MOLECULAR CHARACTERIZATION OF ROTAVIRUS STRAINS USING WHOLE GENOME SEQUENCING REVEALS UNIQUE CHANGES POST-ROTAVIRUS VACCINE INTRODUCTION IN ZAMBIA

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Introduction

Structure of Rotavirus
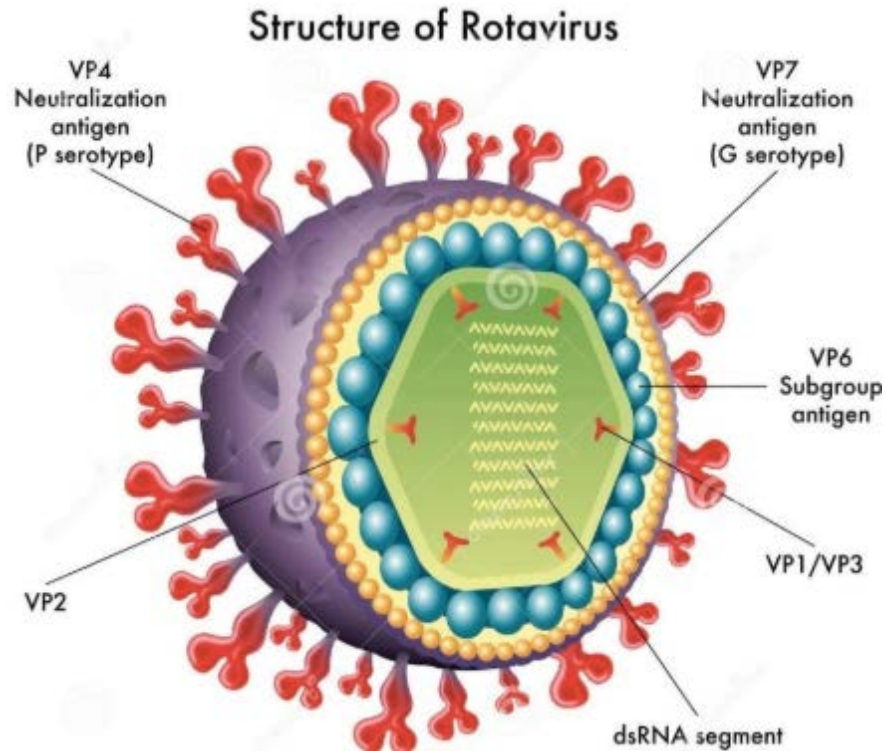
- Whole genome classification of RV.
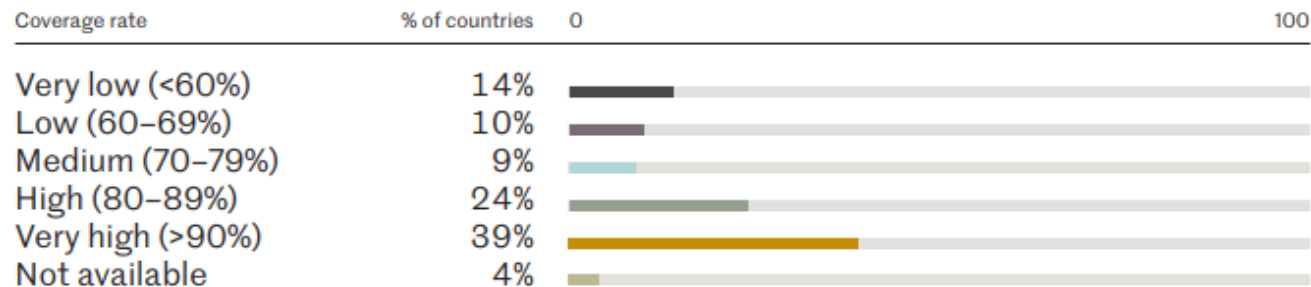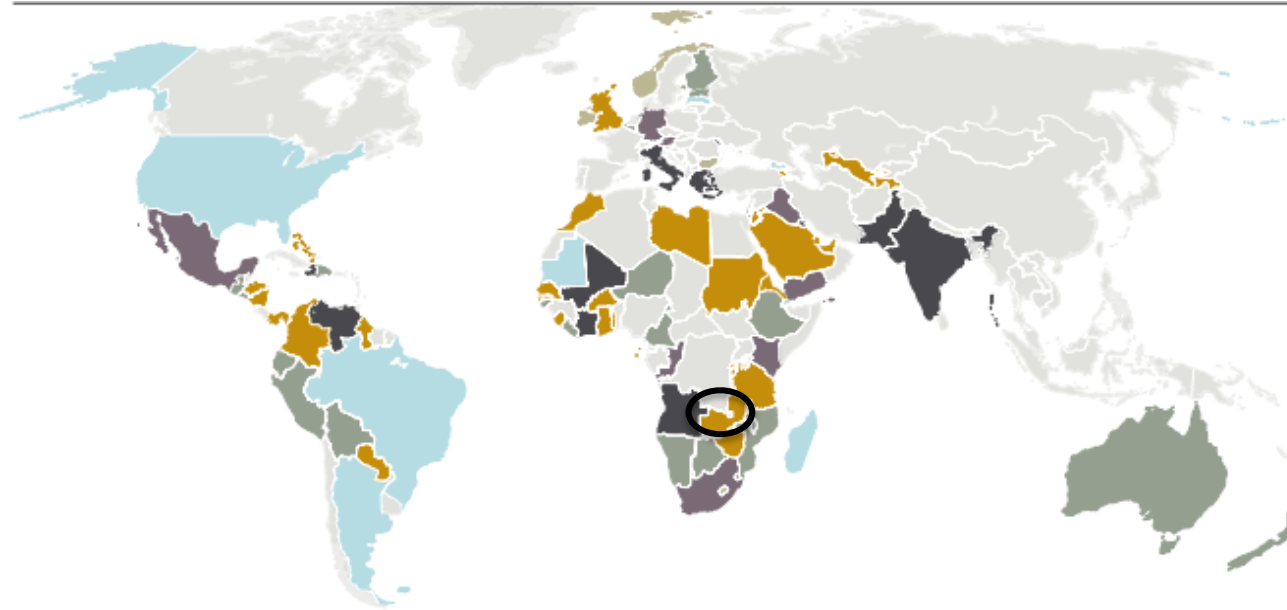
- Demonstrate origin of strains, reassortment events, zoonosis.

