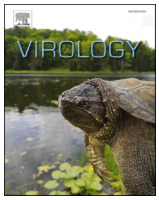
Virology 604 (2025) 110409

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/virology



Enteric pathogenicity characterization of emerging parainfluenza virus 5 in western China

Minting Ni [a,b,1], Shengyu Lin [a,c,1], Yongheng Shao [a,d], Jiao Tang [a,e], Shuxian Li [a,f], Chen Tan [a], Zhenli Gong [a], Hongbo Li [a,f], Jintao Wang [b], Guangliang Liu [a,**], Jianing Chen [a,*]

[a] State Key Laboratory for Animal Disease Control and Prevention, College of Veterinary Medicine, Lanzhou University, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, 730000, China
[b] College of Animal Science and Veterinary Medicine, Heilongjiang Bayi Agricultural University, Daqing, China
[c] College of Animal Sciences, Fujian Agriculture and Forestry University, Fuzhou, Fujian, China
[d] College of Veterinary Medicine, Gansu Agricultural University, Lanzhou, Gansu, China
[e] College of Veterinary Medicine, Shanxi Agricultural University, Jinzhong, Shanxi, China
[f] College of Veterinary Medicine, Xinjiang Agricultural University, Urumqi, Xinjiang, 830052, China

ARTICLE INFO

Handling Editor: Dr. Jasmine Tomar

Keywords:
Parainfluenza virus
Pathogenicity
Intestine

ABSTRACT

Parainfluenza virus 5 (PIV5) is a member of the *Paramyxoviridae* family and causes respiratory symptoms in various animal species. Although the virus has been frequently detected among fecal samples, no study has described its infection of the intestine. Recently, diarrhea with low mortality has spread on pig farms in Gansu, China. Next-generation sequencing confirmed the emergence of PIV5 among the samples. The PIV5 strain was then successfully isolated and characterized in vitro. Further animal tests revealed that PIV5 can result in respiratory symptoms and mild diarrhea in piglets. Immunohistochemical staining confirmed PIV5 infection resulted in steatosis and contributed to diarrhea. A retrospective investigation revealed that the number of cases of PIV5 infection has increased since 2020. Overall, our study is the first to present data indicating that PIV5 infection leads to diarrhea. Although it has low pathogenicity, PIV5 may pose a potential threat to pig production in China.

1. Introduction

Parainfluenza virus (PIV) is an important human and animal pathogen of the central nervous and respiratory systems. It is an enveloped, single-stranded, negative-sense RNA virus belonging to the genus Respirovirus within the *Paramyxoviridae* family (Walker et al., 2021). Its full-length genome is approximately 15.2 kb long and consists of 5′UTR-NP-V/P-M-F-SH-HN-L-3′UTRs (Henrickson, 2003; Vainionpää and Hyypiä, 1994). PIV can be divided into five different genotypes according to phylogenetic analysis. Each genotype shares a nucleotide homology less than 65% compared to other genotypes. Among them, the detection ratio of PIV5 has sharply increased over the past decade (Truong et al., 2023).

PIV5, formerly known as simian virus 5 (SV5), was first isolated from primary kidney cell cultures of rhesus and cynomolgus monkeys (Hull et al., 1956). However, the following epidemiological studies revealed that monkeys were not reservoirs for PIV5 (Atoynatan and Hsiung, 1969). In contrast, accumulating evidences have demonstrated that PIV5 circulates among humans and animals, suggesting a potential challenge to the health of both humans and animals (Charoenkul et al., 2021; Hierweger et al., 2020; Lee and Lee, 2013; Liu et al., 2015; Wang et al., 2019; Xie et al., 2020; Zhai et al., 2017).

Although PIV5 has been frequently identified in the respiratory tract of both animals and humans, its manifestation in pigs varies from that in other species. PIV5 is frequently detected in fecal samples from diarrheal piglets but is rarely detected in other species (Ibrahim et al., 2022; Jiang et al., 2018; Lee and Lee, 2013; Lee et al., 2013; Singh et al., 2022). Importantly, the systemic impact of PIV5 on swine health is largely unknown. Information regarding PIV5 infection in the porcine digestive system is also limited.

* Corresponding author. 1 Xu-Jia-Ping, Yan-Chang-Bu, Cheng-Guan District, Lanzhou, Gansu, 730046, China.
** Corresponding author.
E-mail addresses: LiuGuangliang01@caas.cn (G. Liu), chenjianing@caas.cn (J. Chen).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.virol.2025.110409
Received 9 October 2024; Received in revised form 10 January 2025; Accepted 14 January 2025
Available online 15 January 2025

In this study, PIV5 was identified in pig farms with diarrhea via next-generation sequencing (NGS). A PIV5 strain was then successfully isolated and subsequently identified as the causative agent of diarrhea via further animal tests. We believe that this study contributes to the further understanding of PIV5.

2. Materials and methods

2.1. Sample collection and next-generation sequencing (NGS)

In 2021, piglets approximately 14 days old from a pig farm in Lanzhou, Gansu, presented diarrhea and polypnea. None of porcine epidemic diarrhea virus (PEDV), transmissible gastroenteritis virus (TGEV), porcine deltacoronavirus (PDCoV), porcine rotavirus (PoRV), or African swine fever virus (ASFV) strains were detected. Therefore, the collected samples were centrifuged at 2000 rpm for 15 min, and the clarified supernatant was collected for next-generation sequencing by Shanghai Tanpu Biotechnology Co., Ltd. Briefly, RNA and DNA were both extracted from the samples and prepared for library construction with Nextera XT reagents (Illumina, USA). The library was finally normalized, and loading for sequencing was performed with a NovaSeq 6000 (Illumina, USA).

2.2. Cells and virus isolation

PK-15 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Sigma, Germany) supplemented with 5% calf bovine serum (CBS) (Sigma, Germany). For virus isolation, PK-15 cells were inoculated with the filtered samples at 37 °C for 1 h. The cells were then cultured with Opti-MEM (Invitrogen, USA) and observed daily for 3 days to monitor the development of the cytopathic effect (CPE). At 3 days post infection (dpi), the cells were subjected to three freeze–thaw cycles. The supernatant was then collected and passaged.

2.3. Electron microscopy

The supernatant of PIV5-infected cells was collected and centrifuged at 12,000 rpm for 15 min to remove cell debris, followed by centrifugation at 100,000×*g* for 2 h at 4 °C. The pellet was resuspended in PBS and purified via sucrose density gradient centrifugation. The obtained virions were diluted with PBS and spotted onto Formvar-coated grids for negative staining with phosphotungstic acid. The grids were finally subjected to Hitachi electron microscope observation (HT7700, Japan) at 80 kV.

2.4. Plaque assay

PK-15 cells were seeded into 12-well plates. When grown to 100% confluence, the cells were infected with viral stock. One hour post infection, the cells were overlaid with 0.80% low melting point agarose (Sigma, Germany) in DMEM containing 5% CBS (Sigma, Germany). The cells were then cultured for another 48 h at 37 °C. To visualize plaques, the cells were stained with 1% crystal violet in ethanol.

