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Rotavirus Vaccine Breakthrough With Vaccine-Modified Disease in a Sibling Cluster of Equine-Like G3P[8] Infection

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ABSTRACT

Rotavirus remains one of the major causes of childhood gastroenteritis despite the widespread introduction of rotavirus vaccines. Here, we report a sibling cluster of three children hospitalized with fever, vomiting, and diarrhea. Two unvaccinated siblings (11 years 9 months and 10 years 1 month) developed severe gastroenteritis with profuse watery diarrhea and prolonged hospitalization, while the youngest sibling (3 years 4 months), fully vaccinated with the RotaTeq vaccine, developed milder symptoms and had a shorter duration of hospitalization. Disease severity assessed using the modified Vesikari score indicated severe disease in the unvaccinated siblings (scores 16 and 11) and moderate disease in the vaccinated child (score 10). Rapid antigen testing followed by molecular analysis confirmed equine-like G3P[8] rotavirus infections in all three patients. Epitope analysis of the VP7 protein identified four nonsynonymous substitutions (T87S, N213T, K238D, and D242A) within antigenic regions compared with the RotaTeq G3 vaccine strain. The reduced disease severity in the vaccinated sibling suggests partial protection conferred by the RotaTeq vaccine despite breakthrough infection and highlights the importance of continued molecular surveillance of circulating rotavirus strains.

1 | Introduction

Rotavirus remains one of the leading causes of morbidity and mortality in children under 5 years of age, especially in low- and middle-income countries (LMICs), despite the wide coverage of rotavirus vaccines [1]. Rotavirus vaccination has reduced severe disease and hospitalizations [2]; however, breakthrough infections are increasingly reported [3]. Equine-like G3P[8], which likely originated from reassortment between human and horse rotaviruses, carries a distinct VP7 (outer capsid glycoprotein) from contemporary human G3 strains and may compromise existing protection conferred by the vaccines [4]. Since its emergence in 2013, equine-like G3P[8] has progressively replaced previously dominant genotypes [5–7], with breakthrough infections reported even in countries with wide vaccination coverage [3, 4]. Such reporting is lacking in Malaysia as rotavirus vaccine has not been incorporated into the national immunization programs, and the uptake rates remain uncertain despite the vaccines being available in private hospitals [8, 9]. Case-by-case reporting is crucial for evaluating vaccine performance in real-world settings. This report describes a breakthrough rotavirus infection in a fully vaccinated child and compares the clinical severity with that of unvaccinated siblings infected by equine-like G3P[8] rotavirus in a sibling cluster.

2 | Materials and Methods

2.1 | Study Setting and Patients

Three siblings from the same household (Patients A, B, and C) were admitted to the pediatric ward at the Borneo Medical Centre, Kuching, Sarawak, Malaysia, in February 2025 with fever, vomiting, and diarrhea. All were Malaysian nationals of Chinese ethnicity (Table 1).

2.2 | Sample Collection and Diagnostic Testing

Fresh diarrheal stool samples were collected and tested for rotavirus and adenovirus using Bioline Rota/Adeno Rapid Test (Abbott, USA). Rotavirus vaccination history of the patients was recorded.

2.3 | Molecular Genotyping and Sanger Sequencing

Molecular genotyping of the rotavirus VP7 (outer capsid glycoprotein) and VP4 (spike protein, also known as the protease-sensitive protein) genes was performed using reverse transcription–polymerase chain reaction (RT-PCR) followed by Sanger sequencing using the ABI BigDye Terminator Cycle Sequencing Kit v3.1 on an ABI PRISM 3730xl Genetic Analyzer (Applied Biosystems). The nucleotide sequences generated were analyzed using MEGA X software and deposited in GenBank under the accession numbers PV553303-PV553305, PV795953-PV795955.

2.4 | Epitope Analysis

The VP7 nucleotide sequences were translated into amino acid sequences using MEGA X software. The epitope analysis was performed using UCSF ChimeraX software (PDB IDs: 4V7Q and 3FMG).

2.5 | Transmission Electron Microscopy

Rotavirus particles were adsorbed onto a copper grid (Spec: FF300-Cu; Brand: EMS), negatively stained using uranyl acetate replacement stain (Brand: EMS), and visualized using TEM (Model: JEOL JEM 1230) at (A) 10,000× and (B) 30,000× magnifications. The morphology of rotavirus was identified as wheel-like appearance and a diameter of approximately 70 nm.

