

Genome-Wide Analyses of G1P[8] and G2P[4] Rotaviruses Circulating Before and After Rotavirus Vaccine Introduction in Ghana, Malawi and South Africa.

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Study Aims

-To investigate if vaccine introduction created an immunological pressure that impacts the emergence and evolution of G1P[8] and G2P[4] rotavirus strains

-Potential evolution of antigenic escape strains.

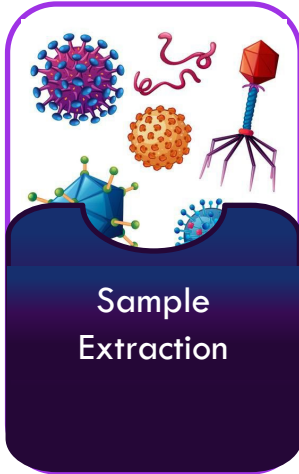
-Globally, at the time of vaccine introduction G1P[8] and G2P[4] were dominant genotypes – not necessarily in Africa.

-G1P[8] is fully homotypic to Rotarix.

-G2P[4] is fully heterotypic to Rotarix.

Sample Processing and Next Generation Sequencing

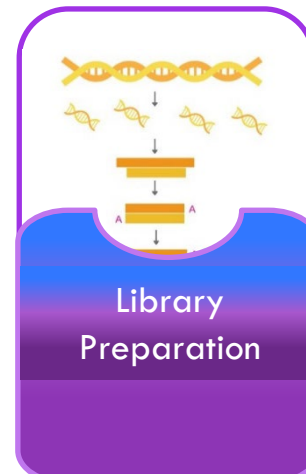
Step 01



Step 02



Step 03



Step 04



Bioinformatic Data Analysis

Data Analysis

- Genome assembly.



Phylogenetic Analysis

- IQTree.



TEMPEST

- Temporal signals of each gene dataset.

Bayesian Reconstruction

- Estimate rates of evolution.
- Time to most recent common ancestor (tMRCA).
- Reconstruct relative genetic diversity.



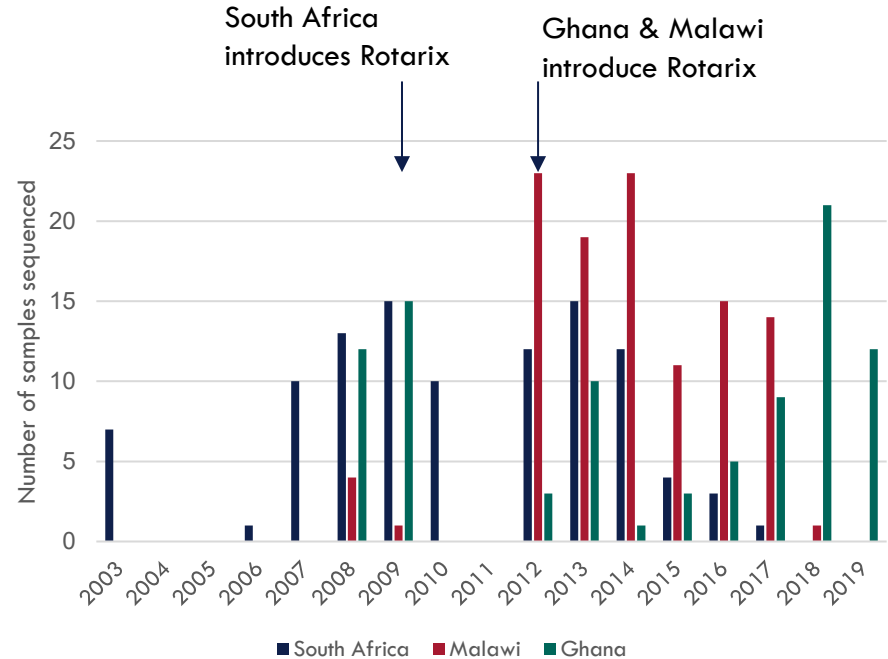
Introduction – G1P[8] and G2P[4]

- **G2P[4] sequences were generated**

- South Africa (2003 – 2017)
- Malawi (2008 – 2018)
- Ghana (2008 – 2019)

