

Genomic Analysis of G8P[4] Rotavirus Strains Exhibit DS-1-Like Constellation with Evidence of Radical Changes in Important Epitope Sites

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Introduction



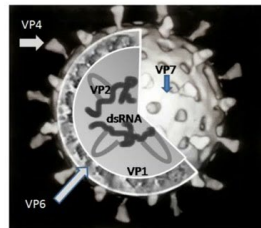
ROTAVIRUS 
#1 cause of severe diarrhea
kills 200,000/year

Symptoms

- non-bloody diarrhea → dehydration
- vomiting
- fever



Rotavirus



Highly Contagious
Common among kids

Before Vaccination -
100 % population



Symptoms:

Severe watery
diarrhea



Vomiting



Fever

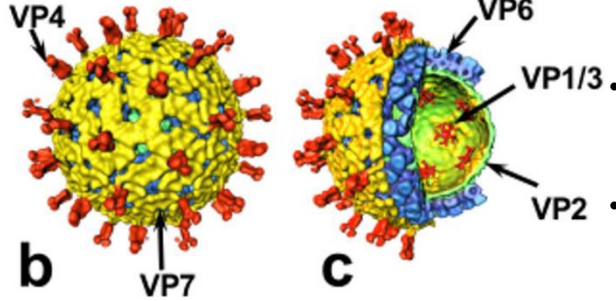
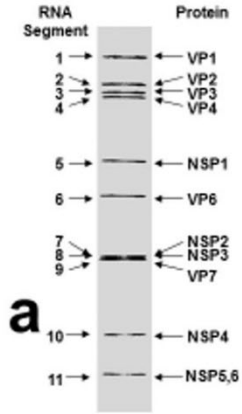


Abdominal
Pain

Dehydration!
If Left Untreated
Kid can Die

Incubation Period: 2 Days
Symptoms lasts:
3-8 Days

Introduction

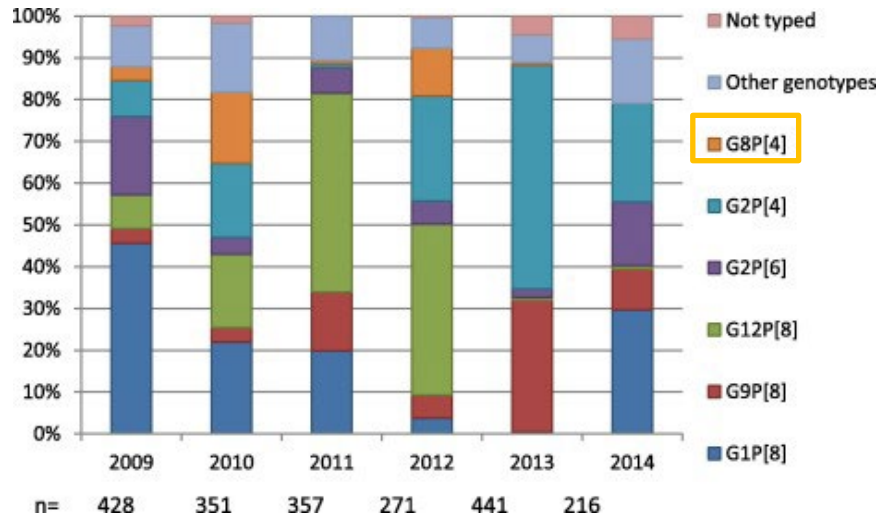


Genome	Gene	Product	Genotype Name
	g1	VP1	RNA polymerase
	g2	VP2	Core shell
	g3	VP3	RNA-capping Methyltransferase
	g4	VP4	Protease-activated spike
	g5	NSP1	interferon Antagonist
	g6	VP6	Intermediate capsid shell
	g7	NSP3	Translation regulation
	g8	NSP2	octameric NTPase
	g9	VP7	outer-shell Glycoprotein
	g10	NSP4	Enterotoxin
	g11	NSP5/6	pHosphoprotein

- Double-stranded RNA (dsRNA) virus that belongs to the *Sedoreoviridae* family.
- The viral genome consists of 11 segments encoding six VP and five/six NSPs.
- RVA Conventional genotyping is based on the VP4 (**P-type**) and VP7 (**G-type**) outer proteins.
- Currently, 42 G types and 58 P types, group A rotavirus have been characterised.
 - **G1P[8], G2P[4], G3P[8], G4P[8], G9P[8] , and G12P[8]** RVA strains.
- Whole genome classification is based on 11 segments Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx (x- genotype number).
 - Wa-like, DS-1, and AU-like genome constellation.

