Whole genome analysis of rare and/or novel rotavirus strains post-Rotarix® introduction in Zambia

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VIROLOGY

*"**Decide in your heart of hearts what really excites and challenges you, and start moving your life in that direction. Every decision you make, from what you eat to what you do with your time tonight, turns you into who you are tomorrow, and the day after that. Look at who you want to be, and start sculpting yourself into that person. You may not get exactly where you thought you'd be, but you will be doing things that suit you in a profession you believe in. Don't let life randomly kick you into the adult you don't want to become**."*

Chris Hadfield, Commander, Expedition 35, International Space Station

Dedication

This dissertation is dedicated to my late grandmother, my sister, my mother, and my fathers.

Declaration

I, Wairimu Makena Maringa, declare that the Master's Degree Research dissertation that I herein submit for the Master's Degree qualification in Medical Science with specialisation in Virology at the University of the Free State is my independent work, and that I have not previously submitted it for any other qualification at another institution of higher education or elsewhere.

4th June 2021

Wairimu Makena Maringa

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List of abbreviations/acronyms

Abbreviation/Acronym	Full form
(+) RNA	Positive-sense RNA
(-) RNA	Negative-sense RNA
aa	Amino acid
ACDH	Arthur Davidson Children's Hospital
AiCc	Akaike Information Criterion (corrected)
ARSN	African Rotavirus Surveillance Network
BLAST	Basic Local Alignment Tool
bp	Base pairs
cDNA	Complementary DNA
CIDRZ	Centre for Infectious Disease Research in Zambia
COVID-19	Coronavirus disease 2019
DDBJ	DNA Data Bank of Japan
DLP	Double-layered particle
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleoside triphosphates
DPRU	Diarrhoeal Pathogens Research Unit
dsRNA	Double-stranded RNA
EDTA	Ethylenediaminetetraacetic acid
EIA	Enzyme Immuno Assay
eIF	Eukaryotic translation initiation factor
ELISA	Enzyme-linked Immunosorbent Assay
EMBL	European Molecular Biology Laboratory
ER	Endoplasmic reticulum
FDA	Food and Drug Administration
GAVI	Global Alliance for Vaccines and Immunisation
gDNA	Genomic DNA
GISAID	Global Initiative on Sharing All Influenza Data
HS	High Sensitivity
HSREC	Health Sciences Research Ethics Committee
HT1	Hybridisation buffer
Ig	Immunoglobulin
LAT	Latex Agglutination Test
$LiCl_2$	Lithium chloride
LLR	Lanzhou lamb rotavirus vaccine
MERS	Middle East Respiratory Syndrome Coronavirus
mRNA	Messenger RNA
NaOH	Sodium hydroxide
NCBI	National Centre for Biotechnology Information
NGS	Next Generation Sequencing
NHGRI	National Human Genome Research Institute
nM	nanomolar
NPM	Nextera® PCR Master mix
NSP	Non-structural protein

NT	Neutralisation Tagment buffer
nt	Nucleotide
ORF	Open reading frame
ORS	Oral rehydration solution
PABP	Poly A binding protein
PAED	Programme for Awareness and Elimination of Diarrhoea
PAGE	Polyacrylamide gel electrophoresis
PC	Polymerase complex
pM	picomolar
PRF	Poliomyelitis Research Foundation
Q-score	Phred quality score
RCWG	Rotavirus Classification Working Group
RNA	Ribonucleic acid
RNase	Ribonuclease
RSB	Resuspension buffer
RV1	Rotarix®
RV5	RotaTeq®
RVA	Group A rotavirus
RVB	Group B rotavirus
RVC	Group C rotavirus
RVD	Group D rotavirus
RVE	Group E rotavirus
RVF	Group F rotavirus
RVG	Group G rotavirus
RVH	Group H rotavirus
RVI	Group I rotavirus
RVJ	Group J rotavirus
RT-PCR	Reverse transcriptase polymerase chain reaction
SARS-CoV-2	Severe acute respiratory syndrome Coronavirus 2
SMU	Sefako Makgatho University
ssRNA	Single-stranded RNA
TBE	Tris borate EDTA
TLP	Triple-layered particle
UFS-NGS	University of the Free State, Next Generation Sequencing Unit
UN	United Nations
UNICEF	United Nations International Children's Emergency Fund
UTH	University Teaching Hospital
ViPR	Virus Pathogen Resource
VP	Viral protein
WBG	World Bank Group
WGS	Whole genome sequencing
WHO	World Health Organization
WHO/AFRO	WHO Regional Office for Africa
WHO-RRL	WHO rotavirus Regional Reference Laboratory
ZMOH	Zambian Ministry of Health

Publications

Molecular Characterisation of a Rare Reassortant Porcine-Like G5P[6] Rotavirus Strain Detected in an Unvaccinated Child in Kasama, Zambia.

Maringa WM, Mwangi PN, Simwaka J, Mpabalwani EM, Mwenda JM, Peenze I, Esona MD, Mphahlele MJ, Seheri ML, Nyaga MM.

Pathogens 2020, 9(8), 663; https://doi.org/10.3390/pathogens9080663 - 17th August 2020.

Whole genome analysis of human rotaviruses reveals single gene reassortant rotavirus strains in Zambia.

Maringa WM, Simwaka J, Mwangi PN, Mpabalwani EM, Mwenda JM, Mphahlele MJ, Seheri ML, Nyaga MM.

Journal: Viruses. Manuscript ID: viruses-1264641. Under review: submitted 1st June 2021.

Conference presentation

Presentation title: Whole genome sequencing identifies idiosyncratic changes post-rotavirus vaccine introduction in Zambia.

W.M Maringa, P.N Mwangi, M.T Mogotsi, S.P Rasebotsa, J. Simwaka, N.B Magagula, K. Rakau, M.L Seheri, M.J Mphahlele, J.M Mwenda, M.M Nyaga.

Type of presentation: Oral presentation.

Name of Conference: Virology Africa 2020.

Location: Radisson Blu Hotel Waterfront, Cape Town, South Africa.

Date: 10th to 14th February 2020.

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Wairimu M. Maringa, Julia Simwaka, Evans M. Mpabalwani, Martin M. Nyaga

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Date: 26th to 27th August 2021

Abstract

Background

Group A rotaviruses (RVA) cause acute diarrhoea in children under the age of five years. In sub-Saharan Africa, limited studies have been conducted on RVA at whole genome level, particularly post-vaccine implementation. Even though strain oscillation has been documented in Zambia since the countrywide rollout of Rotarix® vaccine in 2013, the approach primarily utilised conventional methods to characterise the outer capsid proteins (VP7 and VP4) of RVA strains. However, analysing the remaining genome-encoded proteins contributes to a better understanding of mechanisms driving genetic diversity in rotaviruses. This study undertook whole genome analysis of the rare and/or novel reassortant strains from Zambia during the post-vaccine era.

Methods

Archived samples selected from a WHO-Regional Office for Africa surveillance project (n=133) were sent to the Next Generation Sequencing unit, University of the Free State (Bloemfontein, South Africa). The surveillance project aimed to characterise RVA at a whole genome level in Zambia. These samples had been conventionally genotyped at the WHO-Regional Reference Laboratory located in the Diarrhoeal Pathogens Research Unit at the Sefako Makgatho University (Pretoria, South Africa). The transfer was facilitated by a Material Transfer Agreement (MTA:NGS Unit, UFS(1)).

Viral RNA was extracted from the samples, followed by cDNA synthesis and DNA library preparation. Whole genome sequencing was done on the Illumina® MiSeq to generate 300 bp x 2 paired end reads. FASTQ reads were obtained from the MiSeq, *de novo* assembled on Geneious® Prime and subjected to a quality trim before the genotype constellations were determined using the Virus Pathogen Database and Analysis Resource (ViPR). Strains from the post vaccine-period (2013-2016) that exhibited any form of atypical characteristics were selected for further investigation. The genotypes of the strains (n=5) were confirmed using BLAST, followed by pairwise alignments and bioinformatic analysis on various tools and software.

Results

A rare reassortant porcine-like human strain (RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4724/2014/G5P[6]) with the constellation G5-P[6]-I1-R1-C1-M1-A8-N1-E1-H1 typically found in porcine strains was identified in a sample collected from a child with gastroenteritis who resided in Kasama, Zambia. All the genes of this strain were seen to cluster only among porcine and putative porcine-

like human strains on a phylogenetic level. The sequence identities (95.7%-98.0%) were consistent with the phylogenetic relationships observed. Moreover, reassortment analysis demonstrated the genetic similarity between the Zambian G5P[6] strain and other porcine-like human strains, thus acknowledged that the strain may have arisen due to animal-human interactions.

Furthermore, four reassortant strains were identified in samples taken from four children who resided in different areas of Ndola and Lusaka, Zambia. The children experienced moderate to severe gastroenteritis. Two strains had the constellation G1-P[8]-I1-R1-C1-M1-A1-N2-T1-E1-H1, while the other two strains had the constellations G2-P[8]-I2-R2-C2-M2-A2-N2-T2-E2-H2 and G2-P[4]-I2-R2-C2-M2-A2-N1-T2-E2-H2. One of the strains (RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4749/2014/G2P[8]) was divergent from other global reference strains in the VP4 and VP1 encoding genes on both nucleotide and amino acid level. Moreover, several amino acid changes were observed in the antigenic sites of the VP4 of the divergent Zambian strain in relation to Rotarix® and global reference strains.

Conclusion

Our findings add to the growing global evidence of strains generated through reassortment and/or zoonotic transmission. Further, current rotavirus vaccines do not contain genotypes such as the G5 and P[6], and such animal-like strains may have an impact on vaccines. These findings emphasise the need for continuous active surveillance and analysis of circulating RVA in Zambia at whole genome level.

Key words

Constellation, genogroup, next generation sequencing, phylogenetic, rare strain, reassortment, Rotarix®, rotavirus, whole genome, Zambia.

Chapter one: Introduction

1.1. Preamble

Group A rotaviruses (RVA) are among the most common causes of viral-induced diarrhoea and mortality in children under five years around the world (Clark *et al*., 2017; Tate *et al*., 2016). Approximately 215,000 rotavirus-related deaths occurred in children under five years globally in 2013 (Tate *et al*., 2016). While the virus presents a widespread distribution regardless of environmental conditions or socioeconomic status, the outcome and consequences of rotavirus disease differ significantly between high-income and low-income countries (O'Ryan *et al*., 2005; Troeger *et al*., 2018). Higher mortality is seen in developing countries, due to factors such as, but not limited to, poor access to medical care, poor sanitation, lack of access to non-contaminated water, overcrowding, and malnutrition (O'Ryan *et al*., 2005).

To combat the disease burden associated with rotavirus infections, Rotarix® and RotaTeq® were licensed for use and are currently in use in more than 100 countries globally including Zambia, with most low-income countries having financial backing from the Global Alliance for Vaccines and Immunisation (GAVI) (GAVI, 2020; IVAC, 2021; Rota Council, 2020a). Two additional vaccines, ROTAVAC® and ROTASIIL®, were later licensed and prequalified for use in India and a few selected countries (IVAC, 2021; WHO, 2021a). Post-licensure studies have revealed significant reductions in rotavirus-associated hospital admissions and deaths (Aliabadi *et al*., 2019; Burnett *et al*., 2020; Shah *et al*., 2017; Troeger *et al*., 2018). However, rotaviruses are still of great clinical and epidemiological importance especially in developing countries, where rotavirus-associated hospitalisations and deaths continue to be significantly reported (Kim *et al*., 2017).

In Zambia, diarrhoea was found to be the third major cause of mortality in children below the age of five, after malaria and pneumonia, whereby approximately ten million diarrhoeal episodes were reported in 2009 (ZMOH, 2009). Up to a third of diarrhoeal cases are as a result of rotavirus infection (Chilengi *et al*., 2015). Furthermore, rotavirus was found to be the number one pathogen according to a study conducted to investigate enteric pathogens responsible for moderate to severe diarrhoea in Zambian children following vaccine introduction (Chilengi *et al*., 2015; Chisenga *et al*., 2018). However, following the nationwide rollout of Rotarix® vaccine in 2013, significant declines in rotavirus-associated hospitalisations and mortality were observed (Mpabalwani *et al*., 2016, 2018).

With the establishment of rotavirus surveillance systems such as the African Rotavirus Surveillance Network (ARSN) (Mwenda *et al*., 2014), the development of sophisticated sequencing technologies (Goodwin *et al*., 2016; Heather and Chain, 2016; Metzker, 2010) and analysis software (Frazer *et al*., 2004; Kearse *et al*., 2012; Tamura *et al*., 2013), new insights into the landscape of circulating rotaviruses have

been gained. Whole genome sequence analysis led to the discovery that human RVA can be divided into genogroups based on their most likely host species of origin: Wa-like (G1P[8] prototype with a genotype 1 constellation), DS-1-like (G2P[4] prototype with a genotype 2 constellation) and AU-1-like (G3P[9] prototype with a genotype 3 constellation) (Heiman *et al*., 2008; Matthijnssens *et al*., 2008a; Nakagomi *et al*., 1989). Human strains belonging to these genogroups are believed to have common ancestors with animal rotaviruses with associations to porcine, bovine, and feline strains, respectively (Matthijnssens *et al*., 2008a).

Close interactions between animals and humans, as well as increased worldwide migration can introduce new virus variants into new host populations through mechanisms such as zoonotic transmission and/or reassortment (Jones *et al*., 2008; Pybus *et al*., 2015). Post-vaccine epidemiological surveillance studies indicated that strains with G-type G1-G4, G9 and G12, and P-type P[4] and P[8] are the most predominant cause of rotavirus disease in humans globally (Dóró *et al*., 2014; Santos and Hoshino, 2005). These predominant strains may cause almost 100% of infections during rotavirus seasons in developed countries, with a few cases as a result of uncommon G- and P- types (Bányai *et al*., 2012; Dóró *et al*., 2014; Iturriza-Gómara *et al*., 2011; Payne *et al*., 2011). In contrast, novel or rare G-P types, or strains with mixed-gene constellations are frequently documented in developing countries. These strains likely occur primarily due to reassortment events, either between genogroups or between animal and human strains (Dóró *et al*., 2015; Jere *et al*., 2018; Martella *et al*., 2010; Mwangi *et al*., 2020; Nyaga *et al*., 2018). For instance, rare G5P[7] and G5P[8] strains were identified in Cameroon (Esona *et al*., 2004, 2009). Additionally, surveillance carried out by the ARSN in the post-vaccine era noted that unusual G1P[6], G2P[6], G3P[6], and G8P[6] strains circulated in sub-Saharan African countries at significant frequencies (Seheri *et al*., 2018). Rare strains have a prevalence of less than 0.2%, whereas unusual strains have a prevalence of 0.2-2.0% (Bányai *et al*., 2012). However, to validate this assumption, it is important to carry out molecular characterisation of these strains by performing whole genome sequencing and analysis of the sequenced genes in comparison to reference-selected data from the GenBank.

Furthermore, several intergenogroup reassortant strains have been documented in various countries globally (Fujii *et al*., 2014; Ghosh *et al*., 2011; Komoto *et al*., 2016; Nakagomi *et al*., 2017; Sadiq *et al*., 2019; Yamamoto *et al*., 2014; Zeng *et al*., 2020). Most human RVA with G1P[8], G3P[8], G4P[8], and G9P[8] bear the Wa-like constellation, while G2P[4] strains have the DS-1-like constellation (Matthijnssens and Van Ranst, 2012). Intergenogroup reassortment is hypothesised to occur among locally circulating strains, resulting in strains bearing both Wa-like and DS-1-like gene segments and unusual G-P combinations such

as G1P[4] and G2P[8] (Dóró *et al*., 2015; Gentsch *et al*., 2005). For example, a G2P[8] strain with a DS-1-like constellation was identified in Thailand (Komoto *et al*., 2016).

In Zambia, G-types G1 and G2 along with P-types P[4], P[6], and P[8] were predominant in the post-vaccine period. Unusual G8P[4] and G8P[6] strains were also observed albeit at low frequencies (Simwaka *et al*., 2018). In lieu of the documentation of such strains, whole genome sequencing and analysis is necessary to determine the constellations and phylogenetic attributes of such strains and to understand the evolutionary processes involved. Furthermore, there is limited knowledge on data relevant to circulating strains at a whole genome level in Zambia post-vaccine introduction. To address this knowledge gap, this study identified and analysed reassortant strains at a whole genome level and determined their genome constellations, phylogenetic attributes in relation to other strains, and reassortment events that occurred.

1.2. Problem statement

Despite an upsurge of new data and reports on RVA from epidemiological studies worldwide, majority of these data and reports were based on conventional genotyping techniques such as reverse transcriptase polymerase chain reaction (RT-PCR), which only provides information on the outer capsid gene segments, VP7 (G-type) and VP4 (P-type). As a result, limited knowledge still exists on RVA at a whole genome level, especially in many developing countries. Analysis that focuses only on the outer capsid proteins leaves out important information about the rest of the gene segments, thus may not be sufficient to determine the overall genetic diversity, genomic relatedness, and mechanisms of genetic diversity such as point mutations, interspecies transmission, and/or reassortment that may have occurred in RVA strains.

Global RVA studies conducted in children have identified G1-G6, G8-G12, G14, G20, and G26 G-types in combination with P[1]-P[11], P[14], P[15], P[19], P[24], P[25], P[28], and P[40] P-types (Bányai *et al*., 2012; Do *et al*., 2017; Dóró *et al*., 2014, 2015; Mandal *et al*., 2016; Medici *et al*., 2015; Rahman *et al*., 2003; Rojas *et al*., 2019; Shoeib *et al*., 2020; Takatsuki *et al*., 2019). Several G-types and P-types, such as the G5 and the P[6], are common to animals (Dóró *et al*., 2015; Ghosh and Kobayashi, 2014; Martella *et al*., 2010). Through conventional genotyping, the P[8] in combination with G1, G3, G4, G9, and G12, and the G2 in combination with P[4] were demonstrated to be the most common genotype combinations worldwide (Bányai *et al*., 2012; Gentsch *et al*., 2005; Matthijnssens *et al*., 2010; Santos and Hoshino, 2005).

Because of their segmented genome, rotaviruses are vulnerable to the individual mixing of gene segments during co-infection (Estes and Greenberg, 2013; Gentsch *et al*., 2005; Ghosh and Kobayashi, 2011; Kirkwood, 2010; Santos and Hoshino, 2005). Conventional genotyping-based studies have sporadically

reported unusual, rare and/or novel reassortant strains in human populations worldwide following RVA vaccine implementation, most notably in sub-Saharan African countries (Gikonyo *et al*., 2020; Guerra *et al*., 2019; João *et al*., 2020; Lartey *et al*., 2018; Letsa *et al*., 2019; Mhango *et al*., 2020; Seheri *et al*., 2018). For instance, the globally prevalent G9 and G12 strains are thought to have emerged in human populations through reassortment (Laird *et al*., 2003; Rahman *et al*., 2007; Unicomb *et al*., 1999). However, in comparison to other parts of the world, the number of whole genome studies conducted in sub-Saharan Africa is lower (Jere *et al*., 2018; Mokoena *et al*., 2020; Mwangi *et al*., 2020; Rasebotsa *et al*., 2021; Strydom et al., 2019; Wandera *et al*., 2019). Simwaka *et al*. (2018) employed conventional genotyping in Zambia to identify strains that circulated post-vaccine implementation. However, there is a scarcity of whole genome data of Zambian RVA. In order to address the whole genome data gap in Zambia, as well as three terms of reference for the technical service agreement between World Health Organization (WHO) and the University of the Free State-Next Generation Sequencing unit (UFS-NGS) (**Appendix 1**), this study utilised next generation sequencing (NGS) techniques and existing bioinformatic tools to analyse and characterise reassortant strains that circulated in Zambia after Rotarix® implementation on a whole genome level.

