

Outbreak Characteristics and Whole-Genome Analysis of the First Domestic G4P[2] Group C Rotavirus Epidemic

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Article

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Abstract

Background

Acute gastroenteritis outbreaks caused by Group C Rotavirus (RVC) frequently occur in closed populations such as universities and pose a threat to public health. However, the etiological characteristics and genomic features of domestic RVC outbreaks remain largely unclear. This study performed etiological detection and genetic analysis on 32 anal swab samples collected from patients during a university outbreak.

Methods

Total nucleic acids were extracted from the samples. Group C rotavirus (RVC) was detected using reverse transcription polymerase chain reaction (RT-PCR). Whole-genome capture sequencing was performed on PCR-positive samples to determine the viral genotype and analyze its genetic characteristics.

Results

RVC nucleic acid was detected in 25 out of the 32 samples (78.1%). Using whole-genome capture sequencing technology, complete genomic data were successfully obtained for 13 out of the 25 RVC-positive strains. Genotyping revealed that all 13 outbreak strains (designated as the JNTQ strain) belonged to the G4P[2] genotype.

Conclusions

This study confirms that the acute gastroenteritis outbreak at the university was caused by the G4P[2] genotype RVC, with a relatively wide infection range. The 13 obtained complete genome sequences confirmed the clonal identity of the outbreak strains, providing crucial data support for precise outbreak tracing and molecular epidemiological studies. This study demonstrates that combining PCR screening with whole-genome sequencing technology during cluster outbreak investigations can efficiently identify pathogens and elucidate transmission chains.

1.Introduction

Rotavirus is one of the major pathogens causing acute gastroenteritis worldwide, posing a particular threat to infants and young children. Its primary transmission route is fecal-oral, with clinical manifestations mainly including diarrhea, vomiting, and fever. Severe cases may lead to dehydration and electrolyte imbalance, seriously compromising public health safety [1, 2]. Based on antigenic differences

in the internal capsid protein VP6, rotaviruses are classified into 12 species(A-L)[3], among which groups A, B, and C have been confirmed to infect humans and cause acute gastroenteritis.

Among these three species, Group A Rotavirus (RVA) is the most extensively studied, being the leading cause of severe diarrhea and death in children under five years old and the primary target of current rotavirus vaccines [4]. In contrast, Group C Rotavirus (RVC) was first detected in swine in 1980 and was not identified in human diarrheal patients until 1982 [5, 6]. Unlike RVA, the epidemiological characteristics of RVC are notably distinct: it is not a dominant pathogen in infant diarrhea but can infect individuals across all age groups, particularly in sporadic cases and cluster outbreaks among adults and school-age populations [7, 8]. Currently, RVC exhibits a sporadic or endemic distribution globally, with outbreaks or sporadic cases reported in countries including China, Japan, South Korea, India, Brazil, Italy, and Hungary [9–11]. However, its true disease burden is likely significantly underestimated due to limitations in detection methods and incomplete surveillance networks. Moreover, molecular traceability research on RVC cluster outbreaks affecting adults and school-age populations in China remains scarce, lacking whole-genome characterization data for outbreak strains. This gap hinders the ability of primary disease control institutions to conduct precise traceability and targeted prevention during such outbreaks.

Virologically, RVC belongs to the *Reoviridae* family. It is a non-enveloped, double-stranded RNA virus with an 11-segmented genome encoding six structural proteins (VP1-VP4, VP6, VP7) and five non-structural proteins (NSP1-NSP5) [12]. Serotype classification is primarily based on antigenic differences in the outer capsid protein VP7 (G-type) and the spike protein VP4 (P-type). Currently, various G and P genotypes have been reported globally, with G4P[2] being a relatively prevalent combination in human RVC infections in recent years [13]. Notably, RVC has a broad host range and has been detected in various animals, including pigs and cattle. Molecular epidemiological studies indicate a close genetic relationship between human and animal-derived RVC strains, suggesting potential zoonotic transmission and gene reassortment risks—events that could be key drivers of RVC evolution and genetic diversity [14, 15].

