

Article

Porcine Rotavirus A G3P[6] with a Putative Novel G3-XII Lineage and P[6]-Ig Sublineage Associated with Neonatal Diarrhea in Southern Brazil

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Abstract

Neonatal diarrhea remains a significant threat to piglet health, resulting in substantial economic losses worldwide. Among the viral pathogens associated with this condition, rotavirus (RV) has been extensively reported in Brazil; however, lineage-level classification of circulating strains remains limited. This study aimed to characterize G and P genotypes of porcine RV field strains associated with diarrhea in piglets in Southern Brazil. A total of 10 fecal samples were collected by field veterinarian from diarrheic suckling piglets aged 1 to 14 days and analyzed by RT-PCR for the detection of RV species A, B, C, and H. RV species A (RVA) was detected in 90% (9/10) of the samples, while no other RV species were identified. Genotyping based on the VP7 and VP4 genes revealed a single G3P[6] genotype combination in all RVA-positive samples. Nucleotide (nt) and deduced amino acid (aa) sequence analysis revealed high genetic similarity among strains, with values of up to 99.3% for nt and 98.0% for aa of the VP7 gene and 100% for the VP4 gene (nt and aa). Phylogenetic analysis indicated that the VP7 sequences clustered with Brazilian G3 strains, forming a distinct group consistent with a novel lineage (putative G3-XII), whereas VP4 sequences supported a new sublineage (putative P[6]-Ig). These findings demonstrate low genetic variability of RVA field strains in this neonatal diarrhea outbreak, suggesting the circulation of a single viral population. They also emphasize the importance of continuous molecular surveillance to gain a deeper understanding of viral evolution and transmission dynamics in swine populations.



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1. Introduction

Neonatal diarrhea is recognized as a major cause of high morbidity and mortality in young animals, including piglets, resulting in significant economic losses for the pig industry [1]. This multifactorial and multietiological syndrome involves predisposing

individual, management, and environmental factors, as well as determining factors, such as viruses, bacteria, and protozoa [2].

Porcine rotavirus A (PoRVA) is one of the primary pathogens responsible for diarrhea in piglets during the first weeks of age [3]. Belonging to the *Sedoreoviridae* family and the *Rotavirus* genus, these non-enveloped viruses are approximately 80 nm in diameter. Their icosahedral capsid consists of three concentric protein layers (inner, intermediate, and outer). This structure provides high environmental stability and resistance to adverse conditions [4].

The rotavirus (RV) genome consists of 11 segments of double-stranded RNA, which encode 12 proteins: six structural (VP1–VP4, VP6, and VP7) and six non-structural (NSP1–NSP6) [4,5]. Based on the VP6 protein, RV is classified into 11 species [4]. The outer layer of the RV capsid consists of two structural proteins. The VP7 gene encodes a glycoprotein (G genotype), and the VP4 gene encodes a protease-sensitive protein (P genotype); these proteins neutralize antibodies and are used for the binary classification system to characterize RV strains [4].

Rotavirus alphagastroenteritidis (previously named RVA) is the most common RV species associated with neonatal diarrhea in piglets worldwide [6,7], followed by Rotavirus betagastroenteritidis (previously named RVB), Rotavirus tritogastroenteritidis (previously named RVC), and Rotavirus aspergastroenteritidis (previously named RVH) [4,8]. PoRVA infection is often associated with coinfections with different genotype (VP7 and VP4) combinations [3] or with other enteropathogens, including other RV species (RVB, RVC, and RVH) [1,8–13] as well as bacterial agents such as *Escherichia coli* and protozoa like *Cryptosporidium* spp., which can worsen the clinical outcome [14,15].

Based on the molecular characteristics of the VP7 and VP4 genes, RVA strains are classified into G and P genotypes; to date, 12 G genotypes (G1–G6, G8–G12, and G26) and 20 P genotypes (P[1]–P[11], P[13], P[19], P[22], P[23], P[26], P[27], P[32], P[34], and P[49]) have been reported in PoRVA strains [1,16,17].

