

Isolation, Identification, and Comprehensive Genomic Characterization of a Bovine Rotavirus G10P[11] Strain in China

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Abstract

Rotavirus is a principal infectious agent of diarrhea in both animals and humans, causing acute gastroenteritis with symptoms such as abdominal pain, fever, nausea, and vomiting. Bovine rotavirus (BRVA) not only causes illness in cattle but also poses a risk to human health through direct transmission or reassortment among different virus strains. Here, quantitative RT-PCR for the NSP5 gene was applied to pinpoint BRVA in six fecal specimens from calves. A positive sample was identified and further examined using MA104 cells to isolate the virus. The isolate was then identified through RT-PCR, indirect immunofluorescence assays, and transmission electron microscopy. Genotyping and phylogenetic examination of the entire genome were performed via standard bioinformatics methods. A unique G10P[11] rotavirus strain, named SHH2023001, was identified in a fecal sample from a calf suffering from severe diarrhea in Shanghai, China. Whole genome sequencing revealed a unique genomic configuration of G10-P[11]-I2-R2-C2-M2-A11-N2-T6-E2-H3 for the 11 segments. Notably, these segments likely result from reassortment events involving rotavirus strains from humans, cattle, sheep, and horses. This study is the first to report the G10P[11] genotype constellation in Shanghai, highlighting the genetic diversity and potential for interspecies transmission of calf rotavirus.

Key words: bovine rotavirus, G10P[11], isolation, next-generation sequence, sequence analysis

Introduction

Rotavirus (RV), initially identified in the United States, is a zoonotic causative agent that triggers severe diarrhea and dehydration in children under five as well as in young mammals and birds (Li et al. 2024a; Devi et al. 2025). RV poses a substantial disease burden, particularly in low- and middle-income countries. In China, RV accounts for 32% to 50% of hospitalizations due to diarrhea in children under five (Li et al. 2024c).

Neonatal calf diarrhea (NCD) is a widespread issue and the leading cause of mortality in dairy calves prior to weaning (Urie et al. 2018). It causes signif-

icant economic effects on the cattle industry through stunted growth, death, and treatment costs (Huang et al. 2025). In cow-calf beef operations, managing calves with diarrhea is challenging (Smith 2012). The causes of NCD are complex, involving enteric pathogens as well as environmental conditions, immunity, and nutrition (Louge et al. 2023).

Rotavirus A (RVA) is the primary disease-causing agent of NCD (Niu et al. 2024). RVA infects animals and humans, causing acute gastroenteritis (AGE) with symptoms like abdominal pain, fever, nausea, and vomiting (Qin et al. 2022). Moreover, studies have shown that bovine rotavirus (BRVA) can spread to humans,

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either directly or through successive genetic shuffling events during the strains' evolutionary process (Komoto et al. 2016; Cho et al. 2022). Therefore, controlling the virus is vital for public health. BRVA is widespread on dairy farms in China, and active viral particles were found in drinking water sources for calves (Castells et al. 2018).

RVA is a non-enveloped virus with an 11-segmented RNA genome. These segments range from 667 to 3,302 nucleotides and encode the structural proteins VP1 to VP4, VP6, and VP7, as well as the non-structural proteins NSP1 to NSP5/6 (Strydom et al. 2021). RV is divided into G and P types by sequence analysis of VP7 and VP4. In 2008, the whole-genome system of Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx (x = genotype number) was introduced by the RVVV Classification Working Group. This system uses the order of VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5/6 to trace the strain's origin (Elkady et al. 2021).

To date, the following genetic profiles of RVA strains have been reported in cattle: G1-6, G8-10, G12, G15, G18, G21, G24 for the G types, and P[1], P[5]-[8], P[11], P[17], P[21], P[23], P[29], P[33], P[35], P[38] for the P types (Louge et al. 2023). Among them, the most common G/P combinations are G6P[5], G6P[11], and G10P[11] (Papp et al. 2013). In China, the G6, G8, and G10 genotypes, as well as the P[1], P[5], P[7], and P[11] genotypes have been detected in cattle. Recent studies show that G6 and P[5] are common in dairy calves in some Chinese regions, with G6P[5] as the dominant genotype combination (Yan et al. 2020; Cheng et al. 2021; Elkady et al. 2021). Given the limited data regarding the prevalence and molecular traits of BRVA, this study aims to isolate and characterize BRVA strains from gastrointestinal disorders to facilitate RVA gastroenteritis management in Shanghai.

Experimental

Materials and Methods

Specimen collection. During a significant outbreak of NCD, six fecal specimens were obtained from calves aged 5 to 7 days experiencing a single episode of diarrhea at a bull breeding station. The samples were stored at -80°C in the Shanghai Municipal Center for Animal Disease Diagnosis Laboratory, and subsequently resuspended in phosphate-buffered saline (PBS) (Gibco™,

Thermo Fisher Scientific Inc., USA) at a 1:5 (w/v) ratio. The suspension was spun at $11,000\times g$ for 10 min.