Currently, global public databases lack sufficient whole-genome sequences of RVC, which significantly constrains in-depth understanding of its genetic evolution patterns, transmission dynamics, and epidemic potential. Within China's public health surveillance system, rotavirus monitoring remains primarily focused on Group A, while routine surveillance for non-group A rotaviruses such as RVC has not been comprehensively established. This deficiency makes outbreaks caused by such viruses highly prone to under-detection or misdiagnosis, potentially leading to wider transmission. This study investigates an acute gastroenteritis outbreak caused by RVC at a university in China. The event involved a relatively large number of individuals, exhibited a complex pathogen composition, and featured a rare case of viral-bacterial co-infection. This study characterizes the outbreak strains through molecular detection and genetic analysis, thereby supplementing data on the prevalence and genetic characteristics of RVC in China. It provides a scientific basis for precise prevention and control of future similar cluster outbreaks and for improving surveillance systems. Additionally, it offers domestic

experimental data for in-depth research on cross-regional transmission and gene reassortment mechanisms of RVC, filling a research gap in this field within China.

2.Materials and Methods

- Ethical compliance
- All methods were performed in accordance with the relevant guidelines and regulations of the Chinese Center for Disease Control and Prevention. This study was conducted in compliance with the Declaration of Helsinki.

2.1.Sample Collection and Processing

- The study subjects were 32 diarrhea patients reported during an acute gastroenteritis outbreak at a university in September 2025. Anal swab specimens were collected aseptically from each patient. Specimens were immediately transported to the laboratory, placed into sampling tubes containing 3 mL of physiological saline, vortexed to homogenize the suspension, and then subjected to nucleic acid extraction. All experimental procedures strictly followed Biosafety Level 2 (BSL-2) laboratory regulations.

2.2. Nucleic Acid Extraction and Multiplex Pathogen PCR Screening

Total nucleic acids (DNA and RNA simultaneously) were extracted from the 32 anal swab specimens using the BD MAX automated nucleic acid purification system, strictly following the manufacturer's instructions. Nucleic acids were eluted in 60 µL of RNase-free water. Concentration and purity were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA) to ensure suitability for subsequent experiments.

Broad-spectrum pathogen screening was performed on the extracted nucleic acids using a multiplex real-time PCR detection kit for 25 diarrheal pathogens (Shuoshi Biotechnology). RT-PCR reactions were conducted on a real-time fluorescence quantitative PCR system, with reaction mixtures and cycling parameters strictly set according to the kit's standard protocol. Results were interpreted using the accompanying software.

2.3. Whole-Genome Capture Sequencing of Group C Rotavirus

Reverse transcription, DNA fragmentation, end repair, and 3'-end dA-tailing were performed using a rotavirus whole-genome enrichment library preparation kit (Hongwei Tetes, HWKF-TW0262). Libraries were constructed by ligating corresponding adapters and performing PCR amplification. The constructed libraries were hybridized with rotavirus capture probes to obtain full-length rotavirus genome capture

libraries. PE150 high-throughput sequencing was conducted on the Salus Pro sequencing platform (Sailu Biotech) using the Salus Pro Sequencing Reagent Kit (PRM-PE150-80M, Sailu, SL-S00155), with a sequencing data volume of at least 0.3 Gb.

2.4. Bioinformatics Analysis

Sequence alignment was performed on the quality-controlled data using BWA software (version 0.7.17-r1188). The reads were initially aligned against a reference genome set downloaded from NCBI, encompassing all type reference sequences for this species. The sequence exhibiting the highest nucleotide identity with the samples was selected as the reference sequence. Based on this reference sequence file, a consensus sequence was generated using the consensus module of BCFtools (version 1.21), ultimately yielding the assembled full-length genome sequence.