In Brazil, several associations of G and P genotypes of PoRVA have been described in piglets with neonatal diarrhea, particularly those associated with the G3 genotype (such as G3P[6], G3P[7], G3P[13], G3P[22], and G3P[23]) and P[6] (such as G4P[6], G5P[6], G9P[6], and G11P[6]), evidencing a high genetic variability of circulating PoRVA strains [3,18–20].

The classification of strains into lineages and sublineages was determined according to nt sequence similarity and their clustering patterns in the phylogenetic tree. A novel lineage within the G genotype was defined using a nt similarity threshold of >90% [21]. Similarly, P[6] lineages and sublineages were assigned based on >90% nt sequence similarity, supported by phylogenetic topology and bootstrap values [18,22]. Currently, 11 lineages are described for the G3 genotype (I–XI), highlighting the wide genetic diversity of this genotype in different hosts and geographic regions [23]. Similarly, the P[6] genotype also exhibits high genetic variability, with five (I–V) lineages already described, in addition to sublineages (Ia–If), which were defined based on phylogenetic analyses of the VP4 gene [18]. In Brazil, descriptions of lineages and sublineages are limited, particularly for swine. There has been no research on the PoRVA G3 genotype, and for the P[6] genotype, only Lorenzetti et al. [18] reported molecular characterization, classifying the P[6] strains into sublineages Ie and If.

PoRVA infection in pigs causes not only piglet mortality but also growth retardation, increased treatment costs, and productivity losses. This study aimed to molecularly characterize the G and P genotypes of PoRVA field strains involved in an outbreak of diarrhea in piglets aged ≤ 14 days, and evaluate the genetic variation among strains using phylogenetic analyses.

2. Materials and Methods

2.1. Diarrheic Fecal Samples

Diarrheic fecal samples were collected from 10 piglets (six piglets aged 1 to 7 days and four piglets aged 8 to 14 days) from a PoRVA unvaccinated pig herd with 8000 sows located in Santa Catarina State, Southern Brazil, in September 2024. The fecal samples were sent by the veterinarian responsible for the herd's health program to the laboratory for diagnosis of RV infection during an outbreak of neonatal diarrhea.

2.2. Rotavirus Detection

The fecal samples were subjected to nucleic acid extraction using a combination of phenol/chloroform/isoamyl alcohol (25:24:1) and silica/guanidine isothiocyanate methods and were stored at -80°C until processing [24,25].

Reverse transcription-polymerase chain reaction (RT-PCR) was performed, targeting the amplification of 876 bp of the VP4 gene and 1062 bp of the VP7 gene of RVA [26,27], 434 bp of the NSP2 gene of RVB [28], 1043 bp of the VP7 gene of RVC [29], and 817 bp of the VP7 gene of RVH [30]. Sequenced samples of RVA, RVB, RVC, and RVH from previous studies were used as positive controls [3,8], and sterile ultrapure water aliquots were used as negative controls in all the RT-PCR reactions.

Other relevant enteric viruses, including Porcine Epidemic Diarrhea Virus (PEDV), Transmissible Gastroenteritis Virus (TGEV), and Porcine Deltacoronavirus (PDCoV), were not tested because Brazil is considered free of these pathogens. In addition, bacterial and protozoan enteric agents were not assessed in this study.

2.3. Sequencing and Phylogenetic Analysis

The amplicons of the VP7 and VP4 genes of RVA obtained by RT-PCR assays were purified using the Wizard[®] SV Gel and PCR Clean-up System kit (Promega[®], Madison, WI, USA). The PCR products were quantified with a Qubit[®] fluorometer using the Quant-iT dsDNA BR Assay Kit (Invitrogen[™], Eugene, OR, USA). Nucleotide (nt) sequencing was performed on the ABI 3500 Genetic Analyzer platform with the BigDye[®] Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems[®], Foster City, CA, USA) using the same primers as in the RT-PCR assays.

Nucleotide quality analysis and contig assembly of RVA sequences were performed using PHRED and CAP3 software, respectively (<http://lbi.cenargen.embrapa.br/phph/> accessed on 19 May 2026). The resulting sequences were then compared to those in the GenBank database using BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>, accessed on 19 May 2026). Additionally, the nt and deduced amino acid (aa) sequence identity matrix among sequences was constructed with BioEdit software, version 7.7.1 [31]. The phylogenetic trees were generated in MEGA 11 [32] using the neighbor-joining method with the Kimura 2-parameter model to construct trees based on nt sequences and the Poisson model to construct trees based on deduced aa sequences. Bootstrap probabilities were calculated using 1000 replicates.