1.3. Significance of the study

In surveillance programs worldwide and sub-Saharan Africa, G- and P-typing has been deemed sufficient for monitoring of RVA epidemiology. However, given that rotavirus has eleven genome segments, approximately 82 percent of the rotavirus genome is often not analysed by investigating just two out of eleven segments, thus restricting our understanding of the full genome constellation and phylogenetic attributes of the other segments, as well as the evolutionary processes that could have occurred across the entire genome. Whole genome sequencing and analysis using various bioinformatic tools is yet to be adapted for surveillance analysis of RVA circulating in Zambia. This study utilised an Illumina® NGS technique to adequately characterise novel and/or rare reassortant RVA that circulated in Zambia on a whole genome level, thus overcoming the inadequacy of G-P typing in providing insights into the constellation, phylogenetic and genetic diversity of circulating RVA strains. In addition, sequenced data was submitted to the public database, the National Centre for Biotechnology Information (NCBI), and will provide additional reference sequence data for future genomic studies on strains from Zambia.

1.4. Research aim

The aim of this study was to determine the sequence and phylogenetic attributes of rare and/or novel reassortant strains post-Rotarix® introduction in Zambia.

1.5. Research objectives

1) To analyse the whole genome constellations of rare and/or novel rotavirus strains after vaccine introduction in Zambia.
2) To determine phylogenetic relationships of rare and/or novel reassortant genome constellations detected in Zambia in the post-vaccine era.

1.6. Dissertation organisation

This dissertation consists of five chapters each prefaced with a preamble that provides an overview of the chapter and how it relates to the aims and objectives of the study.

Chapter one provides a general introduction to rotavirus, problem statement, the aims and objectives and significance of the study.

Chapter two provides an extensive review of current literature on rotavirus epidemiology, classification and structure, vaccines, strain diversity and mechanisms of evolution relevant to this dissertation.

Chapter three is structured to address both objectives of this study as an original article published online in *Pathogens* (Available online, 17th August 2020, https://doi.org/10.3390/pathogens9080663), which reports the first rare reassortant porcine-like G5P[6] strain in Zambia. The whole genome constellation was determined and the relatedness to other strains was assessed. The methodology section in this chapter has been expanded to include the general materials and methods used as well as the specific aspects of analysis performed.

Chapter four is in the format of a publishable article that reports on four reassortant strains identified in Zambia. The article has been submitted to the special issue 'Gastroenteritis Viruses 2021' of the journal Viruses (manuscript number: viruses-1264641). The methodology section only includes aspects of analysis that are different to what was described in chapter three.

Chapter five is a general summary of the dissertation.

Chapter two: Literature review

2.1. Preamble

This chapter gives an overview of rotaviruses based on what is relevant to the topic of this dissertation. A significant proportion of this chapter will focus on rotavirus epidemiology, the characteristics of rotaviruses, rotavirus vaccines and the impact of vaccination, mechanisms of genetic diversity of rotaviruses with a focus on reassortment, as well as studies that performed whole genome sequencing to investigate reassortment in rotavirus, and a review of rotavirus research conducted in Zambia.

2.2. Rotavirus discovery

Rotaviruses were discovered in Australia by Dr Ruth Bishop and colleagues in 1973 through electron microscopy. The virions were observed in the duodenal mucosa of children who had acute gastroenteritis. The wheel-like structure of the virus led to the term 'Rotavirus' whereby rota means wheel in Latin (Bishop, 2009; Bishop *et al*., 1973, 1974; Flewett *et al*., 1973). The virus was seen to be similar to those viruses that had already been identified in neonatal mice, calves, and vervet monkeys (Adams and Kraft, 1963; Malherbe and Harwin, 1963; Mebus *et al*., 1969). Rotaviruses are now recognised as the causative agent of diarrhoea in the young ones of many mammals and avian species (Ramig, 2004).

2.3. Epidemiology

2.3.1. Burden of rotavirus diarrhoeal disease (pre-vaccine era)

Diarrhoeal diseases, particularly in developing countries, are a significant cause of morbidity and mortality in young children. RVA is the number one viral causative agent of diarrhoea in children globally. Every child experience diarrhoea due to RVA infection by the age of five, irrespective of socio-economic status hence rotaviruses are commonly referred to as 'democratic viruses' (Hoshino and Kapikian, 2000; Parashar *et al*., 1998, 2003; PATH, 2018a).

Between 1986-2000, RVA was responsible for about 111 million diarrhoeal episodes, 2 million hospitalisations, and 440,000 deaths globally, of which 90% of these deaths occurred in sub-Saharan Africa (Parashar *et al*., 2003; Sanchez-Padilla *et al*., 2009; Tate *et al*., 2012). Hospital-based surveillance across African countries showed that RVA accounted for 21-56% of acute diarrhoeal hospitalisations (Abebe *et al*., 2014; Enweronu-Laryea *et al*., 2014; Khagayi *et al*., 2014; Mayindou *et al*., 2016; Nakawesi *et al*., 2010; Weldegebriel *et al*., 2018). The possible explanations for high mortality rates in low-income countries include limited access to hydration therapy, limited access to healthcare, and comorbid conditions such as malnutrition (Crawford *et al*., 2017). When determining the disease burden, medical and socioeconomic factors should be considered. Rotavirus-related events result in an increase in medical

expenses (hospital visits, hospital stays, medication, and laboratory diagnostic tests) and non-medical expenses (transportation to and from the hospital), family disruptions such as loss of work to take care of the sick child, parental stress, and reduced quality of life (Gray *et al*., 2008; Rheingans *et al*., 2009).

2.3.2. Seasonality

The seasonality of RVA infections varies by geographical regions (Patel *et al*., 2013). It is speculated that factors such as genotype diversity, geographical location and climate all influence the seasonality of rotavirus infections (Patel *et al*., 2013). However, no unifying explanation as to why certain regions experience year-round disease while other regions experience seasonal disease has been established (Patel *et al*., 2013). In temperate climate regions, rotavirus infection is highly seasonal, with the highest activity occurring during winter months (Pitzer *et al*., 2009). In contrast, regions with tropical climates experience rotavirus infections throughout the year, with peaks during the cold and dry months (Levy *et al*., 2009).

2.3.3. Clinical features

Rotavirus is highly contagious and requires as little as 100 viral particles to actuate infection (Graham *et al*., 1987). The virus is shed in the stools of diseased children and spreads via the faecal-oral route (CDC, 2019). Rotaviruses may also be transmitted spatially through respiratory droplets (Fragoso *et al*., 1986). Further, rotavirus has been shown to escape the intestinal tract according to a study conducted by Blutt *et al*. (2003), whereby rotavirus antigen was present in the serum of infected children. Rotavirus was also found in the cerebrospinal fluid of a child who presented with symptoms of central nervous disease associated rotavirus gastroenteritis (Iturriza-Gómara *et al*., 2002). Additionally, rotavirus infection has been associated with encephalitis and autoimmune diseases such as type 1 diabetes and celiac disease (Ballotti and de Martino,2007; Honeyman *et al*., 2000; Ihira *et al*., 2020; Stene *et al*., 2006).

Rotavirus replicates in the enterocytes of the small intestine, causing permanent cell damage and prevents the effective uptake of nutrients and water. The secretory crypt cells proliferate to compensate for the damage caused by the virus which leads to the secretion of fluids into the lumen of the gut. This excessive fluid secretion manifests in the form of diarrhoea (Parashar *et al*., 1988; Vesikari *et al*., 1984; Widdowson *et al*., 2005). Illness occurs after 1-3 days in the form of non-bloody watery diarrhoea, vomiting, fever, dehydration, and death in very extreme cases (CDC, 2019; Kapikian *et al*., 1983; Rodriguez *et al*., 1977). These symptoms typically resolve within 4-7 days (Cortese and Parashar, 2009).

Most symptomatic infections have been reported to occur between the age of three months and two years, with a peak incidence occurring between the age of seven and 15 months (Dennehy, 2013). Neonatal and adult infections are less common and often have no symptoms (Anderson and Weber, 2004; Bishop *et al*., 1983). The relatively mild illness in adults may be as a result of the previously acquired immunity (Hrdy, 1987). However, severe disease can still occur especially in immunocompromised patients (Anderson and Weber, 2004). Neonates and infants, on the other hand, may be protected due to maternal antibodies (Chan *et al*., 2011; Haffejee, 1991).

2.3.4. Immunity to rotavirus infections

The ubiquitous nature of rotavirus can be attributed to the short incubation period of the virus which allows infection to be established before proper immune responses are generated, which then results in shedding of the virus in excessive amounts, more than what is required to produce infection (Franco and Greenberg, 2009). Due to this, individuals can contract rotavirus disease more than once in their lifetime, as it is difficult for hosts to generate 'sterile immunity' against the virus. However, immunity increases with each episode of infection thus subsequent infections are usually mild or even asymptomatic (Franco *et al*., 2006).

The protective effect of natural infection was first observed in a three-year study conducted on new-borns (Bishop *et al*., 1983). Although neonatal rotavirus infection did not provide protection against re-infection, it was observed that disease was much less severe when re-infection occurred (Bishop *et al*., 1983). Further, a study on Mexican infants demonstrated that protection against severe disease increased with each rotavirus infection. The infants appeared to acquire complete immunity against severe disease after two rotavirus infections (Velázquez *et al*., 1996). A similar study was conducted on an Indian birth cohort, which demonstrated about 80% protection against rotavirus disease after three infections (Gladstone *et al*., 2011). Other studies conducted in Guinea-Bissau and Egypt also demonstrated the protective effect of natural infection (Fischer *et al*., 2002; Reves *et al*., 1989).

Innate immune responses are triggered rapidly in a primary infection (Angel *et al*., 2012). Various studies in humans and animal models (rats, mice, pigs, rabbits, lambs, and calves) have been conducted to understand immune responses (mucosal and systemic) after rotavirus infection (Desselberger and Huppertz, 2011). In a study that used mice, it was shown that rotavirus induced type I and type III interferon responses that decrease viral replication (Angel *et al*., 2012; Pott *et al*., 2011).

On the other hand, adaptive immune responses are usually brought about after innate immune responses or in a secondary infection. In the case of rotavirus, it is mainly a mucosal response (Franco *et al*., 2006; Uhnoo *et al*., 1988). The main antibody found on mucosal surfaces such as the gastrointestinal, urogenital, and respiratory tracts is the secretory immunoglobulin (Ig) A (Corthésy and Spertini, 1999; Glass *et al*., 2006). Local immunity in the small intestines mediated by Ig A is thought to be critical since rotavirus infections occur in the gastrointestinal tract (Glass *et al*., 2006). However, gut immunity is difficult to measure and appears to be short-lived (Dennehy, 2008). The exact role of cellular immune responses for protection in humans is unclear. Rotavirus infection has been shown to poorly induce cytotoxic T cells (Jaimes *et al*., 2002). T helper cells may be important both for the elimination of infection and the establishment of immune memory (Franco and Greenberg, 1999; VanCott *et al*., 2001).

Protection against rotavirus infection was found to be serotype-specific and related to the levels of neutralising antibodies against the specific virus. It was discovered that neutralising antibody levels of 1/128 or greater provided protection against disease (Chiba *et al*., 1986). Subsequently, homotypic and heterotypic antibody responses were found in children following primary infection thus indicating the presence of cross-reactive neutralising epitopes (Arias *et al*., 1994). Older children tend to have heterotypic responses and have pre-existing rotavirus-specific antibodies (O'Ryan *et al*., 1994).

The VP7 and VP4 proteins have been shown to elicit Ig A and Ig G neutralising antibodies which may directly inhibit infection by blocking specific epitopes required for attachment and penetration to a host (Aoki *et al*., 2009; Desselberger and Huppertz, 2011; Ludert *et al*., 2002). During primary infection, VP7 was shown to be serotype-specific, while VP4 was more heterotypic (Gorrell and Bishop, 1999). Additionally, non-neutralising antibodies can be directed against the VP6, VP2, NSP2, and NSP4 proteins. Antibodies directed against these proteins are present mostly in individuals recovering from illness (Colomina *et al*., 1998; Desselberger and Huppertz, 2011; Johansen *et al*., 1999; Kirkwood *et al*., 2008; Svensson *et al*., 1987). The VP6 is the immunodominant antigen in the antibody response to RVA infection. Both Ig A and Ig G antibodies against VP6 antigen are produced following natural infection and are indicators of immunity after infection (Caddy *et al*., 2020; Svensson *et al*., 1987).

2.3.5. Risk factors for rotavirus infections

Although rotavirus has a 'democratic' nature, some children are at a higher risk of life-threatening disease due to rotavirus infection than others. According to both the Global Enteric Multi Centre Study (GEMS) and the Malnutrition and Enteric Disease (MAL-ED) study, rotavirus was seen to be pathogenic, causing moderate to severe diarrhoea (Kotloff *et al*., 2019; Platts-Mills *et al*., 2015).

Children living in socioeconomically underdeveloped areas are more prone to succumbing to severe diarrhoea and even death, than those who live in economically developed areas (O'Ryan *et al*., 2005). Similarly, serious illness and death rates are more prevalent in under-developed countries than in developed countries (Tate *et al*., 2016; Troeger *et al*., 2018). This may be attributed to factors such as poor sanitation and therefore higher risk of faecal-oral transmission, overcrowding, use of contaminated food and water, malnutrition and other deficiencies, living in close proximity to domestic animals, and poor health infrastructure (O'Ryan *et al*., 2005).

Age and sex are also considered to be risk factors. Infections in children aged between 3-24 months are more likely to be severe than in older children or adults (Chrystie *et al*., 1978; Dennehy, 2008; Pérez-Schael *et al*., 1984; Wenman *et al*., 1979). Additionally, previous studies demonstrated higher rotavirus prevalence in males than females (Banerjee *et al*., 2006; Gomwalk *et al*., 1990; Newman *et al*., 1999; Nguyen *et al*., 2004; Velázquez *et al*., 1996).

2.3.6. Diagnosis and management

Clinical symptoms brought about by rotavirus infection may be indistinguishable from symptoms caused by other enteric pathogens via clinical examination only. Laboratory testing of faecal specimen is thus necessary to confirm the diagnosis (Maldonado and Yolken, 1990; Parashar *et al*., 2013). Being that rotavirus infections usually lead to a massive shedding of virus particles in faeces, negative-staining electron microscopy was initially utilised for diagnosis due to the high number of virus particles and the distinctive wheel-like shape of the virus (Bishop *et al*., 1974). The Latex Agglutination Test (LAT) was used to detect the presence of rotavirus antigens (Pai *et al*., 1985). Later on, polyacrylamide gel electrophoresis (PAGE) was used for the identification of electrophoretic patterns of rotavirus gene segments (Herring *et al*., 1982).

The previously mentioned methods were replaced by antigen-based assays such as Enzyme-linked Immunosorbent Assay (ELISA) and Enzyme Immuno Assay (EIA) which are more sensitive, less time consuming and give specific diagnosis (Brandt *et al*., 1981; Rubenstein and Miller, 1982). The RT-PCR method is commonly used for identification of rotavirus from stool samples and genotyping, and is more sensitive and specific than immunoassays (Amar *et al*., 2007; Buesa *et al*., 1996; Gentsch *et al*., 1992; Gouvêa *et al*., 1990; Iturriza-Gómara *et al*., 2011; Pang *et al*., 2004). Gouvêa *et al*. (1990) first performed a new method of RT-PCR amplification of the genome segment encoding VP7 (G-typing) using type-specific primers. Subsequently, an RT-PCR typing method to identify distinct VP4 encoding types was developed (Gentsch *et al*., 1992). Finally, rotavirus characterisation is performed using sequence-

independent amplification or sequence-dependent approaches, along with Sanger sequencing, and more recently through NGS combined with bioinformatic analysis (Bányai *et al*., 2017; Chieochansin *et al*., 2016; Mihalov-Kovács *et al*., 2015; Monini *et al*., 2014; Sashina *et al*., 2020; Tagbo *et al*., 2019).

Rotavirus disease does not have a specific therapy. Dehydration as a result of vomiting and diarrhoea is prevented by replacing fluids and electrolytes in the body. The degree of dehydration is first assessed, and fluid replacement is performed accordingly (Dennehy, 2013). Mild dehydration and vomiting are treated by rehydration therapy using an oral rehydration solution (ORS) to restore adsorption of sodium and water in the body (WHO, 2005). In the case of severe dehydration or where vomiting may prevent proper administration of ORS, intravenous rehydration therapy is used. Zinc is often administered to supplement ORS, as it helps to reduce the duration and severity of illness (WHO, 2005).

2.3.7. Prevention and control

Rotaviruses contain a glycoprotein on their outer capsid and a triple-layered particle (TLP) which makes them highly stable in the environment (Estes and Cohen, 1989). Using disinfectants such as 70% ethanol on surfaces un-coats the TLP structure of rotavirus and fragments the capsid proteins hence prevents rotavirus transmission (Estes *et al*., 1979). Additionally, given the fact that rotavirus spreads via the faecal-oral route, avoiding contact with infected patients as well as potentially contaminated food and water can aid in preventing transmission (WHO, 2005). Other interventions such as routine handwashing, improvement of sanitation, water supply and hygiene may also facilitate prevention of disease transmission. These practises do not, however, prevent the spread of rotavirus adequately. Therefore, vaccination is the best and most effective way to prevent infection and disease due to rotavirus (Rota Council, 2020a). Essential healthcare workers and caregivers should practice frequent hand washing and wear protective clothing before going into contact with infected patients (Rao, 1995).

2.4. Rotavirus morphology, genome organisation, proteins, and replication

2.4.1. Morphology

Rotaviruses are non-enveloped. A mature virus particle contains three capsid layers surrounding 11 genome segments of double-stranded ribonucleic acid (dsRNA) (**Figure 2.1**). Each genome segment, except segment 11 codes for one protein (Estes and Greenberg, 2013). Six proteins (VP1-VP4, VP6, and VP7) are structural viral proteins that form the capsid layers, while the other five or sometimes six are non-structural proteins (NSP1-NSP5/6) that support other functions such as replication, genome assembly, and stimulation of viral expression (Estes and Greenberg, 2013).

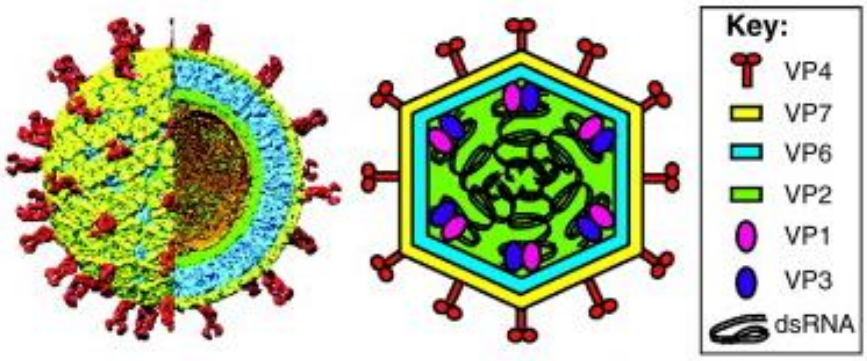


Figure 2.1. Diagrammatic representation of rotavirus architecture and morphology. On the left is a cryo-electron micrograph image of a mature rotavirus TLP. The middle and right panels show a rotavirus illustration with icosahedral symmetry and a colour-coded key, respectively. The proteins and ds-RNA are coloured according to the key provided on the right panel. Image obtained from (McDonald and Patton, 2011) with permission (**Appendix 2**).