2.5. Immunofluorescence assay

PK-15 cells seeded in 96-well culture plates were infected with viral stock. At 72 h after inoculation, the cells were fixed with 4% paraformaldehyde for 30 min and then permeabilized with 1% Triton X-100. After being blocked with 5% skim milk for 1 h, the cells were incubated with a mouse anti-PIV5-nucleocapsid monoclonal antibody (1:2000 dilution) (Qianxun, China) for 1 h, followed by incubation with a DyLight 488-conjugated goat anti-mouse IgG antibody (1:500 dilution) (Biodragon, China) for 1 h. The cell nucleus was stained with DAPI (Bide, China). The cells were then observed under a fluorescence microscope (TE2000U; Nikon) with a video documentation system.

2.6. Phylogenetic analysis of PIV5

The complete genome sequence of PIV5 was uploaded to the GenBank database (accession no. PP189887.1). The parainfluenza virus reference strains were downloaded from GenBank and listed in Supplementary Table S1. The sequences of PIV5 and the reference strains were aligned via multiple alignment via ClustalW (MEGA7) (Katoh and Standley, 2013). Phylogenetic trees based on the complete genome and individual genes were constructed via the maximum likelihood (ML) method, with the best-fitting evolutionary model suggested by the program following 1000 bootstrap replicates. The genetic distance was calculated via the Tamura–Nei model.

2.7. Animal experiments

All twelve piglets used in this study were pathogen free of PIV5, PEDV, TGEV, PDCoV, rotavirus and ASFV, which were detected via PCR analysis. The sows and piglets were also antibody free of PIV5, as proven by the ELISA method developed in our laboratory. The 7-day-old piglets were randomly divided into two groups, and artificial feed was replaced with milk. After the piglets acclimatized to the environment, one group of piglets was inoculated with an isolated virus (10^6 $TCID_{50}$ per pig) orally, while the control group was orally inoculated with an equal volume of DMEM. Clinical symptoms were observed daily. At 3 or 7 days post infection, three pigs from each group were euthanized for viral titer determination and microscopic lesion assessment. The animal experiment was conducted in compliance with the Animal Ethics Procedures and Guidelines of Laboratory Animals, approved by the Animal Administration and Ethics Committee of Lanzhou Veterinary Research Institute (LVRI) of the Chinese Academy of Agricultural Sciences (Permit No. LVRIAEC-2024-003).

2.8. Histopathology and immunohistochemical staining

For histopathological examination, tissue sections were stained with hematoxylin and eosin. For immunohistochemical testing, tissue sections were dewaxed and dehydrated. After deparaffinization, the sections were treated with 3% H_2O_2 for 8 min, followed by washing with distilled water and microwave treatment in citrate buffer for 20 min at 99 °C. The sections were then blocked with 5% BSA and further incubated with mouse anti-PIV-nucleocapsid antibodies (1:1000 dilution) (Qianxun, China), followed by incubation with biotinylated goat anti-mouse antibodies (1:500 dilution) (Abbkine, China) for 30 min at room temperature. The sections were treated with 3,3′-diaminobenzidine tetrahydrochloride chromogen (Beyotime, China), counterstained with hematoxylin, and visualized with a light microscope.

2.9. Real-time PCR analysis

For the determination of PIV5 titers in different tissues, RNA was extracted with RNAiso Plus (Takara, Japan) and then reverse transcribed into cDNA with HiScript II RT SuperMix (Vazyme, China). Real-time PCR analysis was performed in a Bio-Rad CFX96 system with TransStart Probe qPCR SuperMix (TransGen, China). The samples were incubated at 94 °C for 30 s, followed by 40 cycles at 94 °C for 5 s and 60 °C for 30 s. The sequences of the primers and probe used were as follows: PIV-F (5′-TGGCGAAGGCCGTAACC-3′), PIV-R (5′- CAGCGTCTCATTTCGGAGTCT-3′), and Probe (5′-FAM- CCCCGGATACTGCTGTAMRA-3′).

3. Results

3.1. NGS revealed that PIV was involved in diarrhea

In April 2021, diarrhea caused by unknown pathogens broke out on a

pig farm in Gansu Province, China. Most of the suffering piglets were within 14 days old, and very few piglets died. PEDV, TGEV, PDCoV, rotavirus or ASFV were not detected in the samples via PCR analysis. Therefore, diarrhea samples were collected for next-generation sequencing to identify potentially associated pathogens. The workflow is described in Fig. 1A. With the removal of ribosomal RNA, host contamination and bacterial sequences, the remaining reads were finally mapped to PIV5, phages and porcine retrovirus (Fig. 1B). Both phages and porcine retroviruses are endogenous viruses that are maintained in most pigs. The retained reads of PIV5 were also highly distant from those of the other two strains. Further RT−PCR validation confirmed the existence of PIV5 among the diarrheal samples (Fig. 1C). Therefore, PIV5 was taken as the main target related to the outbreak of diarrhea among the pigs.

3.2. Isolation and identification of PIV5

The filtered samples were then inoculated into PK-15 cells for virus isolation. The supernatant was collected from cells that displayed a visible cytopathic effect (CPE) and then passaged 20 times in a blinded manner. The viral stock was then purified via a plaque assay and formed clear plaques approximately 0.5–1.2 mm in diameter (Fig. 2A). RT−PCR analysis revealed that the purified virus was positive for PIV5 (Fig. 2B). The infection of PIV was confirmed with mab against NP by IFA analysis. The positive signals were distributed throughout the cytoplasm (Fig. 2C). TEM revealed enveloped paramyxovirus-like particles approximately 150–200 nm in diameter (Fig. 2D). The growth characteristics of PIV were also described in PK-15 cells. The growth kinetics curve revealed that the viral titers peaked at 60 hpi, at approximately $10^{6.33}$ $TCID_{50}$/mL (Fig. 2E). Collectively, these data suggest that the PIV strain was successfully isolated.

3.3. Phylogenetic analysis of PIV5

To characterize the genetic characteristics of the isolated PIV5 strain, the whole genome was amplified (Fig. 3A) and sequenced with the 15

