing major economic loss to goat and sheep farms. Viral infection, stress conditions, and secondary infection resulted in severe clinical signs in sheep and goats (Haanes et al., 1997; Maidana et al., 2012.).

CPIV3 was confirmed one of the special pathogens causing respiratory disease in sheep and goats (Jizong Li, et al., 2020.). In this study, we first reported a novel lineage of CPIV3 TX01 strain isolated from sheep. The alignment of genome sequences and phylogenetic analysis indicated that the CPIV3 TX01 strain was different from the reported CPIV3 lineages and appeared to be a potentially new genotype. According to the current BPIV3 genotyping, the three genotypes A (BPIV3a) and B (BPIV3b) and genotype C (BPIV3c) were grouped. Therefore, we hypothesized temporarily to divide the CPIV3 into two genotypes, namely, CPIV3a and CPIV3b. As well, the TX01 strain was classified as genotype B (CPIV3b), which distinguished from JS2013 lineage.

In 2018, the presence of CPIV3 infection in sheep was investigated, the positive rate of CPIV3 antibody was 59.3% (1793/3026). The antigen-positive rate was 21.5% (401/1863)(Mao et al., 2019) . Here, we summarized the available publications and illustrated the areas of CPIV3 infections in China (Figure 5).Genbank accession numbers are the following: MK091103, MK091093, MF693117, KT215610, MF693178, MF683167, MK091096, MK091094, MK091095, MK091101, MK091102, MK091097, MT756864, MK091099, MK091098, MK091100.

In the CPIV3 TX01 strain animal study, the typical clinical signs of the infected sheep were depression, cough, sneezing, nasal discharge, tachypnea, and fever. Viremia was detected in the sheep in 7, 14, 21 days .Virus shedding was confirmed by RT-PCR from nasal swabs during 2 - 21dpi. The results are consistent with previous reports, except that rectal temperatures of the goats with JS2013 infected remained normal, with no significant differences observed between the inoculated and non-treated groups (Li,W. et al., 2014). Pathological examination results indicated that most of the virus-inoculated sheep showed mild to moderate gross lesion in lungs, with diffuse purple consolidation. The results of histopathologic examinations founded

that the lungs had mild to moderate thickened alveolar walls, decreased alveolar space, increased amounts of inflammatory cellular infiltrate congestion, and macrophage infiltration (Figure 3). In addition, the originally infected virus was isolated from the lung of virus-inoculated sheep using MDBK cell, the result indicated that CPIV3 TX01 was the main causative agent in this experimental infection. The pathogenicity caused by the CPIV3 strain in goats and guinea pigs have been reported, (Li,W. et al., 2016; Fei Hao et al., 2019). But it is still not clear whether sheep-derived PIV3 strain had pathogenicity in goats.

This study was reported a novel lineage of CPIV3 that originated from sheep in China which demonstrated high virulence to sheep. The risks of cross-transmission between sheep and goat and the evolution mechanisms, including genomic changes driven by the adaptation of CPIV3 to new hosts, are suggested.

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CONFLICT OF INTEREST

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTION

WW designed and initiated the study. MYH participated in the design and conducted most of the experiments in the study and drafted the manuscript. WY ,WJL took the animal experiment and collected samples. ZXH carried out virus isolation. WYZ, CCX, FC, WSR and YH participated in the the molecular genetic studies and analysed the data. WW and LGR participated in revised manuscript. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

Eisa, M., Karrar, A. E., & Abdel Rahim, A. H. J. B. V. J. (1979). The occurrence of antibodies to parainfluenza 3 virus in sera of some domestic animals of the Sudan. British Veterinary Journal, 135(2), 192-197.https://doi.org/10.1016/s0007-1935(17)32941-x

Fischer-Tenhagen, C., Hamblin, C., Quandt, S., FroLich, K. J. J. w. d. (2000). Serosurvey for selected infectious disease agents in free-ranging black and white rhinoceros in Africa. Journal of Wildlife Diseases,36 (2), 316-323.https://doi.org/0.7589/0090-3558-36.2.316

Hao, F., Wang, Z., Mao, L., Yang, L., & Jiang, J. J. M. P. (2019). The novel caprine parainfluenza virus type 3 showed pathogenicity in Guinea pigs. MicrobialPathogenesis,134,103569. https://doi.org/10.1016/j.micpath.2019.103569

Li, W. , Mao, L. , Cheng, S. , Wang, Q. , Huang, J. , & Deng, J. , et al. (2014). A novel parainfluenza virus type 3 (piv3) identified from goat herds with respiratory diseases in eastern china. Veterinary Microbiology, 174(1-2), 100-106.https://doi.org/10.1016/j.vetmic.2014.08.027

