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RESEARCH ARTICLE

Isolation, Identification and the Evaluation of Pathogenicity of G9 Porcine Rotavirus in Guangxi, China

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ABSTRACT

Porcine rotavirus is a significant pathogen that causes rotavirus sickness in both humans and animals. G9 rotavirus, in particular, is thought to be a new rotavirus that has lately spread over the world between humans and pigs. However, the understanding of the pathogenicity of G9 rotavirus is limited to date. In the current study, a G9 porcine rotavirus strain was isolated in the fecal samples of diarrheal piglets from a large-scale pig farm located in Guangxi, China. Then, the virus was identified by western blotting, and the production dynamics of the virus were confirmed by an indirect immunofluorescence test with a viral titer of $10^{7.46}$ $TCID_{50}$/mL. Based on the VP7, VP4 and VP6 genes, phylogenetic analysis and sequencing data indicated that the GX9579 strain was of genotype G9P [23]I5. In addition, to explore the pathogenicity of the isolated GX9579 strain, animal tests were performed herein. Fecal viral shedding was detected, and fecal excretion was the highest at 10^5 copies/μL 24h after the challenge and then gradually decreased. Histopathological test results suggested the intestinal villous arrangement of infected piglets became shortened, partially shed, and broken, intestinal villous epithelial cells shed into the intestinal lumen, and the villi propria lamina became hyperemic with severe bleeding and submucosal edema. Positive signals were seen throughout the intestinal villi's epithelial cells and the small intestine glands, according to immunohistochemical studies. These results provide reference data for further studies on the epidemiology and pathogenesis of G9 swine rotavirus.

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INTRODUCTION

One of the major viruses that induces acute viral gastroenteritis in young animals is rotavirus (RV) (Miyabe *et al*., 2020). It was first isolated from calf diarrhea feces in 1969 (Mebus *et al*., 1969). Subsequently, in the UK, incidences of diarrhea in babies and early children due to RV were documented in 1973 (Bishop *et al*., 1973). In 1975, porcine rotavirus was detected in diarrheal piglet feces (Rodger *et al*., 1975). Pigs are animal reservoirs of zoonotic RV, and piglets are vulnerable to acute gastroenteritis caused by RV infection from the time of lactation until weaning (Vlasova *et al*., 2017). Piglets infected with rotavirus initially show symptoms such as depression, vomiting, and refusal to feed. With the progression of the disease, they excrete large amounts of yellow-white watery manure, exhibit continuous weight loss and emaciation, and finally dehydrate to death (Vlasova *et al*., 2017). Currently, the porcine rotavirus disease has become a global endemic, including humans and mammalian animals (Do *et al*., 2014). This has major ramifications for public health in addition to causing the global pig business to suffer enormous financial losses.

11 dsRNA fragments make up the rotavirus genome; these include non-structural proteins (NSP1–NSP5/NSP6) and structural proteins (VP1–VP4, VP6 and VP7) (Patton *et al*., 2006; Abass *et al*., 2022). Eight groups (RVA−RVH) of the virus are identified based on the features of the VP6 serological response and the varying migration rates of the rotavirus genome fragments in polyacrylamide gel electrophoresis (Shoja *et al*., 2022). RVA-RVC and RVH are prevalent in both human and mammalian species. However, pigs are susceptible to RVA–RVC, RVE, and RVH. Among these, RVA has the highest infection rate and

is a principal cause of acute diarrhea and dehydration in pigs (Kumar *et al*., 2022).

According to the double typing approach, rotavirus A is categorized into G and P types based on the genetic makeup and antigenic properties of the structural proteins VP7 and VP4, respectively (Fukuda *et al*., 2020). There are now 42 Type G and 58 Type P cases recorded globally (Malakalinga *et al*., 2022). Globally, G1–G4, G9, and G12 are regularly seen, frequently in conjunction with P [4], P [6] and P [8] in Human rotavirus (HRV) (Zhou *et al*., 2015). In recent years, G9 RVA has been identified as a circulating strain exchanged between humans and pigs globally, It has become endemic in multiple countries, such as China (Shi *et al*., 2012, Wang *et al*., 2018), South Korea (Kim *et al*., 2012), Germany (Wenske *et al*., 2018), and Brazil (Silva *et al*., 2015).

Herein, we obtained a G9 porcine RVA strain named GX9579 and analyzed and explored the genetic and evolutionary relationships among its VP4, VP6, and VP7 genes. In addition, piglets were inoculated with GX9579 to evaluate the pathogenicity of this strain.

MATERIALS AND METHODS

Sample and virus isolation: In this study, fecal samples were taken in 2022 from 12 piglets from a major Guangxi pig farm that had diarrhea. Using RT-PCR, twelve samples were examined for the presence of the pig rotavirus (RVA), infectious gastroenteritis virus (TGEV), and porcine epidemic diarrhea virus (PEDV). After three freeze-thaw sessions at -80°C, stool samples that were found to be exclusively positive for RVA were chosen, combined with 0.01 M PBS buffer, and centrifuged at 12,000 rpm for five minutes at 4°C. 500 µL of the mixture was seeded onto 90% monolayer Marc-145 cells after the supernatant was filtered through a 0.22 µm filter and prepared with 30 µg/ml trypsin solution (Gibco, ThermoFisher, China) at 37°C for 1.5 hours. After adsorption at 37°C and 5% CO_2 for 2 h, PBS was washed and a maintenance medium containing 8 µg/ml trypsin was added (Gibco, ThermoFisher, China).