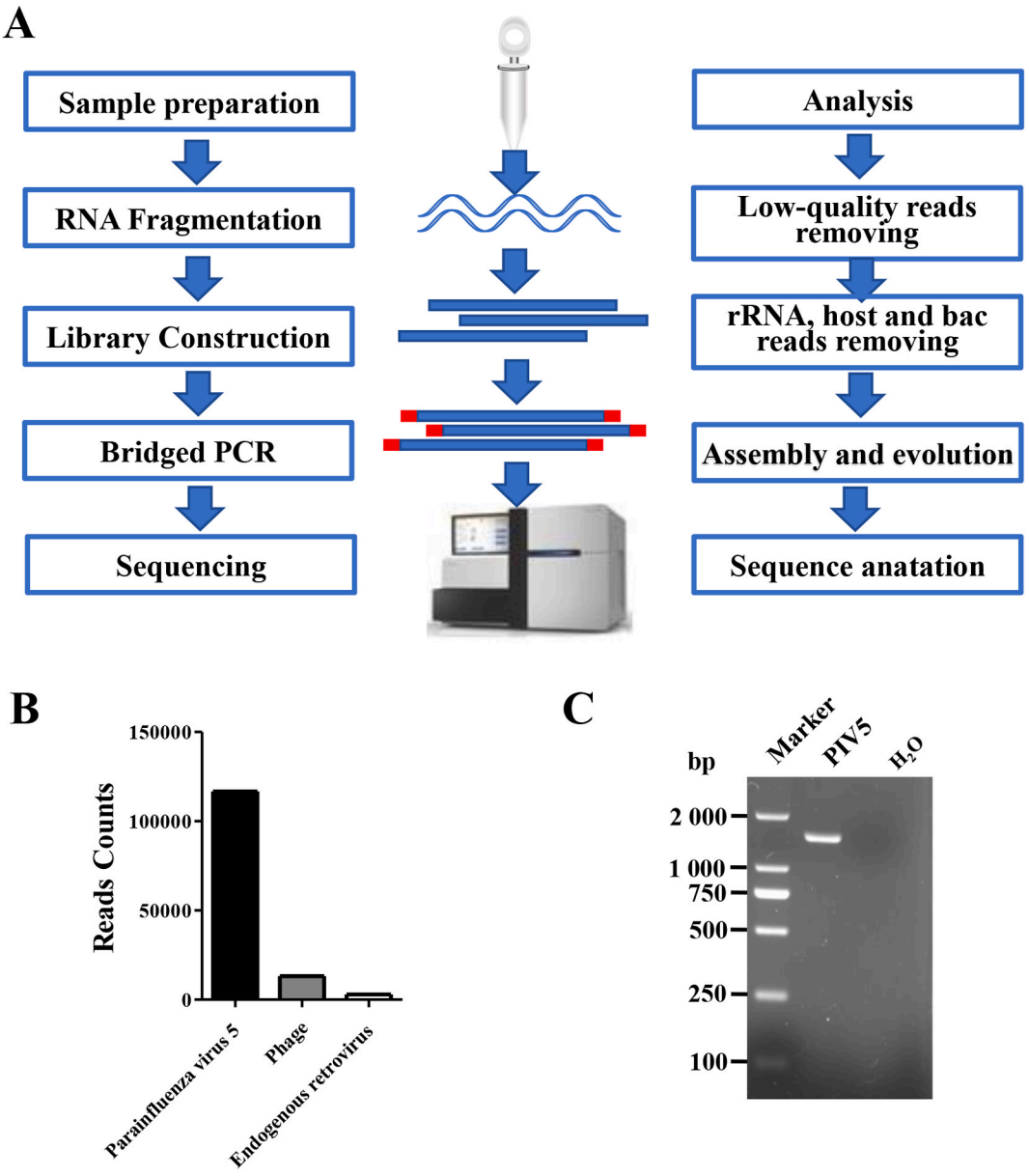


Fig. 1. Identification of PIV5 among diarrheal samples via NGS. (A) Workflow of next-generation sequencing for virome analysis. (B) Statistical results of the virus-associated read counts obtained via NGS. (C) RT−PCR results confirmed the presence of PIV5 in diarrhea samples.

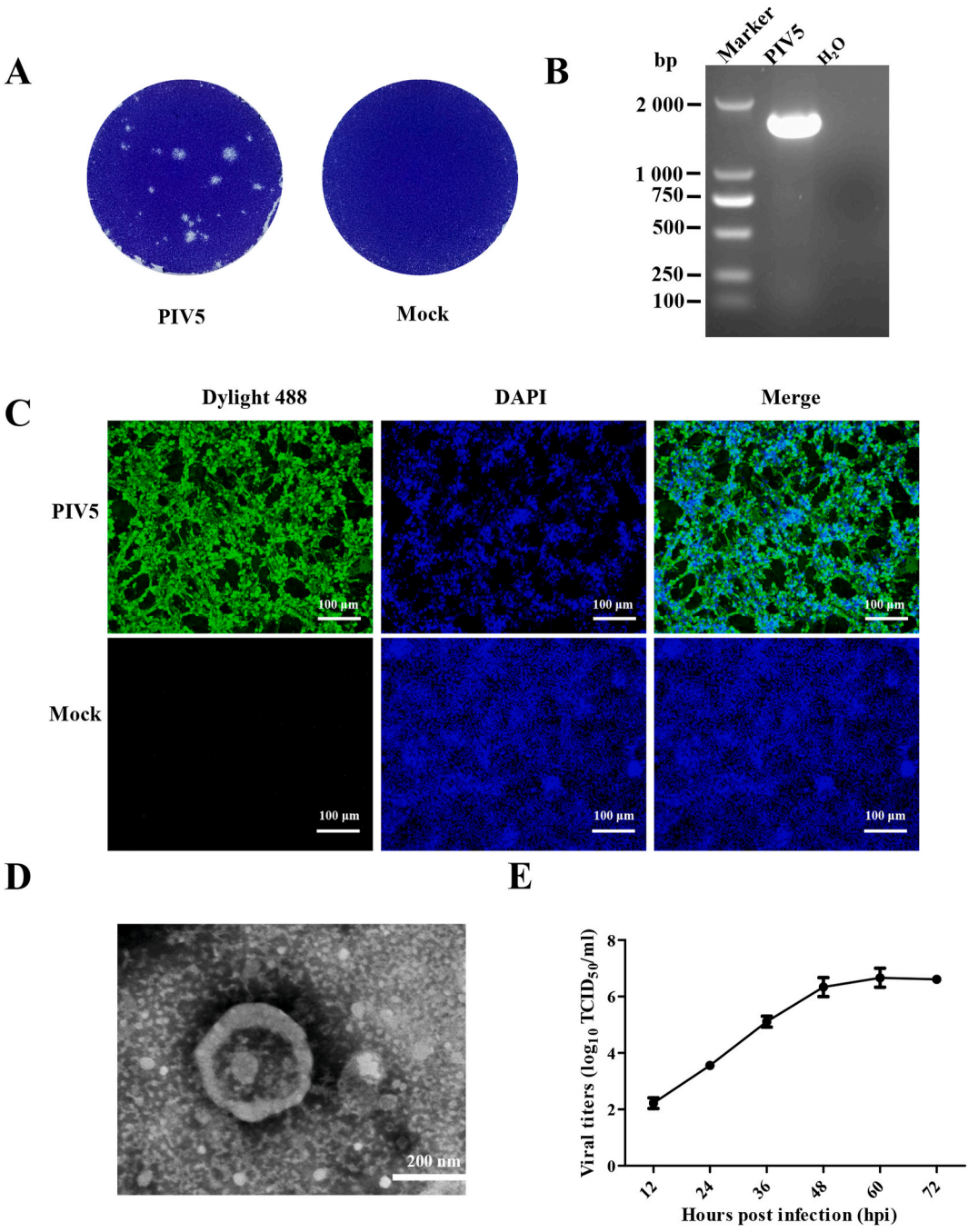


Fig. 2. Isolation and characterization of PIV5 isolate. (A) Plaque assay of the purified PIV5 isolate in PK-15 cells. (B) RT–PCR detection of PIV5 in the purified viral stock. (C) IFA analysis of PIV5-infected cells and mock-treated cells. (D) Electron microscopy image of purified PIV5 virions. (E) Growth kinetics curve of PIV5 in PK-15 cells.

pairs of primers listed in Table 1. The assembled genome sequence was uploaded to the GenBank database (Accession No. PP189887.1) and named LZ-1/2021. (Fig. 3B). On the basis of all the complete genomes of PIV5 uploaded to GenBank, the phylogenetic tree further revealed that PIV5 could be divided into four groups (I, II III and IV). The Group I strains infected various wild species, whereas the Group II strains infected only dogs and pigs. The group III strains included primates as the main host, with only one exception, which was isolated from a horse. Group IV contains PIV strains with swine as the main host. Importantly, the isolated PIV5 was clustered into Group III, which was far related to other porcine parainfluenza viruses (Fig. 3B). However, phylogenetic analysis of the HN and L genes revealed that the LZ-1/2021 strain was closely related to the CPI+ and CPI- strains, which were isolated from dogs and clustered into Group IV. The F and M genes of LZ-1/2021 were classified as W3A strains belonging to Group III. For the P gene, the above three strains were closely related (Fig. 3C). These results suggest that the isolated PIV5 strain shares high genetic diversity.