Trimers of VP7, usually arranged with a T=13 symmetry, and 60 multimeric spikes of VP4 form the outer capsid (Prasad *et al*., 1990). The intermediate capsid also exhibits T=13 symmetry and is composed of VP6 surrounding a VP2 core shell (McClain *et al*., 2010). One of the distinguishing features of a rotavirus particle is the presence of aqueous channels (132 in number) that penetrate through the intermediate, VP6, and outer, VP7, capsid layers. Aqueous material and biochemical substrates can pass through these channels (Estes and Greenberg, 2013; McClain *et al*., 2010; Pesavento *et al*., 2006). Lastly, VP1 and VP3 are attached to the inner surface of the VP2 shell (McClain *et al*., 2010; Prasad *et al*., 1996).

2.4.2. Genome organisation

Within the core, is the genome that consists of eleven segments of dsRNA (Estes and Greenberg, 2013). Each segment begins with a 5' guanidine that is followed by a 5' non-coding region (**Figure 2.2**) that includes a set of conserved sequences. An open reading frame (ORF) codes for proteins and is followed by the 3' non-coding region. Each segment of the genome has one ORF. However, genome segment seven, nine and ten have an additional in-phase ORF, while segment eleven contains an additional out-of-phase ORF (Estes and Greenberg, 2013).

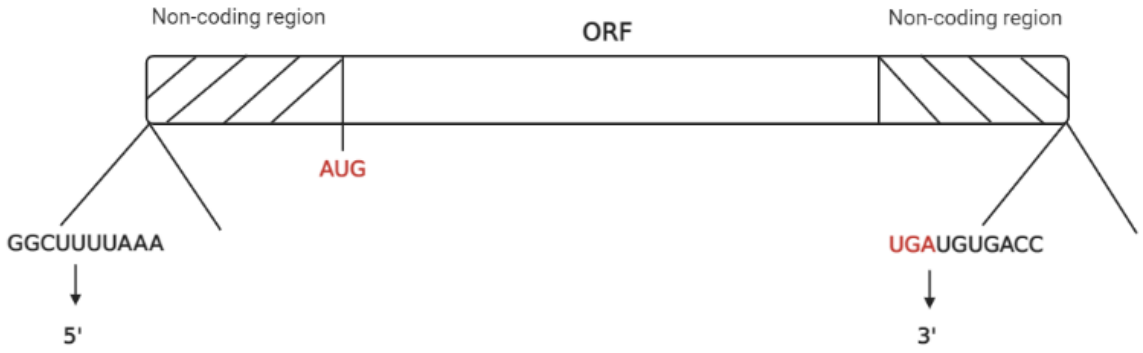


Figure 2.2. General structure of a rotavirus genome segment. Image was created on 18.01.2021 on BioRender. Adapted from Estes and Greenberg, 2013.

The ORF begins with a start codon (AUG) and ends with a stop codon (UGA) that marks the beginning of the 3' termini. The 3' termini begin with a non-coding region with another set of conserved sequences and ends with two 3' terminal cytidine residues. The lengths of the 5' and 3' non-coding regions differ for different genome segments (Estes and Greenberg, 2013; Estes and Cohen, 1989). Almost all rotavirus segments end with the consensus sequence UGUGACC-3'. These consensus sequences together with the 5' terminal sequence (5'-GGCUUUUAAA) contain signals that are important for various viral processes (transcription, translation, and packaging of the genome segments) (Estes and Greenberg, 2013).

2.4.3. Proteins and their functions

Due to their differences in size, rotavirus segments have different migration patterns when subjected to PAGE. Segment one to four are of high molecular weight, segment five and six are middle-sized, segment seven, eight and nine form a distinctive triplet pattern, and segment ten and eleven are the smallest in size (Estes and Greenberg, 2013). **Figure 2.3** illustrates the migration patterns of the eleven segments. Genome segment eleven (NSP5) migrates faster and furthest in comparison to the other ten segments because of its small size (approximately 664 base pairs in DS-1-like rotaviruses). In some strains (often in Wa-like rotaviruses), genome segment eleven is around 821 base pairs hence migrates between segment nine (VP7) and ten (NSP4) which are approximately 1062 and 751 base pairs, respectively (Estes and Greenberg, 2013).

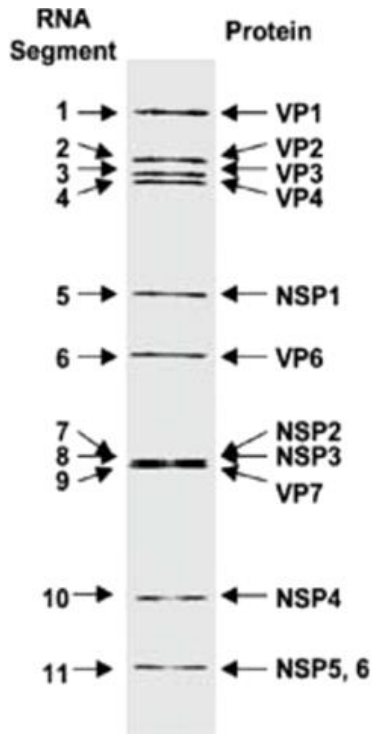


Figure 2.3. PAGE visualisation showing the migration patterns of the eleven segments and their respective proteins. The segments are numbered according to their migration. Because of their nearly identical molecular weights, segment seven, eight, and nine tend to migrate closely. Image obtained from (Pesavento *et al*., 2006) with permission (**Appendix 3**).

2.4.3.1. Structural viral proteins

The VP1 is a core protein that acts as an RNA-dependent RNA polymerase for the virus. It aids in binding of single-stranded RNA (ssRNA) and forms a complex with VP3. The VP2 is located in the core and aids in binding of RNA. It is also needed for replicase activity of the VP1. The VP3 is located in the core. It activates the activity of guanylyl transferase and methyltransferase. Guanylyl transferase catalyses the formation of a 5' cap during post-transcriptional modification of messenger RNA (mRNA) (Estes and Greenberg, 2013; Pesavento *et al*., 2006). The VP4 is located on the outer capsid. It aids in attachment to a host cell during viral entry (Ludert *et al*., 1996). The VP4 is susceptible to proteolysis, which increases viral infectivity and facilitates entry of the virus to the host. It is proteolytically cleaved into: VP8* (amino acids 1-247) and VP5* (amino acids 248-776) cleavage products that remain associated with the virion (Arias *et al*., 1996; Kaljot *et al*., 1988). The VP4 is also a P-type neutralisation antigen that leads to production of neutralising antibodies and hemagglutination. The VP4 determines the host specificity and virulence of

the virus (Estes and Greenberg, 2013; Pesavento *et al*., 2006). The VP6 is the intermediate region. It is important for the viral transcription process. The VP7 is located on the outer capsid. It is a G-type neutralisation antigen and leads to production of neutralising antibodies (Estes and Greenberg, 2013; Pesavento *et al*., 2006).

2.4.3.2. Non-structural proteins

The NSP1 inhibits interferon response during infection and also aids in RNA binding (Graff *et al*., 2002). The NSP2 aids in viral RNA synthesis and packaging as well as assembly of viroplasms. The NSP2 also appears to interact with VP1 polymerase. Additionally, NSP2 aids in ssRNA binding, helix destabilising activities, and exhibits magnesium-dependent nucleoside triphosphatase, RNA triphosphatase and nucleoside diphosphate kinase activities and is therefore a multi-functional enzyme (Estes and Greenberg, 2013; Kumar *et al*., 2007; Taraporewala *et al*., 1999; Taraporewala and Patton, 2011; Valenzuela *et al*., 1991; Vasquez-Del Carpio *et al*., 2006). The NSP3 binds with mRNA at the 3' end, aids in translation of viral mRNA and is also responsible for shutting down of host-cell protein synthesis through antagonism of the poly A binding protein (PABP). The NSP3 interacts with eukaryotic translation initiation factor (eIF) 4G and evicts PABP from eIF 4E which leads to increased translation of viral products. The virus is able to circulate its mRNA through these interactions and enhances viral protein synthesis by the host cell machinery (Groft and Burley, 2002; Piron *et al*., 1988; Vende *et al*., 2000). The NSP4 is a transmembrane protein that is synthesised in the endoplasmic reticulum (ER). It is an enterotoxin thus capable of inducing secretory diarrhoea. The NSP4 is also essential for replication and morphogenesis of the virus. Lastly, expression of NSP4 leads to an increase in cytosolic calcium which is important for the replication and assembly of viral proteins, therefore NSP4 is a viroporin (Estes and Greenberg, 2013; Estes *et al*., 2001; Greenberg and Estes, 2009; Hyser *et al*., 2010). The NSP5 directs the formation of viroplasms with NSP2 via a C-terminal helical domain present in the NSP5. The C-terminal also directs the binding of NSP5 to NSP6 (Sen *et al*., 2007; Torres-Vega *et al*., 2000). The NSP5 has also been shown to interact with VP1, VP2, and NSP2 (Arnoldi *et al*., 2007). NSP6 is a binding protein with an affinity for ssRNA and dsRNA. It is not encoded in all rotaviruses, and for the strains that encode it, it is translated from an ORF that is out-of-phase from that of the NSP5 in segment 11 (Mattion *et al*., 1991; Rainsford and McCrae, 2007; Torres-Vega *et al*., 2000).

2.4.4. Replication cycle

The virus interacts with the host at all stages of the replication cycle. These stages (illustrated in **Figure 2.4**) include entry into the cell, transcription, translation, synthesis and packaging of the genome, and finally, exit of the virus out of the cell (Estes and Greenberg, 2013).

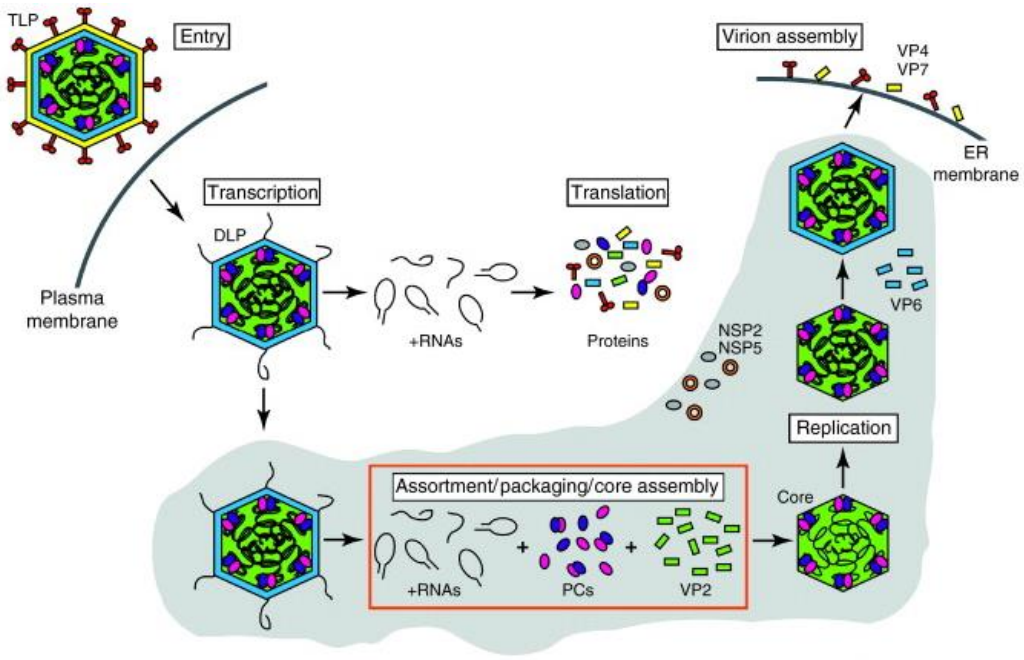


Figure 2.4. Diagram showing the key features of the replication cycle. Image obtained from (McDonald and Patton, 2011) with permission (**Appendix 2**).

Rotaviruses affect mature enterocytes in the villi of the small intestines, where they replicate. Replication takes place in the cytoplasm of infected cells, in viroplasms, and in the ER (Estes and Greenberg, 2013; Parashar *et al*., 1998). The attachment/spike protein (VP4) is cleaved into VP8* and VP5* by gastrointestinal trypsin-like proteases before entry of the virus into a host cell (Baker and Prasad, 2010; Lopez and Arias, 2006). The virus attaches to the host cell by the VP8*, resulting in uncoating of the VP7, loss of the outer capsid and induced membrane penetration by the VP5*. Consequently, a double-layered particle (DLP) that is transcriptionally active is formed in the cytosol. A DLP comprises the intermediate capsid protein and the core proteins (Benureau *et al*., 2005; Denisova *et al*., 1999; Dormitzer *et al*., 2004; Estes and Greenberg, 2013; Kaljot *et al*., 1988; Ludert *et al*., 1987; Wolf *et al*., 2011).

The loss of the outer capsid is immediately followed by transcription of capped but non-polyadenylated (+) RNAs by the VP1 and VP3 polymerase complexes (PC) that are usually attached to the inner layer of the VP2, using the (-) strands of the dsRNA segments as templates (Lu *et al*., 2008; Mansell and Patton, 1990; McDonald and Patton, 2011; Patton *et al*., 1997). The VP1-VP3 PC are transcriptionally active and exhibit enzymatic activities (methylase, transcriptase, and guanyl transferase) which aid in synthesis of 5' capped (+) RNAs. Each segment is transcribed by a specific PC (Gorziglia and Esparza, 1981; Mason *et al*., 1980; McCrae and McCorquodale, 1982). The (+) RNAs have a dual role either as mRNA for synthesis of protein or as templates for (-) RNA synthesis during genome replication (Lawton *et al*., 2000; Lu *et al*., 2008). To synthesise protein, rotaviruses use the host cell translation mechanism. The NSP3, which acts as a PABP because viral RNA is not polyadenylated, facilitates protein synthesis (Kabcenell and Atkinson, 1985; Vende *et al*., 2000).

Viral replication, genome packaging, core assembly and DLP assembly takes place in viroplasms, formed by NSP2 and NSP5 (Fabbretti *et al*., 1999). The VP6 present at the periphery of viroplasms mediates the conversion of cores to DLPs. First, a pre-core is formed, after which the VP2 and VP6 are added, resulting in a DLP (Gallegos and Patton, 1989). A DLP may support the synthesis of additional (+) RNA or may migrate to the ER where it may acquire VP7 and VP4 outer capsid proteins (Estes and Greenberg, 2013; González *et al*., 2000). The NSP4 plays an important role in the assembly of the outer capsid. It accumulates near the viroplasms in the ER. The NSP4 C-terminus acts as an intracellular receptor, whereas the N-terminus stretches into the lumen of the ER where it forms intramolecular disulphide bonds (Bergmann *et al*., 1989; Taylor *et al*., 1993, 1996; Tian *et al*., 1996). It is hypothesised that by binding the DLPs and VP4 using the C-terminus, NSP4 mediates budding of the particle from the viroplasms. The observation that DLPs cannot bind to the ER membrane in the absence of NSP4 supports this hypothesis (Au *et al*., 1989, 1993). Furthermore, the affinity of VP6 for NSP4 facilitates DLP migration to the ER (Meyer *et al*., 1989; Taylor *et al*., 1996).

When DLPs interact with neighbouring NSP4, the ER membrane deforms, allowing the DLP-VP4-NSP4 complex to bud out of the ER, forming a transient envelope for the particles that is later replaced by a thin layer of protein as they move into the ER. The layer is subsequently removed, and the ER-retained VP7 assembles onto the particle, locking VP4 into place and forming a TLP (Estes and Cohen, 1989; González *et al*., 2000; Stirzaker *et al*., 1987; Trask and Dormitzer, 2006). Finally, via direct cell lysis or a budding-like process, progeny virions are discharged from the infected cell (Altenburg *et al*., 1980; Gardet *et al*., 2006).

2.5. Rotavirus classification

Rotaviruses are pathogens of the *Reoviridae* family (Estes and Greenberg, 2013). A total of nine established (RVA-RVD, F-J) rotavirus species have been described since the discovery of rotavirus, based on the genetic characteristics of the VP6 (ICTV, 2021). The RVA are important in the medical and veterinary fields, as they cause infections in humans and animals (mammals and birds) (Bányai *et al*., 2017; Estes and Greenberg, 2013; Matthijnssens *et al*., 2012; Mihalov-Kovács *et al*., 2015). The RVE species is not well established as it was reported only in the United Kingdom over three decades ago, and has since been removed from the classification system (Chasey *et al*., 1986; Vlasova *et al*., 2017). **Table 2.1** shows the different rotavirus species and hosts in which they have been identified. The VP6 based system of classification complemented the traditional classification methods that were based on the clinical, morphological, and serological characteristics of strains (Matthijnssens *et al*., 2012).

Table 2.1. The different rotavirus species and hosts in which they have been identified

Rotavirus species	**Hosts in which species has been identified**
A	Different mammals and birds
B	Mammals (Humans, cattle, sheep, goats, and pigs)
C	Mammals (Humans, cattle, dogs, pigs, goats, and immature ferrets)
D	Birds
E	Pigs
F	Birds
G	Birds
H	Mammals (Humans and pigs)
I	Dogs, cats
J	Bats

Obtained and modified from (Ghosh and Kobayashi, 2014).

Further, the VP7 and VP4 are binary classified according a serotypic classification system into G (glycoprotein) and P (protease-sensitive) types, respectively. Both proteins elicit neutralising antibodies (Estes and Greenberg, 2013).

2.5.1. The whole genome classification system

The G and P type classification only focuses on two out of 11 segments, and therefore does not provide complete information necessary to assess genetic diversity and to study RVA evolutionary pathways such as reassortment. The development of a complete sequence-based classification system in 2008, in which specific genotypes are assigned to each gene segment according to established nucleotide percent cut-off values, overcame this limitation (**Table 2.2**). In this system, Gx-Px-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx represents the gene segments VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5, and x stands for genotype number (Matthijnssens *et al*., 2008b, 2011).

Table 2.2. Nucleotide % cut-off values for the 11 gene segments and their respective genotypes.

Protein	Gene segment	Percentage (%) identity	Currently identified genotypes	Full name of the genotype (acronym in bold)
VP7	9	80	39G	**G**lycosylated
VP4	4	80	55P	**P**rotease-sensitive
VP6	6	85	30I	**I**nner capsid
VP1	1	83	26R	**R**NA-dependent RNA polymerase
VP2	2	84	22C	**C**ore protein
VP3	3	81	22M	**M**ethyltransferase
NSP1	5	79	37A	Interferon **A**ntagonist
NSP2	8	85	26N	**N**TPase
NSP3	7	85	26T	**T**ranslation enhancer
NSP4	10	85	30E	**E**nterotoxin
NSP5/6	11	91	26H	P**H**osphoprotein

Table obtained and modified from (Matthijnssens *et al*., 2008b).

As of March 2021, the Rotavirus Classification Working Group (RCWG) has identified 39G, 55P, 30I, 26R, 22C, 22M, 37A, 26N, 26T, 30E, and 26H human and animal genotypes (**Table 2.2**) (RCWG, 2021). The RCWG also came up with a standardised nomenclature system for individual strains which is: RV group/species of origin/country of identification/common name/year of identification/G- and P-type (Matthijnssens *et al*., 2011). Combined with modern sequencing techniques, bioinformatics software and gene-specific phylogeny, the whole genome classification system has helped to describe the genotype

constellation of strains and various evolutionary processes such as interspecies transmission and reassortment between human strains of different genogroups or between human and animal strains, which often leads to the generation of new strains (Matthijnssens *et al*., 2008a; Matthijnssens and Van Ranst, 2012).