Multiple sequence alignments for each gene segment and concatenated sequences were performed using MEGA-X software[16]. After aligning the concatenated segments (VP1-VP2-VP3-VP4-VP6-VP7-NSP1-NSP2-NSP3-NSP4-NSP5), a neighbor-joining (NJ) phylogenetic tree was constructed using MEGA-X. The tree was tested using the Bootstrap method with 1000 replicates. Recombination breakpoints were identified using SimPlot software v.3.5.1 and RDP5 (Recombination Detection Program) v.5.39 [17]. The extracted recombinant loci regions were used for constructing phylogenetic trees. All phylogenetic trees were visualized using iTOL v7 software [18].

3. Results

3.1. Whole-Genome Capture Sequencing Analysis

In this outbreak investigation, targeted capture sequencing was performed on 25 RVC nucleic acid-positive samples, successfully obtaining complete genomic data for 13 RVC strains (designated JNTQ14, JNTQ16, etc.). All 13 specimens generated more than 6.75 million sequencing reads, with coverage of the rotavirus genome exceeding 99%. Coverage for the 11 individual gene segments ranged from 90.6% to 100.0%. The average sequencing depth was sufficient for high-confidence variant detection, which guaranteed the reliability of subsequent evolutionary analysis and recombination event detection.

3.2. Phylogenetic Analysis

To elucidate the origin and transmission relationships of the outbreak strains, phylogenetic trees were constructed based on concatenated whole-genome sequences and individual gene segments. To clarify the genetic relatedness of the 13 RVC strains with other global RVC strains, phylogenetic trees incorporating all 11 genomic segments and the concatenated full-genome sequence were built. The concatenated sequence phylogenetic tree revealed that the 13 RVC strains and other global RVC strains could be divided into three main clusters: non-human-derived sequences formed a separate cluster (marked in green); the strains obtained from this event (marked in red) clustered with strains from Europe and Asia. Notably, most strains within this cluster originated from Asia (primarily South Korea

and Japan). All 13 RVC strains (marked in red) clustered with human-derived sequences and did not group with animal-derived sequences. Furthermore, all 13 strains belonged to the G4P[2] genotype and formed a clade with the PR713 strain isolated in Italy in 2012 (Fig. 1). The phylogenetic tree based on the concatenated whole-genome sequence further demonstrated that the 13 outbreak strains formed a tight monophyletic group, strongly suggesting that the outbreak was most likely caused by a single dominant clone. Interestingly, this clone did not cluster with more temporally or geographically proximate Asian strains (e.g., the Yunnan strain from China, epidemic strains from Japan/South Korea) but was located on the same terminal evolutionary branch as the 2012 Italian PR713 strain, showing high genomic sequence identity (Fig. 1). This finding suggests that the outbreak strain and the Italian PR713 strain likely share a recent common ancestor.

Legend (Fig. 1): A neighbor-joining phylogenetic tree was constructed based on concatenated whole-genome sequences. The analysis included human-derived RVC sequences available in the NCBI database and nine non-human-derived RVC sequences. The tree was generated using MEGA-X with 1000 bootstrap replicates. The scale bar represents nucleotide substitutions per site. (Color coding for strain groups is embedded within the figure.)