3.4. *PIV5 was pathogenic to suckling piglets*

To determine whether the isolated PIV5 was the causative agent of diarrhea, six 7-day-old piglets negative for PIV5, PEDV, TGEV, PDCoV, rotavirus and ASFV were orally inoculated with 10^6 $TCID_{50}$ of PIV5. Another six piglets were inoculated with an equal volume of DMEM as the control. Among all the tested animals, three from each group were euthanized at 3 dpi, and the rest were euthanized at 7 dpi. All piglets

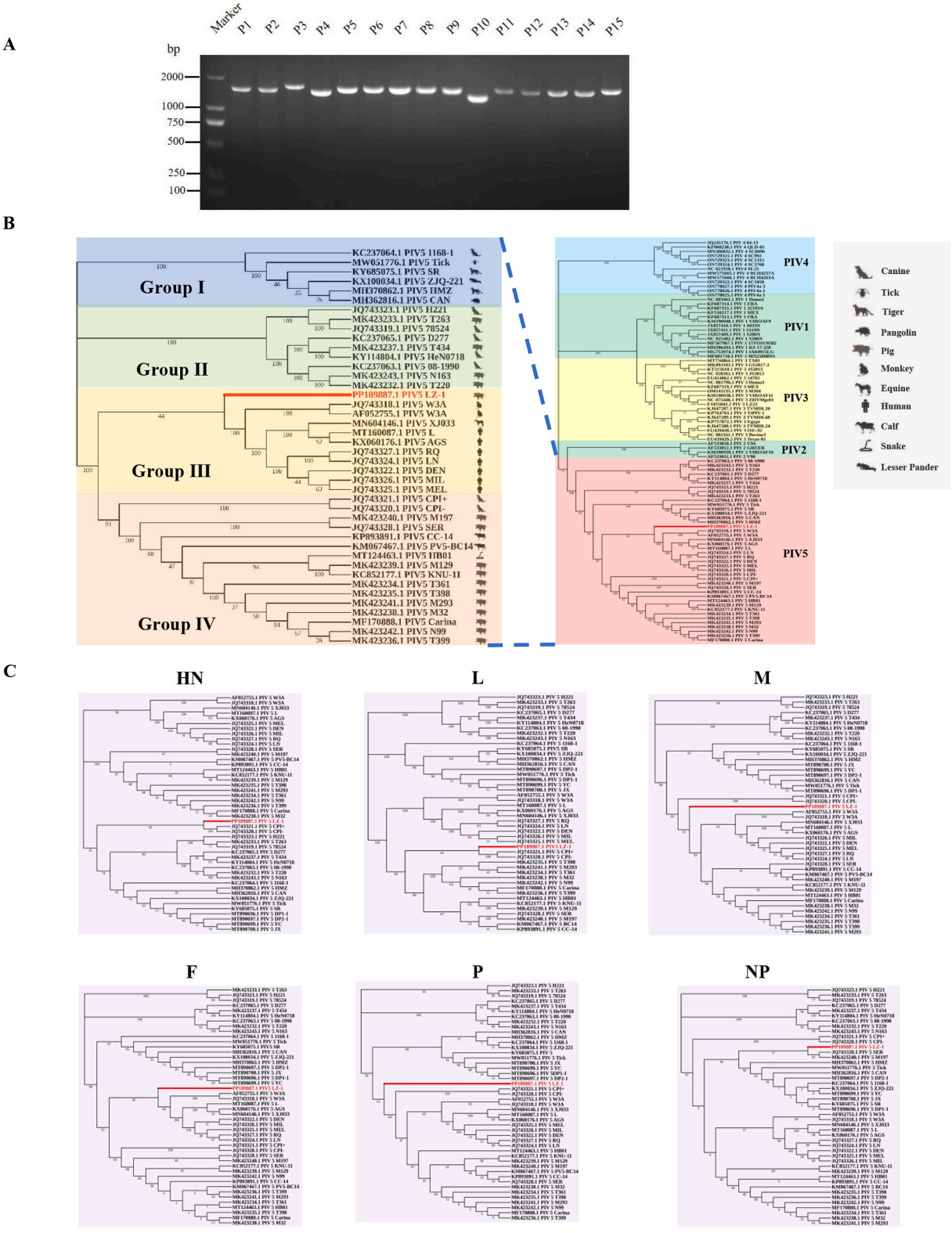


Fig. 3. Phylogenetic analysis of the PIV5 LZ-1/2021 strain. The complete genome of the PIV5 LZ-1/2021 strain was amplified with 15 pairs of specific primers. (B) Phylogenetic analysis based on the complete genome of parainfluenza virus was conducted via MEGA7.0 software. (C) Phylogenetic trees were constructed on the basis of the structural genes of PIV5. The phylogenetic trees were constructed via the maximum-likelihood method with 1000 bootstrap replicates. The PIV5 strain isolated in this study is indicated in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Primers used for the amplification of PIV5 complete genome.

Primer	Sequence (5′-3′)
PIV5 1F/1R	ACCAAGGGGAAAACGAAATAGT/TCCTCTTCCAATGGTGGCAT
PIV5 2F/2R	TCAGGAGAGCTAACAAAGCTA/TGATTCATCCGAGAGTCCAA
PIV5 3F/3R	TGCAGAGGCAAAGATCCAAGA/TGGACAGTAAGTCACTGACAGA
PIV5 4F/4R	ACACTGGTATGACACTGTACT/TGAAGGACGATATGGGTTCGT
PIV5 5F/5R	TGGAAAGGGTGGATGGAGCT/AGGTGCATCTTGTCAAGTTACCT
PIV5 6F/6R	ACACAATCACTAGGAACGGCA/TCTTCTGCAACCATTGTAGTGT
PIV5 7F/7R	ATCTGTCTTGGATCGTTAGGT/AGCAGAGAGCAGCAACCATCT
PIV5 8F/8R	ACCTAGTTTCATTCCAACTGCCA/ACCAAGCGTTGAGCTAAGTT
PIV5 9F/9R	TGGATCAGAAGCAACCTTCACT/ACACATAGACTCGCGTACCT
PIV5 10F/10R	AGCACTTACCTGAATCCTCTGA/ACTACGCATTAGCCGTAAGTGA
PIV5 11F/11R	TCAGGTGAGTATCTACATGATGA/TCCCTATCCAGGAGAAATCT
PIV5 12F/12R	TTGCAACTACTGTCATGAGGT/ACATGATGCACCAGTGTGCA
PIV5 13F/13R	ATCCTCCTATCCGTGTACCGTA/TGTGGGGTTAGCTTAAGACT
PIV5 14F/14R	TTCCGACGAGCTATCAACCT/TTGAATAGAGCATCTTCTCCTAGA
PIV5 15F/15R	TGAGGACTTCATCCCTCTAT/ACCAAGGGGAAAACCAAGAT