After 5 passages of isolation and adaptation, the virus was titrated on Marc-145 cells, and the virus was serially diluted 10-fold with 8 replicates for each dilution. After 4~5 days of inoculation, $TCID_{50}$ was calculated according to the Reed and Mench method.

Vp6 protein expression analysis by immunofluorescence assay and Western blot: The virus was introduced into a monolayer of Marc-145 cells at a multiplicity of infection (MOI) of 1. The cells are fixed with 4% paraformaldehyde for 30 minutes at 24 hours after infection. Subsequently, it was incubated for one night at 4°C with a rabbit anti-rotavirus VP6 polyclonal antibody (1:400 dilution) and then for thirty minutes at 37°C with sheep anti-rabbit IgG that had been FITC-labeled (Abclonal Technology, Wuhan, China). The resultant cells were then observed under a fluorescent microscope after being treated with DAPI for 10 minutes at room temperature. Our lab maintained a polyclonal antibody against the rabbit rotavirus VP6.

In 6-well plates, Marc-145 cells were cultured and exposed to the virus. After 48 hours, protein lysate RIPA (Beyotime Biotechnology, Shanghai, China) was used to remove the whole cell protein. The proteins were then electrophoresed on a 12% SDS-polyacrylamide gel, transferred to PVDF membranes using the electrotransfer method at 4°C, blocked for 2 hours with 5% skim milk powder (Bioprimacy, Wuhan, China), and then incubated for 2 hours at 37°C with a secondary antibody (Abclonal Technology, Wuhan, China) and overnight at 4°C with the primary antibody (rabbit anti-rotavirus VP6 polyclonal antibody, 1:1000 dilution). Lastly, the ECL western blotting substrate (Vazyme, Nanjing, China) was used to generate the color.

VP7, VP4 and VP6 sequence determination and analysis: Using the magnetic bead approach, the viral nucleic acid extraction kit (Harbin Guosheng Biotechnology Co., Ltd., Harbin, China) was utilized to extract viral RNA from 300 µL of the virus. The manufacturer's instructions were followed to synthesize the cDNA using the FastKing RT Kit (with gDNase) from Tiangen, Beijing, China. The final cDNA was kept in storage at -20°C. After being obtained by RT-PCR, the VP7, VP4, and VP6 gene segments were transferred to Liuhe Huada Gene Technology Co., Ltd. in Beijing for sequencing. Table 1 lists the primer pairs that were developed to amplify VP7, VP4, and VP6 cDNAs.

Multiple nucleotide sequences were aligned using ClustalW in Lasergene (version 7.1, DNASTAR Inc., Madison, WI, USA) to further analyze the VP7, VP6, and VP4 sequences of GX9579. A phylogenetic tree of the VP7, VP6 and VP4 sequences was created in MEGA (version 5.0, Mega Limited, Auckland, New Zealand) using the neighbor-joining method. The branch reliability was assessed using bootstrap values derived from 1000 alignment repetition calculations.

Pathogenicity experiment: Six piglets with 1-day-old colostrum were obtained from a large-scale pig farm in Guangxi. Before the experiments, it was confirmed using PCR that they tested negative for rotavirus. The trial piglets were split into two groups at random, with three pigs in each group, and the infected group was orally administered 2 mL ($10^{7.46}$ $TCID_{50}$/mL) of porcine rotavirus GX9579 suspension. The control group simulated infection with the same dose of DMEM medium. Both groups were fed commercial milk powder every 2 to 3 hours. Following the challenge, rectal samples were taken every day, and the piglets were monitored for clinical signs.

Five days following the fake infection, Piglet weighing and a clinical necropsy were carried out. Euthanasia was performed on the fifth day. The tissue was collected in order from the stomach, duodenum, jejunum, ileum, cecum, and colon. The tissue was stored at 4% in paraformaldehyde buffer. Samples that have been liquid nitrogen flash-frozen are then kept in storage at -80°C.

Real-time RT-PCR quantification of viral RNA load: Follow the steps above to obtain cDNA from stool samples and intestinal tissue. TaqMan real-time PCR was performed using primers and probes for the VP6 gene to quantify viral RNA load (VP6-F: CACCTTCAAGAGA AGATAAC; VP6-R: CTGATTAAGATCGGATACCAG; VP6-probe: TTAGCCTGGTCCTCACTTAATCAAC). TaqMan real-time PCR reactions were performed using Applied Biosystems (Thermo Scientific, Waltham, MA, USA).

Table 1: Primers used to amplify VP4, VP6, and VP7 gene fragments

Gene	Primer sequence (5'→3')	Product size	Accession No.
VP4-F	GGCTATAAAATGGCTTCGCTAATTT	2362 bp	MH137266
VP4-R	GGTCACATCCTTTAGAAGCTACTTAT		
VP6-F	GGCTTTTAAACGAAGTCTTCGACAT	1356 bp	EU372759
VP6-R	GGTCACATCCTCTCACTACATCATT		
VP7-F	GGCTTTAAAAGAGAGAATTTCC	1062 bp	MG066590
VP7-R	GGTCACATAAAACAATTCTAATCT		