3. Results

In nine (90%) of the 10 diarrheic fecal samples analyzed by RT-PCR, partial amplification of the RVA VP7 (G genotype) and VP4 (P genotype) genes was successfully achieved. All samples tested negative for RVB, RVC, and RVH. Based on nt and deduced aa sequence analyses of the G (VP7) and P (VP4) genotypes, all RVA field strains were classified as G3P[6] genotype combination.

The VP7 gene analysis revealed high sequence identity among the RVA field strains, with up to 99.3% nt and 98.0% deduced aa sequence identities. It was possible to classify

the G genotypes identified in the PoRVA field strains into four distinct nt sequence patterns. According to the nt sequence percentage, in each pattern, the strains share 100% nt sequence identity. Specifically, pattern 1 comprised four strains, #FS602 to #FS605, all isolated from piglets at 1 to 7 days of age. In contrast, pattern 2 included three strains; of these, #FS606 and #FS607 were obtained from 1- to 7-day-old piglets, whereas #FS608 was isolated from an 8- to 14-day-old piglet. Moving to pattern 3, this category consisted of a single strain, #FS609, derived from an 8- to 14-day-old piglet. Finally, pattern 4 likewise comprised a single strain, #FS610, also isolated from a 14-day-old piglet. One representative strain from each pattern was selected to use in the phylogenetic analysis.

The Brazilian PoRVA field strains ranged from 91.0% to 95.3% nt sequence identity and 94.7% to 97.3% deduced aa sequence identity among them, grouping them together in the same cluster and forming a new branch in G3 genotype, supporting a novel putative G3 lineage XII (Figures 1 and S1). Compared with other G3 lineages (I–XI), the PoRVA strains from this study exhibited 76.8% to 88.7% nt sequence identity and 89.4% to 96.0% deduced aa sequence identity (Table 1).

Table 1. Comparative matrix of nucleotide (nt) and deduced amino acid (aa) sequence identities of the four patterns of nt sequences of G3 genotype RVA from fecal samples collected from 1- to 14-day-old suckling piglets compared with representative strains from established G3 lineages available in GenBank.

G3 Lineage **	Nt Pattern 1 (#FS605)	Nt Pattern 2 (#FS606)	Nt Pattern 3 (#FS609)	Nt Pattern 4 (#FS610)
	% nt (% aa)	% nt (% aa)	% nt (% aa)	% nt (% aa)
G3-I	86.5–88.4 (90.7–92.0)	86.8–88.7 (91.6–92.9)	86.3–88.2 (90.7–92.0)	86.6–88.5 (91.6–92.9)
G3-II	79.1–79.7 (91.6–92.0)	79.4–80.0 (92.5–92.9)	79.0–79.6 (91.6–92.0)	79.3–79.9 (92.5–92.9)
G3-III	78.0–78.8 (90.3–92.0)	78.2–79.1 (91.1–92.9)	77.8–78.7 (90.3–92.0)	78.4–79.3 (91.1–92.9)
G3-IV	78.1–78.8 (89.4–90.3)	78.4–79.1 (90.3–91.1)	78.0–78.7 (89.4–90.3)	78.5–79.3 (90.3–91.1)
G3-V	86.0–87.0 (92.5)	87.0–87.3 (93.3)	86.6–86.9 (92.5)	86.9–87.2 (93.3)
G3-VI	78.2 (89.4–89.8)	78.5 (90.3–90.7)	78.1 (89.4–89.8)	78.7 (90.3–90.7)
G3-VII	78.2–78.7 (89.8–90.7)	78.5–79.0 (90.7–91.1)	78.1–78.5 (89.8–90.3)	78.4–78.8 (90.7–91.1)
G3-VIII	81.0–81.2 (92.5)	81.3–81.5 (93.3)	80.9–81.0 (92.0)	81.2–81.3 (93.3)
G3-IX	81.6–81.2 (90.7–91.1)	81.8–81.9 (91.1–91.6)	81.5–81.6 (90.7–91.1)	81.6–81.8 (91.1–91.6)
G3-X	87.9 (95.1)	88.2 (96.0)	87.8 (94.7)	88.4 (96.0)
G3-XI	76.9 (91.6)	77.2 (92.5)	76.8 (91.6)	77.4 (92.5)
G3-XII *	91.2–95.0 (95.1–96.4)	91.4–95.3 (96.0–97.3)	91.0–94.8 (94.7–96.0)	91.3–95.1 (96.0–97.3)

* Suggested new G3 lineage. ** These are the same strains from Figure 1, which shows their access numbers.

