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Short Communication

Identification of a novel intra-genotype reassortant G1P[8] rotavirus in Italy, 2021

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ABSTRACT

Objectives: Rotaviruses G1P[8] are epidemiologically relevant and are targeted by vaccines. The introduction of vaccines has altered rotavirus epidemiology. Hospital-based surveillance conducted in Sicily, Italy, showed a progressive decline in rotavirus prevalence since 2014, along with an increasing vaccine coverage (63.8% in 2020), and a marked decrease in circulation of G1P[8] strains. Surprisingly in 2021, G1P[8] viruses accounted for 90.5% (19/21) of rotavirus infections. This study aimed to understand if the increased activity of G1P[8]'s was related to virus-related peculiarities.

Design: In 2021, 266 patients <15 years of age were hospitalized with acute gastroenteritis (AGE) and included in rotavirus surveillance. Viral proteins (VP7 and VP4) genotyping and sequence data were generated from all rotavirus-positive samples. The genetic makeup of G1P[8] rotaviruses was investigated by full-genome sequencing.

Results: Peculiar G1P[8] rotaviruses, with VP7 and VP4 belonging to novel sub-lineages, circulated in 2021, accounting for 76.2% (16/21) of all rotavirus infections. On full-genome analysis, the novel G1P[8] variant displayed an intra-genotype (Wa-like) reassortant constellation, involving G12 and G1 strains, into a unique arrangement never observed before. The novel G1P[8] variant showed peculiar amino acid substitutions in 8-1 and 8-3 epitopes of the VP4 with respect to the Rotarix strain.

Conclusions: Prompt identification of virus variants circulating in the human population is pivotal to understanding epidemiological trends and assessing vaccine efficacy.

Introduction

Rotavirus group A (RVA) is a major etiological agent of acute gastroenteritis (AGE) in children and animals worldwide [1]. RVA is classified in G-types and P-types based on the two outer capsid proteins, viral protein (VP)7 and VP4, both eliciting neutralizing antibodies and, currently, at least 42 G-types and 58 P-types have been identified (https://rega.kuleuven.be/cev/viralmetagenomics/virus-classification/rcwg). Historically, G1P[8] represents the prevalent genotype, targeted by vaccines (Rotarix and RotaTeq). RVAs are characterized by a high genetic variability and full-genome analyses allow the investigation of their genetic constellations [2].

Vaccines, available since 2006, significantly altered RVA epidemiology. Hospital-based surveillance conducted in Sicily, Italy, showed that G1P[8] RVAs of different variants/sub-variants predominated for almost 30 years [3,4] but, after Rotarix's introduction in June 2012, they steadily declined as vaccine coverage increased and reached 63.8% in 2020 and 62.8% in 2021 [5]. Surprisingly in 2021, this epidemiological trend was reversed as G1P[8] accounted for 90.5% of RVA infections in children. This study aimed to understand if the increased activity of G1P[8] in 2021 was related to virus-related peculiarities.

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Methods

Samples collection

From 1 January to 31 December 2021, a total of 266 stool samples of patients <15 years of age hospitalized with AGE at the Children's Hospital "G. Di Cristina" in Palermo, Sicily, were screened for RVA infection as previously described, and RVA-positive samples were submitted to VP7 and VP4 sequence analyses [3,4] (accession numbers OQ846742-OQ846776).

Full-genome sequencing of three G1P[8] strains, Hu/RVA/PA207/2021/ITA, Hu/RVA/PA222/2021/ITA, and Hu/RVA/PA225/2021/ITA, from the 2021 RVA season, was performed using MinION flow-cell (MIN106 R9.4, ONT™) and run via MinKNOW v.18.05.5 [6] (accession numbers OQ815902-OQ815912 for strain Hu/RVA/PA222/2021/ITA, PP211172 to PP211182 for strain Hu/RVA/PA207/2021/ITA and PP211183 to PP211193 for strain Hu/RVA/PA225/2021/ITA).