Histopathological observations and immunohistochemistry (IHC): The tissues of the duodenum, jejunum and ileum were preserved for 48 hours in a 4% paraformaldehyde buffer, and the intestinal tissue was sectioned, HE-stained, and immunohistochemically stained. The immunohistochemical procedures were conducted as follows: the paraffin sections were dewaxed and subjected to heat-induced antigen retrieval using a citrate repair solution (pH 6.0). Subsequently, the sections were then blocked using goat serum and a peroxidase blocker (Gene Tech, Shanghai, China) after being cleaned with PBS (Boster Biological Technology, Wuhan, China). Following overnight incubation at 4°C with the primary antibody (polyclonal antibody against porcine RVA VP6, diluted at 1:400), a sheep anti-rabbit/murine secondary antibody (Gene Tech, Shanghai, China) was incubated on the slices for half an hour at room temperature. The slices were then stained with 3,3'-diaminobenzidine (Boster Biological Technology, Wuhan, China) and sequentially treated with hematoxylin and 0.5% hydrochloric acid. Finally, the slices were sealed using neutral glue (Servicebio, Wuhan, China).

RESULTS

Virus isolation, growth curves, and tissue Culture Infectious Dose (TCID50) assays: Five RVA-positive fecal samples were inoculated into Marc-145 cells after trypsin activation and one strain of the virus was isolated after 3 consecutive blind passages, it showed a typical cytopathic effect (CPE), which was mainly manifested as cell shrinkage, large slump shedding and death, and reticulation (Fig. 1). Continuing through passage 5, the virus successfully adapted to Marc-145 cells and the growth curve was determined. The 4-h viral titer was at a low level, and the virus entered the rapid growth stage between 4 and 12h. The virus titer reached a peak ($10^{7.46}$ $TCID_{50}$/mL) at 12h, and then the viral titer was maintained at a high level.

Detection of porcine rotavirus by IFA and Western blotting: To confirm VP6 expression, perform Western blot 48 h after viral infection. The results show that the protein collected from Marc-145 cells infected with GX9579 reacts with rabbit anti-RVA VP6 polyclonal antibody (Fig. 2B). In addition, the cells fluoresced green at 24 and 48 hours after GX9579 infection (IFA results show, Fig. 2A). These results indicate that the isolated virus is RVA.

Sequence analysis of VP7, VP4 and VP6: The genotype of the porcine RVA strain GX9579 is G9P [23]I5. Among the VP7 genes, GX9579 shows the closest relationship to the SCJY-5 strain (MH898992) in southwest China and the HB-1 strain (MZ165507) in central China, with a nucleotide sequence homology of 96.06%. The homology with the American-derived MRC-DPRU1255 strain (KJ752929) is 94.36%. Concerning the VP4 genes, GX9579 exhibits the highest homology with the SCYB-C2 strain (MT198751) in southwest China and the PGRV16 strain in Brazil (KC254751), at 95.3 and 93.61%, respectively. The GX9579 strain's VP6 gene genotype is I5, which demonstrates a homology of 97.05 and 97.01% with the HeNNY-01 strain (MW575221) in central China and the SCLS-3 strain (MK597975) in southwest China respectively.

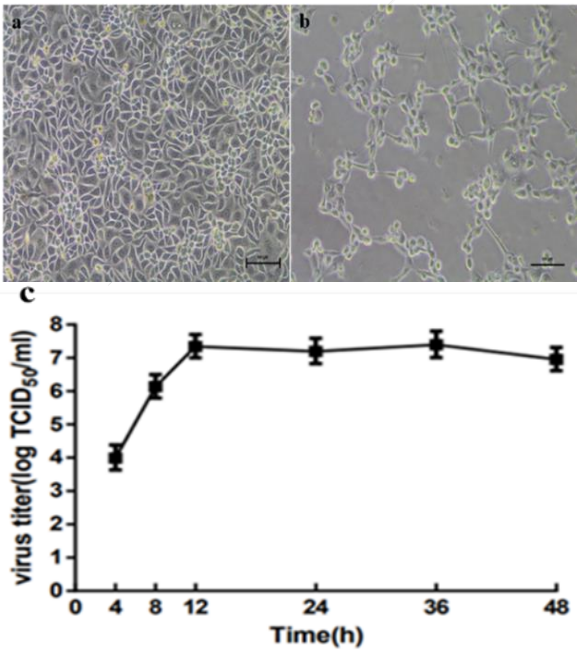

Fig. 1: Cytopathological changes and growth curves of Marc-145 cells infected with GX9579; (a): Normally cultured Marc-145 cells; (b): virus-infected Marc-145 cells. Magnification 100×;(c): Data were expressed as the mean ±standard deviation for every three replicates.

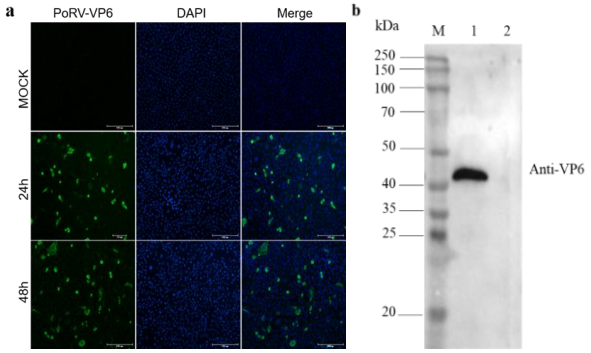

Fig. 2: Immunofluorescence and western blotting analysis of porcine rotavirus; (a): Immunofluorescence. RVA fluoresces green. No RVA fluorescence signal was detected in the control group. Magnification 200×; (b): Western blot identification results of RVA VP6 protein. M: Protein marker; 1: Proteins extracted from 5th generation cell culture media; 2: Protein extracted from cells not infected with GX9579.