Genogroups were previously used to describe RVA, based on RNA-RNA hybridisation assays (Nakagomi *et al*., 1989). RVA are classified into three genogroups (two major and one minor), each represented by a reference strain (**Table 2.3**). For RVA of the same genogroup, a high degree of genetic relatedness was observed (Matthijnssens *et al*., 2008a; Matthijnssens and Van Ranst, 2012; Nakagomi *et al*., 1989).

Table 2.3. The three genogroups and their respective constellations.

Genogroup	**Constellation**										
	VP7	**VP4**	**VP6**	**VP1**	**VP2**	**VP3**	**NSP1**	**NSP2**	**NSP3**	**NSP4**	**NSP5**
Wa-like (major)	G1	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
DS-1-like (major)	G2	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
AU-1-like (minor)	G3	P[9]	I3	R3	C3	M3	A3	N3	T3	E3	H3

The colours green, red, and yellow are used to distinguish between the different constellations. Green is the Wa-like, red is the DS-1-like, and yellow is the AU-1-like.

The Wa-like genogroup tends to have the G1P[8] (prototype), G3P[8], G4P[8], G9P[8], and G12P[8] G- and P- combinations, whereas the DS-1-like and AU-1-like genogroup includes G2P[4] and G3P[9] as the prototypes, respectively (Matthijnssens *et al*., 2008a). Human Wa-like RVA strains share the genotype 1 with porcine RVA in the VP1-VP3, NSP2-NSP5/6 gene segments. This observation supports the suggestion that a common origin is shared by human Wa-like and porcine rotaviruses. Similarly, human DS-1-like and AU-1-like RVA share genes in their backbone with bovine and canine/feline RVA, respectively (Gauchan *et al*., 2015; Matthijnssens *et al*., 2008a). **Table 2.4** provides a summary of common human, porcine, bovine, and feline G-P types.

Table 2.4. Prevalent G-and P- specificities in various host species.

Host species	G-P type
Human	G1-G4, G9, G12, P[4], P[6], P[8]
Porcine	G3-G5, P[6], P[7], P[13]
Bovine	G6, G8, G10, P[1], P[5], P[11]
Feline	G3, G6, P[3], P[9]

The table was compiled from (Dóró *et al*., 2015; Ghosh and Kobayashi, 2014; Martella *et al*., 2010; Papp *et al*., 2013; Vlasova *et al*., 2017).

2.6. Rotavirus vaccines

Even though natural infections confer first-line protection against severe illness, preventative measures are necessary. The main objective of rotavirus vaccines is to avert death and severe disease and alleviate the disease burden especially in low-resource countries. Rotavirus vaccines are designed to mimic protection that is usually conferred by natural infection.

2.6.1. First-generation vaccines (non-human strains as vaccines)

Researchers in the mid-1970s discovered that previous animal infections protected laboratory animals from infection with human rotavirus strains, which sparked rotavirus vaccine development (Zissis *et al*., 1983). For this reason, it was believed that live attenuated animal strains could copy immunity conferred by natural infection when given orally to humans and could curb severe disease. This led to the development of the first vaccine based on a Jennerian approach, a technique that was used to develop human smallpox vaccines (Hoshino and Kapikian, 1994; Kapikian *et al*., 1992). The vaccine was based on a bovine rotavirus vaccine strain 4237 (G6P6[1]) that was originally derived for Nebraska Calf Diarrhoea Virus (NCVD) strain of bovine rotavirus (Vesikari *et al*., 1985). Clinical trials of this vaccine showed variable efficacy thus the vaccine was discontinued in 1987 (Hanlon *et al*., 1987; Lanata *et al*., 1989; Ruuska *et al*., 1990; Santosham *et al*., 1991; Vesikari *et al*., 1985). Subsequently, the development of another bovine vaccine was started. It was derived from the strain WC3 (G6P9[5]) isolated from a calf (Clark *et al*., 1988). This vaccine was seen to elicit neutralising antibodies in vaccinated patients. However, it displayed low and inconsistent protection against disease (Bernstein *et al*., 1990; Clark *et al*., 1988; Georges-Courbot *et al*., 1991).

Further, a vaccine based on a G3 strain isolated from a rhesus monkey with acute diarrhoea was developed as an alternative to the 4237 bovine rotavirus vaccine on the assumption that it shared neutralisation specificity with G3 strains of human origin (Christy *et al*., 1988; Stuker *et al*., 1980). The vaccine was found to be highly reactogenic with variable efficacy (Christy *et al*., 1988; Gothefors *et al*., 1989; Pérez-Schael *et al*., 1990; Rennels *et al*., 1986).

Lastly, is the Lanzhou lamb rotavirus (LLR) vaccine, the only animal-derived vaccine that is still in use. The LLR vaccine, produced at the Lanzhou Institute in China, was first isolated in 1985 and is a G10P[12] live-attenuated ovine strain vaccine (Li *et al*., 2015; Zhen *et al*., 2015). Since 2000, this vaccine has only been licensed for use in China, and by 2014, approximately 60 million doses had been administered to children (Fu *et al*., 2007, 2012). So far, there have been no reports of negative side effects from the LLR vaccine, and it has been shown to confer partial protection against rotavirus disease (Fu *et al*., 2007, 2012).

Up to this point, these were considered as first-generation vaccine candidates, which later gave rise to second generation vaccines (Bresee *et al*., 1999; 2005).

2.6.2. Second generation vaccines (human and human-animal reassortant vaccines)

Because the first-generation zoonotic vaccine candidates failed to consistently offer protection against rotavirus disease caused by human strains, focus was directed towards formulation of human and human-animal reassortant vaccines. Second generation vaccines include RotaShield® and four WHO-prequalified vaccines (Rotarix®, RotaTeq®, ROTAVAC®, and ROTASIIL®) (Bresee *et al*., 2005; Dennehy, 2008; Rota Council, 2020b).

The first licensed reassortant vaccine, RotaShield® (Wyeth Laboratories, United States), was created by co-infecting cell cultures with the G3 strain that was obtained from a rhesus monkey and human strains G1, G2, and G4 (Estes and Cohen 1989). The immunology aspects of the rhesus G3 was similar to that of human G3 (Bines *et al*., 2009). RotaShield® offered 70-100% protection against severe disease and after several trials in Finland, the United States, and Venezuela, it became the first licensed rotavirus vaccine in 1998 (Bernstein *et al*., 1995; Joensuu *et al*., 1997; Pérez-Schael *et al*., 1997; Rennels *et al*., 1996; Santosham *et al*., 1997). However, the vaccine was discontinued the following year after it was linked with cases of intestinal obstruction (CDC, 1999; Murphy *et al*., 2001; Patel *et al*., 2009).

2.6.3. Rotavirus vaccines with WHO-prequalification

In 2006, WHO recommended the use of Rotarix® and RotaTeq® in Europe and the Americas after showing good efficacy against severe disease during clinical trials (WHO, 2007). This recommendation was

extended to all countries worldwide after a review of clinical trial data from Africa and Asia, as well as post-licensure data from the Americas (WHO, 2009). Rotarix® also referred to as RV1 (GlaxoSmithKline Biologicals, Belgium) and RotaTeq® (RV5; Merck & Co. Inc, USA) obtained WHO-prequalification in 2009 and 2008, respectively (WHO, 2021a). At present, these two vaccines are the most widely used in national immunisation programs worldwide (**Figure 2.5**) (IVAC, 2021). Subsequently, ROTAVAC® (Bharat Biotech, India) and ROTASIIL® (Serum Institute of India, India) achieved WHO-prequalification in 2018 (WHO, 2021a). The four vaccines are live-attenuated and administered orally (Rota Council, 2020b).

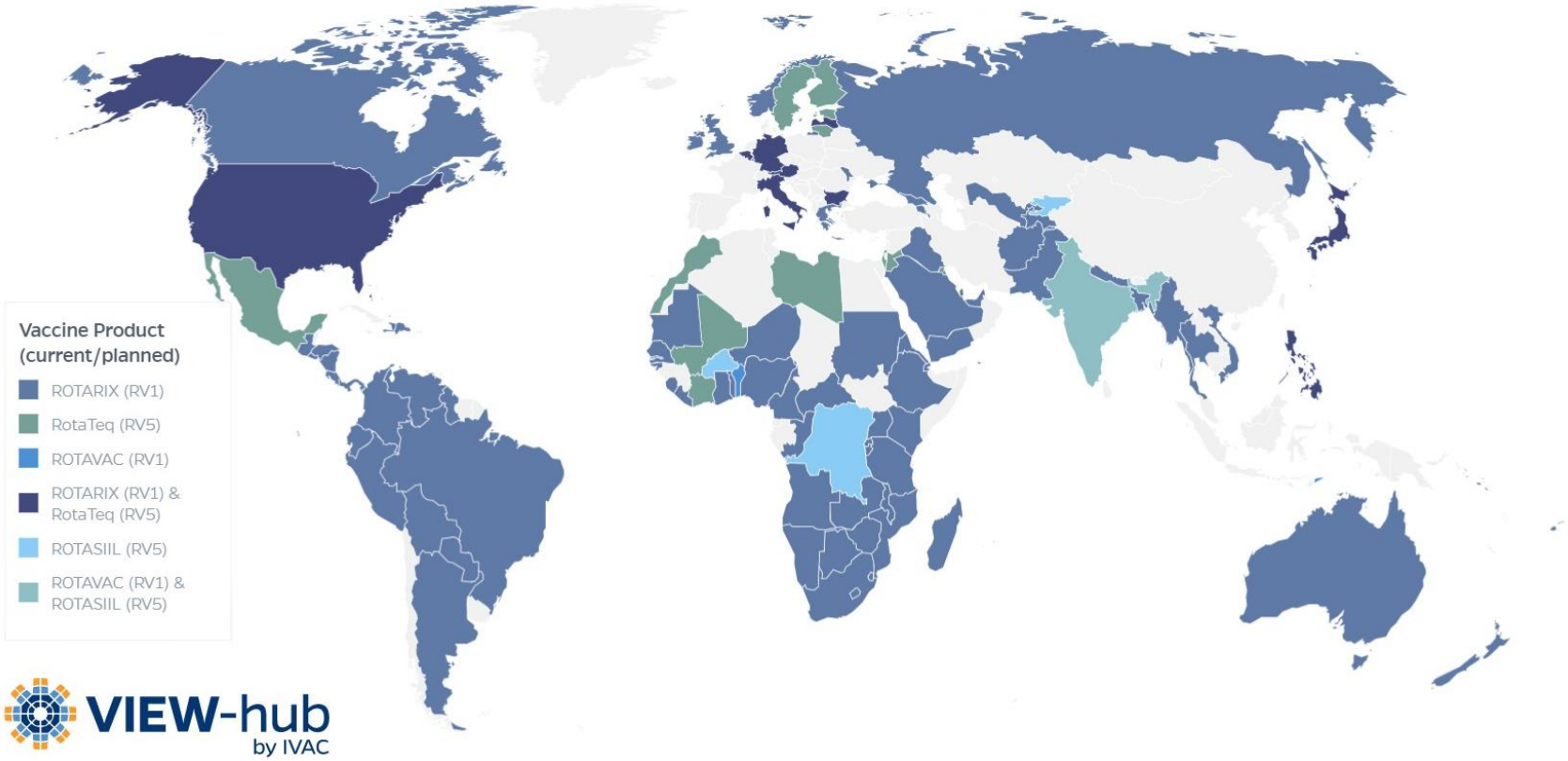


Figure 2.5. World map showing the use of the four WHO-prequalified vaccines in various countries as of 10th May 2021. Image obtained from (IVAC, 2021) with permission (**Appendix 4**).

While some countries with the highest disease burden such as Ethiopia, India, and the Democratic Republic of Congo have incorporated rotavirus vaccines into their immunisation programs, other countries such as Chad, Egypt, Nigeria, and Somalia have yet to introduce the vaccines (IVAC, 2021; Rota Council, 2017; WHO; 2017). These countries face hurdles such as large birth cohorts, the inability to get support from GAVI, and purchasing costs and logistics in storage and transportation for countries that are not eligible for GAVI support (Deen *et al*., 2018; Kallenburg *et al*., 2016; Simpson *et al*., 2007). Established in 2000, GAVI provides financial aid to low-income countries on condition that they take on the financial responsibility once GAVI's support has ended (Saxenian *et al*., 2011).

As of 10th May 2021, 110 countries globally have incorporated rotavirus vaccines into their immunisation programs, out of which 53 countries have support from GAVI (**Figure 2.6**) (IVAC, 2021). Worldwide coverage according to the 2019 WHO-United Nations International Children's Emergency Fund (UNICEF) estimates of national immunisation coverage is greater than 80% in 63 countries and less than 80% in 33 countries (WHO, 2020).

2.6.3.1. RotaTeq®/RV5

The RV5 is a three-dose vaccine comprised of five reassortant rotaviruses, which are, human G1-G4 and P[8] on a bovine backbone of the parental strain WC3 (G6P7[5]). The first dose is given between 6-12 weeks, with subsequent doses administered at intervals of 4-10 weeks (Clark *et al*., 1996; Dennehy, 2008; Heaton *et al*., 2005; Vesikari *et al*., 2005; WHO, 2013). The Food and Drug Administration (FDA) approved RV5 for use in the United States by in 2006, and is currently used in more than 20 countries globally (**Figure 2.6**) (Dennehy, 2008; IVAC, 2021).

2.6.3.2. Rotarix®/RV1

The RV1 is a two-dose vaccine containing the human G1P[8] strain. The vaccine was derived from the human strain 89-12 (Bernstein *et al*., 1998; Dennehy, 2008). RV1 is first given at six weeks, while the second at least four weeks apart but not later than 24 weeks (WHO, 2013). Rotarix® was first licensed for use in Mexico and subsequently in the United States in 2004 and 2008, respectively (Dennehy, 2008). Even though RV1 contains only G1 and P[8] specificities, significant protection against G2, G3, and G9 was demonstrated in various efficacy studies (O'Ryan and Linhares, 2009; Ruiz-Palacios *et al*., 2006; Vesikari *et al*., 2007; Ward and Bernstein, 2009).

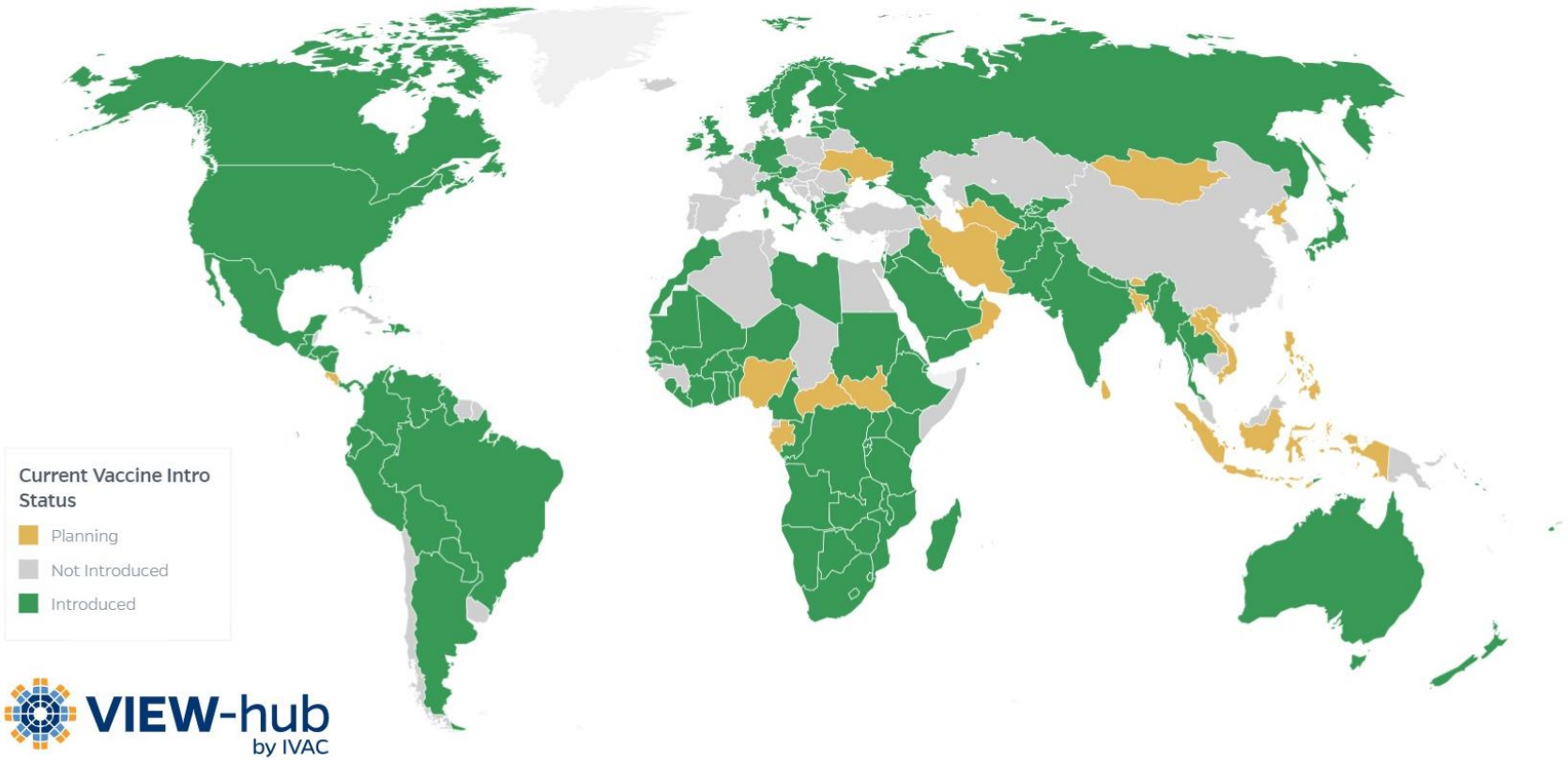


Figure 2.6. World map showing rotavirus vaccine introduction as of 10th May 2021. Image obtained from (IVAC, 2021) with permission (**Appendix 4**).

Taking into account the need for affordable vaccines especially in low-resource settings, two additional vaccines, ROTASIIL® and ROTAVAC® were developed and attained WHO-prequalification in 2018 (PATH, 2018b; WHO, 2018b, 2018c; WHO, 2021a).

2.6.3.3. ROTAVAC®

Licensed in India in 2014, ROTAVAC® was derived from strain 116E (G9P[11]), a naturally attenuated human neonatal strain that was originally obtained in 1988 from an asymptomatic infant (Bhandari *et al*., 2014; Das *et al*., 1993; WHO, 2014). The vaccine is given in three doses at intervals of four weeks. The first dose is administered at six weeks and the last dose not later than 32 weeks (Bharat Biotech, 2019). Currently, ROTAVAC® is used in Benin, India, and Timor-Leste (IVAC, 2021).

2.6.3.4. ROTASIIL®

ROTASIIL®, licensed in India in 2016, is a three-dose human-bovine reassortant vaccine comprised of five specificities (G1-G4 and G9). It is unique in that it is heat-stable hence can stay unrefrigerated for up to 18 months at 40°C and 36 months at 25°C. This is advantageous especially in low-resource settings where access to refrigerators and/or power supply may be a challenge (Anil *et al*., 2018; Kulkarni *et al*., 2017; Naik *et al*., 2017). The countries that have implemented ROTASIIL® into their immunisation programs are Burkina Faso, the Democratic Republic of Congo, India, and Kyrgyzstan (IVAC, 2021).