- Ability to determine genetic relationship among RV strains.

- Assess impact of vaccine pre and post-vaccine introduction.

VP7	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5
Gx	Px	Ix	Rx	Cx	Mx	Ax	Nx	Tx	Ex	Hx

Study rationale and motivation

- WHO/AFRO in partnership with UFS-NGS unit.
- Zambia rolled out Rotarix® in Nov 2013.

Coverage rate	% of countries	0	100
Very low (<60%)	14%		
Low (60–69%)	10%		
Medium (70–79%)	9%		
High (80–89%)	24%		
Very high (>90%)	39%		
Not available	4%		

2017-WHO-UNICEF Rotavirus vaccine coverage.

Aim

To perform **whole genome characterization** of rotavirus strains collected during pre- and post-rotavirus vaccination introduction in Zambia.

Objectives

- To perform **whole genome sequencing** of rotavirus strains on a maximum of 150 specimens from Zambia.

- Conduct **phylogenetic analysis** of rotavirus strains which determine the circulation of rotavirus vaccine strains.

- Assess the data to determine whether there is evidence of **vaccine pressure** to guide further analysis.

Materials and methods

Stool collection → **G/P typing** → **Whole genome sequencing**

Zambia

SAMRC Diarrhoeal Pathogens Research Unit

Next Generation Sequencing Unit

Quantification of viral RNA

cDNA synthesis and quantification

DNA library preparation and validation

Normalization and pooling of samples

MiSeq™ sequencing

Data analysis

Raw reads in fastq format files

Genome assembly

Genotyping

Sequence and phylogenetic analysis

Protein modeling

Results
Whole genome constellations

Table 1:Whole genome constellations of pre-vaccine Zambian RV strains

No. of samples (37)	Strains	VP7	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5
5	G1P[8]	G1	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
2	G9P[6]	G9	P[6]	I1	R1	C1	M1	A1	N1	T1	E1	H1
6	G9P[8]	G9	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
6	G12P[6]	G12	P[6]	I1	R1	C1	M1	A1	N1	T1	E1	H1
5	G2P[4]	G2	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
10	G2P[6]	G2	P[6]	I2	R2	C2	M2	A2	N2	T2	E2	H2
2	G8P[4]	G8	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
1	G8P[6]	G8	P[6]	I2	R2	C2	M2	A2	N2	T2	E2	H2