The gene sequences of the GX9579 strain's VP7, VP6, and VP4 have been deposited in the GenBank database under the accession numbers OQ411031, OQ411032 and OQ411033, respectively.

Clinical Symptoms and histopathological changes in infected piglets: Within 12h post-inoculation (hpi), the piglet's mental state began to deteriorate, body temperature gradually increased, appetite decreased and the piglets developed diarrhea symptoms and excreted yellow loose feces. With the aggravation of the degree of infection, diarrhea continued to worsen 12 to 30 h after inoculation and yellow watery feces spontaneously ejected out of the anus of the piglets. At 48–96 hpi, the piglets were dehydrated and on the verge of death, and their abdomen was sunken before death. Only one piglet in the infected group survived after 120 hpi. The piglets in the control group showed no clinical symptoms and their feces were normal. The results showed that the infection with RVA GX9579 in 1-day-old piglets with missing breast milk caused 100% (3/3) morbidity, and the case fatality rate was 66.67% (2/3). The primary pathological changes in the gastrointestinal tract were observed after autopsy. The stomach of the piglets in the infected group was full, and a significant amount of undigested yellow-white curd in the stomach was found. The small intestine was bulging and dilated, and the intestinal walls of the jejunum and part of the ileum were significantly thinned and transparent. Gastric mucosa and mesenteric capillary dilation along with hyperemia were seen and the contents of the intestine were watery. However, the gastrointestinal tracts of the piglets in the control group were normal (Fig. 4A).

Duodenum, jejunum, and ileal tissues were taken for histological analysis. Viral infection severely damages the gastrointestinal system, especially the small intestine, according to histopathological examination. The duodenal villi of the infected piglets were partially ruptured, and necrotic and exfoliated cell fragments were mixed with fibrin-like substances in the intestinal lumen and adhered to the tip of the intestinal villi or shed into the lumen. Severe loss of jejunal villi was observed. Nearly no complete intestinal villi structure was present and propria hyperemia and edema occurred. The ileal villi became short, thick and disorganized (Fig. 4B). Immunohisto-chemical analysis showed that brownish-yellow RVA-positive signals were detected in the duodenum, jejunum, and ileum, and these signals were mainly distributed in the intestinal villus epithelial cells and small intestinal glandular cells. More RVA-positive signals were observed in the jejunum than those in the duodenum and ileum. However, no positive signals were found in the intestines of piglets in the control group (Fig. 4C).