2.6.4. Nationally licensed vaccines

Two oral attenuated vaccines, LLR (see section 2.6.1) and Rotavin-M1 are licensed for domestic use in China and Vietnam, respectively. Rotavin-M1 was formulated from a G1P[8] strain (KH0118-2003) isolated from a child who was hospitalised with acute gastroenteritis in Vietnam (Anh *et al*., 2012; Le *et al*., 2009).

2.6.5. Rotavirus vaccine candidates under development

Two rotavirus vaccine candidates are currently under development with the aim of tackling the overall challenges posed by rotavirus disease and improving vaccine effectiveness especially in developing countries (Kirkwood *et al*., 2019).

2.6.5.1. RV3-BB

Given that rotavirus infection occurs at an earlier age in children living in low-income settings (Gladstone *et al*., 2011; Steele *et al*., 2016), the administration of current vaccines from the age of six weeks may be too late to protect some infants. The RV3-BB was developed as a vaccine to be given at birth. Because of this, the vaccine offers a greater safety advantage since the risk of intussusception is extremely low in

new-borns (Danchin *et al*., 2013; Kirkwood *et al*., 2019). RV3-BB is an oral, naturally-attenuated vaccine containing a G3P[6] neonatal strain isolated from an asymptomatic infant in Australia in the late 1970s (Bishop *et al*., 1983; Burke *et al*., 2019; Kirkwood *et al*., 2019). This strain is biologically unique in that it replicates in the neonatal gut despite the presence of maternal antibodies, it offers heterotypic protection for up to three years in neonates, and is currently the only vaccine with the P[6] VP4 protein (Burke *et al*., 2019; Kirkwood *et al*., 2019) which has been shown to circulate widely in high-mortality regions in Africa (Nyaga *et al*., 2018) and some parts of Asia (Bányai *et al*., 2012).

2.6.5.2. UK-BRV

This vaccine is a live-attenuated bovine-human reassortant vaccine comprised of VP7 genes of human strains on a bovine G6P[5] backbone. Originally, the vaccine contained G1-G4 G-types, but later, G8 and G9 were added due to their emergence in various parts of Africa and Asia (Hoshino *et al*., 1997; Kapikian *et al*., 2005; Kirkwood *et al*., 2019). Several manufacturers in China, Brazil and India are currently formulating the UK-BRV vaccine (Kirkwood *et al*., 2019).

2.6.6. Impact of Rotarix® and RotaTeq® vaccination globally and in sub-Saharan Africa

Post-market surveillance of the Rotarix®/RV1 and RotaTeq®/RV5 vaccines showed a slight increased risk of intussusception following administration of the first dose (WHO, 2013). However, this risk is significantly lower than the previously licensed vaccine, RotaShield®. Both vaccines have been reported to offer similar protection against homotypic and heterotypic rotavirus strains (Leshem *et al*., 2014; WHO, 2013). Mortality strata data from different countries between 2006 and 2016 revealed that RV1 and RV5 were effective against rotavirus disease, with effectiveness ranging between 57%-85% for RV1 and 45%-90% for RV5 (Jonesteller *et al*., 2017; Leshem *et al*., 2014; Soares-Weiser *et al*., 2019). Vaccine performance is moderate in low-resource countries compared to richer and more developed countries. Potential reasons for this observation could be due to differences in disease epidemiology (strain distribution, seasonality, younger age of first infection, and mortality risks) and various host factors (malnutrition, interference by neutralising antibodies present in breast milk, high levels of natural immunity at younger ages owing to natural infection, and presence of other enteric pathogens) (Bresee *et al*., 2005; Cunliffe *et al*., 2014; Jonesteller *et al*., 2017; O'Ryan *et al*., 2015; Soares-Weiser *et al*., 2019).

A tremendous reduction in the number of rotavirus-associated deaths has been documented since the incorporation of RV1 and RV5 in immunisation programs globally. There was a decrease from approximately 528,000 deaths in 2000 to 215,000 deaths in 2013 was observed, followed by a further decline to 128,000 deaths (Tate *et al*., 2016; Troeger *et al*., 2018). Furthermore, it was estimated that

rotavirus vaccination in 72 GAVI eligible countries would avert 2.4 million deaths in children between 2011 and 2030 (Atherly *et al*., 2012). Substantial reductions in hospital admissions due to rotavirus disease have also been recorded following rotavirus vaccine introduction (Aliabadi *et al*., 2019; Burnett *et al*., 2020). Between 2006-2019, a median reduction of 59% in rotavirus-associated hospitalisations was observed among children under five globally (Burnett *et al*., 2020). The change in rotavirus-associated mortality has mirrored the global decrease in diarrhoea-associated mortality since rotavirus is typically the number one aetiologic agent of diarrhoeal cases in under five-year-old children. This is supported by the fact that both diarrhoeal hospitalisations and mortality declined by 36% globally (Burnett *et al*., 2020).

The ARSN, initiated in June 2006, has played a major role in establishing the burden of disease and impact of vaccination in sub-Saharan Africa (Mandomando *et al*., 2017; Mwenda *et al*., 2010, 2014, 2019). In 2016 alone, approximately 21,000 deaths and 130,000 hospitalisations were prevented in 29 countries that had implemented rotavirus vaccines by the end of 2015 (Shah *et al*., 2017). It was projected that 48,000 deaths and 270,000 hospitalisations would be prevented annually if all African countries implemented rotavirus vaccines into their immunisation programs at coverage levels similar to other routine infant vaccinations. Therefore, with vaccine implementation into more countries and increasing coverage, the disease burden is in turn projected to decrease (Shah *et al*., 2017). Studies from several early-introducing countries such as Ghana, Malawi, Rwanda, Togo, and Zimbabwe, showed declines of 35%-80% in rotavirus-associated hospitalisations of children under five years (Armah *et al*., 2016; Bar-Zeev *et al*., 2016; Mujuru *et al*., 2017; Ngabo *et al*., 2016; Tsolenyanu *et al*., 2016, 2018). In Burkina Faso, for example, declines of 54%-61% in rotavirus hospitalisations were reported following vaccine introduction (Bonkoungou *et al*., 2018).

Rotavirus vaccines have also been shown to confer indirect protection (herd immunity). Herd immunity applies to immunisation, infection or both, and is defined as the reduction of infection or disease at population level among unvaccinated individuals as a result of vaccinating a proportion of the population (John and Samuel, 2000). This was observed in Malawi whereby rotavirus positivity also decreased in unvaccinated infants, hence showing the indirect effects of vaccination (Bennett *et al*., 2018).

Change in rotavirus seasonality has also been documented post-vaccine implementation. Delays in the start of the rotavirus season, blunting of seasonal peaks and a shorter duration of the season has been documented across different countries (Jani *et al*., 2018; Maphalala *et al*., 2018; Rahajamanana *et al*., 2018; Sanneh *et al*., 2018). For example, rotavirus hospitalisations in Mozambique before vaccine introduction exhibited a seasonal peak between June and September (winter period) in 2014 and 2015. However, this shifted to the period between August and December in 2016, and the peak was later

diminished (de Deus *et al*., 2018). In Rwanda, a significant blunting of the seasonal peak of hospital admissions was observed in all age groups after rotavirus vaccine implementation (Ngabo *et al*., 2016).

2.7. Rotavirus genetic diversity

2.7.1. Mechanisms of rotavirus evolution that promote genetic diversity

The observed diversity in human RVA has been attributed to five mechanisms (reassortment, zoonotic transmission, point mutations, recombination, and genome rearrangements). Among them, reassortment changes the genotype constellation of the virus, whereas point mutations result in changes in the gene sequence which may affect the functions of viral proteins (Bányai and Pitzer, 2016; Ciarlet and Estes, 2002; Ghosh and Kobayashi, 2011; Iturriza-Gómara *et al*., 2001; Kirkwood, 2010; McDonald *et al*., 2009). When the relative significance of these mechanisms is compared, reassortment is arguably the main contributor to genetic diversity, as evidenced by the increasing documentation of rotavirus strains that have arisen through this mechanism (Donato *et al*., 2014; Dong *et al*., 2013; Gentsch *et al*., 2005; Iturriza-Gómara *et al*., 2001; Katz *et al*., 2019; Quaye *et al*., 2018; Rasebotsa *et al*., 2021; Santos and Hoshino, 2005).

2.7.1.1. Point mutations

Point mutations involve base or frameshift substitutions, which occur due to the error prone nature of the viral RNA polymerase (Bányai and Pitzer, 2016). A point mutation is said to occur when there is a change in a single nucleotide at a location in the RNA sequence. These mutations have been shown to vary by genes and genotypes. Base substitutions occur when a single base is replaced by another base. On the other hand, frameshift mutations can occur either through insertion of a base into the RNA sequence or when a nucleotide is deleted from the RNA sequence. At least one mutation occurs per genome replication (Bányai and Pitzer, 2016; Blackhall *et al*., 1996; Ciarlet and Estes, 2002; Donker and Kirkwood, 2012; Matthijnssens *et al*., 2010).

2.7.1.2. Genome rearrangements

Genome rearrangements refer to insertions, partial deletions, and duplication. They occur mainly in non-structural genome segments, especially in segment five, encoding NSP1. Rearrangement of the VP6-encoding segment has also been documented (Bányai and Pitzer, 2016; Desselberger *et al*., 2001; Kojima *et al*., 1996; Méndez *et al*., 1992; Shen *et al*., 1994). Gene duplications are the most common form of rearrangements, whereby the coding region (ORF) remains unaffected but has an extended 3’ end, while deletions are the least common and result in size reduction of segments (Bányai and Pitzer, 2016).

2.7.1.3. Recombination

Similar to reassortment, infection of a single host cell by different rotavirus strains is a requirement for genome recombination to occur (Bányai and Pitzer, 2016; Jere *et al*., 2011; Parra *et al*., 2004; Suzuki *et al*., 1988). Several reports have documented intragenic, intergenotype, intersegmental, inter-lineage, and inter-sub-lineage recombination events in the structural and non-structural genome segments (Donker *et al*., 2011; Esona *et al*., 2017; Hoxie and Dennehy, 2020; Jere *et al*., 2011; Martínez-Laso *et al*., 2009; Parra *et al*., 2004; Phan *et al*., 2007; Woods, 2015).

2.7.1.4. Zoonotic transmission

Zoonotic/interspecies transmission is a key source of rotavirus genetic diversity and involves the introduction of animal genes into human populations. Zoonotic transmission is often coupled with reassortment (Dóró *et al*., 2015; Gentsch *et al*., 2005). Zoonotic genes may be defined as genes originating from animal rotaviruses that may interact with human rotavirus genes to form infectious particles that may spread to human populations (Cook *et al*., 2004). Close proximity to animals is believed to accelerate interspecies transmission, which may lead to the generation of reassortant strains (Bányai *et al*., 2009; Cook *et al*., 2004; Gentsch *et al*., 2005).

Fully zoonotic strains are rarely detected in human surveillance studies, due to host genetic factors. However, due to the identification of animal genotypes in humans in various parts of the world, it was suggested that animals play a crucial role as a potential reservoir of rotavirus infections in humans, thus contributing to diversity (Bányai *et al*., 2012; Cook *et al*., 2004; Dóró *et al*., 2014, 2015). Zoonotic transmission coupled with reassortment is an efficient means of introducing new antigen specificities into human populations. Furthermore, reassortant strains with a human backbone and one or a few genes of animal origin may be more transmissible to new human hosts (Bányai and Pitzer, 2016; Dóró *et al*., 2015).

Animal rotavirus genes can also be transmitted to humans through contaminated surfaces, food, and water. The risk is significantly higher in areas that practise animal farming (Steyer *et al*., 2008). Developing countries report the occurrence of rotaviruses of animal origin at slightly higher rates than developed countries. The increased risk of zoonosis may be due to factors such as poor water supply and sharing of living spaces with animals. Zoonotic genes can cause not only asymptomatic infection but also mild to severe diarrhoea in humans (Cook *et al*., 2004; Martella *et al*., 2010; Palombo, 2002).

2.7.1.5. Reassortment

Reassortment is a mechanism in which cognate genome segments are exchanged between rotaviruses of the same species (e.g., RVA and RVA), thus yielding progeny with new genotype constellations that reflects the mixing of genome segments between parental strains. Rotaviruses of different species (e.g., RVA and RVB) are not known to undergo reassortment (Bányai and Pitzer, 2016; Patton *et al*., 2006). It is hypothesised that reassortant rotaviruses can arise not only through simultaneous infection by two different strains, but also via asynchronous infection whereby one strain infects a host after another has initiated infection (Ramig, 1990, 1997).

The frequency of coinfection is a key factor in the generation of reassortants. Coinfection rates in developing countries may be as high as 20%, while the rates are typically less than 5% in developed countries. It is therefore speculated that the wide genetic diversity documented in developing countries is due to the high rates of coinfection (Patton, 2012). Furthermore, the constant reshuffling of genes through reassortment and coinfections in developing countries may overwhelm the selective pressures that favour the maintenance of typical genotype constellations (Patton, 2012). This could explain the emergence of human RVA that lack the typical Wa-like or DS-1-like constellation as well as RVA with animal-like characteristics, particularly in sub-Saharan Africa (Esona *et al*., 2009; Jere *et al*., 2018; João *et al*., 2020; Mokoena *et al*., 2020; Mwangi *et al*., 2020; Nyaga *et al*., 2014, 2018; Rasebotsa *et al*., 2021; Shoeib *et al*., 2020).

2.7.2. Rotavirus strain prevalence: a global and regional perspective

Global rotavirus surveillance programs have generated important data on the G-P types that are associated with human infections. The G1P[8], G2P[4], G3P[8], and G4P[8] were reported to be the cause of approximately 90% of diarrhoea in children worldwide between 1987 and 2007 (pre-vaccine period), with G1P[8] in particular as the most predominant. Most of these studies were based on conventional (binary) typing (Bányai *et al*., 2012; Beards *et al*., 1989; Gentsch *et al*., 1996, 2005; Santos and Hoshino, 2005). Additionally, G9 and G12 strains in combination with P[8] have become epidemiologically significant worldwide and are considered the fifth and sixth major human RVA genotypes, respectively (Kirkwood *et al*., 2003; Matthijnssens *et al*., 2009, 2010; Rahman *et al*., 2007; 2008; Rodrigues *et al*., 2007; Samajdar *et al*., 2006; Sánchez-Fauquier *et al*., 2006; Steele *et al*., 2003). Similar to the pre-vaccine era, the six G-P combinations (G1P[8], G2P[4], G3P[8], G4P[8], G9P[8], and G12P[8]) were the most prevalent post-vaccine licensure according to a global review conducted between 2007 and 2012 (Dóró *et al*., 2014).

In sub-Saharan Africa, the prevalence of the six globally predominant strains was demonstrated in the 1980s and 1990s in countries such as the Central African Republic, Gambia, Kenya, Nigeria, and South Africa (Avery *et al*., 1992; Georges-Courbot *et al*., 1988; Page *et al*., 2009, 2010; Rowland *et al*., 1985; Steele *et al*., 1995, 2003; Todd *et al*., 2010; Urasawa *et al*., 1987). The same observation was made in the post-vaccine period in various African countries. However, unusual genotypes such as the G1P[6], G2P[6], G3P[4], G3P[6], G8P[4], G8P[6], G9P[4], G10P[6], G12P[4], and G12P[6] were also documented. These findings highlighted the unusually high prevalence of the P[6] genotype that is presumably of porcine origin (Abebe *et al*., 2018; Dóró *et al*., 2014; Gikonyo *et al*., 2020; João *et al*., 2020; Lartey *et al*., 2018; Letsa *et al*., 2019; Mhango *et al*., 2020; Nyaga *et al*., 2018; Seheri *et al*., 2018). Furthermore, G8 and G10 genotypes are presumably of bovine origin (Dóró *et al*., 2015; Ghosh and Kobayashi, 2014).

2.7.3. Rare and/or novel reassortant rotavirus strains: studies based on whole genome sequencing and analysis

Whole genome sequencing (WGS) of RVA provides useful information for better understanding the diversity that arises from interspecies transmissions and/or reassortment. Additionally, WGS aids in the accurate interpretation of the origin of RVA strains and evolutionary patterns (Estes and Greenberg, 2013; Ghosh and Kobayashi, 2011; Matthijnssens *et al*., 2008a; Ramig, 1997; Tsugawa and Hoshino, 2008). With developments in NGS technology and improved bioinformatics tools for data analysis, newer RVA studies have provided evidence of the emergence of novel G-P combinations and RVA strains possessing mixed genotype constellations as a result of zoonotic transmission, animal-human reassortment and/or intergenogroup reassortment. Reassortant strains have been reported worldwide, including in sub-Saharan Africa (Esona *et al*., 2017; Esposito *et al*., 2019; Fukuda *et al*., 2020; Ghosh *et al*., 2011; Hoa-Tran *et al*., 2020; Jere *et al*., 2018; Komoto *et al*., 2016; Nyaga *et al*., 2015; Rasebotsa *et al*., 2021; Sashina *et al*., 2020; Steyer *et al*., 2008; Zeng *et al*., 2020).

Several studies conducted worldwide have identified unusual G-types G5-G6, G9-G11, G14, G20, G26 and P-types P[1]-P[3], P[5]-P[7], P[9]-P[11], P[19], P[23], P[25], P[28], P[40] in children. **Table 2.5** shows the different G-P combinations that have been documented in humans (Agbemabiese *et al*., 2017; Bányai *et al*., 2012; Bwogi *et al*., 2017; Dhital *et al*., 2017; Dóró *et al*., 2015; Esona *et al*., 2009; Hungerford *et al*., 2019; Matthijnssens *et al*., 2009; Mukherjee *et al*., 2011; My *et al*., 2014; Nyaga *et al*., 2018; Quaye *et al*., 2018; Rojas *et al*., 2019; Santos *et al*., 2001).

Table 2.5. A summary of the G-P combinations that have been identified in humans.

	G1	G2	G3	G4	G5	G6	G8	G9	G10	G11	G12	G14	G20	G26
P[1]														
P[2]														
P[3]														
P[4]														
P[5]														
P[6]														
P[7]														
P[8]														
P[9]														
P[10]														
P[11]														
P[14]														
P[15]														
P[19]														
P[23]														
P[24]														
P[25]														
P[28]														
P[40]														

Different colour codes used include: Yellow (most predominant strains reported globally), red (unusual combinations), green (rare strains and/or suspected human-animal reassortants) and white (not reported yet). The table was compiled from (Agbemabiese *et al*., 2017; Bányai *et al*., 2012; Bwogi *et al*., 2017; Dhital *et al*., 2017; Dóró *et al*., 2015; Esona *et al*., 2009; Hungerford *et al*., 2019; Matthijnssens *et al*., 2009; Mukherjee *et al*., 2011; My *et al*., 2014; Nyaga *et al*., 2018; Quaye *et al*., 2018; Rojas *et al*., 2019; Santos *et al*., 2001; Strydom *et al*., 2019).

In Latin America, a significant number of rare and/or reassortant strains have been recorded (de Oliveira *et al*., 2009; Linhares *et al*., 2011). There was an endemic persistence of the G5 porcine genotype in the 1900s, which was exclusively associated with P[6] and P[8] (Gouvêa *et al*., 1994; Mascarenhas *et al*., 1999, 2002). Since then, G5 has been reported in various parts of the world (Ahmed *et al*., 2007; Bok *et al*., 2001a; Chieochansin *et al*., 2016; Esona *et al*., 2009; Komoto *et al*., 2013; Mladenova *et al*., 2012). Porcine rotaviruses typically possess the constellation I5-R1-C1-M1-A8-N1-T1/7-E1-H1 as demonstrated by multiple studies (Martel-Paradis *et al*., 2013; Monini *et al*., 2014; Silva *et al*., 2016; Theuns *et al*., 2015). In

Europe (Bulgaria), the human G5P[6] strain possessed the constellation I1-R1-C1-M1-A8-N1-T1-E1-H1. This constellation was similar to the G5P[6] isolated in Japan, except for the VP6 segment that had the genotype I5 (Komoto *et al*., 2013; Mladenova *et al*., 2012).