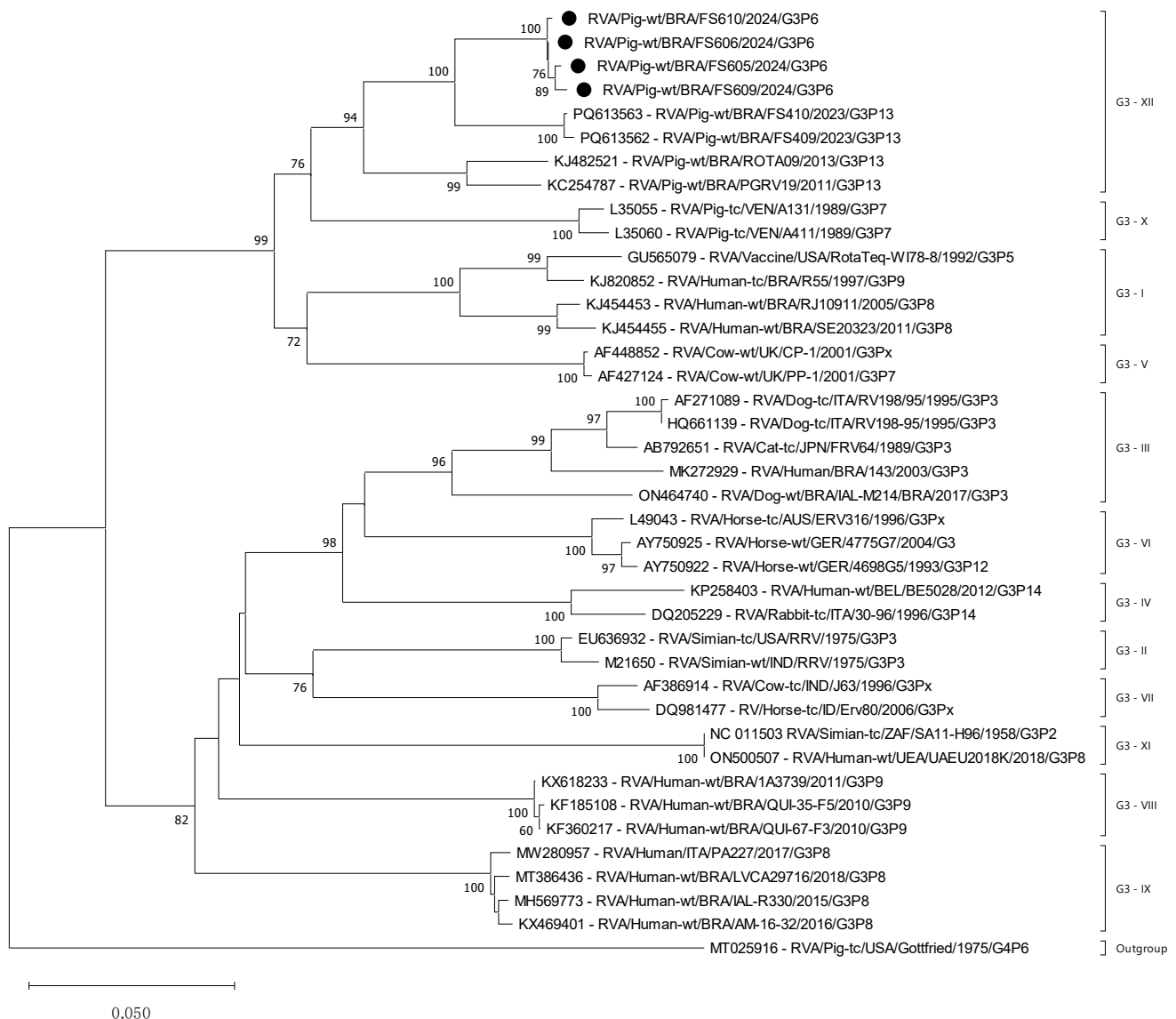


Figure 1. A phylogenetic tree based on nucleotide sequences was constructed using 682 bp of the VP7 gene of the rotavirus A (RVA) G3 genotype. Representative reference strains corresponding to all recognized G3 lineages were included for comparative analysis. The tree was constructed using the neighbor-joining method with the Kimura two-parameter model, and the numbers adjacent to the nodes represent the percentage of bootstrap support for 1000 replicates for the clusters. Bootstrap values less than 50% are not shown. The black circles indicate the PoRVA G3 strains from this study. The PoRVA prototype Gottfried (G4P[6]) vaccine strain was used as an outgroup.

The VP4 gene was completely conserved across all nine PoRVA field strains, exhibiting 100% of nt and deduced aa identities; consequently, only one strain was selected for use in the phylogenetic analysis. Comparing the VP4 gene with the P[6] lineage I sublineages (Ia–If) revealed 84.7% to 89.3% nt and 89.3% to 94.2% of deduced aa sequence identities. The PoRVA field strain genotype P[6] from this study exhibited 92.7% of nt sequence identity and 95.1% of deduced aa sequence identity with the PoRVA FS415 strain from Brazil that was previously described [3], forming the P[6]-Ig sublineage. In relation to P[6] lineages (II–V), it shares 81.2% to 84.7% nt sequence identity and 85.3 to 90.2% deduced aa sequence identity (Table 2).