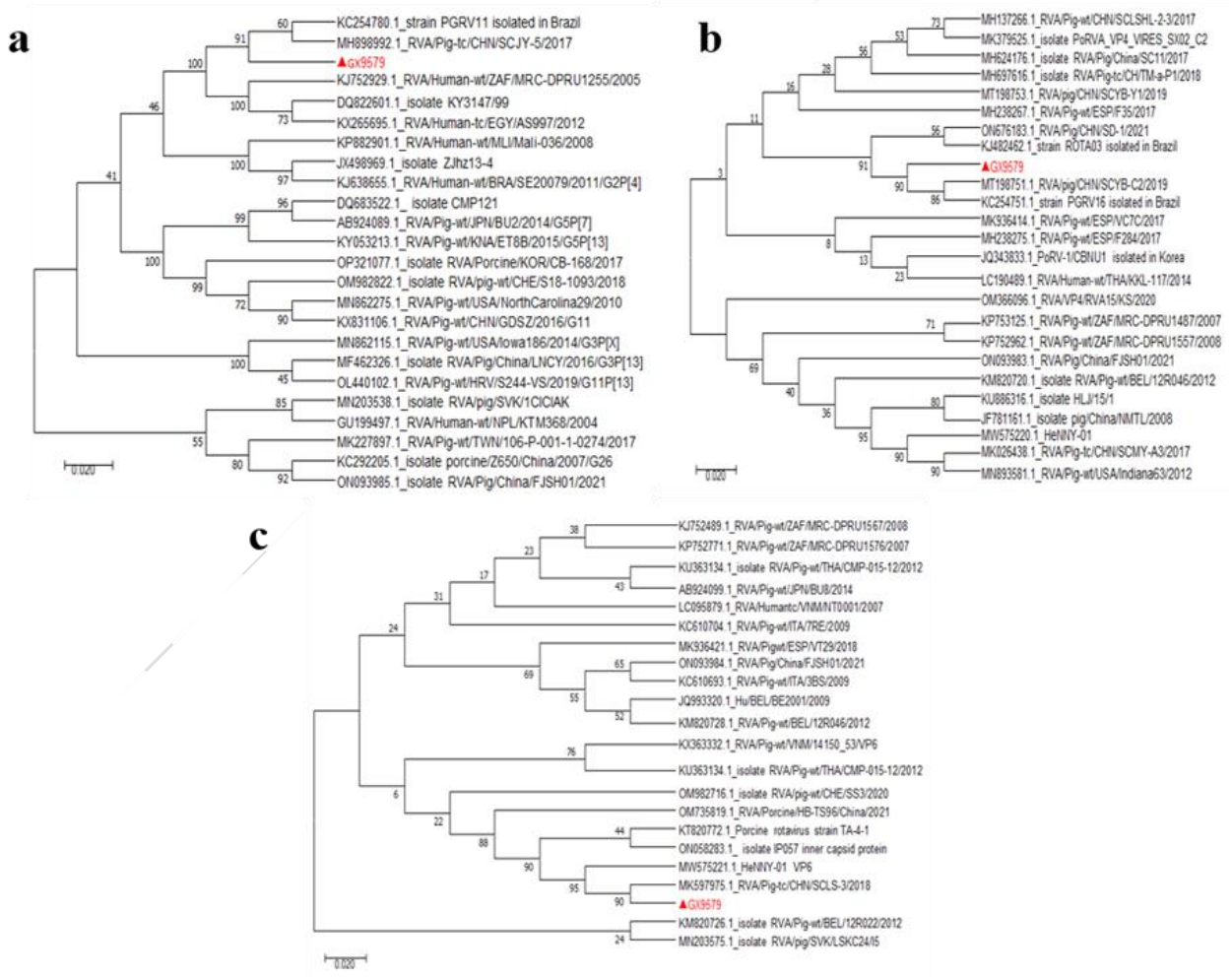

Fig. 3: Nucleotide sequence genetic tree of the VP7, VP4, and VP6 genes of isolates; (a): Genetic evolutionary tree of the VP7 gene of the isolate; (b): Genetic evolutionary tree of the nucleotide sequence of VP4 gene isolate; (c): Genetic evolutionary tree of the VP6 gene of the isolate; ▲Indicates the RVA strain isolated herein.

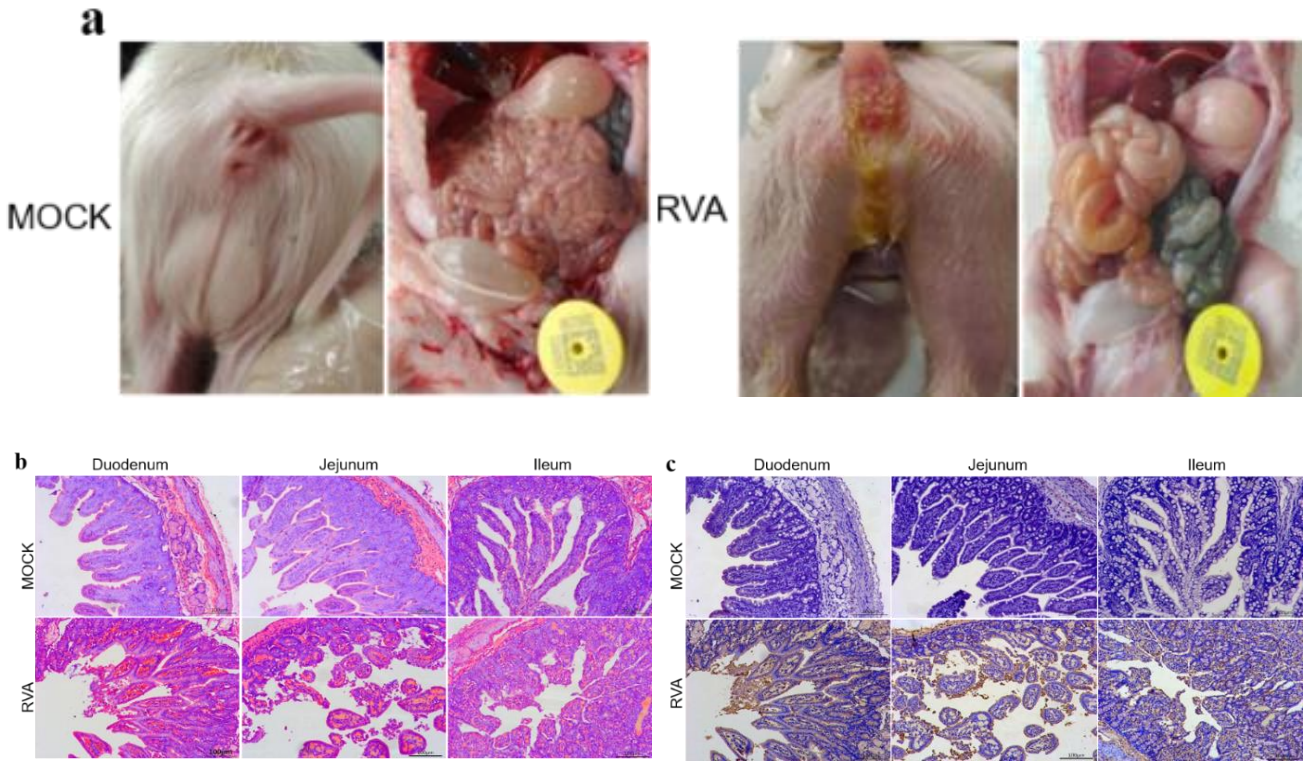

Fig. 4: Clinical signs and histologic changes; (a): Gross lesions infecting the intestinal tract of piglets. Infected piglets excreted thin yellow manure. The small intestine was inflated, and the intestinal wall was significantly thinned and transparent; (b): Examination of intestinal histopathology. The intestinal villi became shorter, partially ruptured, and shed out; the lamina propria was hyperemic and edematous (HE, Magnification 200×); (c): Immunohistochemical staining results of RVA. Numerous RVA-positive signals were observed in intestinal villi epithelial cells (IHC, Magnification 200×)