Introduction

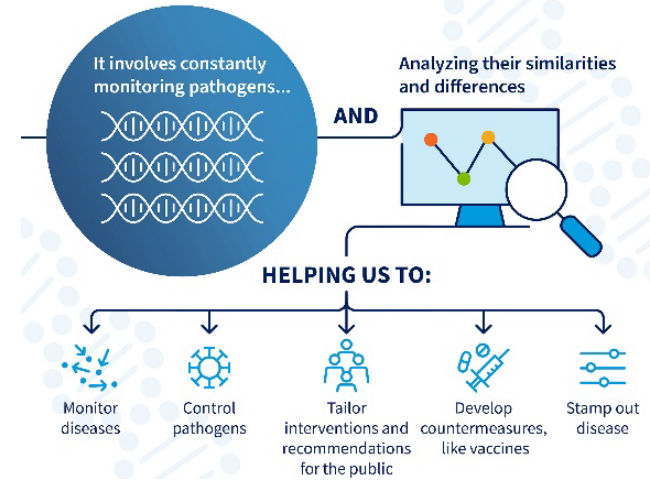
- Following Rotarix (G1P[8]) vaccine introduction in South Africa in August 2009, non-G1P[8] RVA strains were detected at higher frequencies than before.
 - Including G8P[4], detected at 8,2% in the African continent and 6% in South African hospitalisations from 2004 to 2016.
 - Major concern for possible strain replacement in the country, which has been observed previously with the G12 RVA strain.



Introduction

- **G8** RVA, originally a bovine strain, has successfully crossed the animal to human infection barrier, with the strain first reported in South Africa in 2009.
- Multiple studies in Africa have determined the G8 strain as an endemic circulating genotype.
- Despite growing epidemiological significance, no whole genome analysis has been conducted **in South Africa** on the G8P[4] strain, with a few African countries reporting on comprehensive analysis.

What is GENOMIC SURVEILLANCE?



Results

- 21 G8P[4] study strains exhibited DS-1-like genomic backbone, across all 11 segments.
 - No reassortments present.

Table 1. Genotype constellation of South African G8P[4] study strains.

Strain name	VP7	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5
Segment	9	4	6	1	2	3	5	8	7	10	11
DS1-Like Constellations	G2	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-wt/ZAF/UFS-NGS-4076/2009/G8P[4]	G8	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-wt/ZAF/UFS-NGS-4124/2009/G8P[4]	G8	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-wt/ZAF/UFS-NGS-5157/2010/G8P[4]	G8	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-wt/ZAF/UFS-NGS-5380/2010/G8P[4]	G8	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-wt/ZAF/UFS-NGS-5595/2010/G8P[4]	G8	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-wt/ZAF/UFS-NGS-5665/2010/G8P[4]	G8	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-wt/ZAF/UFS-NGS-5671/2010/G8P[4]	G8	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-wt/ZAF/UFS-NGS-5813/2010/G8P[4]	G8	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-wt/ZAF/UFS-NGS-6098/2010/G8P[4]	G8	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-wt/ZAF/UFS-NGS-9189/2012/G8P[4]	G8	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-wt/ZAF/UFS-NGS-9367/2012/G8P[4]	G8	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-wt/ZAF/UFS-NGS-9414/2012/G8P[4]	G8	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-wt/ZAF/UGS-NGS-9431/2012/G8P[4]	G8	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-wt/ZAF/UFS-NGS-9577/2012/G8P[4]	G8	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-wt/ZAF/UFS-NGS-10095/2012/G8P[4]	G8	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-wt/ZAF/UFS-NGS-18276/2017/G8P[4]	G8	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-wt/ZAF/UFS-NGS-18961/2017/G8P[4]	G8	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2

Results

Alignment of antigenic residues in the **VP4** and **VP7** proteins between the reference Rotarix[®] vaccine strain (JN849114/RVA/Vaccine/USA/Rotarix- A41CB052A/1988/G1P[8]) and G8 strains circulating in South Africa during the 2009 – 2021 rotavirus seasons. Variations in amino acids between the vaccine strain and study strains are highlighted in a brown colour.