MA104 cell line culture. Continuous cultures of the MA104 cell line (X-Y Biotechnology Co., Ltd., China) were used. The cells were grown in 75 cm² Nunc flasks using Dulbecco's modified Eagle's medium (DMEM) enriched with 10% fetal bovine serum (FBS) (Gibco™, Thermo Fisher Scientific Inc., USA), 100 U/ml penicillin, and 100 µg/ml streptomycin (Gibco™, Thermo Fisher Scientific Inc., USA). The cells were cultivated at 37°C until confluence was achieved. For virus exposure, cells were transferred to 25 cm² incubation flasks and allowed to reach confluence over 24 h.

Virus isolation and culture. In every sample, 2 ml of fecal material was mixed with 6 ml of PBS containing glass beads in a tube. The samples were clarified by spinning at $2,500\times g$ for 10 min. Following centrifugation, the supernatant was collected and filtered through a 0.22 µm membrane filter (Pall, USA). The filtered supernatant was then stored at -80°C for further use. Each 1 ml filtrate was treated with 15 µg/ml trypsin (Gibco™, Thermo Fisher Scientific Inc., USA) for 1 h at 37°C after mixing, while DMEM serum-free medium was prepared as a negative control (Qin et al. 2022). To prepare the MA104 cell monolayers for infection, the growth medium was removed, and the cells were subjected to one wash with prewarmed serum-free DMEM.

After activation, the samples (or controls) were introduced into a complete coverage MA104 cell culture and then incubated at 37°C in a humidified incubator with 5% CO₂ for 2 h to facilitate virus attachment. The cells were then washed once with 10 ml of prewarmed serum-free DMEM. Subsequently, prewarmed serum-free medium containing 1 µg/ml trypsin was added. The cultures were monitored once a day for 3 to 6 days or until the cytopathic effects CPE was observed. Each sample was processed in triplicate.

For successive passages, infected cell populations underwent freeze-thaw cycles and were subsequently seeded onto freshly confluent cell sheets for extended passage, following the same trypsin treatment. Cells that lacked CPE after five passages were regarded as negative for RVA isolation.

Measurement of viral concentrations. 100 µl of the cell concentration (1×10^5 cells/ml) was transferred into each well of 96-well plates. After propagation at 37°C for 24 h, the cultures were serially diluted 10-fold and added to the plates. A negative control group comprising normal cells was incorporated. Following 2 h of

viral infection, each well received 100 μ l of serum-free DMEM containing 1 μ g/ml trypsin. The plates were then grown for 6 days, with daily observations, after which the TCID₅₀ was measured.

RT-PCR assay for the BRVA isolate. Using a Mag-Pure Viral RNA Kit (Magen Biotech Co., Ltd., China), total RNA was harvested from the cell cultures. The primers for amplifying the VP7 gene of BRVA (forward primer: 5'GGCTTTAAAAGMGAGAATTTC3'; reverse primer: 5'GGGGGTCACATCATACAATTCT3') were produced by Saiheng Biotech (China) based on a previous report (Fujii et al. 2012). For the RT-PCR reactions (Takara Biomedical Technology (Beijing) Co., Ltd., China), a 50 μ l reaction assembly was constructed as follows: 25 μ l of 2 $\times$ 1 Step Buffer, 2 μ l of PrimeScript 1 Step Enzyme Mix, 1 μ l of up-primer (20 μ M), 1 μ l of down-primer (20 μ M), 20 μ l of ddH₂O, and 1 μ l of template RNA.

The RT-PCR amplification conditions were: 50°C for 30 min, 94°C for 2 min, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 2 min, with a final amplification step at 72°C for 10 min. The RT-PCR products were resolved using 1.0% agarose gel electrophoresis, and then sequenced by Saiheng Biotech (China). Each sample was processed in triplicate.

Indirect immunofluorescence (IFA) for BRVA isolate. MA104 cells were challenged with BRVA for 1 h, after which the solution was discarded and the cells were cultured until slight CPEs emerged. The resulting cells were then exposed to 4% paraformaldehyde for 15 min, permeabilized using 0.5% Triton X-100 (Sangon Biotech Co., Ltd., China) for 20 min, followed by blocking with 5% bovine serum albumin (BSA) for 1 h. The cells were cultured overnight with a monoclonal antibody (clone 3C10) (Abnova, China) against the BRVA p42 antigen (1:200). Following this, the cells were cultured with a goat anti-mouse IgG coupled with a green fluorescent compound (Beyotime Biotech Co., Ltd., China) (1:100) at 37°C for 1 h. The cells were washed three times with PBS, then stained with DAPI (Sigma-Aldrich, Merck KGaA, Germany), followed by thorough rinsing with PBS five times. The treated cells were examined by fluorescence microscope (BX53, Olympus, Japan).

Transmission electron microscopy (TEM) observation for BRVA isolate. The CPE-positive cell cultures were spun at 30,000 $\times$ g for 10 min, then subjected to 0.2% phosphotungstic acid staining, and examined using TEM (Li et al. 2024b).

Next-generation sequencing of BRVA isolate. Approximately 50 μ l (220 ng/ μ l) of RNA extracted from the isolates was sent to Shanghai Tanpu Biotechnology Company for sequencing using the Illumina® Novaseq™ PE150 platform. The sequences have been submitted to GenBank.