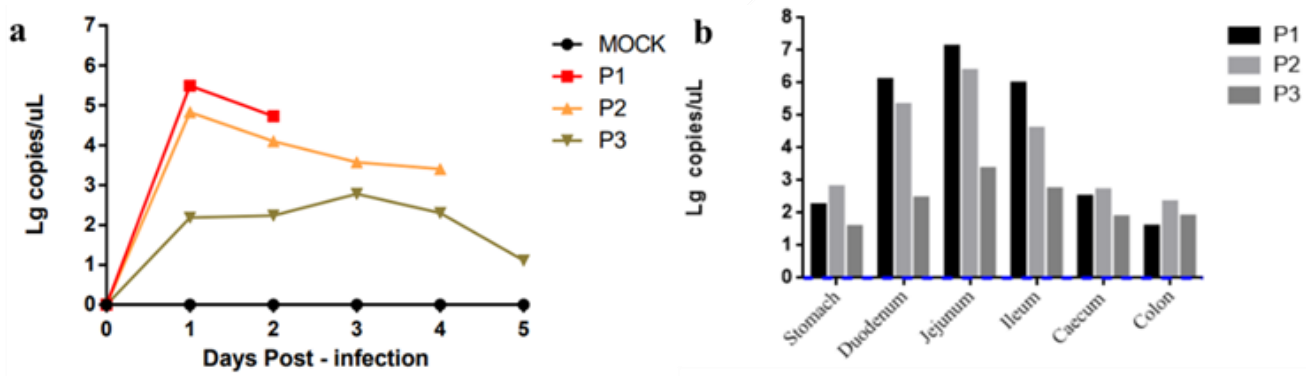

Fig. 5: Viral shedding in feces and the viral RNA load in intestinal tissue; (a): Virus shedding from the feces of the infected piglets; MOCK: Control group; P1–P3: Piglets with RVA infection; (b): Intestinal viral load in the infected piglets; Dashed line in blue: control group

Fecal viral shedding and quantification of RVA RNA in tissues: The results of fecal virus shedding detection showed that the fecal virus shedding in the infected group reached a peak after 1 day, about 10^4 to 10^6 copies/μL, and then gradually decreased. Following infection, the fecal viral shedding of the surviving piglets in the infected group stayed modest, ranging from 10^1 to 10^2 copies/μL. In the control group, no viral shedding was observed (Fig. 5A).

The findings about the detection of viral shedding in intestinal tissues indicated that the virus was present in every intestinal tissue within the infected group, with the jejunum displaying the highest level of shedding, ranging from 10^3 to 10^7 copies/μL. The duodenum and ileum were second, and the viral load was about 10^3 to 10^6 copies/μL. In addition, small amounts of the virus were detected in the stomach, cecum, and colon. However, in the control group's intestines, no virus was found (Fig. 5B).

DISCUSSION

Among the pig A–C, E, and H rotaviruses, porcine A rotaviruses are the most common. Currently, swine RVA strains in various G9 combinations are circulating globally. New strains of G9 in combination with P[23], P[13], P[7], and P[19] dominated several outbreaks of piglet diarrhea in Japan between 2000 and 2010 (Teodoroff *et al*., 2005; Miyazaki *et al*., 2011). Li *et al*. (2022) investigated the enterovirus in pig farms in central and southern China in 2018–2021, showing that the G9 strain became the primary circulating genotype only after 2018. This study identified the obtained porcine RVA GX9579 strain as G9P [23] type. Studies have reported the extensive spread of G9P[23] in pig herds in China (Yan *et al*., 2019). GX9579 strain was compared and analyzed with the strains isolated from various regions, such as GXqz-2 strain (Heilongjiang),

LLP48 strain (Beijing), TM-a strain (Hubei), SD-1 strain (Shandong), SCMY strain (Sichuan) and GDJM1 strain (Guangdong). It was found that the homology of each strain was more than 95% at the nucleotide level. It was speculated that climate, geographical location, and other factors led to different degrees of recombination or mutation of strains in each region.

Currently, increasing reports of RVA isolation appear globally, but relatively only a few reports of the pathogenicity of RVA in pigs, particularly G9 RVA, are observed. Kim *et al*. (2012) evaluated the pathogenicity of the G9P[23] strain using piglets that did not consume breast milk and found no clinical manifestations other than diarrhea. Zhang *et al*. (2015) orally administered the G9P[7] strain of 4-day-old piglets and found yellow watery diarrhea in <24 h after infection. One piglet died 50 hours after infection, and the other two piglets were nearly dying. In this study, G9P[23] type RVA were orally infected with 1-day-old colostrum-deprived piglets, and the infected piglets developed yellow watery diarrhea at approximately 12h. Within 48–96 hp of infection, two piglets died of dehydration, and the other piglets gradually returned to their normal state. However, All infected piglets, according to Wang *et al*. (2018) experienced acute watery diarrhea within 24 hours, and their stools recovered to normal within 72 hours. According to Shao *et al*. (2016) PRV G9P[13] caused fecal viral shedding that lasted for over ten days. In this study, the piglets inoculated with GX9579 could detect rotavirus shedding in feces on days 1–5, which was consistent with the other reports (Azevedo *et al*., 2005). This indicates that rotaviruses with different combinations of genotypes exhibit different infectivity in piglets.