2.6 | Respiratory Pathogen Testing

Since Patient C exhibited more prominent respiratory symptoms, further testing of Patient C was performed using QIAstat-Dx Respiratory Panel (Qiagen, Germany) on combined throat and nasal swabs.

2.7 | Disease Severity Assessment

The severity of gastroenteritis was evaluated using the modified Vesikari score [10]. Scores were calculated based on the available clinical parameters, including the duration and frequency of diarrhea and vomiting, maximum body temperature, and hospitalization status. Data on dehydration status were unavailable and were not included in this scoring. Disease severity was classified as mild (< 7), moderate (7–10), or severe (≥ 11) based on the total score.

3 | Results and Discussion

3.1 | Clinical Presentation

Patient A (11 years 9 months, male) was the first to develop symptoms and had the most severe illness. He had more than ten episodes of vomiting per day for 2 days, more than 10 episodes of diarrhea (profuse watery) per day for more than 10 days, and a peak temperature of 39.2°C. He was hospitalized for 5 days.

Patient B (10 years 1 month, male) developed illness 3 days after Patient A. Patient B had two episodes of vomiting per day for 1 day, one episode of diarrhea (profuse watery) per day for more than 4 days, and a peak temperature of 38.4°C. He was hospitalized for 4 days.

Patient C (3 years 4 months, male) developed illness 1 day after Patient B. Patient C had the mildest symptoms, with only one episode of vomiting per day for 1 day, two episodes of diarrhea (loose stool) per day for 2 days, and a peak temperature of 39.1°C. He was hospitalized for 3 days.

Patients A and B had not received rotavirus vaccination, whereas Patient C had completed the three-dose series of RotaTeq vaccine (Table 1). Disease severity was further evaluated using the modified Vesikari score. The calculated scores were 16 for Patient A, 11 for Patient B, and 10 for Patient C, corresponding to severe disease in Patients A and B and moderate disease in Patient C.

All three patients tested positive for rotavirus and negative for adenovirus using the rapid antigen test. Molecular genotyping identified the strain as equine-like G3P[8] rotavirus. Notably, all three siblings also presented with upper respiratory tract symptoms, including cough and sore throat. Testing of Patient C detected *Chlamydophila pneumoniae*. All patients were empirically treated with oral clarithromycin (Klacid, Abbott, USA) along with fluid replacement and demonstrated clinical improvement.

Rotavirus is one of the leading causes of death in children under 5 years of age worldwide. Infections typically cause severe rotavirus gastroenteritis (RVGE) in this age group [11], while the likelihood of acquiring RVGE decreases with increasing age, likely due to previous exposure or vaccination [12]. However, our finding contradicts this widely reported pattern. In our cohort, the two oldest patients (Patients A and B, aged ≥ 5 years) experienced severe RVGE and prolonged hospitalization. These observations suggest the current definition of high-risk age groups may need to be reconsidered.

Currently, four live oral rotavirus vaccines are World Health Organization (WHO)-prequalified for global use (RotaTeq [Merck, USA], Rotarix [GSK, USA], ROTAVAC [Bharat Biotech, India] and ROTASIIL [Serum Institute of India, India]). The WHO currently recommends the inclusion of rotavirus vaccines in the national immunization programs of all countries [2].

TABLE 1 | Demographic and clinical characteristics of the patients of the sibling cluster hospitalized for rotavirus acute gastroenteritis.

	Patient A	Patient B	Patient C
Demographic			
Gender	Male	Male	Male
Age	11 years 9 months	10 years 1 month	3 years 4 months
Ethnicity	Chinese	Chinese	Chinese
Clinical presentations			
Symptom onset	2025-02-04	2025-02-07	2025-02-08
Fever	39.2°C	38.4°C	39.1°C
Vomiting			
Episodes (per 24 h)	> 10	2	1
Duration (day)	2	1	1
Diarrhea			
Episodes (per 24 h)	> 10	1	2
Duration (day)	> 6	> 4	2
Texture	Profuse, watery diarrhea	Profuse, watery diarrhea	Loose stool
Hospitalization			
Admission	2025-02-05	2025-02-07	2025-02-08
Discharge	2025-02-09	2025-02-10	2025-02-10
Length of stay (day)	5	4	3
Disease severity assessment			
Modified Vesikari score	16 (severe)	11 (severe)	10 (moderate)
Rotavirus vaccination	Unvaccinated	Unvaccinated	Vaccinated with three doses of RotaTeq (2, 3, and 4 months of age)
Rotavirus detection			
Rapid antigen test	Positive rotavirus A	Positive rotavirus A	Positive rotavirus A
Molecular genotyping and Sanger sequencing	Equine-like G3P[8]	Equine-like G3P[8]	Equine-like G3P[8]