Phylogenetic evaluation. The genomic sequences of the SHH2023001 strain obtained from the experiment have been submitted to GenBank with accession numbers PP987320, PP987322, PP987321, PP987325, PP987324, PP987323, PP987330, PP987329, PP987328, PP987327, and PP987326, respectively. The genotypes were determined via BLASTn (<http://blast.ncbi.nlm.nih.gov/>), nucleotide sequence identities were evaluated, and phylogenetic diagrams were assembled in MEGA 7.0 (Kumar et al. 2016) with bootstrap values derived from 1,000 resampling events.

Results

Effects of the isolate on cell morphology. In this study, six supernatant samples were utilized to inoculate MA104 cells (African green monkey kidney cells). By the fifth generation, only one sample exhibited CPE (Fig. 1). Infected cells exhibited rounding and detachment, typical signs of viral-induced cytopathogenicity at 12 h post-infection. In contrast, uninfected control cells maintained normal morphology. Moreover, a more pronounced swelling morphology was evident in the infected cells. After 18 post-infections, CPEs intensified, affecting 80% $\pm$ 4.6% of the cells. Cell detachment and vacuolization increased, with some cells starting to float in the culture solution. The TCID₅₀ determination indicated a concentration of 1.1 $\pm$ 0.05 $\times$ 10^{6.4} TCID₅₀/0.1 ml in the fifth-generation cell culture of the isolate.

Characterization of the isolate as BRVA using RT-PCR. The cell culture was exposed to RNA extraction, followed by RT-PCR analysis. The PCR-amplified fragment was run on a 1.0% agarose gel, showing a target fragment of roughly 1,060 bp (Fig. 2). The presence of a specific segment relative to the control confirmed the isolation of BRVA, which was further verified by sequencing.

Identification of the isolate as BRVA using IFA. To further identify the isolate as BRVA, we performed an IFA using a monoclonal antibody against the p42 antigen of BRVA. MA104 cells infected with BRVA showed specific green fluorescence, while control cells did not (Fig. 3). This confirms that the isolated virus interacted with the monoclonal antibody.

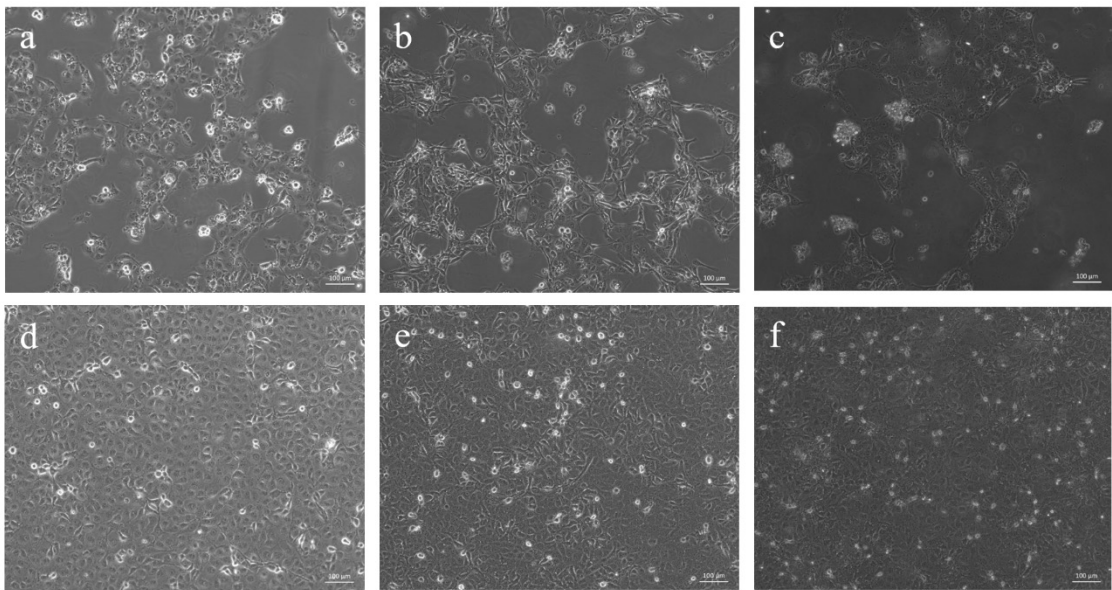


Fig. 1. Inverted phase-contrast microscopy of BRVA-infected MA104 cells (100× magnification). A) Infected, 24 h; B) infected, 48 h; C) infected, 72 h; D) uninfected, 24 h; E) uninfected, 48 h; F) uninfected, 72 h. Cells were cultured in 24-well plates and incubated with or without BRVA for different durations. Cytopathic effects began at 48 h and intensified by 72 h compared to the negative controls. Scale bars represent 100 μm.