3.3. Analysis of Segment-Specific Evolutionary Characteristics

The 13 RVC strains exhibited a high degree of overall genomic sequence consistency with PR713 strain. However, discrepancies in the phylogenetic branching structure of certain gene segments were observed compared with the concatenated sequences. Specifically, the VP1, VP2, VP4, NSP3, and NSP5 gene segments of the 13 RVC strains showed closer phylogenetic relationships with the 2025 Yunnan isolates, with VP1 being particularly closely related to YN02, VP2, VP3 exhibiting proximate relationships with YN01, YN02, and YN03, VP4 showing affinity with YN02 and YN03, NSP3 being closely related to YN03, and NSP5 displaying proximity to YN01 and YN03. In contrast, the VP7 and NSP4 gene segments were more closely related to the 2018 Russian isolate NS18-A1602, while the VP6 and NSP2 gene segments were more closely related to the 2017 Russian isolate NS17-A1035. Regarding the NSP1 gene segment, 7 RVC strains clustered with the 2017 Russian isolate NS17-A1035, while the remaining 6 strains were associated with prevalent Eurasian epidemic strains from Hungary, South Korea, Japan, and other regions (Supplementary Figure S1). In-depth analysis of each gene segment revealed more complex evolutionary characteristics: although the outbreak strains exhibited a clonal pattern overall, different gene segments displayed diverse evolutionary pathways. This phenomenon of "segment-specific phylogenetic relationship inconsistency" provides crucial molecular evidence for viral evolution through genetic reassortment.

3.4. Reconstructed phylogenetic analysis confirms the trans-regional origin of the VP4 gene

In response to the aforementioned findings, SimPlot and RDP5 software were employed to rigorously verify recombination events. The results clearly demonstrated significant recombination breakpoints in

the VP4 gene of 13 RVC strains (located within the 8301 ~ 10481 bp interval of the concatenated genomic sequence). Further phylogenetic relationship validation confirmed that the primary parent of this recombination event was the Korean strain CAU10-312, while the secondary parent was the Japanese strain OS-270 (Figs. 2a and 2b). This discovery explains at the molecular level why the whole-genome backbone of the outbreak strain resembles the Italian strain, yet its VP4 segment exhibits East Asian regional characteristics, conclusively confirming that the outbreak virus was formed through cross-regional viral genetic exchange.

Legend (Fig. 2): (a) Recombination breakpoints were identified using SimPlot v3.5.1 and RDP v5.39. The y-axis shows percent identity between the recombinant and putative parental strains; the x-axis shows genomic position aligned with a 20-nt sliding window. Comparisons between the recombinant and the major parent, and between the recombinant and the minor parent, are shown. (b) Phylogenetic analysis of the VP4 gene recombinant locus. The tree was constructed using the neighbor-joining method with 1000 bootstrap replicates. The scale bar represents nucleotide substitutions per site. (Color coding for strain groups is embedded within the figure.)

4. Discussion

This study successfully resolved the molecular epidemiological characteristics of an acute gastroenteritis outbreak caused by RVC in universities through high-throughput sequencing combined with bioinformatics analysis. The outbreak was caused by a G4P[2] type RVC clone, whose whole genome framework is highly homologous to the early Italian strain PR713 [19]. Its genome was assembled from gene segments of prevalent strains in multiple countries, including China, Russia, South Korea, and Japan, through complex reassortment events [20].

Firstly, the clonal characteristics of the outbreak strain suggest that this epidemic was caused by interpersonal transmission following the introduction of a single infectious source, which is consistent with the fecal–oral transmission pattern of pathogens in closed or semi-closed environments such as schools [21]. Whole genome capture sequencing technology provides key support for confirming the source of the outbreak and the identity of the transmission chain at the genetic level. By obtaining complete genome data, the homology of the outbreak strain sequence can be accurately verified at the molecular level, providing conclusive evidence for identifying the epidemic source and clarifying the transmission chain [22], thereby buying valuable time for timely implementation of prevention and control measures such as lockdowns and disinfection.