Porcine rotavirus can be transmitted through the fecal-oral modes. The virus in the exfoliated feces enters the digestive tract through the oral cavity to colonize the small intestine and is activated by enzymes in the gastrointestinal tract to invade the villi epithelial cells of the small intestinal mucosa. There it replicates and proliferates in large quantities, causing degeneration and necrosis of the infected cells. Consequently, the intestinal villi break and shed out, resulting in malabsorption and diarrhea (Greenberg and Estes, 2009). In the current study, the duodenum, jejunum, and ileum tissue were studied by real-time PCR. The RVA viral load in the jejunum was found to be the highest, followed by that in the duodenum, and the ileum was the lowest. Therefore, it was speculated that the main replication region of isolate GX9579 was in the jejunum. Yuan *et al*. (2002) showed that the ileum, duodenum, and jejunum had the highest viral loads in piglets infected with the human RVA Wa strain. In this study, the damage in the jejunum was more severe than that in the duodenum and ileum, and the VP6 antigen content of RV in the jejunum was significantly higher than that in the duodenum and ileum, as reported by Wang *et al*. (2018). Li *et al*. (2016) infected 5-day-old piglets with G1 and G3 HRV and discovered that the duodenum did not contain the RV VP6 antigen, which was mostly found in the epithelial cells of the jejunum and ileal villi. According to Miao *et al*. (2022) and Zhou *et al*. (2023), rotavirus infections can also lead to extraintestinal manifestations, including respiratory tract infections, indicating a broader organ tropism beyond the intestines. Nelsen demonstrated that RVA localizes in lung tissue's alveolar and interstitial macrophages(Nelsen *et al*., 2022). Notably, the viral load in the lungs of PoRV-infected pigs in this study was significantly lower compared to that in the small intestine, with no observed clinical signs such as coughing. minimal levels of VP6 antigen were detected in kidney, lung, spleen, liver, and other tissues. Furthermore, as reported by Kim *et al*. (2016) the distribution of the VP6 antigen in the small intestine was uniform at 24, 48, and 72 hpi, Therefore, the differences in serotype, strain, and pathogenicity probably contribute to the differences in piglet infection caused by porcine rotavirus.

Conclusions: A strain of porcine rotavirus was identified as having a viral titer of $10^{7.46}$ $TCID_{50}$/mL after it was successfully isolated from diarrheal samples that tested positive for RVA in this study. Based on VP7, VP4, and VP6 gene phylogenetic analysis, the isolated strain GX9579 was found to belong to genotype G9P [23]I5. Additionally, the evaluation of piglet pathogenicity revealed that the isolated GX9579 strain was extremely harmful to young pigs and could colonize the small intestine's epithelial cells, which could result in diarrhea, dehydration, and even death. This work serves as a foundation for further investigation into the pathophysiology of the G9 porcine rotavirus, as well as for the creation of very successful therapeutic interventions and vaccine development.

Authors contributions: Conceptualization: Zhengdan Lin, Jun Tu, Guofu Cheng Data Curation and Methodology: Zhengdan Lin, Jun Tu, Xiuxiu Sun, Xinxin Jin; Formal Analysis and Investigation: Zhengdan Lin, Xi Liu, Li Li, Junjie Yang, Helong Feng, Cunlin Zhan; Funding Acquisition: Guofu Cheng; Supervision: WanpoZhang, Changqin Gu, Xueying Hu, Xiaoli Liu, Ping Qian, Pin Chen, Guofu Cheng; Writing – Original Draft Preparation: Zhengdan Lin, Guofu Cheng; Writing – Review & Editing: Guofu Cheng.

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Declaration of competing interest: There is no conflict of interest in this manuscript.

Ethics statement: This project was approved by the Scientific Ethics Committee of Huazhong Agricultural University (ethics number: HZAUSW-2023–0031).

REFERENCES

Abass G, Dubal ZB, Rajak KK *et al*., 2022. Molecular characterization of porcine rotavirus A from India revealing zooanthroponotic transmission. Anim Biotechnol 33(6): 1073-1085

Azevedo MS, Yuan L, Jeong KI, *et al*., 2005. Viremia and nasal and rectal shedding of rotavirus in gnotobiotic pigs inoculated with Wa human rotavirus. J Virol 79(9): 5428-5436

Bishop RF, Davidson GP, Holmes IH, *et al*., 1973. Virus particles in epithelial cells of duodenal mucosa from children with acute non-bacterial gastroenteritis. Lancet 2(7841): 1281-1283

Do LP, Nakagomi T and Nakagomi O, 2014. A rare G1P[6] super-short human rotavirus strain carrying an H2 genotype on the genetic