Results

Over the study period, RVA infection was detected in 7.9% (21/266) of children hospitalized with AGE (11 males and 10 females, range 2-115 months, mean and median ages of 36 and 24 months, respectively). Overall, 57.1% (12/21) of the patients had not been vaccinated, 14.3% (3/21) had received a single dose, and 9.5% (2/21) had completed the vaccination cycle with Rotarix, while, for four children, the vaccination status was not known. RVA infection occurred 2-6 years after the second dose in the two children with a complete two-shot vaccination schedule, and 1-28 months after vaccination in the three children with a single shot.

Out of the 21 RVA-positive samples, G1P[8] represented the predominant genotype accounting for 90.5% of rotavirus infection (19/21). On phylogenetic analyses of the VP7 gene, all the Italian 2021 G1P[8] strains clustered in lineage II, with, 3/19 strains (15.8%) segregating into sub-lineage IIa together with Rotarix vaccine strain (JN849113) and 16/19 strains (84.2%) falling into a separate sub-lineage. This new sub-lineage differed by more than 1.5% nucleotide (nt) identity from other sub-lineages (IIa-IId) and it was here tentatively designated as sub-lineage IIe following a VP7 lineage classification scheme [7] (Figure 1a).

On phylogenetic analysis of the VP4 gene, the three vaccine-like G1 strains segregated into lineage I with a 99.7-100% nt identity to the Rotarix strain, while all the others fell into sub-lineage IIId. This novel P[8]-IIId cluster, here renamed as IIId', showed 92.8-