were observed daily for clinical manifestations and body temperature. The results revealed that soft stool and slight diarrhea also emerged in infected pigs beginning at 3 dpi (Fig. 4A). Moreover, mild coughing and hard breath were observed in PIV5-infected pigs. For piglets euthanized at 3 dpi, white spots in the lungs of infected piglets were observed, suggesting the emergence of pulmonary consolidation (Fig. 4B). The intestines of infected pigs were also thin-walled, gas-distended, and filled with yellow water (Fig. 4C). However, the pigs recovered at 5 dpi, and no pigs died during the 7 days of observation. The body temperature of all the tested piglets remained stable throughout the entire observation period (Fig. 4D). Nasal and anal swabs were collected daily to determine virus shedding. The results of nasal swab detection revealed that viral shedding started at 1 dpi, peaked at 4 dpi, and was eliminated at 6 dpi. Anal swab detection revealed a similar viral shedding curve. However, the feces still contained limited virus at 7 dpi. The determination of the viral load in different tissues revealed that PIV5 was detected in the spleen, lung, jejunum and ileum at 3 dpi but in the lung, jejunum and ileum at 7 dpi. The viral titers at 3 dpi were much higher than those at 7 dpi. The results revealed that PIV5 was both respiratory and enteric pathogenic in piglets but did not cause severe disease or death.

3.5. Histopathological examination of PIV5-infected pigs

To further explore the pathogenesis of PIV5 infection, H&E analysis was performed on the lung and ileum. The lungs presented with interstitial pneumonia, characterized by alveolar wall thickening and

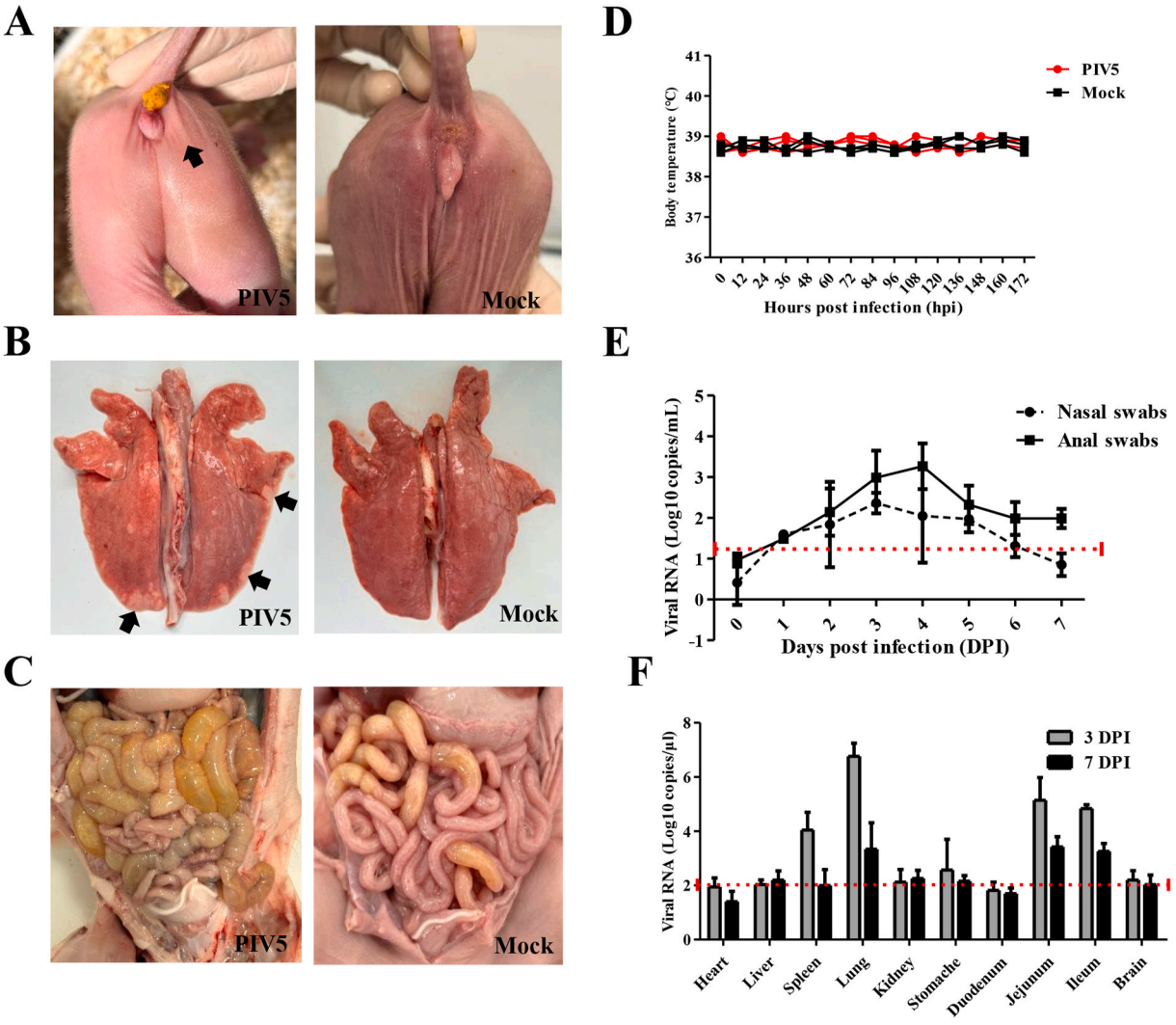


Fig. 4. Pathogenicity evaluation of the PIV5 LZ-1/2021 strain in piglets. (A) Soft stool of PIV5-challenged piglets at 3 dpi. (B) Macroscopic lesions in the lungs of PIV5-challenged piglets and mock-treated piglets. (C) Macroscopic lesions in the intestine of PIV5-challenged piglets and mock-challenged piglets. (D) Body temperature was detected daily in PIV5-challenged piglets and mock piglets. (E) Nasal swabs and anal swabs were collected daily from PIV5-challenged piglets for determination of viral shedding via real-time PCR analysis. (F) Virus loading in different tissues of PIV5-challenged piglets detected by real-time PCR analysis.

pulmonary interstitial fibrosis, which are typical clinical manifestations of viral infection (Fig. 5A). The ileum presented lipid droplets on the edge of the intestinal villi with a decreasing number of follicles, suggesting the occurrence of steatosis (Fig. 5B). No gross lesions were observed in the relevant organs of the control pigs. Immunohistochemical (IHC) staining was also conducted on the harvested tissues to locate the viral antigens. The PIV5 NP antigen can be detected in the lung and ileum. The emergence of positive signals further confirmed that PIV5 targets both the lung and intestine (Fig. 5C and D).