Li, W., Hao, F., Mao, L., Wang, Z., Zhou, T., Deng, J., . . . Lv, Y. J. V. R. (2016). Pathogenicity and horizontal transmission studies of caprine parainfluenza virus type 3 JS2013 strain in goats. Virus Research,223, 80-87. https://doi.org/10.1016/j.virusres.2016.06.021

Li, J. , Zhong, C. , Liao, Z. , Mao, L. , & Zhang, W. . (2020). Bta-mir-98 suppresses replication of caprine parainfluenza virus type 3 through inhibiting apoptosis by targeting caspase-3. Frontiers in Immunology, 11, 1575. https://doi.org/10.3389/fimmu.2020.01575

Luzzago, C., Ebranati, E., Lavazza, A., Besozzi, M., & Lauzi, S. J. A. (2020). Identification and Genetic Characterization of a Novel Respirovirus in Alpine Chamois (rupicapra rupicapra rupicapra). Animals (Basel),10(4), 704. https://doi.org/10.3390/ani10040704

Lyon, M., Leroux, C., Greenland, T., Chastang, J., Patet, J., & Mornex, J. F. (1997). Presence of a unique parainfluenza virus 3 strain identified by RT-PCR in visna-maedi virus infected sheep. Veterinary Microbiology, 57(2-3):95-104. https://doi.org/10.1016/s0378-1135(97)00104-1

Mao, L., Yang, L., Li, W., Liang, P., Zhang, S., Li, J., . . . Diseases, E. (2019). Epidemiological investigation and phylogenetic analysis of Caprine parainfluenza virus type 3 in sheep of China. Transboundary Emerging Diseases,66(3):1411-1416. https://doi.org/10.1111/tbed.13149

Kirsten C,Eberle, John D,Neill,Stephanie K,Venn-Watson, Jodi L,McGill, Randy E,Sacco (2015).Novel Atlantic bottlenose dolphin parainfluenza virus TtPIV-1 clusters with bovine PIV-3 genotype B strains. Virus Genes. 51(2), 198-208. https://doi.org/10.1007/s11262-015-1224-7

Ohkura, T., Minakuchi, M., Sagai, M., Kokuho, T., Konishi, M., Kameyama, K. I., & Takeuchi,K. J. V. (2015). Infection of the upper respiratory tract of hamsters by the bovine parainfluenza virus type 3 BN-1 strain expressing enhanced green fluorescent protein. Virology, 476, 134-140. https://doi.org/10.1016/j.virol.2014.12.015.

Ohsawa, K., Yamada, A., Takeuchi, K., Watanabe, Y., Miyata, H., & Sato, H. J. J. o. V. M. E. (1998). Genetic Characterization of Parainfluenza Virus 3 Derived from Guinea Pigs. Journal of Veterinary Medical Science, 60(8), 919-22. https://doi.org/10.1292/jvms.60.919

Parks, J. B., Post, G., Thorne, T., & Nash, P. J. J. o. t. A. V. M. A. (1972). Parainfluenza-3 virus infection in Rocky Mountain bighorn sheep. Journal of the American Veterinary Medical Association, 161(6), 669-672.

Qiao, D., Janke, B. H., & Elankumaran, S. J. J. o. V. (2010). Complete genome sequence and pathogenicity of two swine parainfluenzavirus 3 isolates from pigs in the United States. Journal of Virology, 84(2), 686-694. https://doi.org/10.1128/JVI.00847-09

Ren JL, Zhu YM, Zhou YH, Lv C, Yan H, Ma L, Shi HF, Xue F.Identification of three antigen epitopes on the nucleocapsid protein of the genotype C of bovine parainfluenza virus type 3. Veterinary Microbiology,178(1-2),61-69. https://doi.org/10.1016/j.vetmic.2015.04.016

Selim, Comakli, Selcuk, & Pathogens, O. J. (2019). Comparative Evaluation of the Immune Responses in Cattle Mammary Tissues Naturally Infected with Bovine Parainfluenza Virus Type 3 and Bovine Alphaherpesvirus-1.Pathogens(Basel,Switzerland),8(1):26. https://doi.org/10.3390/pathogens8010026

Silvina S Maidana, P. M. L., Gustavo Combessies, Maria I Craig, Julian Diodati, Daniela Rodriguez, Viviana Parreno, Osvaldo Zabal, Jose L Konrad, Gustavo Crudelli, Axel Mauroy, Etienne Thiry & Sonia A Romera (2012). Isolation and characterization of bovine parainfluenza virus type 3 from water buffaloes (Bubalus bubalis) in Argentina.Bmc Veterinary Research,8(1). https://doi.org/10.1186/1746-6148-8-83