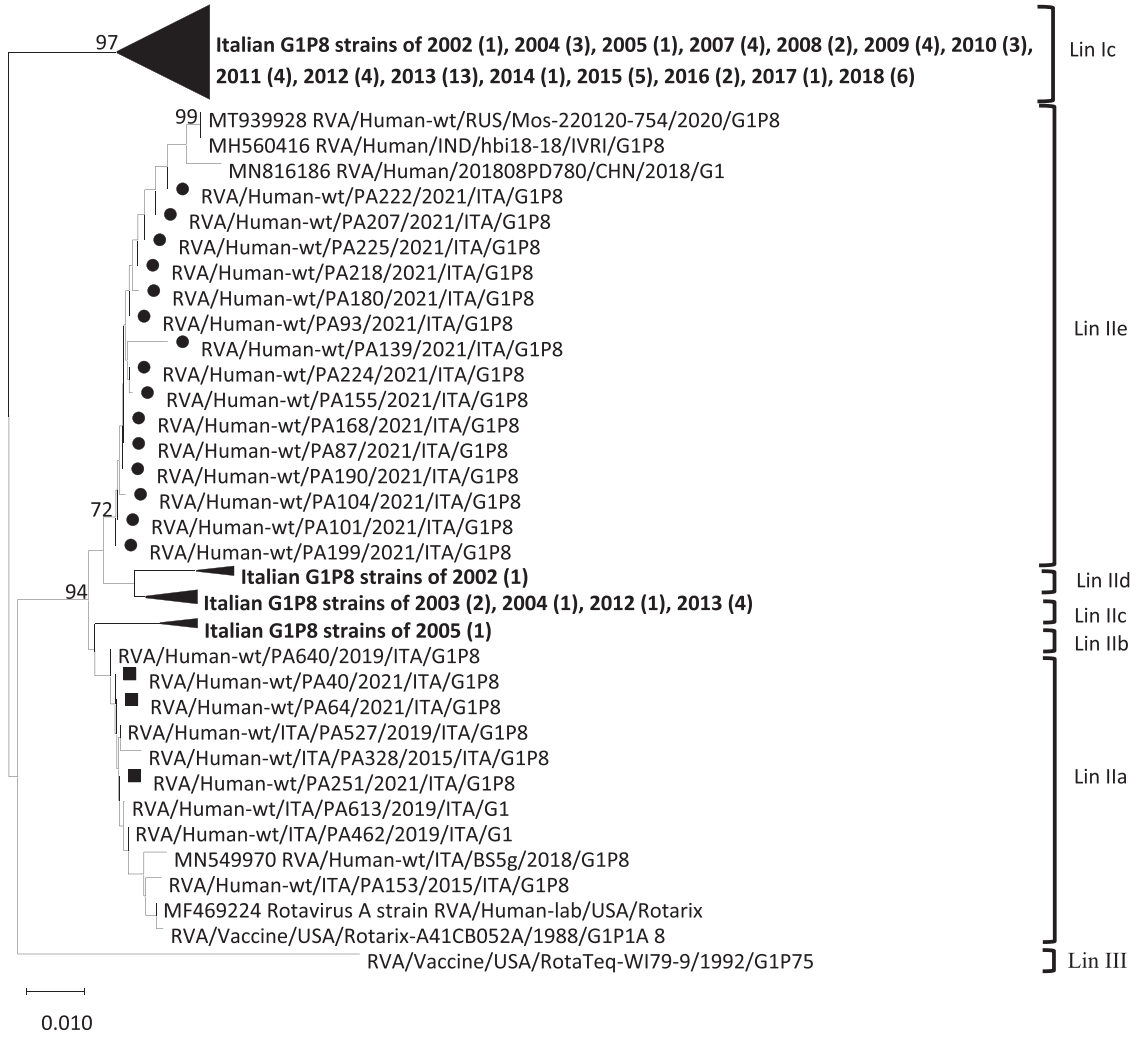

Figure 1. Phylogenetic analysis of partial nucleotide sequences of VP7 (a) and VP4 (b) genes of G1P[8] strains constructed using the Maximum-likelihood method with 1000 bootstrap replicates. Bootstrap values below 70% are not shown. The strains of this study are highlighted with filled circles (novel G1P[8] Palermo variant) or filled squares (Rotarix-like G1P[8]). For graphical simplicity, some closely related strains are grouped in triangles, the height of the triangles is proportional to the number of sequences included.

Figure 1. Continued

Table 1
Amino acid substitutions of VP7 (a) and VP4 (b) neutralizing epitopes of Italian G1P[8] rotaviruses with respect to vaccine strains (Rotarix® and RotaTeq®).

(1a)	**Epitopes**	
	7-1a	**7-2**
	Positions	
Strains (n° sequences tested)	**97**	**147**
RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P1A	E	N
RVA/Vaccine/USA/RotaTeq-WI79-9/1992/G1P7	D	S
G1P[8] Vaccine-like (3)	.	.
G1P[8] variant Palermo 2021 (15)	.	.

(1b)	**Epitopes**						
	8-1			**8-3**			
	Positions						
Strains (n° sequences tested)	**145**	**150**	**195**	**116**	**125**	**131**	**135**
RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P1A	S	E	N	D	S	S	N
RVA/Vaccine/USA/RotaTeq-WI79-9/1992/G1P7	.	.	D	.	N	R	D
G1P[8] Vaccine-like (3)	.	.	.	E	N	R	D
G1P[8] variant Palermo 2021 (16)	G	D	G	E	N	R	D

97.5% nt identity to all other P[8]-III sub-lineages (Figure 1b). Such novel VP7/VP4 combination of sub-lineages might indicate a new G1P[8] variant that was herein termed as "Palermo 2021".

On inspection of the neutralization epitopes of VP7 (7-1a, 7-1b, and 7-2) all Italian G1P[8] strains did not differ from the Rotarix strain, while they differed in two amino acid (aa) residues from RotaTeq strain (Table 1a). Also, the variant Palermo 2021 showed seven aa substitutions in epitopes 8-1 and 8-3 of VP4 (Table 1b).

The full-genome of three representative strains (PA207/2021/ITA, PA222/2021/ITA, and PA225/2021/ITA) of the novel variant Palermo 2021 was obtained, with a mean depth of coverage per segment ranging from 249 to 6280 nt. The three strains differed by as high as 0.3% nt among each other in the various RNA segments and showed a Wa-like genetic backbone (G1-P[2]-I1-R1-C1-M1-A1-N1-T1-E1-H1). However, on sequence comparison with cognate genome segments retrieved from the databases and on phylogenetic analysis, the viruses revealed a puzzled genome com-

position. In particular, the VP1, non-structural protein NSP4, and NSP5 genes were related to G12P[6] RVAs while the rest of the genome segments to G1P[8] strains detected in Belgium, Pakistan, India, and Russia, into a unique arrangement never observed before (Figure 2).

Discussion

G1P[8] are epidemiologically relevant and they are targeted by human vaccines Rotarix and RotaTeq [3,8,9]. Vaccine introduction has been associated with a significant reduction in the rate of RVA infection and has seemingly influenced RVA genetic diversity, likely enacting a strong selective pressure [5,9].