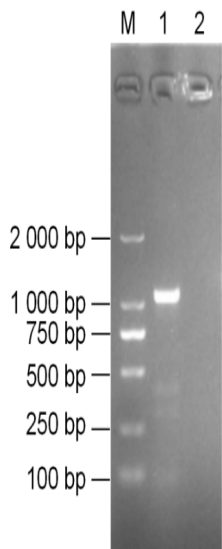


Fig. 2. Results of agarose gel electrophoresis for RT-PCR amplification of BRVA. M – DL 2000 DNA marker; 1 – MA104 cells infected with SHH2023001; 2 – negative control (uninfected MA104 cells). The presence of a specific band at approximately 1,060 bp confirms the successful amplification of the BRVA VP7 gene in the infected sample (lane 1). The negative control (lane 2) shows no amplification, indicating the absence of viral RNA.

Observation of virions under TEM. The morphology of the isolated BRVA strain was observed using TEM. The TEM image unveiled some BRVA virions, with characteristic BRVA particles clearly visible (Fig. 4). The virions presented a wheel-like structure, typical of rotavirus particles, with a diameter of about 70–80 nm.

Genotype pattern analysis. The entire genome of SHH2023001 was successfully sequenced, and the 11 segments, including VP1–VP4, VP6, VP7, and NSP1–NSP5, were submitted to GenBank (accession numbers PP987320–PP987330). The SHH2023001 strain has a

genetic pattern of G10-P[11]-I2-R2-C2-M2-A11-N2-T6-E2-H3 (Table I). In this study, genetic closeness was defined as having a nucleotide identity of at least 90% for the 11 segments. BLASTn analysis showed that five segments (VP7, VP1, NSP2, NSP3, NSP5) were genetically close to human RVA isolates, with nucleotide identities ranging from 95.41% to 97.77%. VP4, VP6, and VP3 segments were genetically close to bovine RVA isolates, with nucleotide identities ranging from 94.25% to 97.20%. VP2 and NSP1 segments were genetically close to sheep RVA isolates, with nucleotide identities of 97.77% and 96.47%, respectively. The

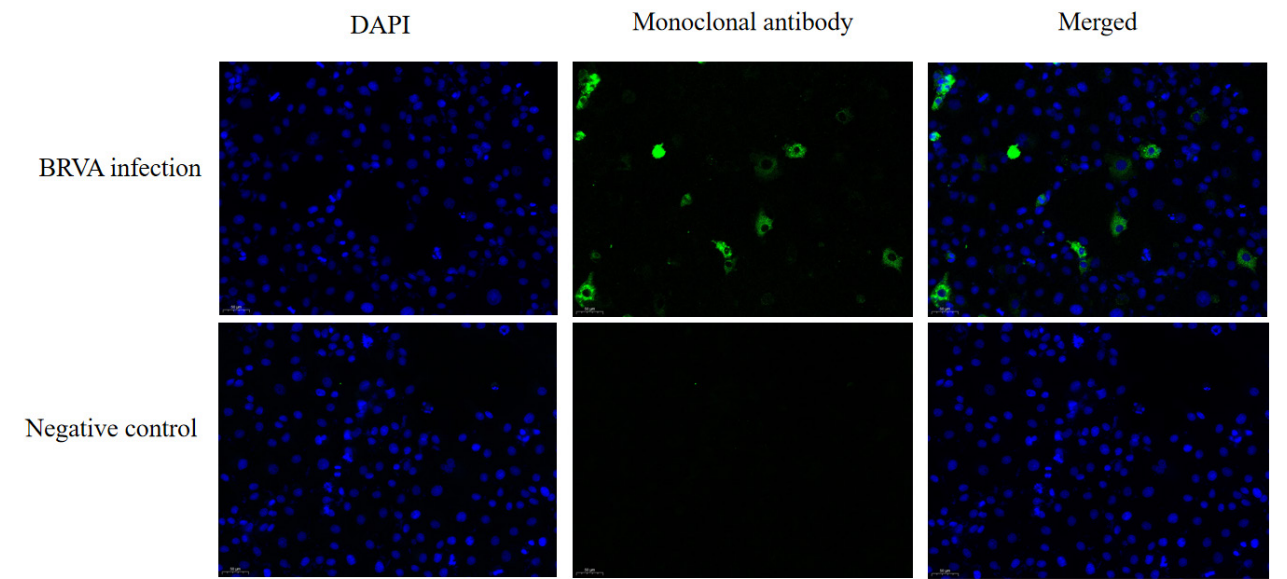


Fig. 3. Virus identification by indirect immunofluorescence assay. MA104 cells grown on coverslips in 6-well plates were infected with BRVA. The cells were fixed and incubated with a monoclonal antibody against the BRVA p42 antigen (1:200) and a goat anti-mouse fluorescent secondary antibody. Images were captured post-infection. BRVA infection (SHH2023001); uninfected (normal cells). Scale bars represent 50 μ m. The signal indicates cytoplasmic sub-cellular localization, which is expected for BRVA infection. The negative control (uninfected) was used to validate the assay, and multiple fields of view were checked for consistency.