Table 2: Whole genome constellations of post-vaccine Zambian RV strains

No of samples (85)	Strains	VP7	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5
25	G1P[8]	G1	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
9	G9P[8]	G9	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
3	G12P[8]	G12	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
31	G2P[4]	G2	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
14	G2P[6]	G2	P[6]	I2	R2	C2	M2	A2	N2	T2	E2	H2
2	G3P[6]	G3	P[6]	I2	R2	C2	M2	A2	N2	T2	E2	H2
1	G8P6]	G8	P[6]	I2	R2	C2	M2	A2	N2	T2	E2	H2

Table 3: Whole genome constellations of reassortant post-vaccine Zambian RV strains

No. of samples (5)	Strains	VP7	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5
2	G1P[8]	G1	P[8]	I1	R1	C1	M1	A1	N2	T1	E1	H1
1	G2P[4]	G2	P[4]	I2	R2	C2	M2	A2	N1	T2	E2	H2
1	G2P[8]	G2	P[8]	I2	R2	C2	M2	A2	N2	T2	E2	H2
1	G5P[6]	G5	P[6]	I1	R1	C1	M1	A8	N1	T1	E1	H1

Homologies

Gene segments	Nucleotide homology range(%)	Amino acid homology range(%)
VP4		
P4	99.6-100	95.9-100
P6	85.1-100	88.6-100
P8	87.7-100	75.0-100
VP7		
G1	76.1-100	80.0-100
G2	94.1-100	97.2-100
G3	97.8-100	97.8-100
G8	98.4-100	97.5-100
G9	91.1-100	94.9-100
G12	96.2-100	97.2-100
VP6		
Wa-like	95.5-100	98.5-100
DS-1-like	97.4-100	99.7-100

Gene segments	Nucleotide homology range(%)	Amino acid homology range(%)
VP1		
Wa-like	88.3-100	96.2-100
DS-1-like	93.7-100	97.9-100
VP2		
Wa-like	96.3-100	90.9-100
DS-1-like	96.4-100	96.9-100
VP3		
Wa-like	94.2-100	95.7-100
DS-1-like	96.4-100	96.9-100
NSP1		
Wa-like	76.1-100	80.3-100
DS-1-like	96.4-100	94.6-100

Gene segments	Nucleotide homology range(%)	Amino acid homology range(%)
NSP2		
Wa-like	82.1-100	89.9-100
DS-1-like	82.2-100	88.3-100
NSP3		
Wa-like	93.9-100	81.6-100
DS-1-like	97.3-100	98.4-100
NSP4		
Wa-like	85.5-100	89.7-100
DS-1-like	86.3-100	92.5-100
NSP5		
Wa-like	88.3-100	92.5-100
DS-1-like	90.1-100	95.1-100