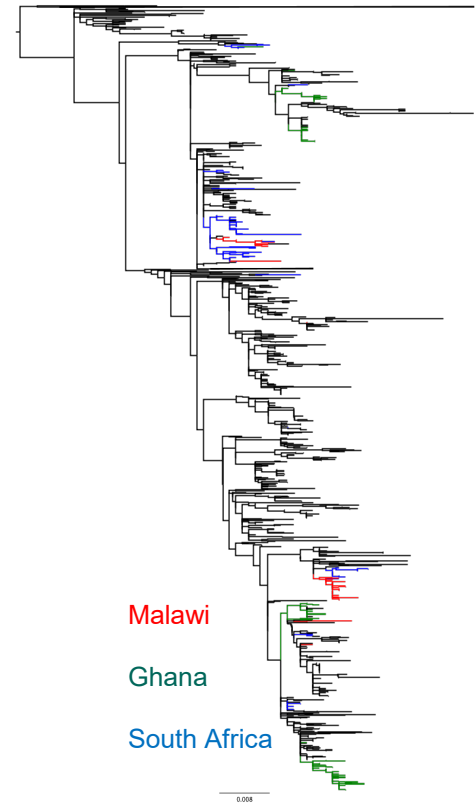
- **G1P[8] sequences were generated**

- South Africa (2002 – 2017)
- Malawi (1998 – 2019)
 - 17 DS-1-like G1P[8]
- Ghana (2008 – 2019)



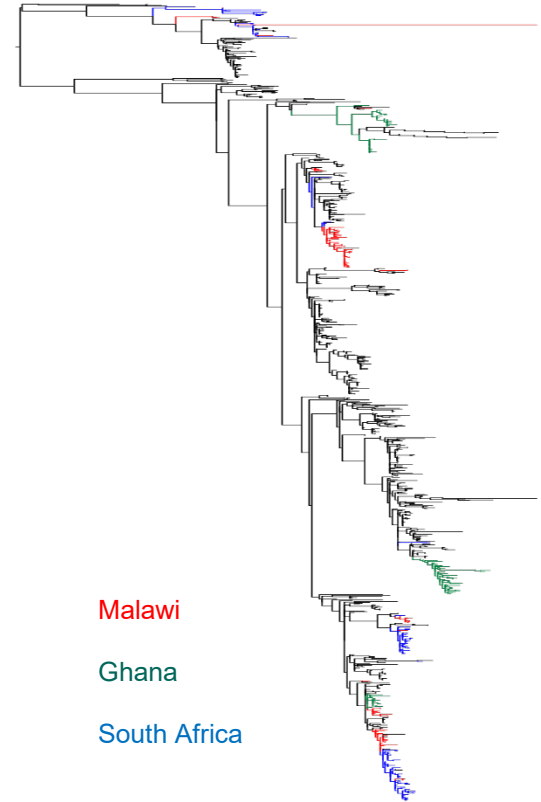
G2 - VP7: co-circulation of lineages

- **Co-circulation of divergent lineages.**
 - Malawi and South Africa have a degree of co-circulation and transmission dynamics.
 - Ghana is distinct.
- **Separate global introductions.**
 - Ghana – Europe / Russia.
 - Malawi and South Africa – India / Kenya.
- **Shift in vaccine-era lineage IVa-1 to Va-3.**
 - Reflective of a global shift in G2.



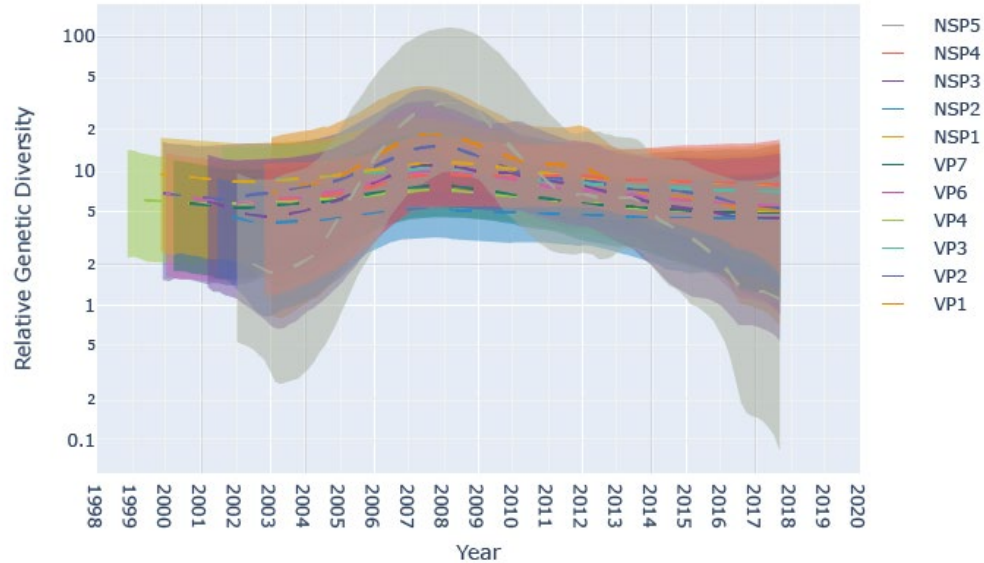
P[4] - VP4: co-circulation of lineages

- Co-circulation of divergent lineages.
- Malawi and South Africa have a degree of co-circulation and linked transmission dynamics.
- Ghana has some distinct lineages and some related to Malawi and South Africa lineages.
- Endemic African lineages of P[4].
- Vaccine-era shift from lineage IVa to Ivb.
 - Reflective of a global shift in P[4].