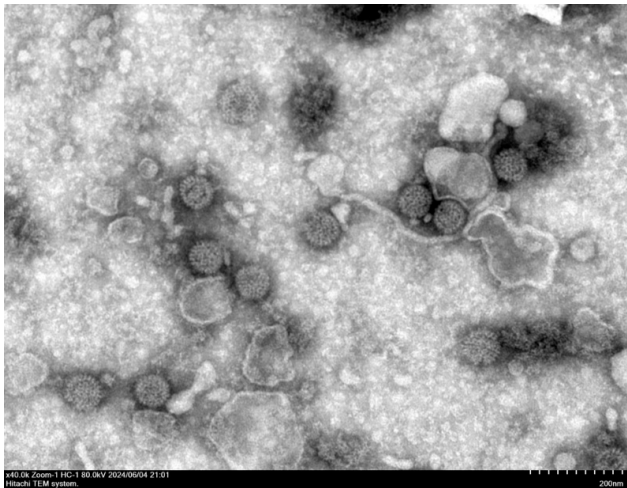


Fig. 4. Transmission electron microscopy (TEM) observation of BRVA-infected MA104 cell cultures. SHH2023001 virions were visualized under TEM (Scale bar = 200 nm).

NSP4 segment was genetically close to a horse RVA isolate, with a nucleotide identity of 97.44% (Table I).

Phylogenetic analyses. Maximum likelihood (ML) trees were established for the 11 genomic fragments of SHH2023001 (Fig. 5A–5F). For VP7, SHH2023001 was closely related to human strain DB2015-066, forming a distinct G10 cluster with 83.0–95.2% nucleotide identity to other G10 strains. For VP4, it closely resembled the American bovine strain B223 (96.6% identity) and grouped with the Japanese bovine P[11] strains (95.6–

96.4% identity). For VP6 (I2), it was closely related to the Korean bovine strain KJ9-1 (97.2% identity) and the Argentinean oyster strain BsAs (96.9% identity), forming a subcluster with bovine G10P[11] strains (93.7–96.3% identity).

In the VP1 (R2) phylogeny, SHH2023001 grouped with the Japanese human strain HKD0825 and nine other human strains (95.0–95.2% identity). For VP2 (C2), it clustered with the Chinese human strain DZ614090 and sheep strain GS2023 (97.2% and 97.8% identity, respectively), but was more distantly related to human strain DS-1 (85.7% identity). In VP3 (M2), it was closely related to bovine strains DQ-75 (94.2% identity) and MPT-93 (88.9% identity), forming a cluster with ten human strains (86.4–91.5% identity).

For NSP1 (A11), SHH2023001 was very closely related to the Chinese human strain DZ614090 (95.9% identity), forming a unique subcluster (Fig. 6A). In NSP2 (N2), it was closely connected to human strains DZ614090 and NT0578 (both 97.0% identity), forming a subcluster with strains SCMY1 and AU109 (Fig. 6B). For NSP3 (T6), it grouped closely with the Hungarian human strain BP1062 (96.3% identity) (Fig. 6C). In NSP4 (E2), it was closely related to human strain 12579, horse strain ERV2 (97.5% identity), and vaccine strain RotaTeq-WI79-4 (96.4% identity) (Fig. 6D). For NSP5 (H3), it clustered between the Chi-