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Phylogenetic analysis

Fig. 1. A phylogenetic tree drawn for the VP7 genes . The Zambian strains are in bold. The scale bar at the bottom indicates a genetic distance of 0.05 nucleotide substitutions per site.
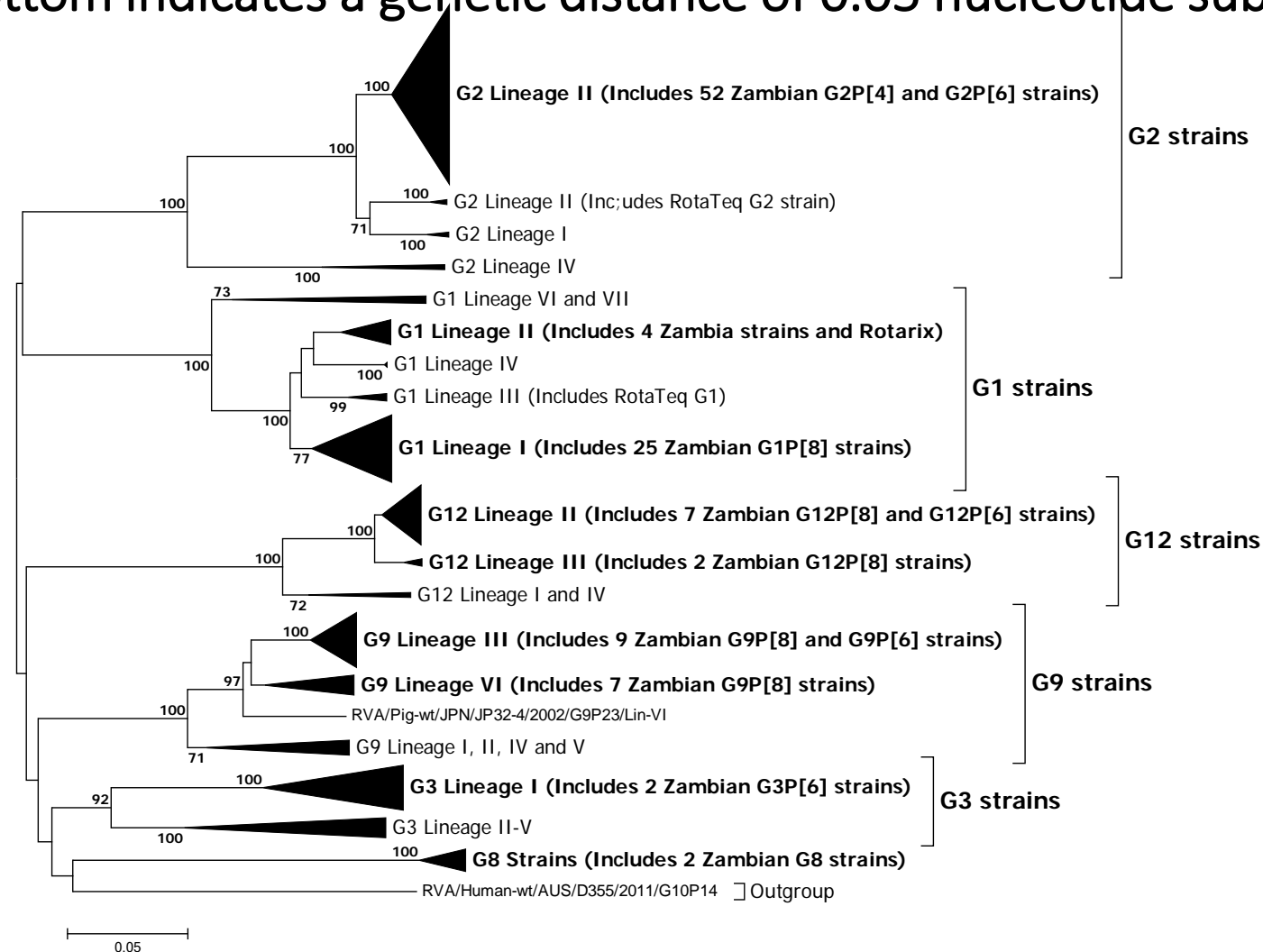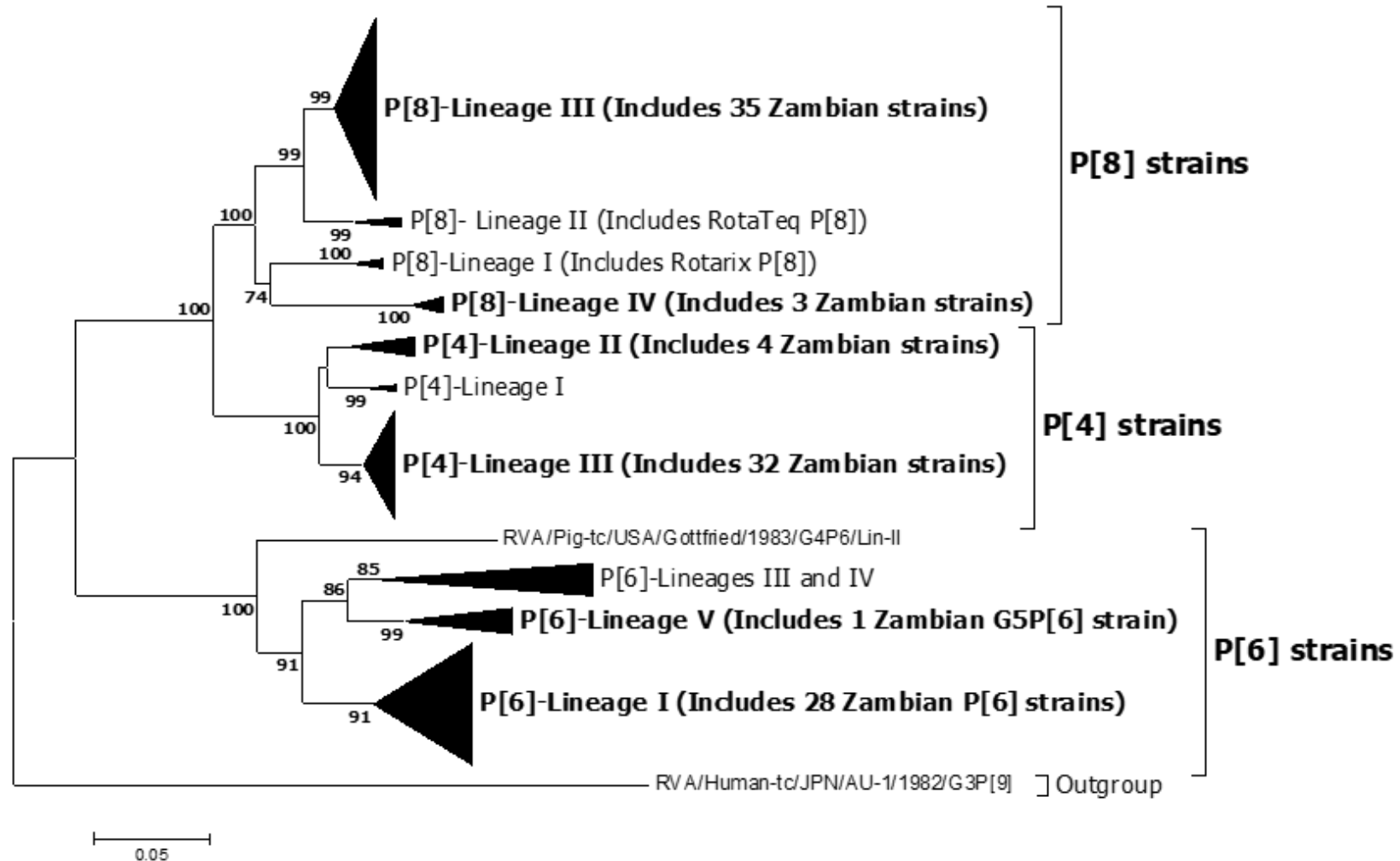
Phylogenetic analysis