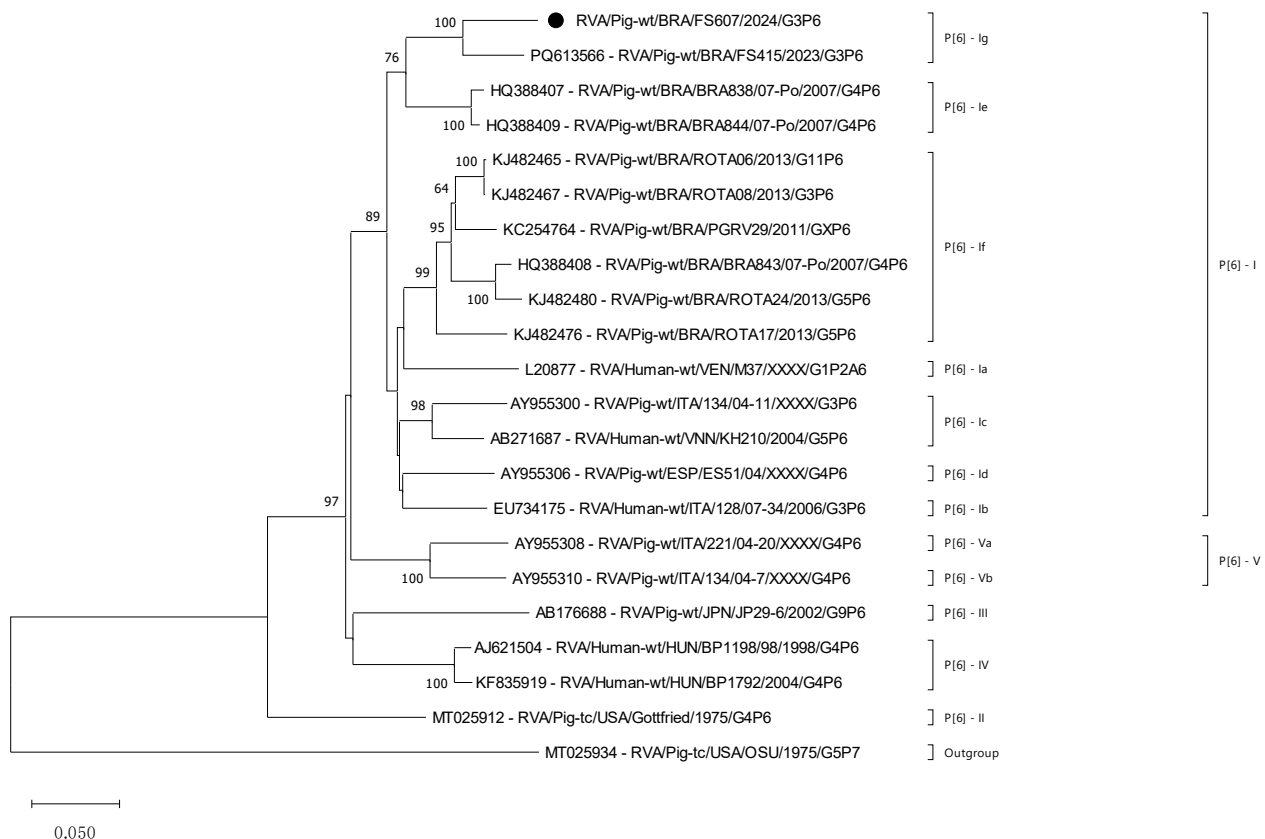


Figure 2. A phylogenetic tree based on nucleotide sequences was constructed using 681 bp of the VP4 gene of the rotavirus A (RVA) P[6] genotype. Representative reference strains corresponding to all recognized P[6] lineages and sublineages were included for comparative analysis. The tree was constructed using the neighbor-joining method with the Kimura two-parameter model, and the numbers adjacent to the nodes represent the percentage of bootstrap support for 1000 replicates for the clusters. The black circle indicates the PoRVA P[6] strain that represents all the positive strains from this study, which exhibited 100% nt identity (PZ381128). Bootstrap values less than 50% are not shown. The PoRVA prototype OSU (G5P[7]) strain was used as an outgroup.

Table 2. Comparative matrix of nucleotide (nt) and deduced amino acid (aa) sequence identities of the P[6] genotype RVA #FS607 strain from a fecal sample collected from a 7-day-old suckling piglet compared with representative strains from established P[6] lineages and sublineages available in GenBank.

P[6] Lineage (Sublineage) **	#FS607	
	Range	
	% nt	% aa
P[6]-I (a)	86.7	92.0
P[6]-I (b)	86.5	92.4
P[6]-I (c)	85.8–87.4	92.0–92.4
P[6]-I (d)	87.5	91.1
P[6]-I (e)	89.0–89.3	93.3–94.2
P[6]-I (f)	84.7–87.8	89.3–92.0
P[6]-I (g) *	92.7	95.1
P[6]-II	81.2	85.3
P[6]-III	82.5	88.8
P[6]-IV	84.4–84.7	90.2
P[6]-V (a)	82.8	85.3
P[6]-V (b)	82.5	87.1

* Suggested new P[6]-I sublineage. ** These are the same strains in Figure 2, which shows the access numbers.