Secondly, the complex genetic background of the outbreak strain is one of the core findings of this study, with its genome exhibiting significant cross-regional genetic fusion characteristics. Phylogenetic analysis shows that the main body of the entire genome of the RVC clone is highly similar to the Italian PR713 strain, suggesting the potential for cross-continental transmission of RVC. Although the specific transmission route remains unclear, this result suggests that the global transmission network of RVC may be more extensive and complex than currently understood [23]. At the same time, multiple gene

segments of the outbreak strain showed obvious regional fusion characteristics: segments such as VP1, VP2, VP4, NSP3, and NSP5 were closely related to the 2025 Yunnan isolate [24], while segments such as VP7 and NSP4 were highly similar to Russian epidemic strains. For the NSP1 segment, 7 of the 13 strains clustered with the 2017 Russian isolate NS17-A1035, and the remaining 6 strains were associated with prevalent strains from Hungary, South Korea, Japan, and other Eurasian countries [25]. The VP4 gene, which encodes the key viral attachment protein, was confirmed by recombination analysis to be a reassortment product of the Korean strain CAU10-312 and the Japanese strain OS-270 [26]. This integration phenomenon of multi-regional gene fragments reflects the frequency of RVC gene exchange in East Asia. A similar cross-regional gene reassortment phenomenon was also reported in the G4P[2] RVC outbreak in Hungary in 2013, where all gene segments except NSP4 of the isolated strains were highly homologous to epidemic strains from China and the Russian Far East, further confirming the universality of cross-regional reassortment in RVC [27].

This study supplements the whole genome data of G4P [2] type RVC in China, enriching the global RVC genetic evolution database. RVC whole-genome sequence resources are scarce in global public databases, and the proportion of Chinese local sequences is extremely low, which severely restricts research on the genetic evolution characteristics of RVC in China. This study successfully obtained complete genome sequences of 13 G4P [2] type RVCs from university outbreaks. This represents the first whole genome study of an adult cluster outbreak of RVCs in China, filling the gap in whole-genome data of locally clustered RVC strains and enriching the global genetic evolution database of G4P[2] type RVCs. It also provides important Chinese samples for international research on cross-regional transmission of RVCs.

The current rotavirus surveillance system in China is centered on Group A, and routine surveillance of non-Group A rotaviruses such as RVC has not yet been incorporated into the national surveillance network, resulting in a serious underestimation of the disease burden of such viruses and significant gaps in epidemic early warning. This study confirms that G4P [2] type RVC can cause large-scale cluster outbreaks in densely populated areas such as universities, and that the strain exhibits cross-regional gene reassortment characteristics. This indicates that RVC has become an important pathogen causing acute gastroenteritis in adult and school-age populations in China and urgently needs to be included in routine surveillance. The 13 complete genome sequences of G4P [2] type RVC obtained in the study provide local sequence data for the establishment of RVC-specific surveillance primers and probe libraries in China, laying the foundation for establishing and improving non-Group A rotavirus surveillance systems, and providing core basis for improving China's full type rotavirus surveillance system.

This study has two limitations. Firstly, the samples were derived from an outbreak event in a single university in Jinan and did not cover RVC epidemic strains from other regions of China, making it difficult to fully reflect the distribution characteristics of RVC genotypes in China. The second issue is the lack of continuous tracking of patients' clinical progress, the inability to establish a direct correlation between virus sequences and patient clinical information, and the inability to explore the pathogenicity of reassortment strains in depth [28]. Based on these limitations, future research can expand sample

coverage and conduct surveys on RVC prevalence characteristics across multiple regions in China. At the same time, we will strengthen the correlation analysis between virus sequences and clinical information, integrate laboratory research with clinical data, and explore in depth the impact mechanism of VP4 gene reassortment on RVC replication efficiency and host adaptability [29]. In addition, the transmission chain of the outbreak in Jinan universities can be further traced to clarify the specific transmission routes of RVC in congregate settings, providing more accurate scientific basis for formulating targeted prevention and control measures [30].