Fig. 2 A phylogenetic tree drawn for the VP4 genes . The Zambian strains are in bold. The scale bar at the bottom indicates a genetic distance of 0.05 nucleotide substitutions per site.

Antigenic site analysis

Figure 3. Alignment of antigenic residues in VP7 between the strains contained in Rotarix® and RotaTeq ® and strains circulating in Zambia pre- and post vaccine era.

	7-1a														7-1b						2-Jul								
	87	91	94	96	97	98	99	100	104	123	125	129	130	291	201	211	212	213	238	242	143	145	146	147	148	190	217	221	264
RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P[8]	T	T	N	G	E	W	K	D	Q	S	V	V	D	K	Q	N	V	D	N	T	K	D	Q	N	L	S	M	N	G
RVA/Vaccine/USA/RotaTeq-WI79-9/1992/G1P[5]	T	T	N	G	D	W	K	D	Q	S	V	V	D	K	Q	N	V	D	N	T	K	D	Q	S	L	S	M	N	G
RVA/Human-wt/ZAM/UFS-NGS-MRC-DPRU16669/2016/G1P[8]	T	T	N	G	E	W	K	D	Q	S	V	V	D	K	Q	N	V	D	N	T	K	D	Q	D	L	S	M	N	G
RVA/Human-wt/ZAM/UFS-NGS-MRC-DPRU1380/2013/G1P[8]	T	N	N	G	E	W	R	D	Q	N	V	V	D	R	Q	N	V	D	N	T	K	D	Q	N	L	S	M	N	G
RVA/Human-wt/ZAM/UFS-NGS-MRC-DPRU2500/2011/G1P[8]	T	T	S	G	E	W	K	D	Q	N	V	V	D	R	Q	N	V	D	N	T	K	D	Q	N	L	S	T	N	G
RVA/Human-wt/ZAM/UFS-NGS-MRC-DPRU2502/2011/G1P[8]	T	T	S	G	E	W	K	D	Q	N	V	V	D	R	Q	N	V	D	N	T	K	D	Q	N	L	S	T	N	G
RVA/Human-wt/ZAM/UFS-NGS-MRC-DPRU4146/2010/G1P[8]	T	T	S	G	E	W	K	D	Q	N	V	V	D	R	Q	N	V	D	N	T	K	D	Q	N	L	S	T	N	G
RVA/Human-wt/ZAM/UFS-NGS-MRC-DPRU4741/2014/G1P[8]	T	T	S	G	E	W	K	D	Q	N	V	V	D	R	Q	N	V	D	N	T	K	D	Q	N	L	S	T	N	G
RVA/Human-wt/ZAM/UFS-NGS-MRC-DPRU4743/2014/G1P[8]	T	T	S	G	E	W	K	D	Q	N	V	V	D	R	Q	N	V	D	N	T	K	D	Q	N	L	S	T	N	G
RVA/Human-wt/ZAM/UFS-NGS-MRC-DPRU4760/2014/G1P[8]	T	T	S	G	E	W	K	D	Q	N	V	V	D	R	Q	N	V	D	N	T	K	D	Q	N	L	S	T	N	G
RVA/Human-wt/ZAM/UFS-NGS-MRC-DPRU4762/2014/G1P[8]	T	T	S	G	E	W	K	D	Q	N	V	V	D	R	Q	N	V	D	N	T	K	D	Q	N	L	S	T	N	G
RVA/Human-wt/ZAM/UFS-NGS-MRC-DPRU4777/2014/G1P[8]	T	T	S	G	E	W	K	D	Q	N	V	V	D	R	Q	N	V	D	N	T	K	D	Q	N	L	S	T	N	G
RVA/Human-wt/ZAM/UFS-NGS-MRC-DPRU4786/2014/G1P[8]	T	T	S	G	E	W	K	D	Q	N	V	V	D	R	Q	N	V	D	N	T	K	D	Q	N	L	S	T	N	G