The proposed new P[6]-I sublineage was also based on the phylogenetic analysis of the VP4 gene, which revealed that the #FS607 strain clustered within a distinct branch of P[6] lineage I, together with the PoRVA strain FS415 that was previously described [3], with consistent nt and aa sequence identity values (Table 2). The high bootstrap support in P6-Ig (100%) and in P[6]-I (89%) for the nt-based tree further reinforces the reliability of this phylogenetic placement (Figures 2 and S2).

The nt sequences for the FS602 to FS610 PoRVA strains obtained in this study were deposited in the GenBank database under accession numbers PZ381123 to PZ381131 (VP4) and PZ381132 to PZ381140 (VP7).

4. Discussion

Notably, this report describes a high incidence of diarrhea in suckling piglets (1–14 days old) caused by a single PoRVA G3P[6] genotype. The low diversity of G and P genotypes, and RVA being the only viral pathogen detected are uncommon in pig herds [33], although they are often observed in infections by bovine RVA in beef and dairy cattle herds in Brazil [34,35].

Analysis of the VP7 gene revealed a higher nt identity among the Brazilian PoRVA G3 strains detected in 2011 [19], 2013 [20], and 2023 [3], reaching up to 91.0%, although these studies did not perform lineage classification. The other RVA G3 strains, which were distributed in other lineages, presented nt identities ranging between 76.9% and 88.5% compared with the PoRVA G3 strains from this study. The nt identity percentages support the proposed new G3 lineage XII.