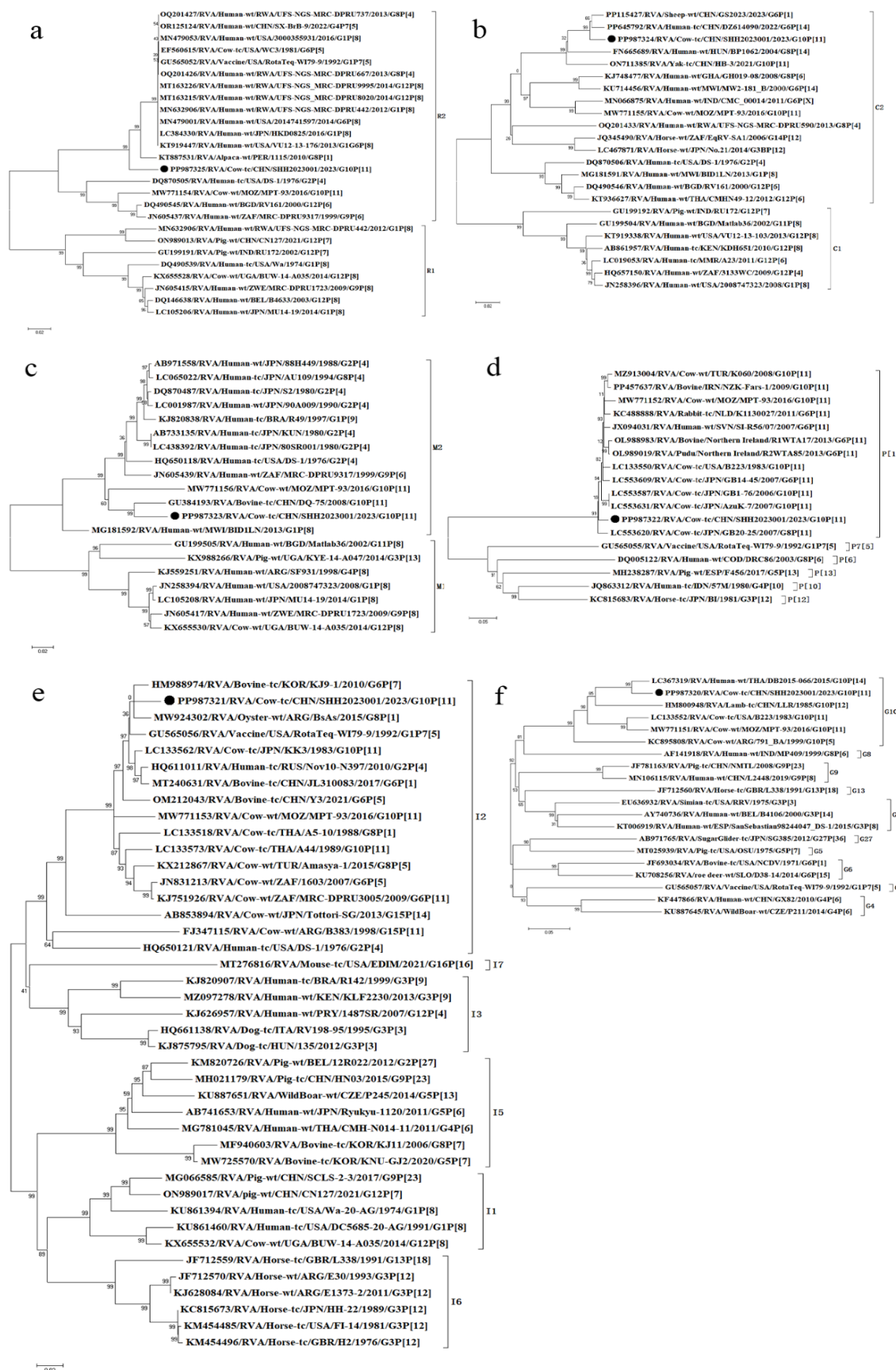


Fig. 5. Phylogenetic analysis of the SHH2023001 strain based on nucleotide sequences of VP1 (a), VP2 (b), VP3 (c), VP4 (d), VP6 (e), and VP7 (f) genes. Trees were constructed using the neighbor-joining method in MEGA 7.0, with bootstrap values (1,000 replicates) above 70 indicated. The scale bar represents nucleotide substitutions per site. Genotypes are shown on the right. Strains detected in this study are marked with a black circle.