3.6. PIV5 detection in field samples

Currently, PIV5 has a global presence and is prevalent in several Asian countries; including Korea and China. however, little information has been provided about its distribution in Northwest China. To evaluate its prevalence, a retrospective investigation was performed with diarrhea samples collected from 2015–2022 in Gansu, China. The results demonstrated that PIV5 was absent among samples collected before 2020. However, 12 out of 67, 9 out of 62, and 5 out of 57 samples tested positive for PIV5 between 2020 and 2022 (Fig. 6A). These data further indicate that PIV5 is related to enteric diseases. The detected regions

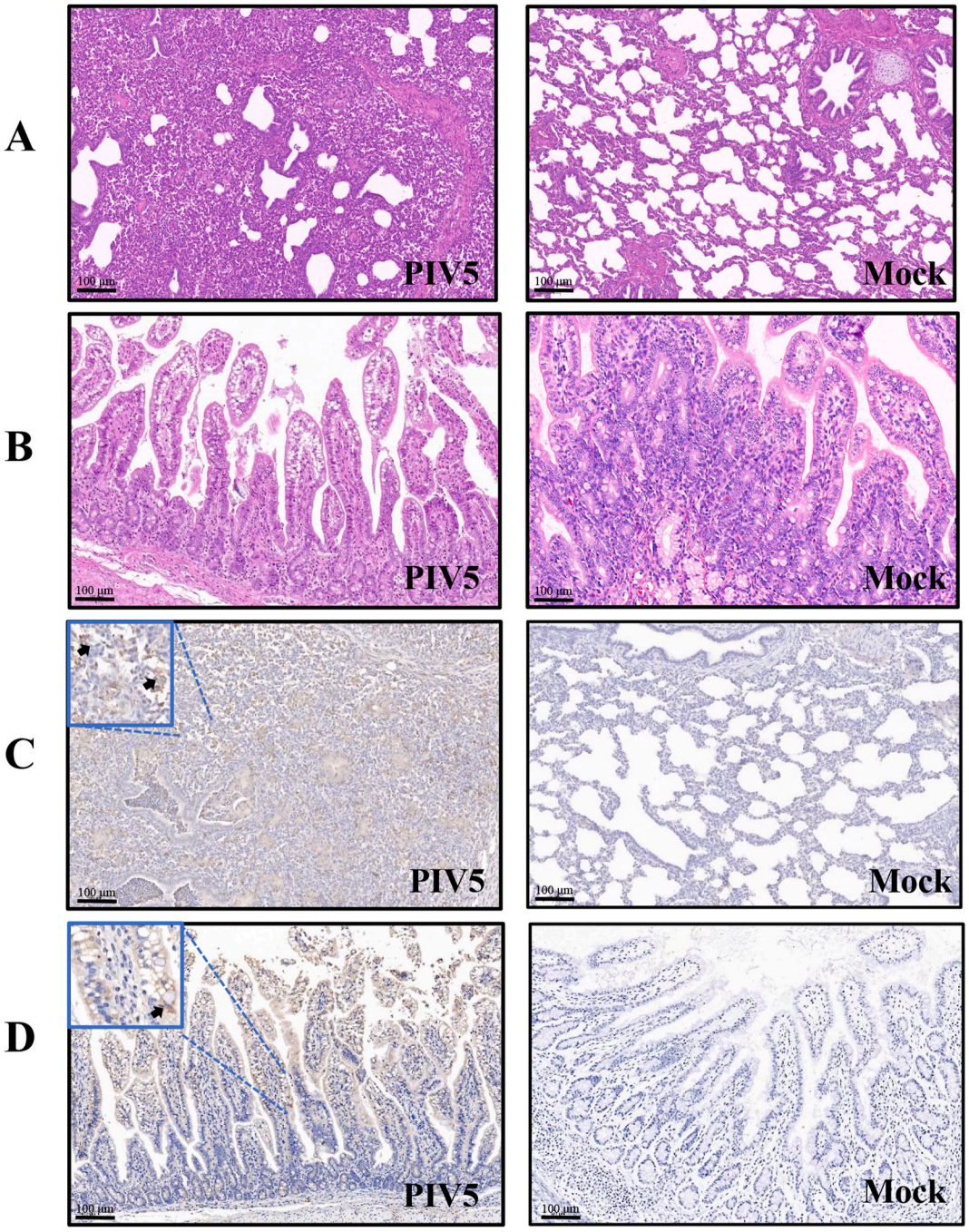


Fig. 5. H&E and IHC examination of PIV5-infected pigs. Piglets were orally inoculated with PIV5 at a dose of 10^6 TCID50 per pig. The animals were sacrificed, and paraffin sections were prepared for H&E or IHC staining. (A) H&E staining of the lungs of PIV5-challenged pigs characterized by interstitial pneumonia compared with mock-infected pigs. (B) H&E staining of the ileum of PIV5-challenged pigs characterized by steatosis compared with mock-infected pigs. (C) IHC staining of the lungs of PIV5-challenged and mock pigs. (D) IHC staining of the ileum of PIV5-challenged and mock pigs. The arrows indicate positive signals.