Table 3. Amino acid variability in G8 VP4 neutralisation epitopes between South African strains and the Rotarix vaccine strain.

Strain	VP8*																VP5*																				
	8-1				8-2				8-3				8-4				5-1					5	5	5	5												
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3	3	3	3	3	3	4	4	4	4	4	3					
Rotarix- A41CB052A/1988/G1P[8]	D	S	Q	E	S	T	N	L	N	N	I	T	A	N	P	V	D	S	S	N	D	N	N	T	N	Y	F	I	W	P	G	R	T	P	E	L	R
UFS-NGS-4076/2009/G8P[4]	D	S	Q	E	S	T	D	L	N	N	I	T	A	S	Q	T	N	N	E	N	N	D	N	T	D	Y	F	L	W	P	G	R	T	P	E	L	R
UFS-NGS-4124/2009/G8P[4]	D	S	Q	E	S	T	D	L	N	N	I	T	A	S	Q	I	N	N	E	N	N	D	D	T	D	Y	F	L	W	P	G	R	T	P	E	L	R
UFS-NGS-5157/2010/G8P[4]	D	S	Q	E	S	T	D	L	N	N	I	T	A	S	Q	I	N	N	E	N	N	D	D	T	D	Y	F	L	W	P	G	R	T	P	E	L	R
UFS-NGS-5380/2010/G8P[4]	D	S	Q	E	S	T	D	L	N	N	I	T	A	S	Q	I	N	N	E	N	N	D	D	T	D	Y	F	L	W	P	G	R	T	P	E	L	R
UFS-NGS-5595/2010/G8P[4]	D	S	Q	E	S	T	D	L	N	N	I	T	A	S	Q	I	N	N	E	N	N	D	D	T	D	Y	F	L	W	P	G	R	T	P	E	L	R
UFS-NGS-5665/2010/G8P[4]	D	S	Q	E	S	T	D	L	N	N	I	T	A	S	Q	I	N	N	E	N	N	D	D	T	D	Y	F	L	W	P	G	R	T	P	E	L	R
UFS-NGS-5671/2010/G8P[4]	D	S	Q	E	S	T	D	L	N	N	I	T	A	S	Q	I	N	N	E	N	N	D	D	T	D	Y	F	L	W	P	G	R	T	P	E	L	R
UFS-NGS-5813/2010/G8P[4]	D	S	Q	E	S	T	D	L	N	N	I	T	A	S	Q	I	N	N	E	N	N	D	D	T	D	Y	F	L	W	P	G	R	T	P	E	L	R
UFS-NGS-6098/2010/G8P[4]	D	S	Q	E	S	T	D	L	N	N	I	T	A	S	Q	I	N	N	E	N	N	D	D	T	D	Y	F	L	W	P	G	R	T	P	E	L	R
UFS-NGS-9189/2012/G8P[4]	D	S	Q	E	S	T	D	L	N	N	I	T	A	S	Q	I	N	N	E	N	N	D	D	T	D	Y	F	L	W	P	G	R	T	P	E	L	R
UFS-NGS-9367/2012/G8P[4]	D	S	Q	E	S	T	D	L	N	N	I	T	A	S	Q	I	N	N	E	N	N	D	D	T	D	Y	F	L	W	P	G	R	T	P	E	L	R
UFS-NGS-9431/2012/G8P[4]	D	S	Q	E	S	T	D	L	N	N	I	T	A	S	Q	T	N	N	E	N	N	D	D	T	D	Y	F	L	W	P	G	R	T	P	E	L	R
UFS-NGS-9414/2012/G8P[4]	D	S	Q	E	S	T	D	L	N	N	I	T	A	S	Q	T	N	N	E	N	N	D	D	T	D	Y	F	L	W	P	G	R	T	P	E	L	R
UFS-NGS-9577/2012/G8P[4]	D	S	Q	E	S	T	D	L	N	N	I	T	A	S	Q	T	N	N	E	N	N	D	D	T	D	Y	F	L	W	P	G	R	T	P	E	L	R
UFS-NGS-10095/2012/G8P[4]	D	S	Q	E	S	T	D	L	N	N	I	T	A	S	Q	T	N	N	E	N	N	D	D	T	D	Y	F	L	W	P	G	R	T	P	E	L	R
UFS-NGS-18276/2017/G8P[4]	D	S	Q	E	S	T	D	L	N	N	I	T	A	S	Q	I	N	N	E	N	N	D	D	T	D	Y	F	L	W	P	G	R	T	P	E	L	R
UFS-NGS-18961/2017/G8P[4]	D	S	Q	D	S	T	D	L	N	N	I	T	A	S	Q	T	N	N	E	N	S	D	D	T	D	Y	F	L	W	P	G	R	T	P	E	L	R
UFS-NGS-19043/2017/G8P[4]	D	S	Q	E	S	T	D	L	N	N	I	T	A	S	Q	T	N	N	E	N	N	D	D	T	D	Y	F	L	W	P	G	R	T	P	E	L	R
UFS-NGS-19184/2017/G8P[4]	D	S	Q	E	S	T	D	L	N	N	I	T	A	S	Q	T	N	N	E	N	N	D	D	T	D	Y	F	L	W	P	G	R	T	P	E	L	R
UFS-NGS-22335/2019/G8P[4]	D	S	Q	E	S	T	D	L	N	N	I	T	A	S	Q	T	N	N	E	N	N	D	D	T	D	Y	F	L	W	P	G	R	T	P	E	L	R
UFS-NGS-23867/2021/G8P[4]	D	S	Q	E	S	T	D	L	N	N	I	T	A	S	Q	I	N	N	E	N	N	D	D	T	D	Y	F	L	W	P	G	R	T	P	E	L	R