Rare strains have also been demonstrated in Asian countries. The rare porcine-like strain G9P[23] with the constellation I5-R1-C1-M1-A8-N1-T1-E1-H1 was documented in Thailand and was suggested to have arisen via interspecies transmission from porcine to human (Komoto *et al*., 2017). The G9 has also been reported in combination with P[13], P[14], and P[19] (Do *et al*., 2017; Mukherjee *et al*., 2011; Shoeib *et al*., 2020; Takatsuki *et al*., 2019; Wu *et al*., 2017). Further, the rare porcine-like human strain, G26P[19], possessing the constellations I12-R1-C1-M1-A8-N1-T1-E1-H1 and I5-R1-C1-M1-A8-N1-T1-E1-H1 was isolated in Nepal and also in Vietnam, respectively. In both instances, the strains were shown to have arisen via interspecies transmission coupled with reassortment events (Agbemabiese *et al*., 2017; My *et al*., 2014).

Rare bovine-like strains such as the G6P[14], G10P[11], and G10P[14] have been reported in countries such as India, Italy, and Thailand (Banerjee *et al*., 2006; Iturriza-Gómara *et al*., 2004; Mandal *et al*., 2016; Medici *et al*., 2015; Quaye *et al*., 2018; Ramani *et al*., 2009; Tacharoenmuang *et al*., 2015, 2018). G10P[11] strains were shown to be human-bovine reassortants (Glass *et al*., 2005; Ramani *et al*., 2009). Whole genome analysis of the G10P[14] strain isolated in Honduras, Italy, and Thailand showed that the strain possessed a typical bovine constellation (I2-R2-C2-M2-A3-N2-T6-E2-H3) (Medici *et al*., 2015; Quaye *et al*., 2018; Tacharoenmuang *et al*., 2018). This strain was also reported in Oceania (Australia) and Europe (Slovenia) whereby it was shown to have arisen via interspecies transmission from bovine to human and/or reassortment events (Cowley *et al*., 2013; Steyer *et al*., 2010).

In sub-Saharan Africa, there is a scarcity of information on rare and/or reassortant strains at a whole genome level. The rare G8P[1], G10P[6], and G10P[8] strains were reported in Ghana and Nigeria (Adah *et al*., 2001; Lartey *et al*., 2018; Letsa *et al*., 2019). In Malawi and Rwanda, reassortant strains that possessed mixed genotype constellations (intergenogroup reassortants) were identified to be circulating post-vaccine implementation (Jere *et al*., 2018; Rasebotsa *et al*., 2021). Similarly, a G3P[4] emerged in Mozambique after rotavirus vaccine was implemented (João *et al*., 2020). Continuous active monitoring and whole genome sequencing of African strains is required for the detection of such strains.

2.8. The Zambian context

2.8.1. Vaccine introduction and impact

Zambia is a lower middle-income country in the sub-Saharan region with a population of about 18 million people and a Human Capital Index of 0.4 on a scale of 0-1 according to statistics by the United Nations (UN) and the World Bank Group (WBG) (UN, 2019; WBG, 2020). Prior to the introduction of rotavirus vaccine, approximately ten million diarrhoeal episodes occurred in Zambian children under the age of five, resulting in 63,000 hospitalisations and 15,000 deaths, making diarrhoea the third-leading cause of mortality in Zambian children. Further, a third of the diarrhoeal episodes were attributable to rotavirus disease (Chilengi *et al*., 2015; Mpabalwani *et al*., 1995; ZMOH, 2009). According to a study conducted in Lusaka between July 2012 and October 2013, rotavirus was the leading enteric pathogen detected among children presenting with diarrhoea (Chisenga *et al*., 2018).

To promote awareness on effective strategies for diarrhoea prevention, the Programme for Awareness and Elimination of Diarrhoea (PAED) was launched in 2012 by the Centre for Infectious Disease Research in Zambia (CIDRZ) in collaboration with the Zambian Ministry of Health (ZMOH). PAED was designed to focus on rotavirus vaccination of at least 180,000 children and carry out campaigns within the community on handwashing and management of diarrhoea (Bosomprah *et al*., 2016; Chilengi *et al*., 2015, 2017). The Interagency Co-ordination Committee reviewed data from clinical trials conducted in various African countries, as well as Zambia's epidemiology and other logistical requirements, in order to devise a strategy for deciding which vaccine to introduce in Zambia. The RV1 was chosen on the basis of the required storage system (immunisation supply chain), which, when compared to RV5, is a third of the space (Armah *et al*., 2010; Chilengi *et al*., 2015; Levy *et al*., 2009; Madhi *et al*., 2010; Mwenda *et al*., 2010; Steele *et al*., 1998).

The RV1 vaccine was gradually implemented in Zambia between January and October 2012 in Lusaka Province, and rolled out across the country in November 2013 (Beres *et al*., 2016; Chilengi *et al*., 2015). The vaccine is given in two doses at six and ten weeks, with no catch-up dose (Mpabalwani *et al*., 2016). Vaccine coverage in Zambia over the years went from 73% in 2014, 82% in 2015, 90% in 2016, 96% in 2017, 91% in 2018 and 90% in 2019 according to WHO-UNICEF coverage statistics (WHO, 2021c).

Routine rotavirus surveillance has been ongoing at the University Teaching Hospital in Lusaka since 2006 (Mwenda *et al*., 2010). Mpabalwani *et al*. (2016, 2018) documented rotavirus disease burden in Zambia during the pre-vaccine period (2009 to 2011) and after RV1 implementation (2013 to 2016). During the pre-vaccine period, rotavirus was detected in 40% of children who were hospitalised due to diarrhoea. A

significant decrease in rotavirus positivity to 30% in 2013, 24% in 2014, 27% in 2015 was observed in the post-vaccine period (Mpabalwani *et al*., 2016, 2018). The greatest reduction was observed in children less than one year, whereby rotavirus positivity declined from 44% before vaccine implementation to 26% after RV1 was implemented (Mpabalwani *et al*., 2016). Overall, a 52% reduction of rotavirus positive children was reported following the introduction of RV1, indicating a positive impact of the use of rotavirus vaccine (Mpabalwani *et al*., 2018).

Changes in the seasonality of rotavirus disease was also observed after RV1 was introduced in Zambia. Rotavirus disease displayed distinct seasonality with two peaks between May-July and September-October prior to RV1 introduction. However, the May-July seasonal peak was dwarfed, while the September-October peak was eliminated in the post-vaccine period (Mpabalwani *et al*., 2016, 2018).

2.8.2. Strain diversity in Zambia

Conventional genotyping-based surveillance reports in Zambia showed that circulating strains fluctuated from year to year before and after RV1 introduction. Between 2006 and 2011, G1P[8], G9P[8], G1P[6], G3P[6], G8P[4], G8P[6], G9P[6] and G12P[6] were detected in Zambia. The G1P[8] was predominant between 2006 and 2008, G9P[8] and G12P[6] in 2009, G9P[8], G1P[6], and G9P[6] between 2010 and 2011 (Mwenda *et al*., 2010; Seheri *et al*., 2014, 2018; Simwaka *et al*., 2018). G2P[4] and G2P[6] strains were only detected in the 2008 and 2010 rotavirus seasons, respectively, at very low frequencies. On the contrary, an epidemic of G2P[4] strains was observed in 2012 after RV1 introduction whereas G2P[6] strains were prevalent from 2013 to 2015. The G1P[8] was present in 2012 and disappeared in 2013 after which it became predominant in 2014 (Simwaka *et al*., 2018). The yearly strain fluctuations in Zambia after RV1 implementation from most to least predominant were as follows: G2P[4], G1P[8], G2P[6] in 2012, G2P[6], G2P[4] in 2013, G1P[8], G2P[6], G2P[4] in 2014 and G2P[4], G2P[6] in 2015 (Simwaka *et al*., 2018). The unusual bovine-like strains, G8P[4], G8P[6], and G8P[8] were also documented in the post-vaccine period, albeit at low frequencies (Simwaka *et al*., 2018).

2.9. Next Generation Sequencing technologies

Next Generation Sequencing (NGS) has enabled researchers to study the entire genomes of various lifeforms such as plants, animals, and microorganisms. NGS technologies were developed to meet the demand for higher sequencing capacity as well as lower cost per nucleotide for large genome sequencing projects (Metzker *et al*., 2010). Further, NGS plays an important role in various virology applications such as identification of novel viruses and/or strains of known viruses and their origin, as well as monitoring of the spread and transmission of outbreaks caused by viral pathogens. This has been demonstrated via

recent experiences with viruses such as Zika, Ebola, Dengue, Chikungunya, severe acute respiratory syndrome (SARS), and Middle East respiratory syndrome (MERS) coronavirus (de Wit *et al*., 2016; Kafetzopoulou *et al*., 2018; Kamelian *et al*., 2019; Quick *et al*., 2016; Sahadeo *et al*., 2017; Shrivastava *et al*., 2018; Wang *et al*., 2020; Zakotnik *et al*., 2019). Identification of viral sequences relies heavily on sequence-based matching of the unknown sequences to known sequences that are available in data banks such as the GenBank hosted by NCBI, the European Molecular Biology Laboratory (EMBL) hosted by the European Bioinformatics Institute, the DNA Data Bank of Japan (DDBJ), the Virus Pathogen Resource (ViPR), and the Global Initiative on Sharing All Influenza Data (GISAID) database (Benson *et al*., 2013; Goujon *et al*., 2010; Mashima *et al*., 2016; Pickett *et al*., 2012; Shu and McCauley, 2017).

The world is currently facing the Coronavirus disease 2019 (COVID-19) pandemic, caused by SARS Coronavirus 2 (SARS-CoV-2). The SARS-CoV-2 was identified in December 2019 and January 2020 in respiratory samples as the causative agent of a cluster of pneumonia cases in Wuhan, China through a combination of Illumina® sequencing and nanopore sequencing (Zhu *et al*., 2020). More than 154 million individual cases of COVID-19 and a death toll of more than three million have been reported as of 7th May 2021 in more than 200 countries (WHO, 2021b). The pandemic triggered the formation of efficient real-time surveillance strategies based on genome sequencing that brought forth over 100,000 complete and partial SARS-CoV-2 genomes in a relatively short time (Chiara *et al*., 2021; Gonzalez-Reiche *et al*., 2020; Meredith *et al*., 2020; Rockett *et al*., 2020). The data obtained made it possible to rapidly track and investigate the virus based on global transmission routes, how quickly the virus adapts as it spreads, and the role of co-infection, leading to rapid development of prophylactic remedies like vaccines (Illumina, 2021a; Lu *et al*., 2020; Zhu *et al*., 2020).

The first sequencing technologies were developed in the 1970s by Frederick Sanger, Allan Maxam and Walter Gilbert (Maxam and Gilbert, 1977; Sanger *et al*., 1977a). Sanger's techniques required fewer toxic chemicals and radioisotopes handling compared to that of Maxam and Gilbert. Due to this, Sanger sequencing techniques were continually improved which later led to the generation of the first human genome sequences, carried out by two competing teams led by Eric Lander and John Craig Venter (Lander *et al*., 2001; Venter *et al*., 2001). However, even after this revolutionary achievement, there was still a need for faster and greater sequencing throughput at affordable costs. As a result, the National Human Genome Research Institute (NHGRI) initiated a funding program aimed at lowering the cost of sequencing. This stimulated the development of NGS technologies (Schloss, 2008).

Launched in 2005, the Roche 454 pyrosequencer (Basel, Switzerland) was the first commercially successful NGS technology. This sequencer employed a bead emulsion amplification strategy. However, due to its inability to compete with other platforms in terms of yield and cost, the 454 pyrosequencer was discontinued (Goodwin *et al*., 2016; Margulies *et al*., 2005). Several other NGS technologies with different physical and chemical sequencing strategies have since been developed by companies such as Pacific Biosciences (California, United States), 10X Genomics (California, United States), Oxford Nanopore technologies (Oxford, United Kingdom), Qiagen (Hilden, Germany), and Illumina® (California, United States). These technologies have enabled the production of huge amounts of data at lower cost-per-kilobase (Goodwin *et al*., 2016; Mardis, 2008, 2011; Metzker, 2010; Quail *et al*., 2012).

Despite the existence of several NGS providers, Illumina® is arguably the current market leader (Goodwin *et al*., 2016; Hernandez, 2018; Van Dijk *et al*., 2014). This is due to its wide range of platforms, high accuracy and throughput (volume of data generated and cost per base pair of this data), high level of compatibility with other platforms as well as the wide range of applications offered both in research and clinical applications (Goodwin *et al*., 2016; Kumar *et al*., 2019). The suite of Illumina® sequencing platforms range from the low throughput fast-turnaround iSeq 100 to the powerful ultra-high throughput NovaSeq 6000 that was recently released (Illumina, 2021b). The standard measure for sequencing run quality on Illumina platforms is their Phred quality score (Q score). Illumina® sequencers produce millions to billions of reads per run with a Q score of 30 and above (an average error rate of 1 per 1000 bases). The high quality of reads produced by Illumina® platforms is another factor that has ensured the company's success (Goodwin *et al*., 2016; Kwon *et al*., 2013; Manley *et al*., 2016; Tan *et al*., 2019). The success of Illumina® can easily be verified by analysing the vast number of globally published clinical and research studies conducted using Illumina® platforms (Kafetzopoulou *et al*., 2018; Mogotsi *et al*., 2020; Nyaga *et al*., 2018; Omasanggar *et al*., 2020; Paulsen *et al*., 2021; Pereira *et al*., 2020; Rockett *et al*., 2020; Thibodeau *et al*., 2020; Yee *et al*., 2021; Zakham *et al*., 2019).

The Illumina® MiSeq platform was used in this study to sequence the whole genomes of rotavirus strains. This platform has a capacity to generate up to 15 Gigabytes of data per run, a simple workflow, the ability to convert deoxyribonucleic acid (DNA) to data within a few hours (fast turnaround), stellar data quality, and a user-friendly software integrated into the sequencer (Illumina, 2021c). The MiSeq employs a sequencing by synthesis method and uses fluorescently labelled reversible terminator nucleotides (Bentley *et al*., 2008). The preparation of DNA libraries is carried out before sequencing, which affixes barcodes/adaptors to DNA fragments of appropriate size. The actual sequencing is conducted on a flow

cell. The DNA libraries are hybridised to the flow cell containing patterned clusters of complementary adaptors. This is usually followed by clonal amplification that produces millions-to-billions of clusters (areas that contain copies of the same DNA) of clonal template DNA that can be sequenced simultaneously (Kumar *et al*., 2019; Liu *et al*., 2012; Mardis, 2008).

2.9.1. Sequence independent amplification for virus discovery

The main advantage of NGS platforms is the ability to sequence DNA samples without any prior knowledge of the sequence for priming (Margulies *et al*., 2005). Before PCR techniques were developed (early 1980s), methods such as simple cloning (parvovirus) and recombinant cDNA library construction (HCV) were utilised in virus diagnostics (Choo *et al*., 1989; Clewley, 1985). Later, primers that were based on the conserved regions of viral genomes were designed to amplify sequences from novel viruses. However, these primers were only useful when searching for a specific type of viral genome (Donehower *et al*., 1990; Wichman and Van Den Bussche, 1992).

Several PCR techniques were then developed in the 1900s which involved ligation of primer binding sites to DNA fragments and sequence enrichment by amplification (Muerhoff *et al*., 1997). One well known such technique is the Sequence-independent single primer amplification (SISPA). SISPA was used to identify viral nucleic acids of unknown sequence at low concentrations. This technique was introduced by Reyes et al. (1991). Lambden and Clarke then developed a SISPA technique for double-stranded RNA (dsRNA) viruses, whereby they demonstrated the feasibility of the technique using a human RVC of 728 base pairs (Lambden and Clarke, 1995). In this method, oligonucleotide that is ligated on the dsRNA is blocked at the 3'end by an amino group and phosphorylated at the 5'end. Following this, RNA is then reverse transcribed to cDNA and amplified by PCR using a complementary primer (Lambden and Clarke 1995; Lambden *et al*., 1992). In SISPA, extracted nucleic acid sample is first pre-treated and purified using a variety of methods, followed by generation of cDNA (if working with RNA) and adapter ligation. A primer that is complementary to the adapters can then be used to amplify the unknown viral sequence, followed by a second round of selective amplification (Allander *et al*., 2004; Ambrose and Clewley, 2006). Since then, several studies have combined random priming approaches with NGS to viral sequences (Blomström *et al*., 2010; Victoria *et al*., 2009).

Chapter three: Rare reassortant porcine-like G5P[6]

3.1. Preamble

This chapter entails a scientific paper entitled '***Molecular characterisation of a rare reassortant porcine-like G5P[6] rotavirus strain detected in an unvaccinated child in Kasama, Zambia***' published in the special issue 'Rotavirus and Rotavirus vaccines' of the Pathogens journal (impact factor 3.492). The paper can be found online using the identifier: https://doi.org/10.3390/pathogens9080663 and is presented here in its entirety with a few minor exclusions, that is, the acknowledgments section, funding, and declaration of conflict of interest by the authors. Furthermore, in addition to what was published in the scientific paper, the section on materials and methods has been extended in depth, and the references were adapted to fit the dissertation style. Additionally, a copy of the abstract page is provided in **Appendix 5**.

Author contributions are as follows: Conceptualisation of the main project (Martin Nyaga, Mphahlele Jeffrey, Mapaseka Seheri, Jason Mwenda), funding acquisition and project administration (Martin Nyaga, Jason Mwenda), facilitation of the samples (Martin Nyaga, Julia Simwaka, Evans Mpabalwani, Mphahlele Jeffrey, Ina Peenze, Mapaseka Seheri, Jason Mwenda), laboratory experiments (**Wairimu Maringa**, Peter Mwangi, Julia Simwaka, Evans Mpabalwani, Martin Nyaga), formal analysis (**Wairimu Maringa**, Mathew Esona, Martin Nyaga), data curation (**Wairimu Maringa**, Peter Mwangi, Martin Nyaga) and writing of the manuscript (**Wairimu Maringa**).

Addressing objective one and two in an overlapping manner, this chapter discusses a rare G5P[6] strain which possessed a unique genotype constellation similar to that of porcine strains. This was the first report of whole genome characterisation of a human G5P[6] rotavirus strain in the African region through the WHO Regional Office for Africa (WHO/AFRO) rotavirus surveillance network. The strain was shown to have likely arisen because of reassortment events between strains of porcine and porcine-like human origin.

3.2. Introduction

Group A rotaviruses (RVA), of the family *Reoviridae*, are the number one viral pathogens causing severe diarrhoea in children below five years of age (Estes and Greenberg, 2013). In 2016, an estimated 128,000 deaths in children below five years were due to RVA infections, 90% of which occurred in developing countries (Tate *et al*., 2016; Troeger *et al*., 2018). Similarly, RVA are the primary cause of acute gastroenteritis in new-born piglets (Martella *et al*., 2010).