In summary, this study has identified G4P [2] type RVC as an important pathogen causing clustered acute gastroenteritis in adult and school-age populations in China, and demonstrated that this strain exhibits characteristics of cross-regional transmission and gene reassortment. These findings provide direction for future prevention and control of RVC and related research and development work. At the prevention and control level, in addition to improving the surveillance systems, strengthening prevention and control in key settings, and port quarantine, it is also necessary to establish a national RVC strain database and gene database, conduct continuous molecular epidemiological surveillance, and track strain variation trends and transmission dynamics in a timely manner to provide scientific support for epidemic early warning and adjustment of prevention strategies. At the research and development level, this study found that the VP4 gene is the core gene segment involved in cross regional-reassortment of RVC, and as a spike protein of the virus, it is a key structural protein for virus adsorption to host cells. This discovery provides an important target reference for RVC vaccine development, laying a molecular foundation for the subsequent development of RVC vaccines against local epidemic strains in China, and also offering a new research direction for the development of RVC antiviral drugs.

5. Conclusion

This study identified the pathogen responsible for the acute gastroenteritis outbreak at a university in Jinan as a G4P[2] RVC clone strain. Whole genome analysis shows that the outbreak strain is a complex reassortant virus with a genetic background that combines global (homologous to the Italian strain) and regional (East Asian reassortment) characteristics. The VP4 gene is derived from a reassortment event of the Japanese and Korean epidemic strains, and is a key evolutionary link in the formation of this outbreak strain. The results of this study suggest that in port quarantine and domestic surveillance efforts, genomic monitoring of rotaviruses, especially non-Group A rotaviruses, should be strengthened. Continuous molecular epidemiological surveillance is of great significance for tracking virus mutations, clarifying transmission dynamics, and promoting subsequent vaccine development.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Jinan Center for Disease Control and Prevention (Ethical Approval Number: JNCDC-2025-020). All methods were performed in accordance with the

relevant guidelines and regulations of the Chinese Center for Disease Control and Prevention. The study was conducted in compliance with the Declaration of Helsinki. All experimental protocols were approved by the institutional committee. The ethics committee waived the requirement for informed consent because the study involved analysis of residual diagnostic specimens that were de-identified and posed no more than minimal risk to participants. All participants were university students aged 18 years or older (adults). No minors were included in this study. The samples used were collected as part of routine outbreak investigation procedures, and all personal identifying information was removed prior to analysis.

Consent for publication

The samples used in this study are routine hospital procedures. We do not use

Competing interests

The authors declare no competing interests.

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Author Contribution

B.Z. and X.G. conceived the study. C.G. and C.M. carried out all the experimental work, data analysis and manuscript preparation. H.L., L.Y., Q.W. and S.Y. assisted with the molecular studies.

Data Availability

The whole-genome sequences of the G4P[2] Group C Rotavirus strains reported in this study have been deposited in the GenBank database under accession numbers PZ518900–PZ519042. All other data generated or analysed during this study are included in this published article and its supplementary information files. Raw sequencing reads are available from the corresponding author upon reasonable request.