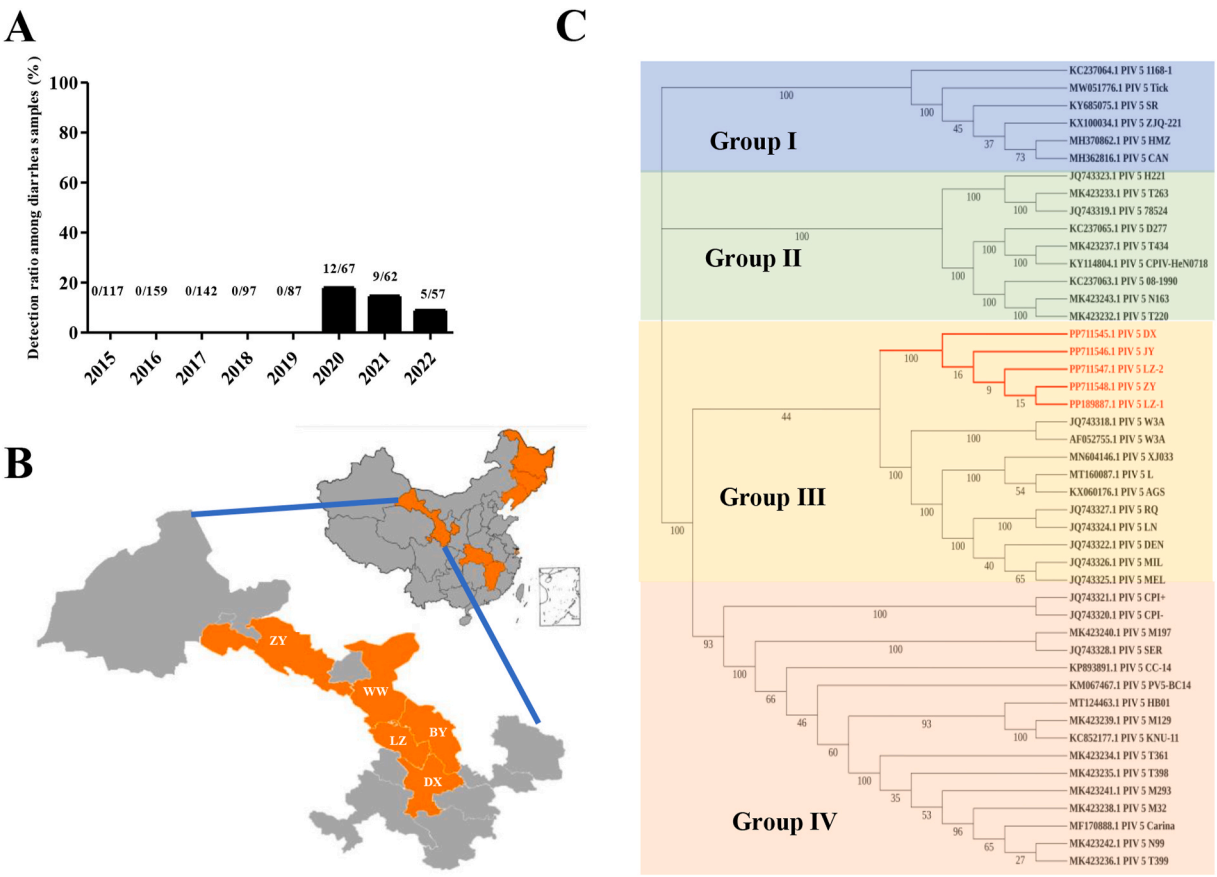


Fig. 6. The retrospective investigation of PIV5 in Gansu, China. The detection ratio of PIV5 between 2015 and 2022 in Gansu, China. (B) The locations of the identified PIV5 strains in Gansu. The yellow straining indicates the positive areas for PIV5. (C) Phylogenetic analysis of sequenced PIV5 detected in Gansu. The red line indicates the sequenced PIV5 strains in this study. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

included Dingxi (DX), Lanzhou (LZ), Zhangye (ZY), Baiyin (BY), and Wuwei (WW), the main pork production areas in Gansu, which all tested positive for PIV5 (Fig. 6B). Four of these detected viruses, which were collected from different regions in Gansu, were then sequenced for the complete genome. All these viruses shared 99% identity with the isolated PIV5 strain and were clustered into the same subclade, which suggested that the PIV5 strains were closely related (Fig. 6C).

4. Discussion

The parainfluenza viruses were first described and isolated from hospitalized children in the 1950s (Branche and Falsey, 2016; Henrickson, 2003; Weinberg, 2006). Later, it was characterized by a wide range of species, including humans, pigs, cattle, poultry and companion animals, which were described as among the most important respiratory pathogens with high morbidity. Genetic analysis of the complete genome revealed that PIV could be further divided into five clades. The pathogenicity of the different clades varied. The porcine parainfluenza viruses were clustered mainly into PIV1 and PIV5. PIV1 was detected only and isolated from respiratory samples, whereas PIV5 was detected in both respiratory and fecal samples (Lau et al., 2013; Li et al., 2022; Park et al., 2019). PIV5 was first identified in 1954 from primary monkey kidney cells and later isolated from different hosts, including humans, pigs, dogs, cats, cattle, hamsters, guinea pigs, pangolins, ticks, lesser pandas and horses (Charoenkul et al., 2021; Hierweger et al., 2020; Lee and Lee, 2013; Liu et al., 2015; Wang et al., 2019; Xie et al., 2020; Yang et al., 2022; Zhai et al., 2017). Although PIV5 can cause severe clinical manifestations and even death in humans and animals, the symptoms are much milder in pigs. The first PIV5 was isolated from pigs co-infected with porcine reproductive and respiratory syndrome virus, which presented with respiratory clinical signs (Heinen et al., 1998). The following tests with different PIV5 strains indicated that mild coughing, minor sneezing, and serous nasal discharge may have emerged in several challenged pigs but were not observed in some pigs in the same study (Lee et al., 2013). Despite strong evidence of viral infection and replication, PIV5 may not play a primary role in porcine respiratory disease but contribute to the porcine respiratory disease complex (PRDC) (Schuele et al., 2021).

Recently, an increasing number of PIV5 strains have been identified and isolated from diarrheal piglets in China (Ibrahim et al., 2022; Jiang et al., 2018; Liu et al., 2015; Wang et al., 2019; Xie et al., 2020; Yang et al., 2022; Zhai et al., 2017). Before 2020, Gansu was not considered as one of the main pork production areas in China and the collected samples within Gansu province were also free of PIV. However, the emerging of ASFV has posed great threatens to the pork production industry in China and asked for new areas free of ASFV. Since then, the pork production industry sharply increased and several of viruses have then been discovered in Gansu. The pathogenicity of PIV5 in the digestive system has not been evaluated. It is also not clear whether PIV5 alone can cause enteric symptoms. In this study, the NGS method was used to detect unknown pathogens in porcine diarrhea samples and identify the PIV5 distribution. The PIV5 strain was then successfully isolated and confirmed by TEM and IFA. Pathology further revealed that this virus may be both respiratory and enteric pathogenic in piglets.

In the present study, PIV5-infected piglets maintained a relatively constant body temperature during seven days of observation. Although mild coughing and hard breath combined with soft stool and slight diarrhea were observed, the manifestations disappeared at 5 dpi. No pigs

died during the observation period. H&E staining revealed pneumonia in the lung and steatosis in the ileum beginning at 3 dpi. Viral titer determination revealed that the viral titers peaked at 3 dpi in the lung and ileum but then sharply decreased. The detection of viral shedding also revealed that the number of viral genome copies increased at 3 and 4 dpi but soon decreased in nasal swabs. These results indicated that the replication of PIV5 is highly restricted by the rapid removal of sand in vivo. Previous studies have also demonstrated that PIV5 can cause neurological disease and encephalitis (Hierweger et al., 2020; Yang et al., 2022). However, it was not observed or detected in this study.

The epidemic investigation of PIV5 has focused mainly on Northeast Asia, including Korea and the northeast of China. All these studies have shown that PIV5 is highly prevalent. The percentage of positive serum samples was greater than 75.5%. Our retrospective investigation revealed that PIV5 was absent among diarrhea samples before 2020, but the positive ratio has increased since then. The sequenced PIV5 genome shared 99% identity with the isolated PIV5 strain, which suggested pandemic potential.

5. Conclusion

In summary, this study described the pathogenicity of a PIV5 strain isolated from diarrheal piglets. An animal test demonstrated that the PIV5 LZ-1/2021 strain can cause coughing and diarrhea in piglets. H&E staining revealed that PIV5 infection led to pneumonia in the lungs and that steatosis contributed to diarrhea. We believe that this study provides solid evidence about the systemic impact of PIV5 on swine health, especially its effects on the intestine, which will facilitate the understanding of PIV5.

CRediT authorship contribution statement

Minting Ni: Formal analysis, Data curation. **Shengyu Lin:** Visualization, Software, Formal analysis, Data curation. **Yongheng Shao:** Data curation. **Jiao Tang:** Data curation. **Shuxian Li:** Formal analysis, Data curation. **Chen Tan:** Data curation. **Zhenli Gong:** Data curation. **Hongbo Li:** Data curation. **Jintao Wang:** Validation. **Guangliang Liu:** Writing – review & editing. **Jianing Chen:** Writing – review & editing, Writing – original draft, Validation, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Funding

This work was supported by the Gansu Natural Science Foundation (22JR5RA027).

Declaration of competing interest

The authors declare that they have no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.virol.2025.110409.

Data availability

All the data that support this study are available upon request.

References

Atoynatan, T., Hsiung, G.D., 1969. Epidemiologic studies of latent virus infections in captive monkeys and baboons. II. Serologic evidence of myxovirus infections with special reference to SV5. Am. J. Epidemiol. 89, 472–479.