Rotaviruses have a distinctive morphology which comprises a nonenveloped, three-layered icosahedral protein shell. The rotavirus genome within the protein shell comprises 11 segments of double-stranded (dsRNA) that encode six structural viral proteins (VP1 to VP4, VP6, and VP7) and five or six non-structural

proteins (NSP1 to NSP5/6) (Estes and Greenberg, 2013). A binary classification system is used to distinguish RVA based on the antigenic properties of the outer shell proteins, VP7 and VP4, that determine the G-genotype and P-genotype, respectively (Estes and Greenberg, 2013). Furthermore, RVA can be separated into two main genogroups and one minor genogroup according to a whole genome classification system, whereby a specific genotype is assigned to the 11 gene segments. These genogroups represent the genotype constellations that are present in most human strains globally (Matthijnssens *et al*., 2008a). Genogroup 1 (Wa-like) bears the constellation I1-R1-C1-M1-A1-N1-T1-E1-H1 and is often associated with the G genotypes G1, G3, G4, G9, and G12 and P genotype P[8]. Genogroup 2 (DS-1-like) includes G2P[4] strains and bears the constellation I2-R2-C2-M2-A2-N2-T2-E2-H2. Lastly, the minor genogroup 3 (AU-1-like) bears the I3-R3-C3-M3-A3-N3-T3-E3-H3 constellation and includes G3P[9] strains (Matthijnssens and Van Ranst, 2012). As of 5th May 2020, the Rotavirus Classification Working Group had identified at least 36 G, 51 P, 26 I, 22 R, 20 C, 20 M, 31 A, 22 N, 22 T, 27 E, and 22 H genotypes (RCWG, 2021). The whole genome classification system has made it possible to analyse and understand the origin of various strains, interspecies transmission, and animal–human reassortment events (Ghosh and Kobayashi, 2011). Human Wa-like strains and porcine rotavirus strains share a common origin, whereas DS-1-like and AU-1-like strains have a common origin with bovine and feline strains, respectively (Matthijnssens *et al*., 2008a).

In humans, G1-G4, G9, and G12 along with P[4], P[6], and P[8] are the most frequently detected, globally (Iturriza-Gómara *et al*., 2011; Matthijnssens *et al*., 2010; Patel *et al*., 2011; Rahman *et al*., 2007). On the contrary, in porcine, predominant genotypes are G3-G5, G9, and G11 along with P[6], P[7], and P[13] (Martella *et al*., 2010; Papp *et al*., 2013). Porcine rotaviruses bear the constellation I5-R1-C1-M1-A8-N1-T1/T7-E1-H1 (Agbemabiese *et al*., 2017; Kim *et al*., 2012; Martel-Paradis *et al*., 2013; Matthijnssens *et al*., 2008a; Monini *et al*., 2014; Silva *et al*., 2016; Theuns *et al*., 2015). While human Wa-like RVA differ from porcine rotaviruses in some gene segments (VP4, VP6, VP7, and NSP1), they both appear to have genotype 1 in the VP1, VP2, VP3, NSP2, NSP3, NSP4, and NSP5 gene segments. Hence, the suggestion that human Wa-like and porcine RVAs have arisen from a common ancestor (Matthijnssens *et al*., 2008a).

The findings that show animals can serve as potential reservoirs for genetically diverse rotavirus strains that can be passed on to humans have elicited a large amount of interest and topics for further research (Dóró *et al*., 2015). Several novel and rare animal-like or animal–human reassortant rotavirus strains have been identified globally (Cowley *et al*., 2013; Komoto *et al*., 2017; Malasao *et al*., 2018; Mukherjee *et al*., 2011; My *et al*., 2014; Quaye *et al*., 2018; Tacharoenmuang *et al*., 2018). The detection of animal strains

in humans is presumed to be as a result of zoonotic transmission, along with reassortment, which contributes to the diversity of circulating RVA (Bwogi *et al*., 2017; Martella *et al*., 2010; Matthijnssens *et al*., 2009). Inter- and intra-genogroup reassortment may occur when multiple RVA simultaneously infect a host. This is attributed to the segmented nature of the rotavirus genome (Estes and Greenberg, 2013; Nyaga *et al*., 2015). It is, therefore, necessary to continuously carry out the monitoring of animal RVA and the role they play in contributing to the diversity of circulating RVA in humans.

The G5, one of the most common porcine genotypes, has sporadically been identified in human populations in Brazil (G5P[X]), Cameroon (G5P[7] and G5P[8]), Argentina (G5P[8]), and the United Kingdom(G5P[X]) (Beards and Graham, 1995; Bok *et al*., 2001; Esona *et al*., 2004, 2009; Gouvêa *et al*., 1994). The P[6] is presumed to be of porcine origin. They have also been identified in human populations (Hwang *et al*., 2012; Lorenzetti *et al*., 2011; Martella *et al*., 2006a; Nyaga *et al*., 2018). The first human G5P[6] strain, LL36755, was detected in a child who had acute gastroenteritis in China in 2007 (Li *et al*., 2008). Other G5P[6] strains were detected in Vietnam, Taiwan, Bulgaria, Japan, and Thailand (Ahmed *et al*., 2007; Chieochansin *et al*., 2016; Duan *et al*., 2007; Hwang *et al*., 2012; Komoto *et al*., 2013). To date, the whole genome of only two human G5P[6] strains—Bulgarian BG620 (nt sequences unavailable in the DDBJ, EMBL, and GenBank data libraries as of 13 August 2020) and Japanese Ryukyu-1120 (full open reading frame, available in GenBank)—have been analysed (Komoto *et al*., 2013; Mladenova *et al*., 2012).

Diarrhoea is a burden for the Zambian healthcare system, with about 33% of the extreme cases being attributable to RVA (Chilengi *et al*., 2015; Mpabalwani *et al*., 1995; ZMOH, 2014). In an attempt to generate disease burden attributable to rotavirus diarrhoea in children, the Zambian Ministry of Health, with support from WHO, launched rotavirus surveillance at the University Teaching Hospital (UTH) in 2006 (Mpabalwani *et al*., 2016, 2018). Surveillance data generated provided evidence of the burden of rotavirus diarrhoea that supported the introduction of the rotavirus vaccine, Rotarix®, as a pilot project in Lusaka, Zambia in 2012, and was later rolled out nationwide in November 2013 (Mpabalwani *et al*., 2016). According to the estimates reported by the World Health Organization (WHO) and the United Nations International Children's Emergency Fund (WHO/UNICEF), rotavirus vaccine coverage in Zambia has been consistently high for the last six years, increasing from 73% in 2014 to 90% in 2019 (WHO, 2021c). Over this period, a sustained and significant reduction in rotavirus-associated hospitalisations and mortality was observed in children under 5 years (Mpabalwani *et al*., 2018).

The African Rotavirus Surveillance Network, coordinated by the World Health Organization Regional Office for Africa (WHO/AFRO), is actively monitoring the diversity and distribution of RVA genotypes in

children hospitalised with acute diarrhoea (Mwenda *et al*., 2014). Initially, the network was established with four countries in 2006, and expanded to 29 countries by the end of 2016 (Mwenda *et al*., 2010, 2017). The Diarrhoeal Pathogens Research Unit at Sefako Makgatho University in Pretoria (South Africa) and the Noguchi Memorial Institute for Medical Research in Accra (Ghana) are the two WHO Rotavirus Regional Reference Laboratories (RRLs) for the network that conducts monitoring of rotavirus epidemiology in Africa (Mwenda *et al*., 2010). The WHO/AFRO is currently supporting the University of the Free State-Next Generation Sequencing (UFS-NGS) unit to undertake rotavirus surveillance of rotavirus strains that circulated in Zambia between 2013 and 2016 at the whole genome level. A G5P[6] strain, UFS-NGS-MRC-DPRU4723, was identified among these strains and was analysed so as to elucidate its origin and evolution. The sample was collected in 2014 from an unvaccinated 12-month-old male hospitalised for gastroenteritis at Arthur Davison Children's Hospital in Ndola, Zambia.

3.3. Methodology

3.3.1. Ethical consideration

This study was approved on the 15th of April 2020, by the Health Science Research Ethics Committee (HSREC), University of the Free State, Bloemfontein, South Africa under ethics number UFS-HSD2020/0277/2104 (**Appendix 6**). The study is a subset of a pilot project that involved whole genome characterisation of samples collected in Zambia in the post-vaccine period as part of the ongoing rotavirus surveillance in the country. The project is supported by the WHO/AFRO (reference 2017/757922-0) in collaboration with the UFS-NGS unit. Ethical clearance for the main project was obtained under ethics number HSREC130/2016(UFS-HSD2016/1082).

3.3.2. Sample collection

The WHO/AFRO has been conducting annual surveillance in Zambia to monitor and document circulating rotavirus strains in the country. Extraction of RNA from stool samples and conventional G- and P- typing was carried out at the WHO rotavirus Regional Reference Laboratory (WHO-RRL) that is based in the Diarrhoeal Pathogens Research unit (DPRU) at the Sefako Makgatho Health Sciences University (SMU). The samples were then transferred to UFS-NGS unit, facilitated by a Material Transfer Agreement (MTA:NGS Unit, UFS(1)). For the pilot project conducted at UFS-NGS, a total of 133 samples were sequenced and only five were not typical RVA strains after whole genome sequencing, which was the inclusion criteria for this study. These five samples were collected in the post-vaccine period (between 2013 and 2016). Given that rotavirus has 11 genome segments, 55 genome segments were analysed in this study.

3.3.3. Demographic information of the G5P[6] sample presented in this chapter

The sample was collected in 2014 from an unvaccinated 12-month-old male at Arthur Davison Children's Hospital (ADCH) in Ndola, a rotavirus surveillance sentinel site. The child had travelled with parents from Kasama, a town in the Northern Province of Zambia, which is approximately 760 km away from Ndola, Zambia. This child was admitted to a paediatric ward at ADCH, with gastroenteritis of four days duration and a history of fever. Frequency of vomiting and diarrhoea was three episodes and two episodes, respectively, in the previous 24 hours. The level of dehydration was assessed as mild and the child received an oral rehydration solution and was discharged after a few days. The stool sample was screened using the enzyme immunoassay (EIA) technique for the presence of RVA antigen in the Virology laboratory in Lusaka. It was randomly picked and sent to the Diarrhoeal Pathogens Research Unit (DPRU), a World Health Organization Rotavirus Regional Reference Laboratory (WHO-RRL) in Pretoria, South Africa as part of the WHO/AFRO annual rotavirus surveillance. Conventional genotyping was carried out at DPRU. Thereafter, the sample was shipped to the UFS-NGS unit for sequencing and whole genome analysis.

3.3.4. Extraction of RNA

RNA extraction from stool was the first step in the processing of samples for NGS. This was performed according to already established methods (Nyaga *et al*., 2018; Potgieter *et al*., 2009). This process can briefly be divided into homogenisation/lysis, phase separation, RNA precipitation, and RNA enrichment (**Figure 3.1**).

First, stool samples (approximately 100 mg of each sample) were suspended in phosphate-buffered saline (Sigma-Aldrich, Saint Louis, Missouri, United States), vortexed briefly (Chiltern Scientific, Wilmington, North Carolina, United States) and incubated for 10 minutes which allowed supernatant to form above the debris. Following this, 300 µl of supernatant was transferred to 2.5 ml Eppendorf tubes (Eppendorf, Hamburg, Germany) and 900 µl of TRIzol/ TRI-Reagent® (Molecular Research Centre, Cincinnati, Ohio, United States) was then added into the 300 µl supernatant. The mixture was vortexed briefly and incubated for 5 minutes at room temperature which ensured that complete lysis occurred in the samples. TRIzol, a mixture of guanidine thiocyanate and phenol, is acidic, inhibits RNase activity, and enables the separation of RNA from DNA and proteins (Brawerman *et al*., 1972; Chomczynski and Sacchi, 1987).

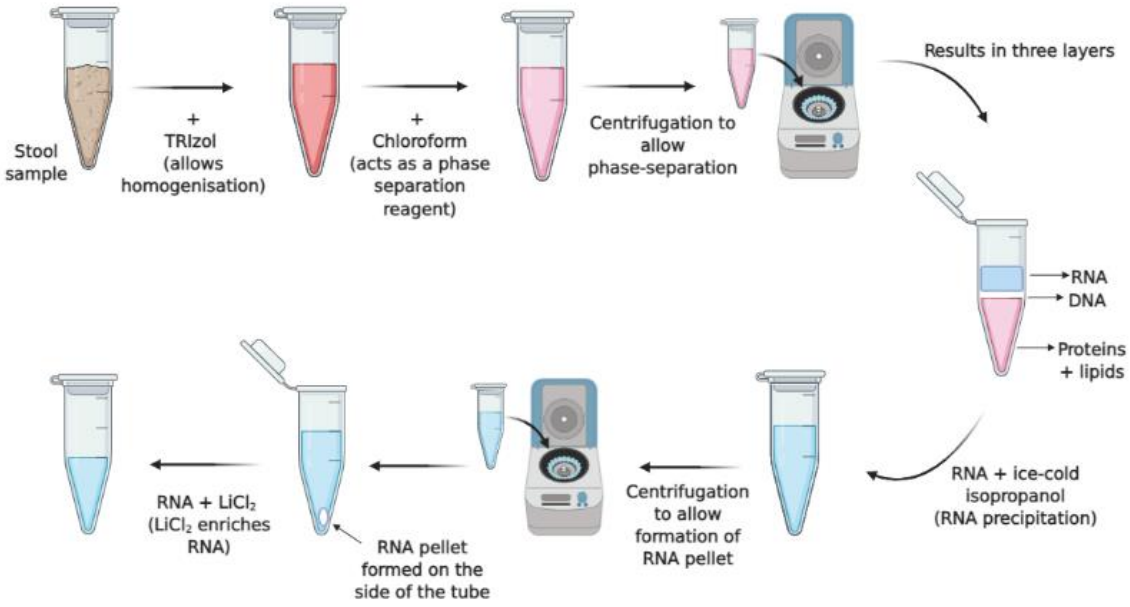


Figure 3.1. Summary of the RNA extraction process. Image created on BioRender on 22.12.2020.

Following incubation, a volume of 300 µl of chloroform (Sigma-Aldrich, Saint Louis, Missouri, United States) was then gently pipetted into the tubes, vortexed briefly and incubated for 5 minutes at room temperature. The tubes were then centrifuged in an Eppendorf microcentrifuge 5424r (Eppendorf, Hamburg, Germany) at 17,300 *x g* for 20 minutes at 4 °C which ensured that phase separation occurred. Chloroform acted as a phase-separation reagent, which caused RNA to be separated away from DNA, protein and lipids thus resulted in three phases: a clear upper aqueous phase (RNA), interphase (DNA) and lower organic phase (proteins and lipids). The RNA in the upper aqueous phase was collected and transferred into new tubes after which 700 µl of ice-cold isopropanol (Sigma-Aldrich, Saint Louis, Missouri, United States) was added to precipitate RNA. The tubes were incubated for 20 minutes to allow precipitation then centrifuged at 17,300 *x g* for 30 minutes at 4 °C. RNA is insoluble in isopropanol; hence a white pellet was formed on the side of the tube. The supernatant was discarded, and the tubes were left to air dry for 10 minutes. To dissolve and recover the dsRNA, a volume of 95 µl elution buffer (buffer EB that contains 10 mM Tris-Cl, pH 8.5) (Qiagen, Hilden, Germany) was added to the tubes, briefly vortexed and left to stand for 10 minutes at room temperature. Precipitation of ssRNA was done using a volume of 30 µl 8 M lithium chloride ($LiCl_2$) (Qiagen, Hilden, Germany) for 16 hours at 4 °C in a water bath in a Tupper wear box. Lithium chloride does not efficiently precipitate DNA or protein and also removes inhibitors of cDNA synthesis hence advantageous for precipitation of RNA (Barlow *et al*., 1963; Cathala *et*

al., 1983). $LiCl_2$ removes ssRNA, thus is a perfect agent for the enrichment of dsRNA viruses like rotaviruses. The integrity of the extracted RNA (5 µl) was then assessed on 1 % Tris Borate ethylenediaminetetraacetic acid (TBE) agarose gel at 100 volts for 90 minutes and visualised using a G:Box UV transilluminator (Syngene, Cambridge, United Kingdom).

3.3.5. Purification of the extracted RNA

A MinElute® gel extraction kit (Qiagen, Hilden, Germany) was used to purify the extracted RNA. To 1.5 ml Eppendorf microcentrifuge tubes (Eppendorf, Hamburg, Germany), 300 µl of buffer QG and 25 µl of the previously extracted dsRNA were added. The mixture was mixed by pipetting, pulse-spun and transferred into MinElute® Spin columns. The spin columns were centrifuged at 11,000 *x g* for 1 minute at 4 °C and the flow through was discarded. Buffer QG provided optimal conditions for binding of RNA to the silica membrane in the spin column. Additionally, the buffer contains a pH indicator for easy observation (yellow for optimal pH, purple indicates high pH i.e., >7.5). PE wash buffer containing ethanol (750 µl) was added to each column, left to stand for 2 minutes at room temperature then centrifuged at 13,300 *x g* for 1 minute at 4 °C. Flow through was discarded and centrifugation was repeated under the same conditions. The columns were placed into new 1.5 ml Eppendorf microcentrifuge tubes (Eppendorf, Hamburg, Germany) followed by subsequent addition of 30 µl elution buffer. The mixture was left to stand for 1 minute at room temperature and then centrifuged at 11,000 *x g* for 1 minute at 4 °C. The columns were then discarded. The integrity of purified dsRNA (5 µl) was then assessed on 1 % TBE agarose gel at 100 V for 90 minutes. The RNA was visualised using a G: Box UV transilluminator (Syngene, Cambridge, United Kingdom).

3.3.6. Quantification of viral RNA

After extraction and purification, the quantity and quality of viral RNA was further evaluated on a BioDrop™ µ-Lite spectrophotometer (Biochrom, Cambridge, United Kingdom). Firstly, the spectrophotometer was programmed to the RNA option under nucleic acids. The sample port on the instrument was then cleaned using 2 µl of nuclease free water (Sigma-Aldrich, St Louis, Missouri, United States), followed by calibration using 2 µl of elution buffer (Invitek Molecular, Berlin, Germany). After this, purified RNA (2 µl) was loaded onto the sample port to determine RNA concentration and the 260/280 absorbance ratio. Wiping of the sample port was done in-between measurements to avoid cross-contamination and minimise inaccuracies. The samples with a 1.8-2.2 absorbance ratio were considered pure for further processing (Desjardins and Conklin, 2010).

3.3.7. Complementary DNA synthesis

Viral RNA was first converted to complementary DNA (cDNA) through a process known as reverse transcription. A Maxima™ H Minus Double-Stranded cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, Massachusetts, United States) was utilised to synthesise double-stranded cDNA from the quantified total viral RNA. The kit was used according to manufacturer's instructions, but with minor modifications.

For synthesis of the first-strand cDNA, the following was performed: a volume of 13 µl of RNA was pipetted (Labnet International, Edison, New Jersey, United States) into a 0.2 ml PCR tube (NEST® Biotechnology, Jiangsu, China) and incubated for 5 minutes at 95 °C in a thermocycler (Labnet International, Edison, New Jersey, United States) whose lid was preheated to 105 °C. This resulted in denaturation of dsRNA, whereby the hydrogen bonds that held together the two strands of RNA were broken and the strands unwound from each other resulting in two separate ssRNA strands. The PCR tubes were then briefly spun on a myFUGE™ mini centrifuge (Benchmark Scientific, Sayreville, New Jersey, United States) which allowed for the condensation of the sample in the tube. Following this, 1 µl of random hexamer primer was added into the PCR tubes, mixed by pipetting up and down, centrifuged briefly, incubated for 5 minutes at 65 °C and then placed on ice. Random hexamer primer consists of short oligonucleotides, usually six nucleotides hence the term 'hexamer'. These oligonucleotides have a random sequence which enables the unbiased/non-specific binding (annealing) of the primer to the RNA template and amplification of all RNA regions. Due to the unbiased binding, random hexamer primer can anneal to all classes of RNA present in a sample. The tube was placed on ice because primers bind to RNA at low temperatures.