Results

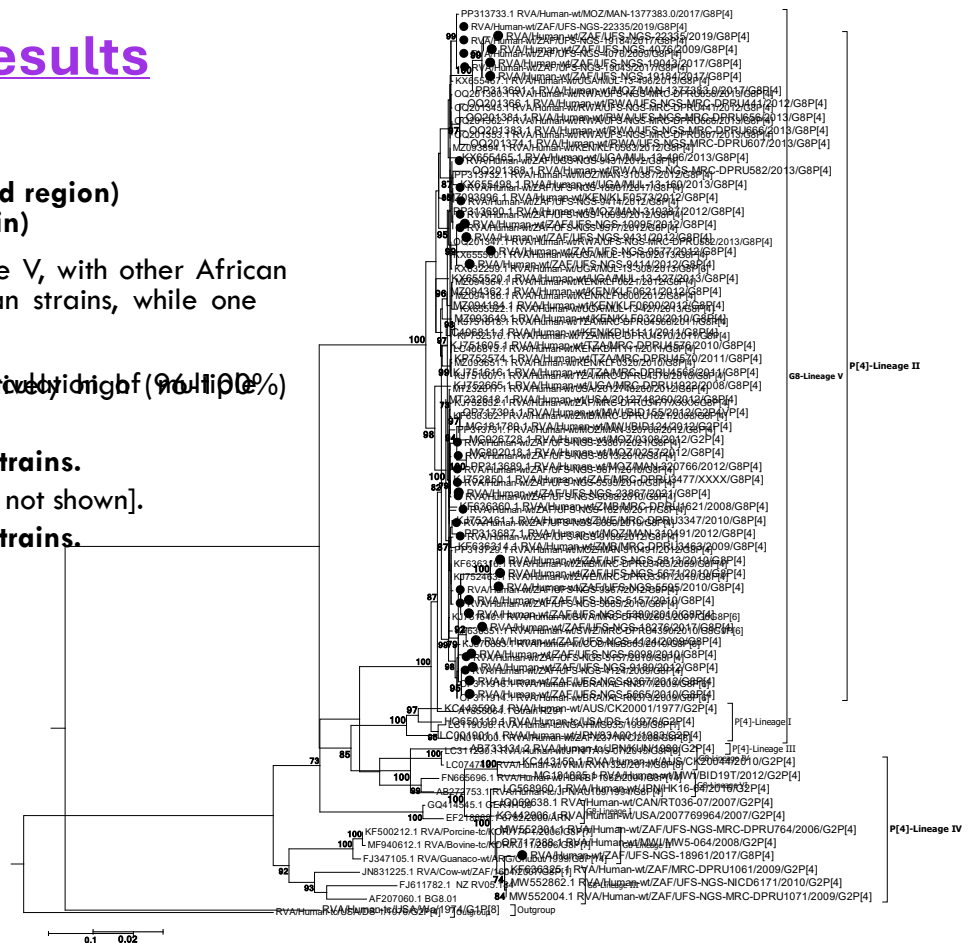
Maximum likelihood phylogenetic tree for VP7 (Outer capsid region) Maximum likelihood phylogenetic tree for VP4 (Spike protein)