Branche, A.R., Falsey, A.R., 2016. Parainfluenza virus infection. Semin. Respir. Crit. Care Med. 37, 538–554.

Charoenkul, K., Nasamran, C., Janetanakit, T., Chaiyawong, S., Bunpapong, N., Boonyapisitsopa, S., Tangwangvivat, R., Amonsin, A., 2021. Molecular detection and whole genome characterization of Canine Parainfluenza type 5 in Thailand. Sci. Rep. 11, 3866.

Heinen, E., Herbst, W., Schmeer, N., 1998. Isolation of a cytopathogenic virus from a case of porcine reproductive and respiratory syndrome (PRRS) and its characterization as parainfluenza virus type 2. Arch. Virol. 143, 2233–2239.

Henrickson, K.J., 2003. Parainfluenza viruses. Clin. Microbiol. Rev. 16, 242–264.

Hierweger, M.M., Werder, S., Seuberlich, T., 2020. Parainfluenza virus 5 infection in neurological disease and encephalitis of cattle. Int. J. Mol. Sci. 21.

Hull, R.N., Minner, J.R., Smith, J.W., 1956. New viral agents recovered from tissue cultures of monkey kidney cells. I. Origin and properties of cytopathogenic agents S.V.1, S.V.2, S.V.4, S.V.5, S.V.6, S.V.11, S.V.12 and S.V.15. Am. J. Hyg. 63, 204–215.

Ibrahim, Y.M., Zhang, W., Werid, G.M., Zhang, H., Pan, Y., Zhang, L., Xu, Y., Li, C., Chen, H., Wang, Y., 2022. Characterization of parainfluenza virus 5 from diarrheic piglet highlights its zoonotic potential. Transboundary Emerg. Dis. 69, e1510–e1525.

Jiang, N., Wang, E., Guo, D., Wang, X., Su, M., Kong, F., Yuan, D., Zhai, J., Sun, D., 2018. Isolation and molecular characterization of parainfluenza virus 5 in diarrhea-affected piglets in China. J. Vet. Med. Sci. 80, 590–593.

Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30, 772–780.

Lau, S.K.P., Woo, P.C.Y., Wu, Y., Wong, A.Y.P., Wong, B.H.L., Lau, C.C.Y., Fan, R.Y.Y., Cai, J.P., Tsoi, H.W., Chan, K.H., Yuen, K.Y., 2013. Identification and characterization of a novel paramyxovirus, porcine parainfluenza virus 1, from deceased pigs. J. Gen. Virol. 94, 2184–2190.

Lee, Y.N., Lee, C., 2013. Complete genome sequence of a novel porcine parainfluenza virus 5 isolate in Korea. Arch. Virol. 158, 1765–1772.

Lee, Y.N., Park, C.K., Kim, S.H., Lee du, S., Shin, J.H., Lee, C., 2013. Characterization in vitro and in vivo of a novel porcine parainfluenza virus 5 isolate in Korea. Virus Res. 178, 423–429.

Li, Y., Yuan, F., Yan, X., Matta, T., Cino-Ozuna, G.A., Fang, Y., 2022. Characterization of an emerging porcine respirovirus 1 isolate in the US: a novel viral vector for expression of foreign antigens. Virology 570, 107–116.

Liu, Y., Li, N., Zhang, S., Zhang, F., Lian, H., Hu, R., 2015. Parainfluenza virus 5 as possible cause of severe respiratory disease in calves, China. Emerg. Infect. Dis. 21, 2242–2244.

Park, J.Y., Welch, M.W., Harmon, K.M., Zhang, J., Piñeyro, P.E., Li, G., Hause, B.M., Gauger, P.C., 2019. Detection, isolation, and in vitro characterization of porcine parainfluenza virus type 1 isolated from respiratory diagnostic specimens in swine. Vet. Microbiol. 228, 219–225.

Schuele, L., Lizarazo-Forero, E., Cassidy, H., Strutzberg-Minder, K., Boehmer, J., Schuetze, S., Loebert, S., Lambrecht, C., Harlizius, J., Friedrich, A.W., Peter, S., Rossen, J.W.A., Couto, N., 2021. First detection of porcine respirovirus 1 in Germany and The Netherlands. Transboundary Emerg. Dis. 68, 3120–3125.

Singh, F., Rajukumar, K., Senthilkumar, D., Venkatesh, G., Srivastava, D., Kombiah, S., Jhade, S.K., Singh, V.P., 2022. First report on coisolation and whole-genomic characterization of mammalian orthorubulavirus 5 and mammalian orthoreovirus type 3 from domestic pigs in India. Arch. Virol. 167, 1529–1545.

Truong, H.T., Nguyen, V.G., Pham, L.B., Huynh, T.M., Lee, J., Hwang, S.J., Lee, J.M., Chung, H.C., 2023. PCR-based detection and genetic characterization of parainfluenza virus 5 detected in pigs in Korea from 2016 to 2018. Veterinary Sci. 10.

Vainionpää, R., Hyypiä, T., 1994. Biology of parainfluenza viruses. Clin. Microbiol. Rev. 7, 265–275.

Walker, P.J., Siddell, S.G., Lefkowitz, E.J., Mushegian, A.R., Adriaenssens, E.M., Alfenas-Zerbini, P., Davison, A.J., Dempsey, D.M., Dutilh, B.E., García, M.L., Harrach, B., Harrison, R.L., Hendrickson, R.C., Junglen, S., Knowles, N.J., Krupovic, M., Kuhn, J. H., Lambert, A.J., Łobocka, M., Nibert, M.L., Oksanen, H.M., Orton, R.J., Robertson, D.L., Rubino, L., Sabanadzovic, S., Simmonds, P., Smith, D.B., Suzuki, N., Van Dooerslaer, K., Vandamme, A.M., Varsani, A., Zerbini, F.M., 2021. Changes to virus taxonomy and to the international code of virus classification and nomenclature ratified by the international committee on taxonomy of viruses (2021). Arch. Virol. 166, 2633–2648.

Wang, X., Chen, W., Xiang, R., Li, L., Chen, J., Zhong, R., Xiang, H., Chen, J., 2019. Complete genome sequence of parainfluenza virus 5 (PIV5) from a sunda pangolin (manis javanica) in China. J. Wildl. Dis. 55, 947–950.

Weinberg, G.A., 2006. Parainfluenza viruses: an underappreciated cause of pediatric respiratory morbidity. Pediatr. Infect. Dis. J. 25, 447–448.

Xie, J., Tong, P., Zhang, A., Zhang, L., Song, X., Kuang, L., 2020. Identification and characterization of the first equine parainfluenza virus 5. Virol. Sin. 35, 245–247.

Yang, M., Ma, Y., Jiang, Q., Song, M., Kang, H., Liu, J., Qu, L., 2022. Isolation, identification and pathogenic characteristics of tick-derived parainfluenza virus 5 in northeast China. Transboundary Emerg. Dis. 69, 3300–3316.

Zhai, J.Q., Zhai, S.L., Lin, T., Liu, J.K., Wang, H.X., Li, B., Zhang, H., Zou, S.Z., Zhou, X., Wu, M.F., Chen, W., Luo, M.L., 2017. First complete genome sequence of parainfluenza virus 5 isolated from lesser panda. Arch. Virol. 162, 1413–1418.