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Figures

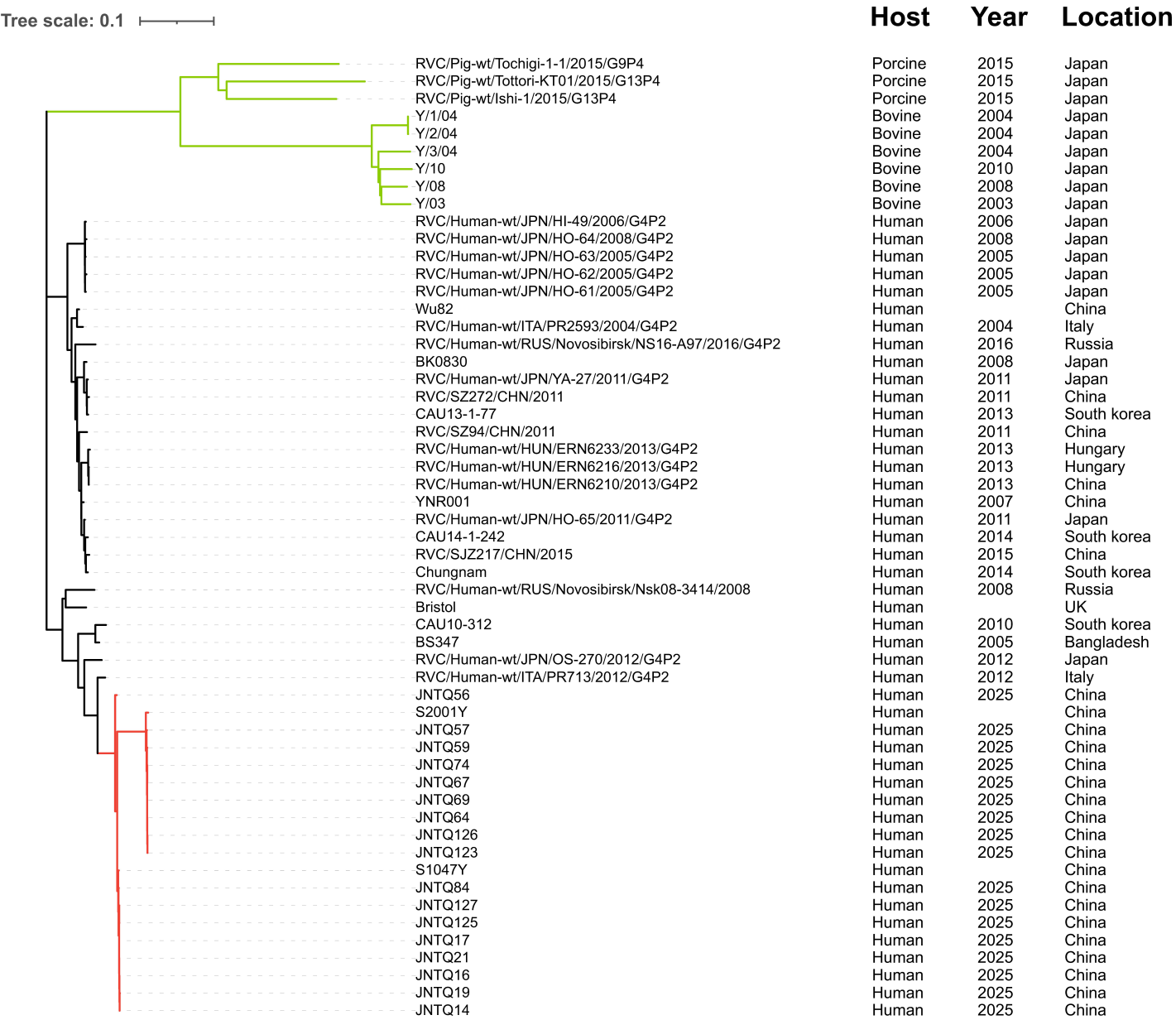


Figure 1

Phylogenetic tree of 13 G4P[2] RVC outbreak strains.

Legend (Figure 1): A neighbor-joining phylogenetic tree was constructed based on concatenated whole-genome sequences. The analysis included human-derived RVC sequences available in the NCBI database and nine non-human-derived RVC sequences. The tree was generated using MEGA-X with 1000 bootstrap replicates. The scale bar represents nucleotide substitutions per site. (Color coding for strain groups is embedded within the figure.)