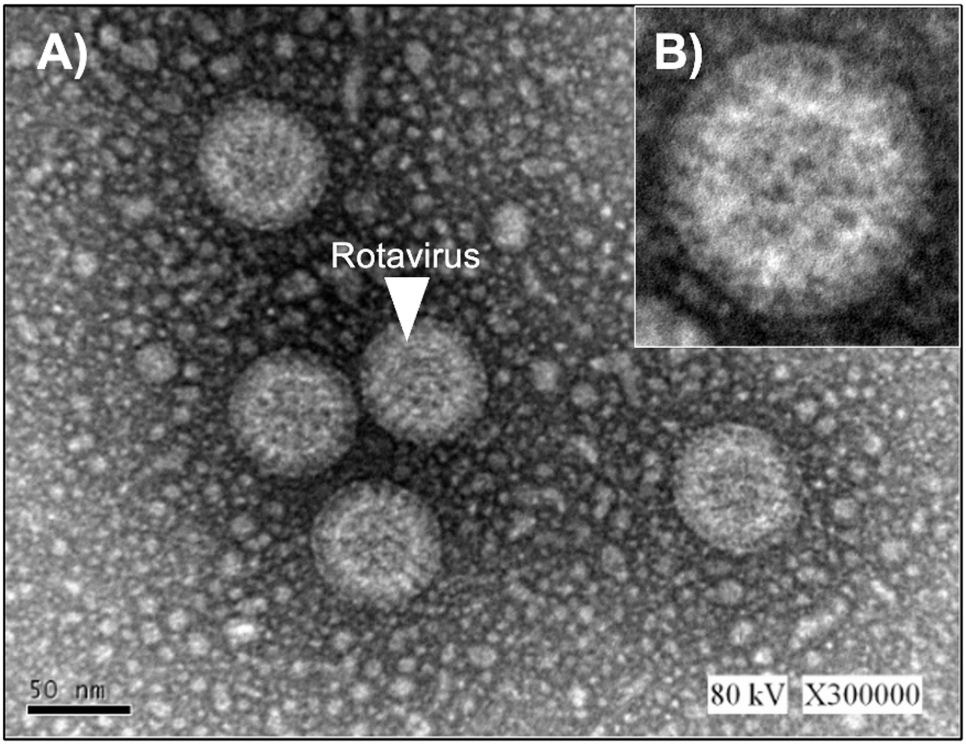

FIGURE 1 | (A) Transmission electron micrograph of rotavirus particles (measuring around 70 nm) at ×30,000 magnification, showing the characteristic wheel-like morphology. (B) VP7 (G protein) represents the outermost structural layer of rotavirus, which serves as a target for neutralizing antibody binding. The bar represents 50 nm.