In Sicily, after the introduction of Rotarix in 2012, a decrease in RVA prevalence and changes in the circulation of G and P genotypes were observed. Since 2014, RVA prevalence decreased (by almost 40%) reaching 6.6% (30/456) in 2018 and 7.9% (21/266) in 2021 [5]. The pre-and post-vaccination patterns of circulation of G1P[8] RVA in the local population have been investigated in detail. Over 35 consecutive years of hospital-based surveillance, G1P[8] strains were constantly detected with fluctuating prevalence over time, and a marked decrease in their circulation in the post-vaccine period (16.9% in 2013-2020) was observed, with respect to the pre-vaccination period (58.3% in 1985-2012) [3,5]. Interestingly in 2021, an unexpected peak of G1P[8] infections (90.5%) were observed, reversing this general trend.

Sequence and phylogenetic analyses of VP7 and VP4 genes of the Italian 2021 G1P[8]s revealed the co-circulation of vaccine-like strains (3/19, 15.8%) and of a novel G1P[8] RVA strain (16/19, 84.2%). This novel G1P[8] clustered apart, in both the VP7 and VP4, from other G1P[8] strains detected previously in Italy and were classified as a novel G1 VP7 sub-lineage IIe and a novel P[8] VP4 sub-lineage IIId' (Figure 1a-b), tentatively named as G1P[8] variant Palermo 2021. In the local population, during a 35-year-long surveillance, the periodical emergence/re-emergence of different VP7 and VP4 lineages/sub-lineages of G1P[8] RVA has been reported [4,5,7,8]. In this time window, lineage IIId was the predominant P[8] sub-lineage, while Rotarix and RotaTeq vaccines have a VP4 of lineage P[8]-I and -II, respectively.

The variant Palermo 2021 showed a conserved Wa-like genetic constellation, although a more detailed analysis revealed multiple possible reassortment events. Most genome segments were genetically related to different G1P[8] strains from Belgium, Pakistan, India, and Russia while the VP1, NSP4, and NSP5 segments were related to G12P[6] RVAs. Also, it was not possible to identify in GenBank a single G1P[8] strain matching the entire