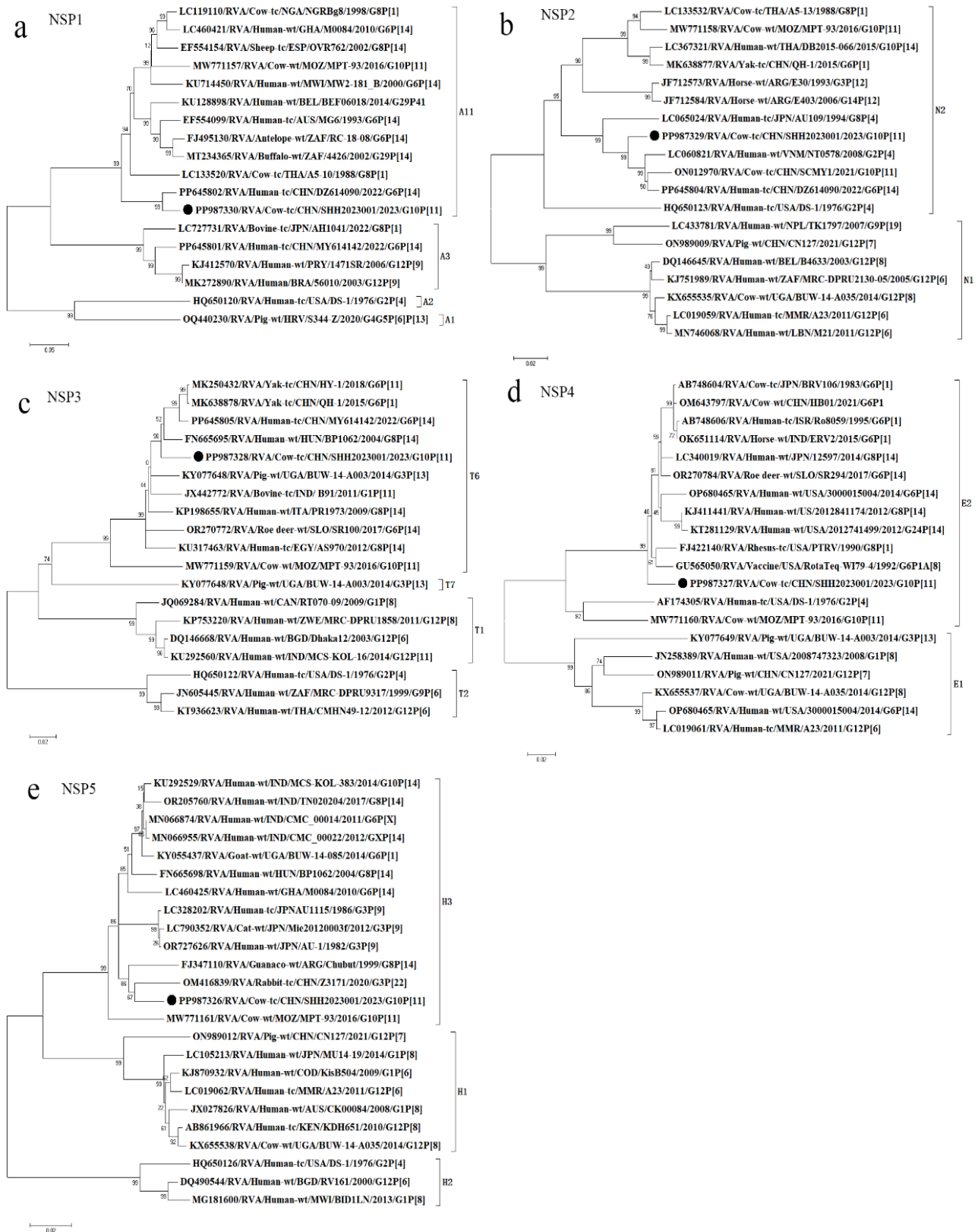


Fig. 6. Phylogenetic analysis of the SHH2023001 strain based on nucleotide sequences of NSP1 (a), NSP2 (b), NSP3 (c), NSP4 (d), and NSP5 (e) genes. Trees were constructed using the neighbor-joining method in MEGA 6.0, with bootstrap values (1,000 replicates) above 70 indicated. The scale bar represents nucleotide substitutions per site. Genotypes are shown on the right. Strains detected in this study are marked with a black circle.

Table I
Nucleotide sequence identity between strain SHH2023001 and other strains for each gene segment.

Gene	Closest strain	Accession No.	Homology (%)	Genotype
VP7	RVA/Human-wt/THA/DB2015-66/2015/G10P[14]	LC367319	95.41	G10
VP4	RVA/Cow-tc/USA/B223/1983/G10P[11]	LC133550	96.62	P[11]
VP6	RVA/Bovine-tc/KOR/KJ9-1/2010/G6P[7]	HM988974	97.20	I2
VP1	RVA/Human-wt/JPN/HKD0825/2016/G1P[8]	LC384330	95.12	R2
VP2	RVA/Sheep-wt/CHN/GS2023/2023/G6P[1]	PP115427	97.77	C2
VP3	RVA/Bovine-tc/CHN/DQ-75/2008/G10P[11]	GU384193	94.25	M2
NSP1	RVA/Sheep-wt/CHN/GS2023/2023/G6P[1]	PP115432	96.47	A11
NSP2	RVA/Human-wt/VNM/NT0578/2008/G2P[4]	LC060821	96.98	N2
NSP3	RVA/Human-wt/HUN/BP1062/2004/G8P[14]	FN665695	96.14	T6
NSP4	RVA/Horse-wt/IND/ERV2/2015/G6P[1]	OK651114	97.44	E2
NSP5	RVA/Human-wt/HUN/BP1062/2004/G8P[14]	FN665698	97.20	H3

nese rabbit strain Z3171 and the Argentinean guanaco strain Chubut (96.3% and 96.1% identity, respectively), forming a unique subcluster, and had 97.2% and 97.1% identity with human strains BP1062 and CMC_00014, respectively (Fig. 6E).

Discussion

RVA infects humans and various animals, causing clinical diseases. In this study, six samples were initially selected following a preliminary assessment of a calf that displayed clinical manifestations of diarrhea in the Shanghai region, and collected from a single farm. The specific criteria included the presence of watery feces and a documented history of recent gastrointestinal symptoms. We successfully isolated a BRVA strain by inoculating suspected samples into MA104 cell monolayers and observing typical RV CPE. The fragmentation of the VP4 protein into VP5 and VP8 facilitates RV entry into susceptible cells (Crawford et al. 2001; Cheng et al. 2021). Thus, we used EDTA-free trypsin to activate BRVA during isolation, along with serum-free medium containing a specific trypsin concentration. From the fifth day, cells showed stable CPE, including swelling, aggregation, shedding, and vacuole formation. TEM revealed RV particles with a wheel-like structure and a diameter of approximately 70–80 nm, matching previous reports (Crawford et al. 2001). We further confirmed the successful isolation of the

SHH2023001 strain by verifying BRVA antigen expression in MA104 cells using an RV-VP6 monoclonal antibody.