RVA/Human-wt/ZAM/UFS-NGS-MRC-DPRU4816/2014/G1P[8]	T	T	S	G	E	W	K	D	Q	N	V	V	D	R	Q	N	V	D	N	T	K	D	Q	N	L	S	T	N	G
RVA/Human-wt/ZAM/UFS-NGS-MRC-DPRU4825/2014/G1P[8]	T	T	S	G	E	W	K	D	Q	N	V	V	D	R	Q	N	V	D	N	T	K	D	Q	N	L	S	T	N	G
RVA/Human-wt/ZAM/UFS-NGS-MRC-DPRU4834/2014/G1P[8]	T	T	S	G	E	W	K	D	Q	N	V	V	D	R	Q	N	V	D	N	T	K	D	Q	N	L	S	T	N	G
RVA/Human-wt/ZAM/UFS-NGS-MRC-DPRU9523/2015/G1P[8]	T	T	S	G	E	W	K	D	Q	N	V	V	D	R	Q	N	V	D	N	T	K	D	Q	N	L	S	T	N	G
RVA/Human-wt/ZAM/UFS-NGS-MRC-DPRU13232/2016/G1P[8]	T	T	S	G	E	W	K	D	Q	N	V	V	D	R	Q	N	V	D	N	T	K	D	Q	N	L	S	T	N	G
RVA/Human-wt/ZAM/UFS-NGS-MRC-DPRU13295/2016/G1P[8]	T	T	S	G	E	W	K	D	Q	N	V	V	D	R	Q	N	V	D	N	T	K	D	Q	N	L	S	T	N	G
RVA/Human-wt/ZAM/UFS-NGS-MRC-DPRU13296/2016/G1P[8]	T	T	S	G	E	W	K	D	Q	N	V	V	D	R	Q	N	V	D	N	T	K	D	Q	N	L	S	T	N	G
RVA/Human-wt/ZAM/UFS-NGS-MRC-DPRU13303/2016/G1P[8]	T	T	N	G	E	W	K	D	Q	S	V	V	D	K	Q	N	V	D	N	T	K	D	Q	D	L	S	M	N	G
RVA/Human-wt/ZAM/UFS-NGS-MRC-DPRU13341/2016/G1P[8]	T	T	S	G	E	W	K	D	Q	N	V	V	D	R	Q	N	V	D	N	T	K	D	Q	N	L	S	T	N	G
RVA/Human-wt/ZAM/UFS-NGS-MRC-DPRU13541/2016/G1P[8]	T	T	S	G	E	W	K	D	Q	N	V	V	D	R	Q	N	V	D	N	T	K	D	Q	N	L	S	T	N	G
RVA/Human-wt/ZAM/UFS-NGS-MRC-DPRU13543/2016/G1P[8]	T	T	S	G	E	W	K	D	Q	N	V	V	D	R	Q	N	V	D	N	T	K	D	Q	N	L	S	T	N	G
RVA/Human-wt/ZAM/UFS-NGS-MRC-DPRU13561/2016/G1P[8]	T	T	S	G	E	W	K	D	Q	N	V	V	D	R	Q	N	V	D	N	T	K	D	Q	N	L	S	T	N	G
RVA/Human-wt/ZAM/UFS-NGS-MRC-DPRU13565/2016/G1P[8]	T	T	S	G	E	W	K	D	Q	N	V	V	D	R	Q	N	V	D	N	T	K	D	Q	N	L	S	T	N	G
RVA/Human-wt/ZAM/UFS-NGS-MRC-DPRU13576/2016/G1P[8]	T	T	S	G	E	W	K	D	Q	N	V	V	D	R	Q	N	V	D	N	T	K	D	Q	N	L	S	T	N	G
RVA/Human-wt/ZAM/UFS-NGS-MRC-DPRU13593/2016/G1P[8]	T	T	S	G	E	W	K	D	Q	N	V	V	D	R	Q	N	V	D	N	T	K	D	Q	N	L	S	T	N	G
RVA/Human-wt/ZAM/UFS-NGS-MRC-DPRU13654/2014/G1P[8]	T	T	N	G	E	W	K	D	Q	S	V	V	D	K	Q	N	V	D	N	T	K	D	Q	D	L	S	M	N	G
RVA/Human-wt/ZAM/UFS-NGS-MRC-DPRU13670/2016/G1P[8]	T	T	N	G	E	W	K	D	Q	S	V	V	D	K	Q	N	V	D	N	T	K	D	Q	D	L	S	M	N	G