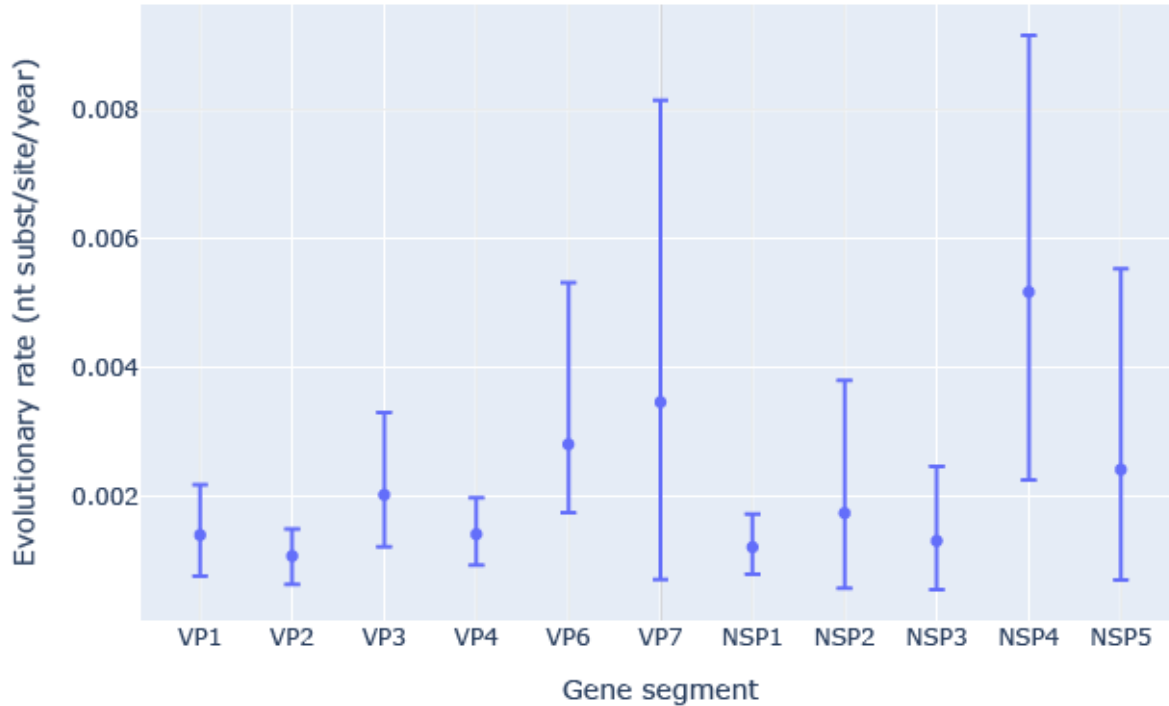
G2P[4] South Africa relative genetic diversity

- Most genes are very stable over time.
- NSP5 – increase in diversity is due to lineage replacement between 2003 and 2010.
- Uncertainty increase from 2016 onwards due to low sample size.



A measure of genetic diversity is given on the y axis with the 95% highest posterior density shown in solid color and the mean as a dashed line.

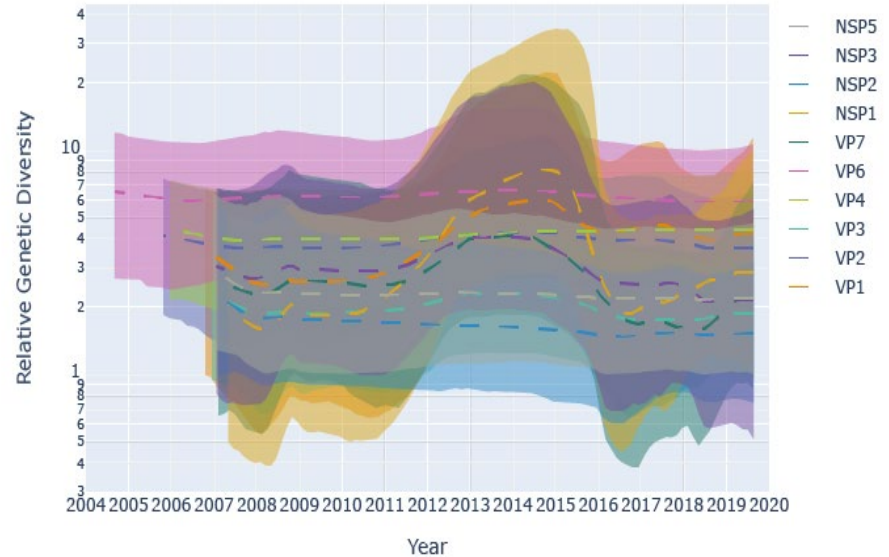
Rates of Evolution – South Africa



- The evolutionary rates are higher than the Malawi dataset despite a similar diversity of strains
- This could indicate more widespread transmission within South Africa
- Some genes have wide 95% highest posterior density