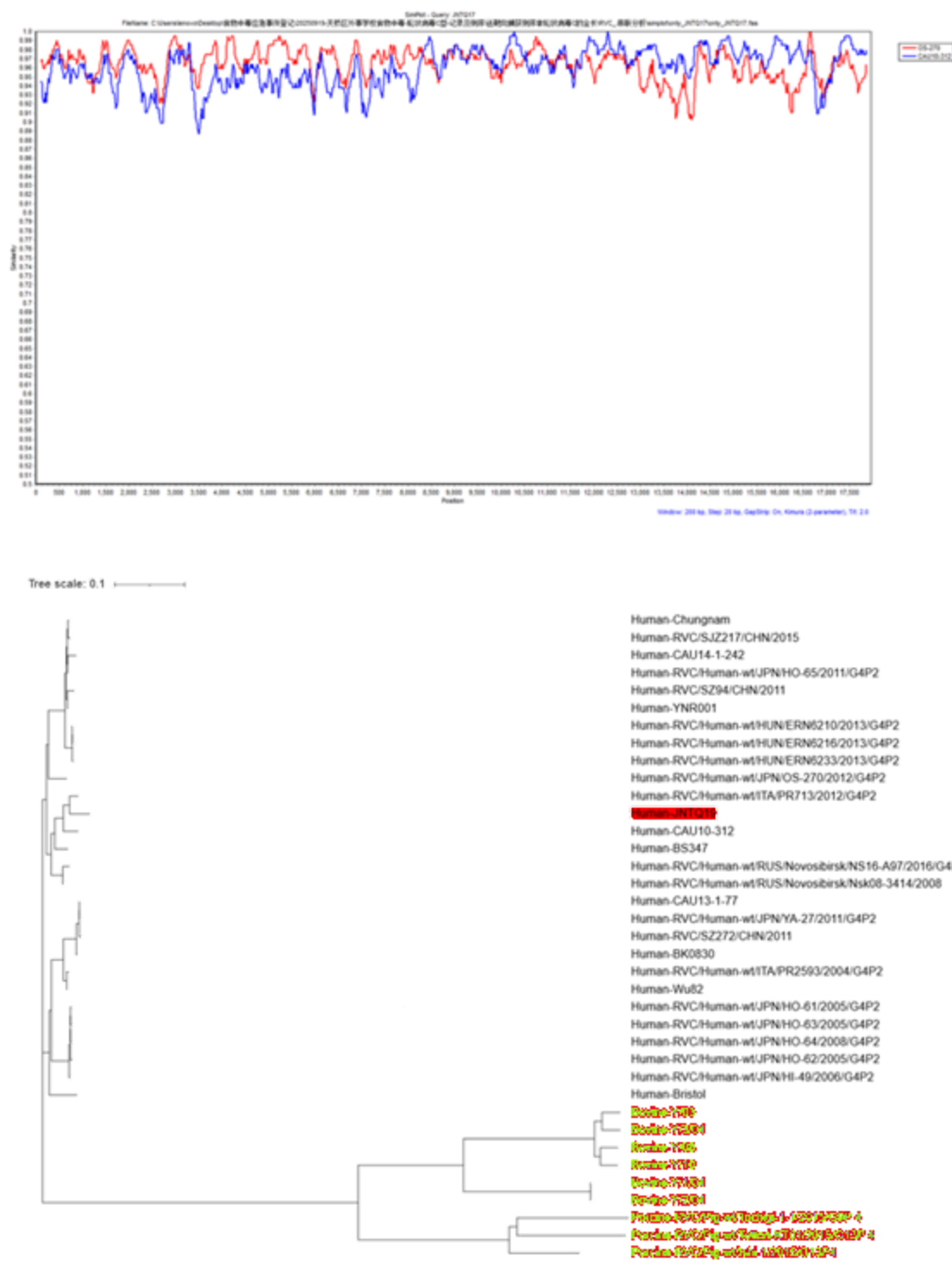


Figure 2

Recombination analysis of RVC VP4 gene.

Legend (Figure 2): (a) Recombination breakpoints were identified using SimPlot v3.5.1 and RDP v5.39. The y-axis shows percent identity between the recombinant and putative parental strains; the x-axis shows genomic position aligned with a 20-nt sliding window. Comparisons between the recombinant and the major parent, and between the recombinant and the minor parent, are shown. (b) Phylogenetic

analysis of the VP4 gene recombinant locus. The tree was constructed using the neighbor-joining method with 1000 bootstrap replicates. The scale bar represents nucleotide substitutions per site. (Color coding for strain groups is embedded within the figure.)

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