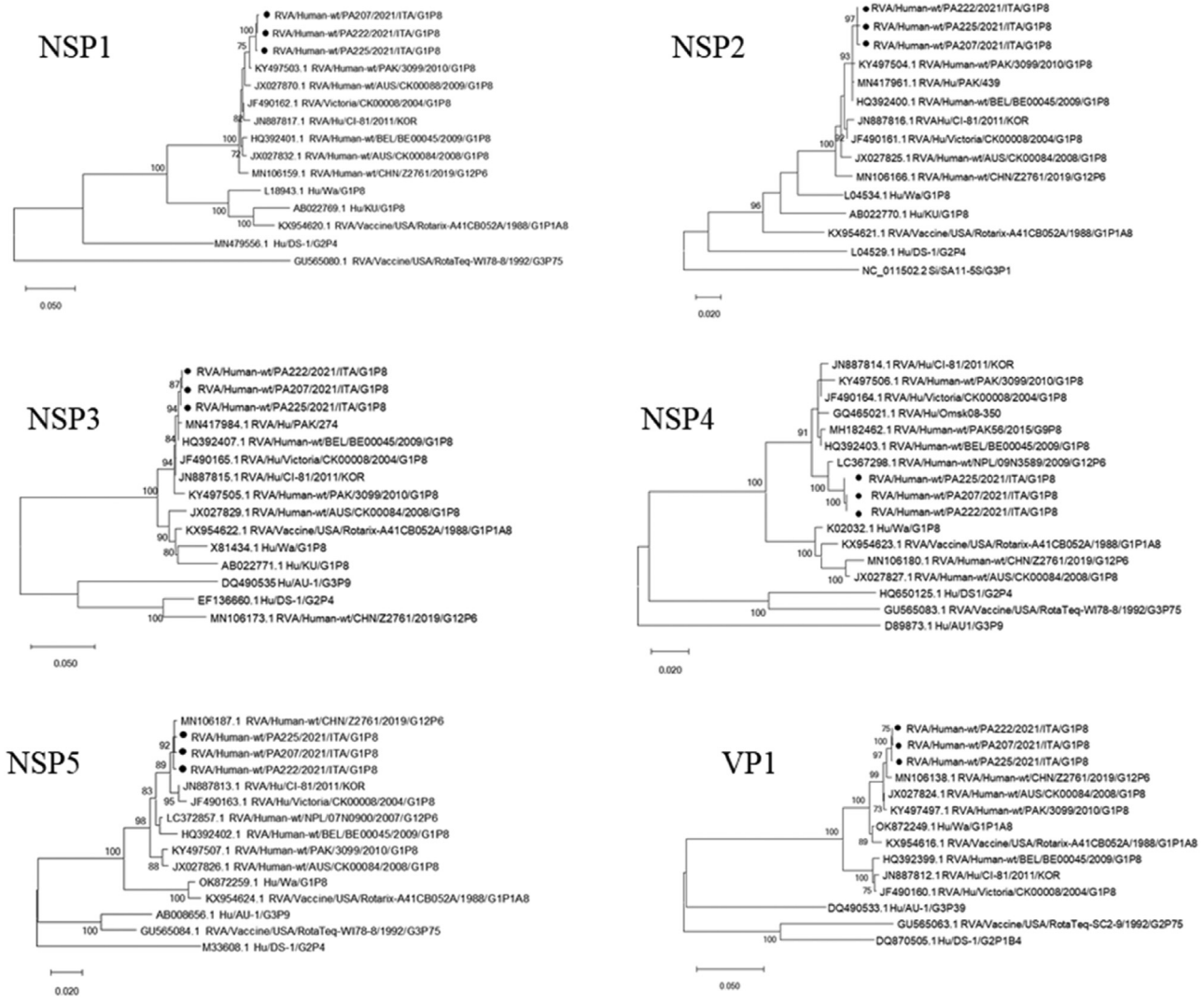

Figure 2. Phylogenetic analyses of the 11 gene segments of the G1P[8] strains constructed using the Maximum-likelihood method with 1000 bootstrap replicates. Bootstrap values below 70% are not shown. The strains of this study (novel G1P[8] Palermo variant) are highlighted with filled circles.