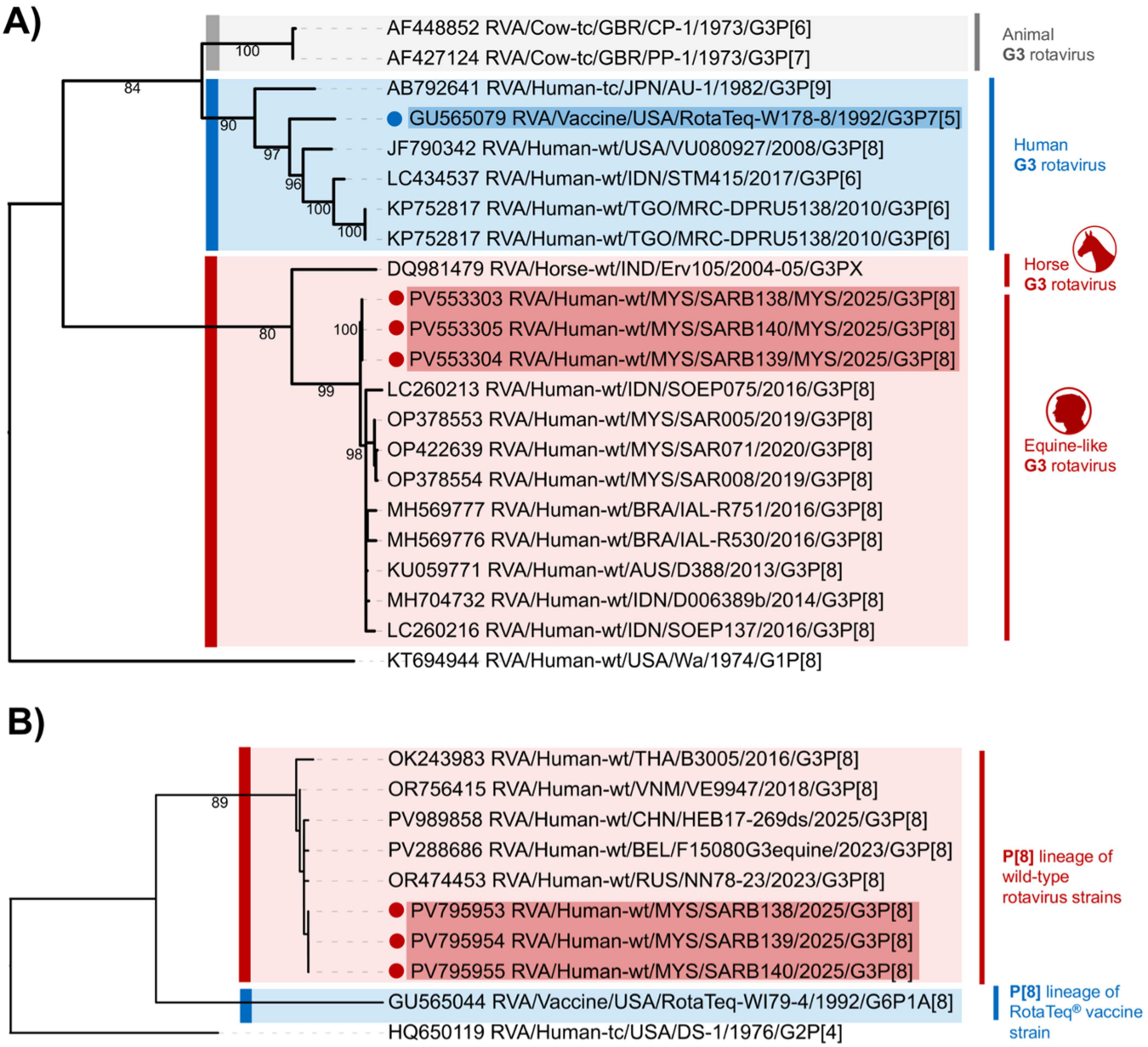

FIGURE 2 | (A) Maximum likelihood phylogenetic tree of G3 rotaviruses, based on the VP7 gene, showing three distinct clades: equine-like G3 (red clade), contemporary human G3 (blue clade), and animal G3 rotavirus (gray clade). Equine-like G3 rotavirus strains isolated in this study (red dots) clustered separately from RotaTeq G3 vaccine strain (blue dot), which clustered together with other human G3 strains. (B) Maximum likelihood phylogenetic tree of P[8] rotaviruses, based on the VP4 gene, showing two distinct clades: P[8] strain of this study (red dots in red clade) clustered separately from RotaTeq P[8] vaccine strain (blue clade).

RotaTeq is a live oral vaccine that protects against gastroenteritis caused by rotavirus genotypes G1, G2, G3, G4, G9, and P[8] [3]. Rotavirus vaccines are nonsterilizing but can reduce disease severity through cross-protection, including against heterotypic strains [13].

Patient C developed a breakthrough rotavirus infection despite completing the full three-dose RotaTeq vaccine series. However, the illness was markedly milder with shorter duration of symptoms and hospitalization, consistent with vaccine-modified disease. Protection in this case may have been partly mediated by shared VP7 epitopes between the human G3 strain in the RotaTeq vaccine and the equine-like G3 detected. VP7 gene encodes a structural protein located on the outermost layer of the rotavirus (Figure 1A,B). Due to its surface exposure, VP7 is a major target for antibody recognition and binding. Changes in its amino acid sequence can change the protein's conformation, potentially reducing the effectiveness of antibody-mediated neutralization [14]. Comparative analysis revealed that the G3 strain in RotaTeq differed genetically from the equine-like G3 of this sibling cluster (SARB138–SARB140) (Figure 2A), with four nonsynonymous mutations detected (T87S, N213T, K238D, and D242A) within the antigenic epitope. Despite equine-like G3P[8] possessing P[8] VP4 gene of human rotavirus, the P[8] in this study clustered separately from that of RotaTeq (Figure 2B), indicating genetic distinctness. Structural mapping of the mutations onto the VP7 antigenic epitopes showed that T87S, N213T, and K238D may influence neutralization escape (Figure 3A,B). However, VP4 is