background of a porcine rotavirus. Infect Genet Evol 21: 334-350
Fukuda S, Tacharoenmuang R, Guntapong R, *et al*., 2020. Full genome characterization of novel DS-1-like G9P[8] rotavirus strains that have emerged in Thailand. PLoS One 15(4): e0231099
Greenberg HB and Estes MK, 2009. Rotaviruses: from pathogenesis to vaccination. Gastroenterology 136(6): 1939-1951
Kim HH, Matthijnssens J, Kim HJ, *et al*., 2012. Full-length genomic analysis of porcine G9P[23] and G9P[7] rotavirus strains isolated from pigs with diarrhea in South Korea. Infect Genet Evol 12(7): 1427-1435
Kim HH, Park JG, Matthijnssens J, *et al*., 2013. Pathogenicity of porcine G9P[23] and G9P[7] rotaviruses in piglets. Vet Microbiol 166(1-2): 123-137
Kumar D, Shepherd FK, Springer NL, *et al*., 2022. Rotavirus Infection in Swine: Genotypic Diversity, Immune Responses, and Role of Gut Microbiome in Rotavirus Immunity. Pathogens 11(10): 1078
Li C, Lu H, Geng C, *et al*., 2022. Epidemic and Evolutionary Characteristics of Swine Enteric Viruses in South-Central China from 2018 to 2021. Viruses 14(7): 1420
Li JT, Wei J, Guo HX, *et al*., 2016. Development of a human rotavirus induced diarrhea model in Chinese mini-pigs. World J Gastroenterol 22(31): 7135-7145
Malakalinga JJ, Misinzo G, Msalya GM, *et al*., 2022. Prevalence and genomic characterization of rotavirus group A genotypes in piglets from southern highlands and eastern Tanzania. Heliyon 8(11): e11750
Mebus CA, Underdahl NR, Rhodes MB, *et al*., 1969. Further studies on neonatal calf diarrhea virus. Proc Annu Meet U S Anim Health Assoc 73: 97-99
Miao Q, Pan Y, Gong L, *et al*., 2022. Full genome characterization of a human-porcine reassortment G12P[7] rotavirus and its pathogenicity in piglets. Transbound Emerg Dis 69(6): 3506-3517
Miyabe FM, Dall Agnol AM, Leme RA, *et al*., 2020. Porcine rotavirus B as primary causative agent of diarrhea outbreaks in newborn piglets. Sci Rep 10(1): 22002
Miyazaki A, Kuga K, Suzuki T, *et al*., 2011. Genetic diversity of group A rotaviruses associated with repeated outbreaks of diarrhea in a farrow-to-finish farm: identification of a porcine rotavirus strain bearing a novel VP7 genotype, G26. Vet Res 42(1): 112
Nelsen A, Lager KM, Stasko J, *et al*., 2022. Identification of Pulmonary Infections With Porcine Rotavirus A in Pigs With Respiratory Disease. Front Vet Sci 9: 918736
Patton JT, Silvestri LS, Tortorici MA, *et al*., 2006. Rotavirus genome replication and morphogenesis: role of the viroplasm. Curr Top Microbiol Immunol 309: 169-187
Rodger SM, Craven JA and Williams I, 1975. Letter: Demonstration of reovirus-like particles in intestinal contents of piglets with diarrhoea. Aust Vet J 51(11): 536
Shao L, Fischer DD, Kandasamy S, *et al*., 2016. Comparative In Vitro and In Vivo Studies of Porcine Rotavirus G9P[13] and Human Rotavirus Wa G1P[8]. J Virol 90(1): 142-151
Shi H, Chen J, Li H, *et al*., 2012. Molecular characterization of a rare G9P[23] porcine rotavirus isolate from China. Arch Virol 157(10): 1897-1903
Shoja Z, Jalilvand S, Latifi T, *et al*., 2022. Rotavirus VP6: involvement in immunogenicity, adjuvant activity, and use as a vector for heterologous peptides, drug delivery, and production of nano-biomaterials. Arch Virol 167(4): 1013-1023
Silva FD, Espinoza LR, Tonietti PO, *et al*., 2015. Whole-genomic analysis of 12 porcine group A rotaviruses isolated from symptomatic piglets in Brazil during the years of 2012-2013. Infect Genet Evol 32: 239-254
Teodoroff TA, Tsunemitsu H, Okamoto K, *et al*., 2005. Predominance of porcine rotavirus G9 in Japanese piglets with diarrhea: close relationship of their VP7 genes with those of recent human G9 strains. J Clin Microbiol 43(3): 1377-1384
Vlasova AN, Amimo JO and Saif LJ, 2017. Porcine Rotaviruses: Epidemiology, Immune Responses and Control Strategies. Viruses 9(3): 48
Wang Z, Lv C, Xu X, *et al*., 2018. The dynamics of a Chinese porcine G9P[23] rotavirus production in MA-104 cells and intestines of 3-day-old piglets. J Vet Med Sci 80(5): 790-797
Wenske O, Rückner A, Piehler D, *et al*., 2018. Epidemiological analysis of porcine rotavirus A genotypes in Germany. Vet Microbiol 214: 93-98
Yan N, Tang C, Kan R, *et al*., 2019. Genome analysis of a G9P[23] group A rotavirus isolated from a dog with diarrhea in China. Infect Genet Evol 70: 67-71
Yuan L and Saif LJ, 2002. Induction of mucosal immune responses and protection against enteric viruses: rotavirus infection of gnotobiotic pigs as a model. Vet Immunol Immunopathol 87(3-4): 147-160
Zhang H, Zhang Z, Wang Y, *et al*., 2015. Isolation, molecular characterization and evaluation of the pathogenicity of a porcine rotavirus isolated from Jiangsu Province, China. Arch Virol 160(5): 1333-1338
Zhou X, Niu JW, Zhang JF, *et al*., 2023. Commentary: Identification of pulmonary infections with porcine Rotavirus A in pigs with respiratory disease. Front Vet Sci 10: 1102602
Zhou X, Wang YH, Ghosh S, *et al*., 2015. Genomic characterization of G3P[6], G4P[6] and G4P[8] human rotaviruses from Wuhan, China: Evidence for interspecies transmission and reassortment events. Infect Genet Evol 33: 55-71