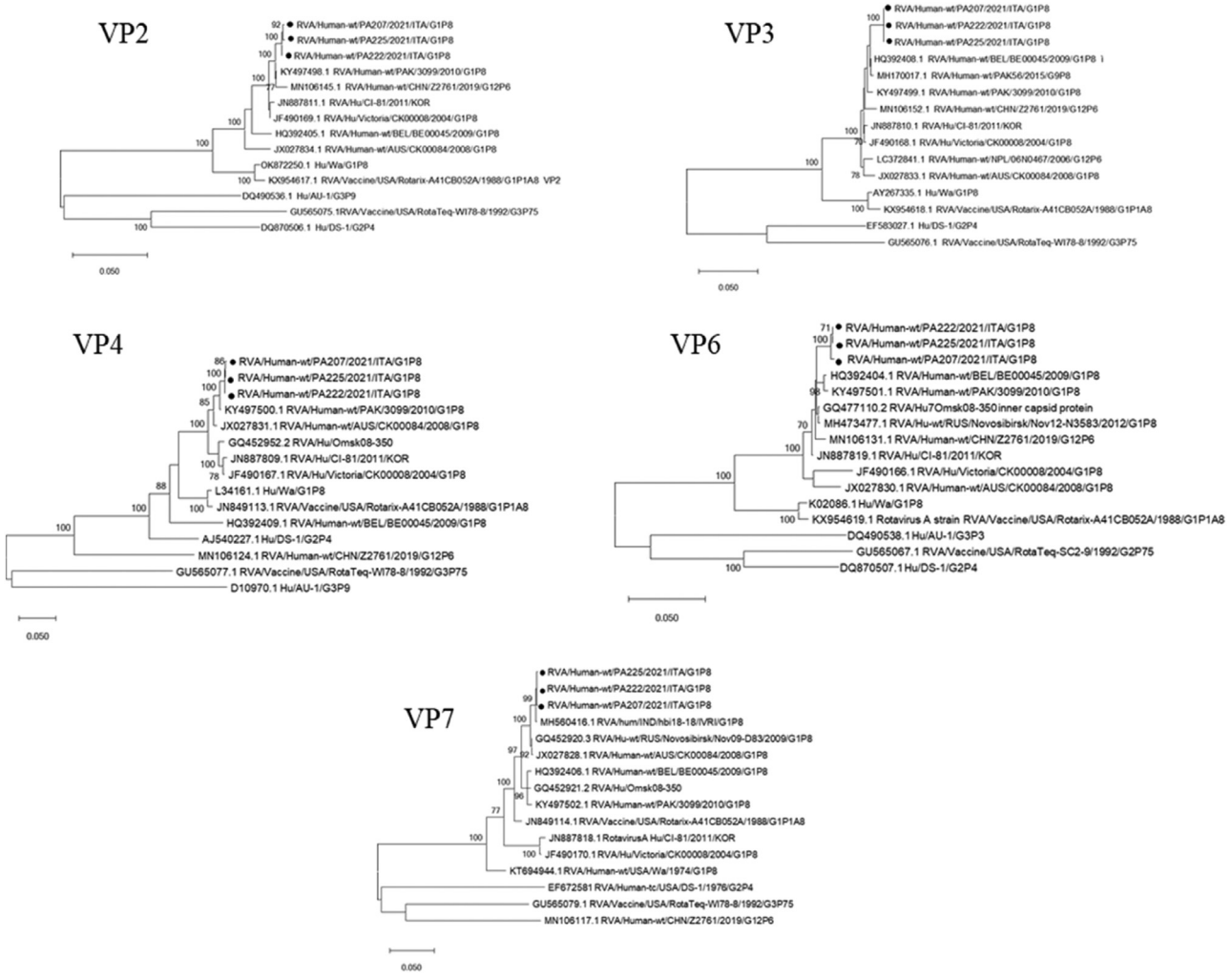

Figure 2. Continued

genome constellation of strains PA207/2021/ITA, PA222/2021/ITA and PA225/2021/ITA. All in all, these results underline the importance of complete genome sequence analysis to investigate the intra-genotype dynamics of the evolution of G1P[8] RVAs [9].

When inspecting the neutralization epitopes of the VP7 and of the VP8* portion of VP4 protein, the variant Palermo 2021 G1P[8] did not differ in the VP7 epitopes from the Rotarix vaccine but it showed seven aa substitutions in the 8-1 and 8-3 epitopes of VP4. The results obtained in vaccine trials have suggested that G1 vaccine strains confer good homotypic protection against G1 strains from the field [10]. Further analyses would be necessary to investigate the role of VP4 variations in modulating RVA fitness.

Of the 16 patients infected with the Palermo 2021 G1P[8] variant, only three patients had a history of vaccination (Rotarix), with two patients having received a complete vaccine regime, while 11 patients were not vaccinated for RVA and for two patients the vaccine status was not available. Of the three children infected with the vaccine-like RVA, two children had received a single vaccine dose, while the vaccination status of the third patient was not available.

The co-circulation of vaccine-related RVA strains in children with AGE has been reported in the literature and it is still unclear whether AGE is caused by the reversion of attenuation of the vaccine virus or the detection of these strains is a serendipitous finding or a result of horizontal transmission.

For instance, one of the vaccinated children was infected with a vaccine-like RVA about 17 months after the vaccine shot, suggesting horizontal transmission of the RVA strain.

Prompt identification of virus variants circulating in the human population is pivotal to understanding epidemiological trends and assessing vaccine efficacy.

Declaration of competing interest

The authors declare no conflict of interest.

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Ethical approval

Ethical approval was granted by the University Hospital Ethical Committee Palermo 1 (No. 02/2017).

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Author contributions

All authors listed contributed significantly to this work and are entitled to authorship.

S. De Grazia. and C. Filizzolo: Conceptualization, Methodology, Data Curation, Writing—Original Draft, Writing— Review and Editing. G.M. Giammanco and V. Martella: Data Curation, Writing—Review and Editing. F. Bonura, M. Pizzo, F. Pellegrini, F. Di Bernardo, and A. Collura: Formal analysis, Methodology, and Technical approaches: All authors have read and agreed to the published version of the manuscript.

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