- South African G8 (VP7) exclusively clustered within lineage V, with other African strains.
- 20 study strains segregated to lineage P[4]-II with African strains, while one strain fell in P[4]-IV with global strains.
- Nucleotide positions and amino acid identities were relatively high (96-100%) [data not shown].

These results suggest the circulation of genetically similar strains.

- NT and AA identities were relatively high (93-100%) [data not shown].

These results suggest the circulation of genetically similar strains.



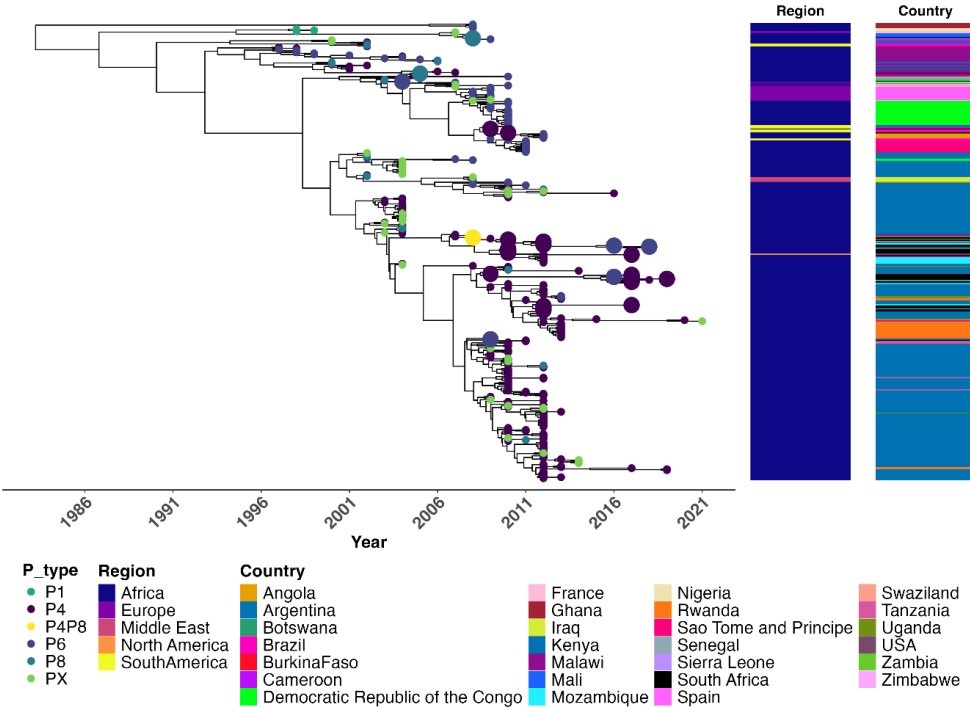
Results



Phylogeny of internal gene segments (VP1-VP3, VP6, and NSP1-NSP5).