The PoRVA G3 sequences available in GenBank were distributed across three (V, IX, and X) lineages. The Thai CMP213 PoRVA strain (accession number: DQ786576) was assigned to lineage V, clustering with bovine RVA G3 strains from the United Kingdom (CP-1 and PP-1). The Brazilian BP64-clone-1 PoRVA strain (accession number: MN201922) grouped within lineage IX, alongside three Brazilian human RVA (HuRVA) G3 strains (LVCA29716, AL-3330, and AM-16-32) and an Italian HuRVA G3 strain (PA227). Two Venezuelan PoRVA strains (accession numbers: L35055 and L35060) formed lineage X. The Thai CMP213 and Brazilian BP64-clone-1 PoRVA strains were excluded from the phylogenetic tree due to their shorter nt sequences compared to those analyzed in this study.

The G3 genotype has a broad host range and is commonly found in species such as cats, dogs, monkeys, pigs, rabbits, horses, and humans [36,37]. Reassortment events and interspecies transmissions have been documented, including porcine–human RVA strains in circulation in Slovenia [38]. Furthermore, the G3 genotype of strain SO1199, which was detected in a pediatric case in Japan, exhibited the highest nt identity compared with PoRVA strains, providing evidence of zoonotic transmission [39]. In the present study, PoRVA G3 strains from Brazil shared up to 88.2% nt identity with the HuRVA strain RJ10911 from Brazil, which were classified within G3 lineage I, representing the highest similarity observed outside the newly proposed G3 lineage XII. The RVA G3 genotype strains available in the database can be classified into eleven (I–XI) lineages using 90% nt sequence identity as the demarcation criterion [21,40].

Phylogenetic analysis of the VP4 gene of the P[6] strains indicates the stability of this genotype within the studied pig herd, as well as high nt and deduced aa sequence identities compared to the RVA/Pig-wt/BRA/FS415/2023/G3P[6] strain [3], which was identified during a neonatal diarrhea outbreak in 2023 on an unvaccinated pig farm also located in the state of Santa Catarina.

In comparison with the PoRVA vaccine strain Gottfried (G4P[6]-II, accession number: MT025912), the P[6] strains identified in this study exhibited the lowest nt sequence identity (81.4%) among all P[6] lineages. A similar pattern was observed with circulating P[6] strains,

which are more closely related to human P[6] strains than to the prototype PoRVA P[6] strain Gottfried [22].

The Brazilian PoRVA P[6] #FS607 field strain exhibited nt identities ranging from 84.7% to 89.3% compared with other strains within the same lineage I. Eight of 10 PoRVA strains belonging to P[6] lineage I are Brazilian strains (BRA838/07-Po, BRA844/07-Po, BRA843/07-Po, ROTA06, ROTA08, ROTA17, ROTA24, and PGRV29) [18–20], one is a Spanish strain (ES51/04) [22], and one is an Italian strain (134/04-11) [22]. Other PoRVA strains are distributed among three P[6] lineages: the PoRVA Gottfried strain (vaccine) in lineage II, the Japan JP29-6 strain [41] in lineage III, and two Italian strains [22] in lineage V.

The nine samples analyzed present four patterns of the G3 genotype (91.0% to 95.3% nt identity) and were identical in the P[6] genotype (100% nt identity), with the VP7 gene less conserved than the VP4 gene. The variability in the genotypes described in RCWG [42] determines more P genotypes ($n = 58$) than G genotypes ($n = 42$). Although, at the lineage level, the variability of G genotype lineages is higher (i.e., G3 lineages I–XI) than in P genotype lineages (i.e., P[6] lineages I–V).

Previous reports from Brazil reported that the G3P[6] genotype is often detected in co-infections with other RV species and/or with different genotype combinations of PoRVA, which are typically associated with diarrhea in piglets [3,20].

Silva et al. [20] identified the G3P[6] genotype over two years through the analysis of 126 fecal samples collected in the states of Mato Grosso do Sul and São Paulo, although the samples were not tested for other RV species. G3P[6] was detected in association with other PoRVA genotypes (G3P[13], G5P[6], G5P[13], G9P[23], and G11P[6]) in suckling and nursery piglets with diarrhea across four distinct herds.