Stevenson, R. G., & Hore, D. E. J. J. o. C. P. (1970). Comparative pathology of lambs and calves infected with parainfluenza virus type 3. Journal of Comparative Pathology,80(4), 613- 618. https://doi.org/10.1016/0021-9975(70)90060-5

Sudhir, Kumar, Glen, Stecher, Koichiro, Biology, T. J. M., & Evolution. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasetsl;, 33(7), 1870–1874. https://doi.org/10.1093/molbev/msw054

Thorsen, J., & Henderson, J. P. J. J. o. W. D. (1971). Survey for antibody to infectious bovine rhinotracheitis (IBR), bovine virus diarrhea (BVD) and parainfluenza 3 (PI3) in moose sera.Journal of Wildlife Diseases, (PI3) in moose sera. Journal of wildlife diseases, 7(2), 93–95. https://doi.org/10.7589/0090-3558-7.2.93

Walker, P. J., Siddell, S. G., Lefkowitz, E. J., Mushegian, A. R., & Davison, A. J. J. A. o. V. (2019). Changes to virus taxonomy and the International Code of Virus Classification and Nomenclature ratified by the International Committee on Taxonom of Viruses (2019). , 164(9), 2417–2429. https://doi.org/10.1007/s00705-019-04306-w

Zarnke, R. L., & Erickson, G. A. J. J. o. W. D. (1990). Serum antibody prevalence of parainfluenza 3 virus in a free-ranging bison (Bison bison) herd from Alaska.Journal of Wildlife Diseases, 26(3), 416–419. https://doi.org/10.7589/0090-3558-26.3.416

Figure legends

Figure 1. Phylogenetic tree based on the complete genome and M gene of the CPIV3 TX01 strain and other PIV3 strains.

(a) The evolutionary analyses based on the full-length genome sequence of the novel isolate and reference respiratory strains showed that TX01 significantly clustered with CPIV3 but formed a separated branch. (b)The evolutionary analyses based on the M gene sequence. The evolutionary analyses were performed using MEGA 7 software. The red circle indicates the novel sequence. Different strains isolated from different species represented by different colors.

Table 1. Comparison of the nucleotide sequences and amino acid sequences between CPIV3 TX01 strain and reference CPIV3 strains.

Figure 2. The rectal temperatures of the sheep infected experimentally with CPIV3 TX01 strain in the infection group and control group.

Figure 3. Gross pathological lesions in the experimentally infected sheep lungs.

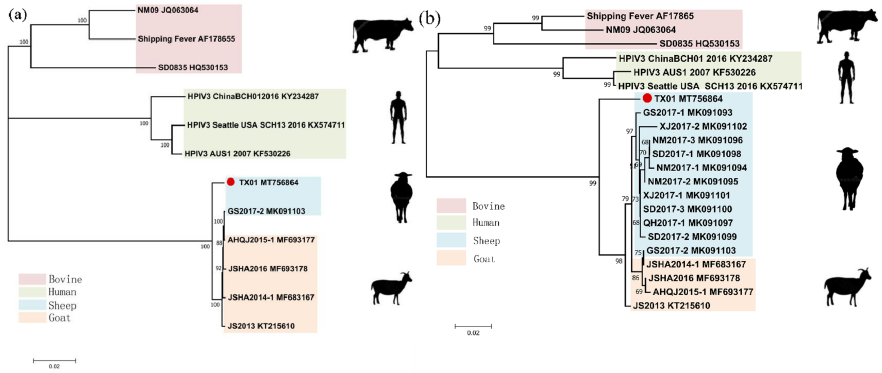
A. Normal control lungs. B. Interstitial lung disease at day 21 poi in one infected sheep. C. A consolidation of 1.0 x 2.0 cm in one sheep lung. D. Consolidations in the pulmonary lobules in one infected sheep.

Figure 4. Histopathological observations in the sheep lungs. (200x).

A.Lung section of sheep of NC group necropsied at 21dpi. B-D.Lung section of one infected sheep showed severe thickened alveoli septa. E. Lung section of one infected sheep showed thickened alveoli septa and expansions of the alveolar interstitium. F. Lung section of one infected sheep showed thickened alveoli septa, congestion, macrophage infiltration and compensatory emphysema.

Figure 5. Distribution map of CPIV3 strains from 2013 to 2019 in China.

The area of the gray color (0) express no CPIV3 strain has been reported in this area. The area of the yellow color (1) express one CPIV3 strain has been reported in this area (QH2017-1, AHQJ2015-1). The area of the blue color (2) express two CPIV3 strains has been reported in this area (GS2017-2, GS2017-1, XJ2017-1, XJ2017-2). The area of the red color (3) express three CPIV3 strains has been reported in this area (JS2013, JSHA2016, JSHA2014-1, NM2017-3, NM2017-1, NM2017-2, SD2017-1, SD2017-2, SD2017-3).



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