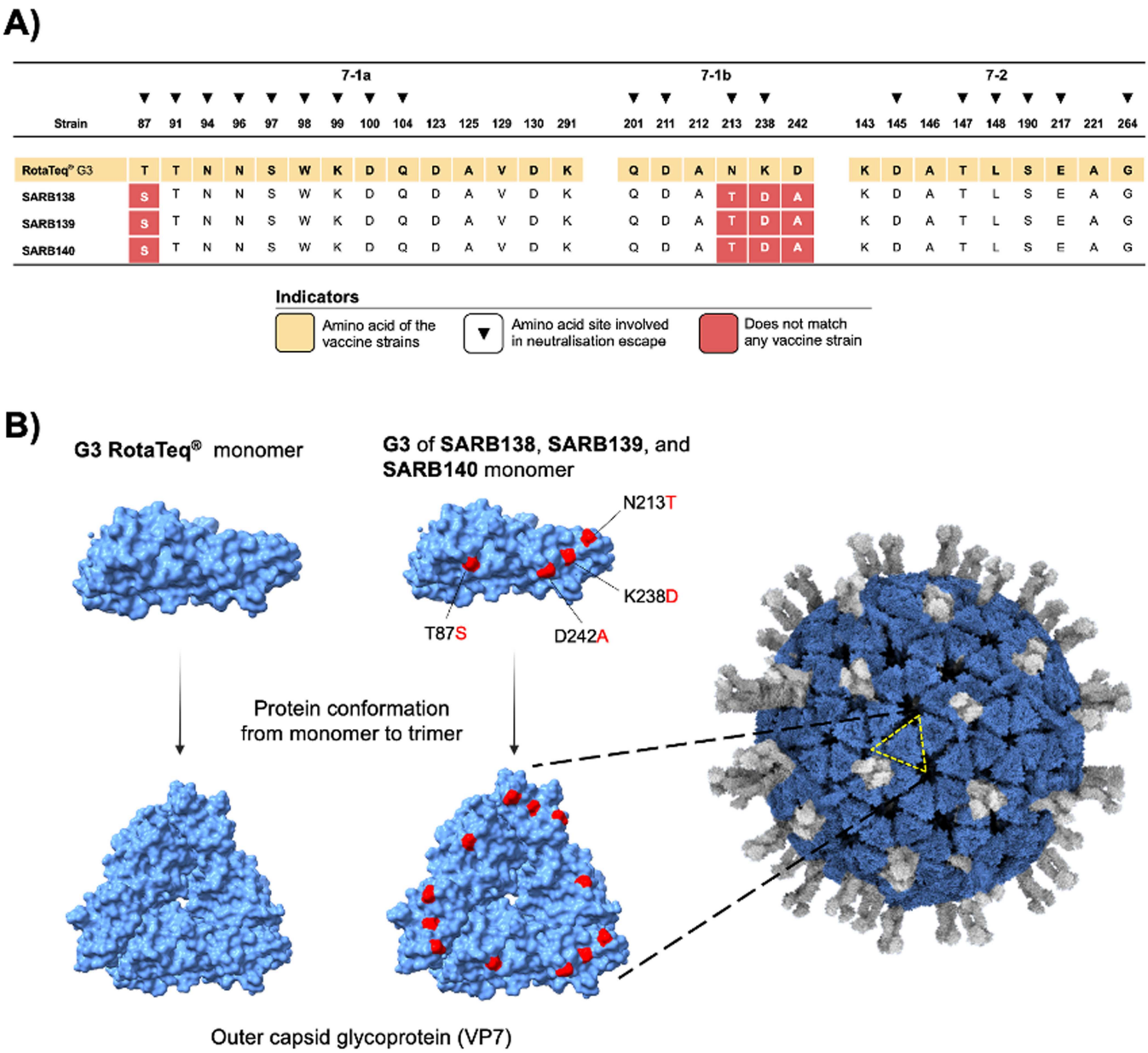

FIGURE 3 | (A) Linear epitope analysis of the equine-like G3 strain of this study versus RotaTeq G3 vaccine strain. VP7 epitope can be divided into three important regions (7-1a, 7-1b, and 7-2), while the equine-like G3 in this study exhibited four mutated amino acids (T87S, N213T, K238D, and D242A) across these regions. Of these, three important positions linked to antibody-neutralization escape were detected (T87S, N213T, and K238D). (B) Conformational epitope analysis showed that the mutations are surface-exposed.