Similarly, Yoshitani et al. [3], in a study also carried out in Santa Catarina, reported the occurrence of G3P[6] in 2- and 3-week-old piglets during a neonatal diarrhea outbreak in a single unvaccinated pig herd. The similarity of the G3P[6] samples in terms of VP4 and VP7 genes could be explained by the persistence of the strain in the nearby pig herds with herd-to-herd transmission. However, the genotype was detected with the other three genotype combinations (G3P[13], G4P[6], and G5P[23]) in the same age interval and was associated with co-infection with RVC in 2-week-old piglets. Considering the previous studies that described the PoRVA G3P[6] genotype, the present study presents low genetic variability.

Differences in production systems and pig herd characteristics may contribute to these constraints. The pig herd analyzed by Yoshitani et al. [3] was composed of 3400 sows, which were not vaccinated and were raised in a complete-cycle system. In the present study, the pig herd comprised 8000 non-vaccinated sows. This pattern differs from the epidemiological scenario commonly described in swine and suggests the circulation of a more homogeneous viral population within the herd.

The G3P[6] genotype combination has also been reported by Martella et al. [22], who identified PoRVA P[6] strains harboring human-like VP4 P[6] gene alleles. The authors analyzed the genetic relationships between porcine and human P[6] rotaviruses by examining 12 PoRVA strains from nursing and weaning piglets in outbreaks of diarrhea, including three from Spain and nine from Italy. These findings highlight the considerable genetic diversity of RVA and underscore the potential for interspecies transmission between porcine and human hosts.

PoRVA represents an important genetic reservoir for the emergence of novel HuRVA strains owing to its extensive genetic diversity and capacity for interspecies transmission [17,37]. The G3P[6] genotype combination appears to be associated with human-to-porcine transmission events, as suggested by the close evolutionary relationship between the VP7 and VP4 genes of HuRVA and PoRVA G3P[6] strains [39]. In this study,

PoRVA G3P[6] strains showed high nt identity compared to other PoRVA strains from Brazil and clustered into a distinct VP7 lineage (G3-XII) and VP4 sublineage (P[6]-Ig).

5. Conclusions

This study reports the detection of a unique PoRVA G3P[6] genotype combination in diarrheic fecal samples from piglets aged 1–14 days from an unvaccinated pig herd located in Southern Brazil, in the absence of co-infection with other RVA genotype combinations (G and P) or other RV species (RVB, RVC, or RVH). The high nt identity among strains indicates low genetic variability. Phylogenetic analysis revealed distinct clustering of VP7 and VP4 sequences, consistent with a suggested novel lineage (G3-XII) and a potential new sublineage (P[6]-Ig), respectively. These findings highlight the importance of continuous epidemiological and molecular surveillance to characterize RV genetic diversity, identify emerging or reemerging genotypes, and monitor changes in circulation patterns, thereby supporting the development of more effective control strategies.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/microbiolres17070122/s1>, Figure S1: A phylogenetic tree based on amino acid (aa) sequences was constructed using 227 deduced sequences of amino acids of the VP7 gene of the RVA G3 genotype. The tree was constructed using the neighbor-joining method with the Poisson model. The black circles indicate the PoRVA G3 strains from this study. Representative reference strains corresponding to all recognized G3 lineages were included for comparative analysis. The numbers adjacent to the nodes represent the percentage of bootstrap support for 1000 replicates for the clusters. Bootstrap values less than 50% are not shown. The PoRVA prototype Gottfried (G4P[6]) strain was used as an outgroup; Figure S2: A phylogenetic tree based on amino acid (aa) sequences was constructed using 226 deduced sequences of amino acids of the VP4 gene of the RVA P[6] genotype. The tree was constructed using the neighbor-joining method with the Poisson model. The black circle indicates the PoRVA P[6] strain from this study. Representative reference strains corresponding to all recognized P[6] lineages were included for comparative analysis. The numbers adjacent to the nodes represent the percentage of bootstrap support for 1000 replicates for the clusters. Bootstrap values less than 50% are not shown. The PoRVA prototype OSU (G5P[7]) strain was used as an outgroup.

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Informed Consent Statement: Not applicable.

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