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Discussion

- Different strains circulating in pre and post-vaccination periods.
  - **Natural annual RV evolution mechanisms**.

- Emergence of distinct lineages or sub-lineages.
  - **Diverse evolutionary mechanisms (Kirkwood, 2010).**

- G2P[4] mono-reassortants carrying Wa-like NSP2 and G1P[8] mono-reassortant carrying DS-1-like NSP2.
  - **NSP2 induces heterotypic antibody responses (Kirkwood *et al*., 2008)**
  - **NSP2 likely target of immunological selective pressure (Bucardo *et al*., 2012)**

- **G5-P[6]-I1-R1-C1-M1-A8-T1-E1-H1** first time being reported in Africa.
  - **Clustered along porcine strains.**

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Conclusion

- **Different strains** circulating during pre and post-rotavirus vaccination periods in Zambia.

- **Reassortant strains** detected during post-vaccination period.

- **Strain surveillance at whole genome level** is warranted.
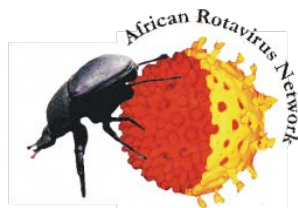
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Future /ongoing work

- **Protein modeling**

- Addressing remaining **Terms of References**
  - Link all the rotavirus whole sequence data to the cases and epidemiological/clinical information.

  - Develop a **strategic plan for future characterization** of rotavirus vaccine strains.

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THANK YOU!
DANKIE!
SIYABONGA!