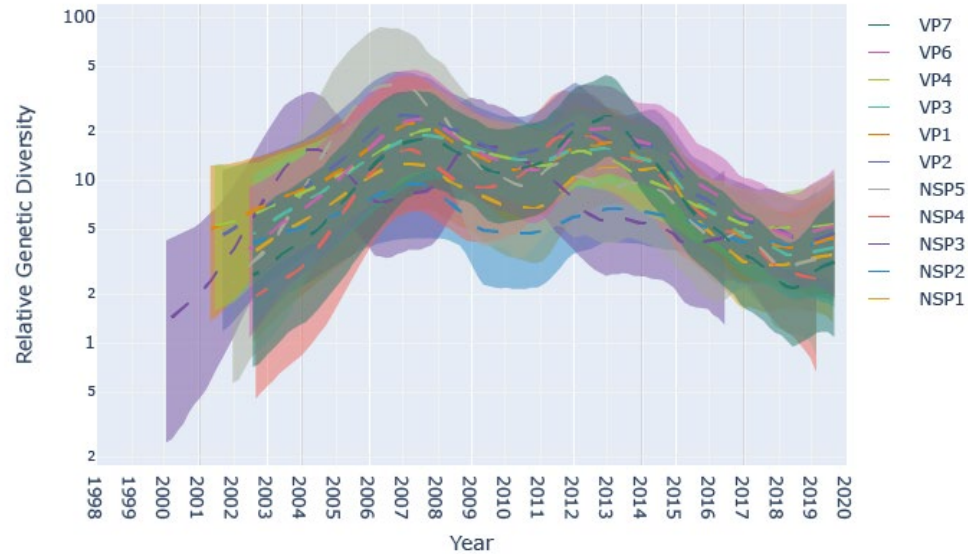
G2P[4] Ghana Relative Genetic Diversity

- Many genes are very stable over time.
- Minor increase between 2012-2013.
 - Associated with increased sampling.
- Bottleneck after mid-2015.
 - Associated with circulation of conserved strains.
- No temporal signal for NSP4.



Combined Dataset

- Increased genetic diversity compared to single-country datasets.
 - A temporal signal for NSP4.
- Strains that don't conform in single-country analysis may conform in combined.
- Evolutionary dynamics may be dominated by a single country.



Selection Analysis

- All genes in all datasets under moderate to strong purifying selection.
- Limited sites detected under positive selection.
- Malawi – no sites under positive selection in any gene.
- Ghana
 - VP2 amino acid position 14 under positive selection.
 - VP3 amino acid 7 under positive selection.

Summary of G2P[4]

- South Africa and Malawi share a similar genetic diversity of G2P[4] strains with contemporary co-circulation of variants resulting in similar evolutionary dynamics.
- There appears to be a higher evolutionary rate and stronger selective pressure in South Africa.
- Ghana has a distinct diversity of G2P[4] strains.
- Minor fluctuations in genetic diversity were observed over time
 - The decrease in genetic diversity occurs in the vaccine era
 - No emergence of divergent variants

GIP[8] Evolutionary Dynamics

Gene	Time of most recent common ancestor	Nucleotide Substitution Rates
VP1	2004.416	1.54E-03
VP2	2005.413	1.34E-03
VP3	2004.653	1.62E-04
VP4	2006.316	2.09E-03
VP6	2006.144	2.53E-03
VP7	2006.337	2.05E-03
NSP1	2005.207	1.85E-03
NSP2	*	*
NSP3	2006.132	2.63E-03
NSP4	2006.135	2.14E-03
NSP5	2006.949	2.21E-03

- Owing to the circulation of distinct lineages in Ghana compared to South Africa and Malawi, strains exhibit distinct evolutionary dynamics
- These strains emerged more recently in Ghana compared to those of South Africa and Malawi
 - *The gene dataset lacks an adequate temporal signal

Summary of G1P[8]

- South Africa and Malawi share a similar genetic diversity of G1P[8] strains with contemporary co-circulation of variants resulting in similar evolutionary dynamics.
- Ghana has distinct evolutionary patterns.
- There appears to be a moderately stronger selective pressure on G1P[8] – more sites observed under positive selection.
- G1P[8] detection decreased in the vaccine era from 2016 onwards.
 - Decrease in genetic diversity.
 - Did not see a sustained emergence of new variants in the vaccine era.

Acknowledgements



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Gates Foundation

Thank you