known to be more conserved than VP7 due to its important roles in viral attachment and cell entry, where mutations can reduce infectivity and lead to negative selection [8]. All three patients shared similar genetic background yet exhibited different clinical outcomes, suggesting the role of vaccination in reducing RVGE severity.

Profuse, watery diarrhea accompanied by fever and vomiting is a typical presentation of RVGE and is mainly caused by enterocyte destruction leading to intestinal malabsorption [15, 16]. In contrast, *C. pneumoniae* is primarily a respiratory pathogen, gastrointestinal symptoms associated with this bacteria are usually mild and nonspecific [17]. In this sibling cluster, the frequent watery diarrhea observed in Patients A and B strongly supports rotavirus as the primary cause of gastroenteritis, consistent with the positive rapid antigen and molecular test results. Although *C. pneumoniae* may have contributed to the respiratory symptoms, its clinical profile is unlikely to explain the severity of the diarrhea in this case. While clarithromycin may cause diarrhea [18], the onset of symptoms before treatment and improvement with fluid replacement are more consistent with rotavirus infection. In contrast, Patient C only passed loose stools, consistent with vaccine-modified disease.

Equine-like G3 strain was hypothesized to have arisen from a zoonotic spillover event, resulting from reassortment of the VP7 gene from horse rotavirus (strain Erv105 in India) into human rotavirus. This event incorporated a horse rotavirus-derived VP7 gene into a human rotavirus intergenogroup backbone [4, 8]. Comparative genetic analysis between the equine-like G3

strain from this sibling cluster and strain Erv105, the proposed ancestral G3 horse rotavirus strain, showed 91.13% genetic similarity, supporting the hypothesis of an equine origin. Vaccine breakthrough of equine-like G3 may be underestimated due to limited genotyping capabilities in the LMICs, despite the high detection rates of RVGE among vaccinated children in these settings [19, 20]. Most cases of acute gastroenteritis are managed clinically without etiologic confirmation, meaning breakthrough infections and emerging or antigenically drifted strains remain largely undocumented. In institutional outbreaks, this underdiagnosis can lead healthcare providers to prematurely rule out rotavirus as a cause in children with documented vaccination, overlooking that vaccinated children may still develop disease and transmit the virus to unvaccinated contacts.

4 | Conclusion

This study represents the first reported case in Malaysia of RotaTeq vaccine breakthrough associated with equine-like G3P[8] rotavirus. This sibling cluster highlights infection with equine-like G3P[8] rotavirus and a breakthrough case in a fully vaccinated child with vaccine-modified disease. While the two unvaccinated siblings experienced severe disease based on the modified Vesikari score (scores 16 and 11), characterized by severe symptoms and prolonged hospitalization, the vaccinated child had only moderate disease (score 10) and a shorter duration of hospitalization. These findings support the protective role of vaccination in reducing disease severity despite breakthrough infection. Given the small sample size and the possibility that *C. pneumoniae* infection may have influenced the clinical presentation, particularly fever, these findings should be interpreted with caution. Continued molecular surveillance and recognition of vaccine breakthrough are therefore important, and these findings further support the incorporation of rotavirus vaccines into national immunization programs in Malaysia and other LMICs.

Author Contributions

Ahmad Syatir Tahar: molecular analysis, interpretation, manuscript drafting. **Eng Joe Ong:** conceptualization, patient care, clinical data collection, manuscript review. **Dewi Mamora:** sample curation, laboratory testing, manuscript review. **Cheng Siang Tan:** conceptualization, data analysis, manuscript drafting, supervision, final manuscript approval.

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The authors have nothing to report.

Ethics Statement

The study protocol was approved by the UNIMAS Medical Ethics Committee (UNIMAS/NC-21.02/03-02(01)).

Consent

Written informed consent for publication of the clinical details was obtained from the patients' parent.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The nucleotide sequences were deposited in GenBank (Accession Numbers PV553303–PV553305, PV795953–PV795955).

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