The extensive genetic diversity of the RV genome complicates RT-PCR characterization due to the lack of universal primers (Li et al. 2024a). To characterize the SHH2023001 strain, we used next-generation sequencing, revealing a genotype profile of G10-P[11]-I2-R2-C2-M2-A11-N2-T6-E2-H3. Recent analyses show that certain VP7 and VP4 gene combinations have become predominant in several Asian countries (Shin et al. 2023). In 2018, BRVA was widespread in Chinese dairy calves, with G6P[1] as the principal genotype, though three G10 strains were found in Xinjiang (Liu et al. 2021). In South Korea, G6P[5] was most prevalent among calves from 2014 to 2022 (Cho et al. 2022; Park et al. 2022). In Bangladesh, G6P[11] was the main genotype (94.4%) among BRVA from diarrhea samples in 2014–2015, with G10P[11] at 5.6% (Hasan et al. 2022). In Bangladesh, G6P[11] was the predominant genotype among BRVA isolated from diarrhea samples between 2014 and 2015 (94.4%), followed by G10P[11] (5.6%) (Uddin et al. 2022). In Uruguay, the predominant G- and P-type combinations isolated from BRVA-infected calves in dairy and beef herds between 2015 and 2018 were G6P[11] (40.4%) and G6P[5] (38.6%), with G10P[11] and G24P[33] detected at much lower rates (Castells et al. 2020). The G6 and P[5] genotypes isolated from Brazilian calves in 2009 were investigated as major causes of diarrhea among

BRVA-vaccinated beef cow herds (da Silva Medeiros et al. 2015). The most prevalent genotype of BRVA strains circulating in Australian calves during 2004 and 2005 was G6P[5] (38.5%) (Swiatek et al. 2010). In several of the countries mentioned, G6P[1], G6P[5], G6P[11], and G10P[11] were the main G- and P-genotype combinations. The G10P[11] strain was first identified in 2008 on a dairy farm in Inner Mongolia, China, marking the first report of a G10P[11] strain isolated from a calf in Shanghai.

Comparison with GenBank sequences showed that the VP7, VP1, NSP2, NSP3, and NSP5 genes of the SHH2023001 strain were most similar to human RV genes. The VP4, VP6, and VP3 genes were most similar to BRVA genes. The VP2 gene was most similar to the sheep strain GS2023, and the NSP4 gene was most similar to the horse strain ERV2. The G10P[11] genotype, originally identified in 1988 in India, is exceptional within neonates and has been linked to outbreaks in neonatal intensive care units and nurseries in Bangalore (1988–1999) and Vellore (2006–2007) (Reju et al. 2022). The phylogenetic analysis (the study of how different species are related through evolution) of SHH2023001 revealed significant genetic relationships with RV strains from various species. Additionally, the phylogenetic trees demonstrate the potential interspecies spread and evolutionary mixing of RV strains, indicating that SHH2023001 clusters closely with strains from both human and animal origins. The zoonotic potential of RV underscores the vital need for a One Health strategy in combating diseases. The detection of human RV sequences in BRVA highlights the necessity for continuous monitoring of RV infections in both human and animal populations. This will allow for the early identification of emerging strains and promote the adoption of targeted strategies to prevent the spread of infections.

In summary, this study conducts an extensive analysis of the BRVA strain SHH2023001, obtained from a calf in Shanghai. The genomic characterization and phylogenetic analysis demonstrate substantial genetic links with rotavirus strains from multiple species. These findings underscore the necessity of continuous surveillance and investigation into the molecular diversity (the variety of genetic material) of rotaviruses. Specifically, local health authorities should consider incorporating targeted surveillance programs to monitor the prevalence of BRVA strain in both human and animal populations. Additionally, the findings show the need for additional research into the efficacy of exist-

ing vaccines against zoonotic strains and the potential for developing new vaccines to address these emerging threats.

While our study provides valuable insights into the molecular characteristics of the SHH2023001 strain, it is essential to recognize its limitations. One major limitation is the limited number of successful isolations, which may narrow the generalizability of our findings. Future investigations should target the isolation and characterization of a larger number of strains from different geographical regions to achieve a more comprehensive picture of the molecular diversity of BRVA. Additionally, further research is imperative to examine the antigenic properties of human RV sequences identified in this study and their potential consequences for vaccine efficacy.

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Abbreviations

AGE – acute gastroenteritis
BRVA – bovine rotavirus
BSA – bovine serum albumin
CPE – cytopathic effect
DMEM – Dulbecco's modified Eagle's medium
FBS – fetal bovine serum
IFA – indirect immunofluorescence
NCD – neonatal calve diarrhea
PBS – phosphate buffered saline
RNA – ribonucleic acid
RT-PCR – reverse transcription-polymerase chain reaction
RV – rotavirus
RVA – rotavirus A
TEM – transmission electron microscopy

Availability of data and material

The nucleotide sequence data of the SHH2023001 strain have been deposited in GenBank. The accession numbers for the genes encoding VP7 (PP987320), VP4 (PP987322), VP6 (PP987321), VP1 (PP987325), VP2 (PP987324), VP3 (PP987323), NSP1 (PP987330), NSP2 (PP987329), NSP3 (PP987328), NSP4 (PP987327), and NSP5 (PP987326) are provided.

Author contributions

Conceptualization: Jian Liu, Weidong Qian, Shixin Huang; Methodology: Xianchao Yang, Qiqi Xia, Guidan Feng, Dequan Yang; Formal analysis and investigation: Ping Xu, Jun Tao, Yuling Ma; Originaldraft preparation: Jian Liu, Wenwei Sheng, Jian Wang, Jun Ma; Writing-review and editing: Shixin Huang, Weidong Qian.

Funding acquisition: Shixin Huang; Supervision: Weidong Qian, Shixin Huang. All authors participated in revising the manuscript and endorsed the final version.

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Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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