- **VP1 [I2]:** Study strains clustered within a single lineage, and NT/AA identities were 86-100%.
- **VP2 [R2]:** Study strains clustered within a single lineage, and NT/AA identities were 86-100%.
- **VP3 [C2]:** Study strains clustered within a single lineage, and NT/AA identities were 92-100%.
- **VP6 [M2]:** Study strains clustered within a single lineage, and NT/AA identities were 87-100%.
- **NSP1 [A2]:** Study strains clustered within a single lineage, and NT/AA identities were 91-100%.
- **NSP2 [N2]:** Study strains clustered within a single lineage, and NT/AA identities were 83-100%.
- **NSP3 [T2]:** Study strains clustered within a single lineage, and NT/AA identities were 94-100%.
- **NSP4 [E2]:** Study strains clustered within a single lineage, and NT/AA identities were 83-100%.
- **NSP5 [H2]:** Study strains clustered within a single lineage, and NT/AA identities were 95-100%.

Results



- South African G8 strains are interspersed with strains from Kenya, Mozambique, and Zimbabwe, indicating potential **transmission events** of the lineage and **regional viral flow**.
- The tree revealed the co-circulation of **multiple sub-lineages** in the sub-Saharan region **persisting over several years**, suggesting the continued circulation or re-introduction of genetically conserved variants over a decade.
- The mean evolutionary rate for the G8 lineage V VP7 sequences was estimated to be 1.491×10^{-3} substitutions per site per year.
 - similar to other G8 strains reported in **Vietnam** and **Argentina**.
 - This moderate substitution rate is consistent with **endemic transmission**, also observed for G12 and G9 evolutionary rates.

Evolutionary analysis. A maximum clade credibility tree (MCC) of G8 (VP7 lineage V, Global and study strains) genotype, estimating the time to the most common recent ancestor, with the time scale represented below the tree.

Discussion


- All study strains exhibited a DS-1-like constellation, consistent with global reports.
 - Co-evolutionary adaptation: these 11 genes have evolved together as a unit, optimising protein-protein interactions for:
 - Efficient **viral replication** in host cells.
 - Successful **transmission** between hosts.
- Phylogenetic analysis revealed the close circulation of study strains with African strains (Kenya, Rwanda, Zambia, Mozambique). High NT/AA identities with these strains.
 - Suggestion: **co-circulation** and **co-evolution** of genetically similar strains in Africa; emphasising genomic surveillance and the deposition of data into public repositories.
- The BEAST analysis of G8 lineage V VP7 genes supports the notion of continued endemic circulation of G8 strains in Africa.
 - Evidenced by stable circulation and persistence of multiple lineages over a decade.
 - Reflects distinct diversity but similar viral lineages; possible multiple re-introductions of highly conserved strains.
- A key finding: Antigenic variations in neutralising epitopes compared to the Rotarix® vaccine strain: antibody structure.
- This antigenic difference, along with known contributing factors such as malnutrition, gut dysbiosis, and others, may induce the potential for these strains to adapt and diversify independently of the vaccine introduced.

Limitations and Conclusion

- **First:** dearth of epidemiological data concerning G8P[4], particularly regarding its temporal distribution, clinical severity, and vaccination status.
- **Second:** structural modelling or neutralisation assays were not performed to directly assess the impacts of antigenic changes observed.
- Despite these limitations, our data provides valuable insights into the molecular characteristics of this underrepresented genotype and its evolutionary history.
- This study presents evidence of continued G8 endemic persistence, regional transmission, and significant antigenic divergence in critical epitope sites compared to the Rotarix® vaccine strain.

Outputs

- M.MedSc degree (*Cum Laude*).



Contents lists available at [ScienceDirect](#)

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Research paper Available 5 September 2025

Whole genome characterisation of DS-1-like G8P[4] rotavirus A strains circulating in South Africa between 2009 and 2021 reveals endemic sub-lineages and evidence of radical epitope changes

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- **Co-supervisor: Dr Celeste Donato**
- **Co-supervisor: Dr Nigel Makoah**



Gates Foundation



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