After the annealing step, 5 µl of 4X First Strand Reaction Mix (similar to a PCR master-mix) and 1 µl of First Strand Enzyme Mix (a mixture of reverse transcriptase and RNase Inhibitor) were added to the tubes in that order and briefly centrifuged. The mixture was first incubated for 10 minutes at 25 °C and then for 2 hours at 50 °C. In this period, reverse transcriptase incorporates deoxynucleoside triphosphates (dNTPs) to generate single-strand cDNA that is complementary to RNA (cDNA:mRNA hybrid). RNase inhibitor in cDNA synthesis was used to inhibit the activity of RNases A, B and C thus prevented degradation of RNA. The reaction was stopped by incubating the mixture for 5 minutes at 85 °C and subsequently cooled on ice. The high temperature deactivates the reverse transcriptase enzyme.

For second-strand cDNA synthesis, the first-strand cDNA that was generated using the steps above was used as a template to generate double-stranded cDNA through a process known as nick translation. The following enzymes were involved in second-strand cDNA synthesis: E. *coli* DNA polymerase I, E. *coli* RNase

H, and E. *coli* DNA ligase (Gubler and Hoffman, 1983; Rigby *et al*., 1977). A volume of 55 µl nuclease-free water, 20 µl 5X Second Strand Reaction mix, and 5 µl Second Strand enzyme was added to the mixture from the first-strand step which amounted to a total volume of 100 µl, centrifuged briefly and incubated for 1 hour at 16 °C. During nick translation, RNase H produces nicks (breaks phosphodiester bonds between nucleotides) in the mRNA strand of the hybrid thus creating a series of 3' binding sites for DNA synthesis. DNA polymerase I recognises the nicks and replaces the gaps with new nucleotides/dNTPs to the 3'end created after a nick in the 5' → 3' direction while simultaneously removing nucleotides. This process repeats over and over as the DNA polymerase I removes existing nucleotides and replaces them with new ones at the site of the nick, resulting in the incorporation of many labelled and unlabelled nucleotides onto the growing sequence, thus leading to the generation of a new complementary strand. A nick remains where DNA polymerase I dissociates, and this is sealed by DNA ligase (D'Alessio and Gerard, 1988; Gubler and Hoffman, 1983; Hermanson, 2013; Okayama and Berg, 1982). Because DNA polymerase has a tendency to carry out strand displacement rather than nick translation at high temperatures, the relatively low temperature of 16 °C provided optimum conditions for nick translation to occur (Eun, 1996). The reaction was halted by addition of 6 µl 0.5 M EDTA. Following this, any residual RNA was removed by adding RNase I (10 µl) to the mixture and the tube was incubated for 5 minutes at 25 °C.

3.3.8. Purification of the double-stranded cDNA

The synthesised double-stranded cDNA was subjected to purification using the MSB® Spin PCRapace purification kit (Invitek Molecular, Berlin, Germany). A volume of 50 µl cDNA was transferred to a 1.5 ml microcentrifuge tube (Molecular Bioproducts, San Diego, California, United States), followed by addition of 250 µl binding buffer and mixed thoroughly by vortexing (Labnet International, Edison, New Jersey, United States) briefly. A spin filter tube (Invitek Molecular, Berlin, Germany) was then placed in a 2.0 ml receiver tube (Invitek Molecular, Berlin, Germany). Into the receiver tube with the spin filter, 300 µl of the mixture was transferred using the P1000 pipette (Labnet International, Edison, New Jersey, United States), incubated for 1 minute at 25 °C then centrifuged (Labnet International, Edison, New Jersey, United States) at 24,000 *x g* for 4 minutes. In this step, cDNA selectively binds to the surface of the spin filter with the aid of the binding buffer. This removes impurities from DNA since the cDNA synthesis mixtures used in the previous step such as dNTPs, primers and enzymes do not bind to the spin filter. Centrifugation enables the impurities to be drawn out of the spin filter column. The purified cDNA was then eluted by use of 12 µl elution buffer (buffer EB containing 10 mM Tris-Cl, pH 8.0) which was added directly to the

centre of the spin filter, incubated for 3 minutes at 25 °C and centrifuged at 18,000 *x g* for 1 minute. Elution buffer provides optimum basic conditions for elution of cDNA.

3.3.9. Quantification of purified cDNA

It was important to quantify cDNA to ensure that reproducible and consistent results are obtained after the preparation of DNA libraries. Accurate and precise quantification of DNA is critical for the efficient use of DNA samples in sequencing (Haque *et al*., 2003; Simbolo *et al*., 2013). Purified cDNA was therefore quantified on a Qubit™ 3.0 fluorometer (Life Technologies by Thermo Fisher Scientific, Waltham, Massachusetts, United States). The Qubit™ fluorometer (Life Technologies by Thermo Fisher Scientific, Waltham, Massachusetts, United States) is an instrument that measures protein and nucleic acid. Various assays that contain sensitive dyes that fluoresce in proportion to the amount of protein, RNA, and DNA are used together with the Qubit™ fluorometer (O'Neill *et al*., 2011).

Fluorometric methods are accurate compared to spectrophotometric/absorbance-based assays. This is because absorbance methods measure any compound that can absorb light at 260 nm including impurities and single-stranded nucleic acid, thus may lead to the overestimation of nucleic acid that is present in a sample. Absorbance methods cannot differentiate between RNA, DNA, or protein and therefore not suitable for downstream NGS processes (Glasel, 1995; Manchester, 1996; O'Neill *et al*., 2011). On the other hand, fluorometric methods, which in this case is the Qubit system uses fluorescent dyes that are sensitive and specific, allowing you to quantify only either RNA or DNA (O'Neill *et al*., 2011). In this study, a Qubit™ dsDNA High Sensitivity (HS) assay kit (Invitrogen™ by Thermo Fisher Scientific, Waltham, Massachusetts, United States) which exhibits high accuracy and precision for pure dsDNA was used. Fluorescent dyes specifically bind to dsDNA and ignores the presence of any contaminating RNA, proteins or single-stranded DNA (Sah *et al*., 2013; Simbolo *et al*., 2013).

A working solution for 10 assays was first prepared by adding 10 µl of Qubit dsDNA HS reagent which usually contains fluorescent dyes and 1990 µl of Qubit HS buffer into a falcon tube (**Figure 3.2**). Following this, 198 µl of the working solution was added into separate sterile Qubit tubes for the cDNA samples, and 190 µl was added into two additional Qubit tubes for the standards. Afterwards, 2 µl of each cDNA sample was then added to the tubes that contained 198 µl working solution, and 10 µl of Qubit standard 1 and 2 were separately added to the tubes that contained 190 µl working solution.

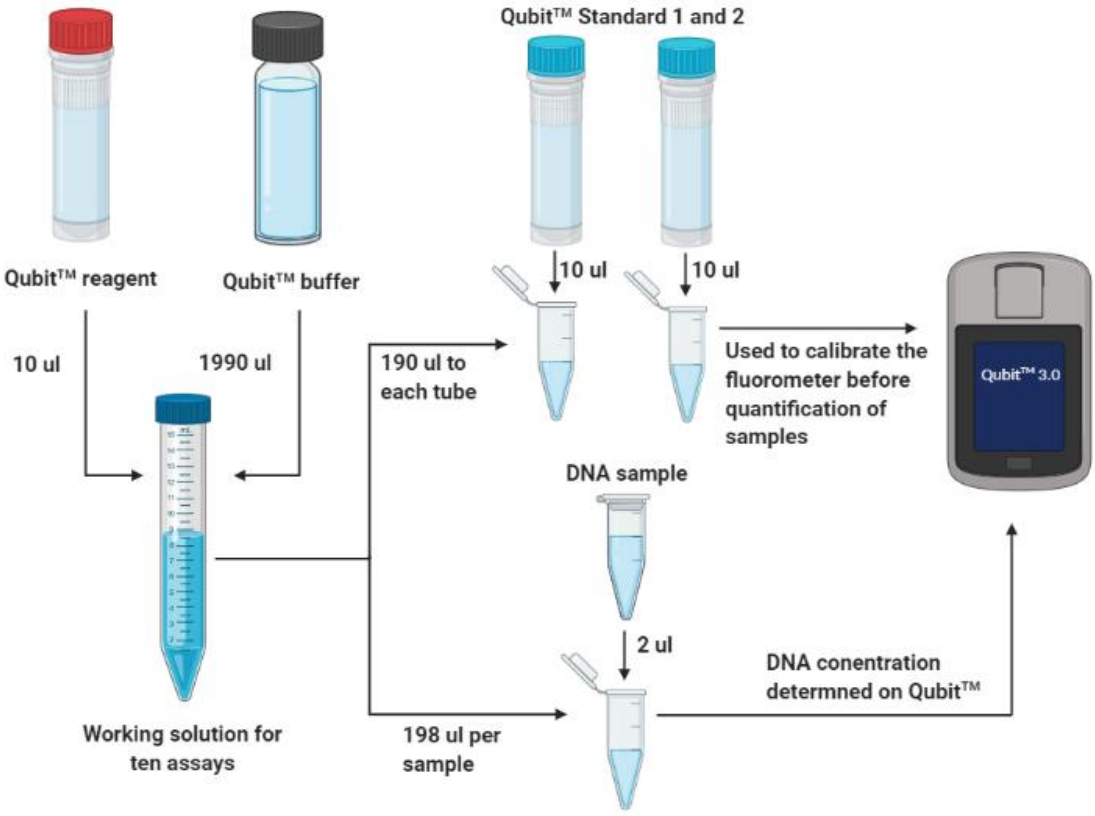


Figure 3.2. Qubit assay procedure for DNA quantification. Image created on BioRender on 22.12.2020.

All the tubes were vortexed (Labnet International, Edison, New Jersey, United States) to ensure thorough mixing and incubated for 2 minutes at room temperature. The HS dsDNA assay was selected on the Qubit fluorometer and the standards were used to calibrate the instrument. After this, concentrations of the DNA samples were determined and recorded in nanogram per microlitre (ng/µl).

3.3.10. Preparation of libraries

Library preparation is the first and critical step in NGS. The purpose of this procedure is to ensure that DNA quality is optimal enough to hybridise to the flow cell (acts as the microfluidic conduit for cluster generation and sequencing reagents), and that individual samples can be identified once sequencing is complete. The workflow entailed fragmentation of DNA into appropriate sizes for sequencing, approximately 300 base pairs (bp), and the addition of index adapters (barcodes/tags) to either end of DNA fragments with functional elements for cluster amplification, multiplexing/pooling, and sequencing. Index adapters are short DNA oligonucleotides, typically 6-8 bp, which contain the primer sites used by the sequencer to generate sequencing reads. The adapters are complementary to the short sequences

present on the surface of an Illumina® flow cell (Illumina, 2020). Generally, indexing serves as a way to identify individual samples when sequenced together (Craig *et al*., 2008; Hoffman *et al*., 2007; Meyer *et al*., 2007). Indexing was followed by size selection and clean-up using magnetic beads, 80 % ethanol and buffer. To ensure that a successful sequencing run was obtained, the quality of the libraries was assessed and quantified, prior to normalisation to equal concentration and pooling. In this study, a Nextera® XT DNA library preparation kit (Illumina®, San Diego, California, United States) was used.

3.3.10.1. Normalisation (standardisation) of starting DNA sample

The aim of this step was to achieve uniform reaction efficiency during fragmentation/tagmentation by standardising the concentration of the generated DNA across samples. Tagmentation of DNA is sensitive to the concentration or amount of input DNA used (Baym *et al*., 2015). The readings obtained after performing Qubit™ were used to carry out appropriate dilution concentration calculations to adjust the previously obtained Qubit™ concentrations to an input DNA concentration of 0.2-0.3 ng/µl. Qubit assay as described previously was performed after dilutions with elution buffer (Qiagen, Hilden, Germany) to confirm the normalised concentrations.

3.3.10.2. Tagmentation/fragmentation of genomic DNA

This process is transposome-mediated, whereby enzymes known as transposases cut DNA into smaller fragments. On a skirted 96-well PCR plate (NEST® Biotechnology, Jiangsu, China), 10 µl of Tagment DNA buffer (TD) was added to the well, followed by addition of 5 µl of normalised genomic DNA (gDNA) and mixed by pipetting up and down. Thereafter, 5 µl of Amplicon Tagment Mix (ATM) was added to the wells that contained the sample and TD and mixed by pipetting up and down. The PCR plate was then sealed with a PCR sealing film (NEST® Biotechnology, Jiangsu, China) and centrifuged at 280 *x g* for 1 minute at 20 °C (Labnet International, Edison, New Jersey, United States) which combined the mixture at the bottom of the well. Immediately after, the PCR plate was transferred to a thermocycler (Labnet International, Edison, New Jersey, United States) with a pre-heated lid (105 °C) and incubated for 5 minutes at 55 °C, then cooled at 10 °C. A volume of 2.5 µl Neutralisation Tagment buffer (NT) was added to the wells and the PCR plate was sealed again with a PCR sealing film (NEST® Biotechnology, Jiangsu, China) and the plate was centrifuged at 280 *x g* for 1 minute at 20 °C (Labnet International, Edison, New Jersey, United States). Neutralisation buffer halted the tagmentation reaction by neutralising the enzyme that fragmented the DNA. Lastly, the PCR plate was incubated for 5 minutes at 25 °C.

3.3.10.3. Library amplification/PCR

In this step, index adapters are ligated to both ends of the DNA fragments (**Figure 3.3**). The DNA fragments with properly ligated indexes are selected for and amplified using a limited-cycle PCR program.

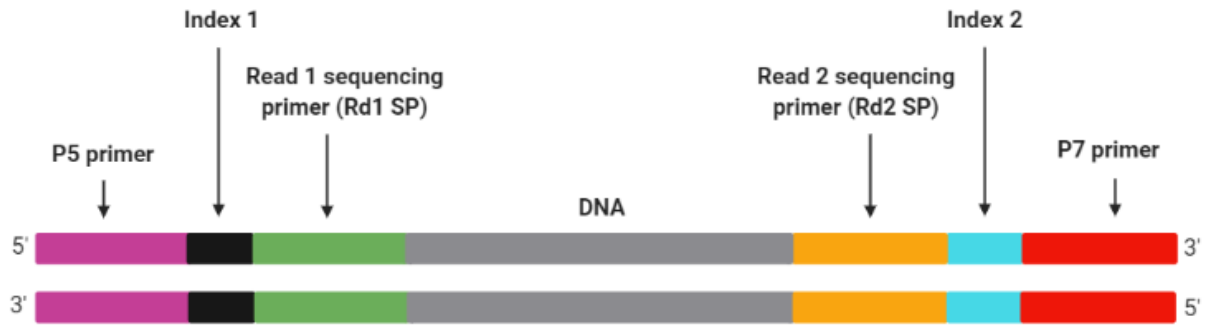


Figure 3.3. DNA insert with index adapters ligated on both ends. P5 and P7 are complementary to Illumina® flow cell oligonucleotides and allow libraries to bind to the flow cell for sequencing. Index 1 and 2 are unique DNA sequences for identification of samples. Rd1 and Rd2 are complementary to the indexes. Created with BioRender on 29.12.2020.

The Illumina® experiment manager software (Illumina®, San Diego, California, United States) was used to assign unique index pairs to each of the samples (**Table 3.1**). This was done to enable identification of pooled samples using their unique index sequence post-sequencing. To the tagmented DNA, 5 µl of Index 1 adapter, 5 µl of Index 2 adapter and 15 µl of Nextera® PCR Master Mix (NPM) was added to each of the samples based on the unique combinations that were created on the Illumina® Experiment Manager.

Table 3.1. Sample sheet with unique index combinations for the five samples.

Sample number	Sample ID	Index 1_ID	Index	Index 2_ID	Index
1	4723	N716	ACTCGCTA	S502	CTCTCTAT
2	4749	N717	GGAGCTAC	S502	CTCTCTAT
3	13232	N718	GCGTAGTA	S502	CTCTCTAT
4	13327	N719	CGGAGCCT	S502	CTCTCTAT
5	13541	N720	TACGCTGC	S502	CTCTCTAT

The plate was covered with the PCR sealing film (NEST® Biotechnology, Jiangsu, China) and centrifuged at 280 *x g* for 1 minute at 20 °C. PCR was then carried out in a thermocycler (Labnet International, Edison, New Jersey, United States) under the following reaction conditions: 72 °C for 3 minutes, 95 °C for 30 seconds, 12 cycles of 95 °C for 10 seconds, 55 °C for 30 seconds, 72 °C for 5 minutes and a hold at 10 °C.

3.3.10.4. Size selection and clean-up of libraries

It was necessary to perform clean-up to remove any unwanted contaminants such as unused index adapters and residual enzymes that may have been present in the samples because of the amplification step above. A bead-based selection and clean-up method was utilised, whereby AMPure XP magnetic beads (Beckman Coulter, Brea, California, United States) were used to purify the libraries while simultaneously isolating the DNA fragment sizes of interest based on their molecular weight. DNA selectively bound onto the beads while the contaminants remained suspended in the solution.

First, the AMPure XP beads were removed from 4 °C and left for 30 minutes to normalise to room temperature. The indexed libraries were centrifuged (Labnet International, Edison, New Jersey, United States) at 280 *x g* for 1 minute at 25 °C to collect condensation, after which 50 µl was transferred from each well to a new skirted 96-well PCR plate (NEST® Biotechnology, Jiangsu, China). The AMPure XP beads were vortexed using a vortex mixer (Labnet International, Edison, New Jersey, United States) for 30 seconds to ensure even distribution of the beads. Thereafter, a sufficient volume of beads was added to a trough (SPL Life Sciences, Pocheon-si, Korea), from which 30 µl was transferred and added to each well containing 50 µl of the indexed libraries using a P200 multichannel pipette (Labnet International, Edison, New Jersey). The plate was then sealed using a PCR sealing film (NEST® Biotechnology, Jiangsu, China), shaken on a multi-microplate genie shaker (Scientific Industries, Bohemia, New York, United States) at 280 *x g* for 2 minutes, then incubated for 5 minutes at 25 °C without shaking. This allowed the genomic material to bind to the magnetic beads. Following incubation, the plate was placed on a magnetic stand (PerkinElmer, Waltham, Massachusetts, United States) until the liquid became clear (approximately 2 minutes). The beads with the bound genomic material settled at the bottom, leaving a clear liquid at the top. The supernatant (clear liquid) which contained the impurities was discarded using a multichannel pipette (Labnet International, Edison, New Jersey, United States) set at 100 µl. Freshly prepared 80 % ethanol (200 µl) was added to each well containing the beads while still placed on the magnetic stand and incubated for 30 seconds. Here, the magnetic beads with bound DNA were washed with the 80 % ethanol (Merck KGaA, Darmstadt, Germany) hence removing impurities. Since DNA is not soluble in ethanol, any other molecules apart from DNA were washed away from the beads. The wash was repeated, followed

by removal of residual ethanol. The beads were allowed to air dry for 10 minutes while still mounted on the magnetic stand. Following this, the plate was taken off the magnetic stand and 52.5 µl of Resuspension buffer (RSB) was added to each well to elute the DNA. The plate was sealed using a PCR sealing film (NEST® Biotechnology, Jiangsu, China) and shaken on a multi-microplate genie shaker (Scientific Industries, Bohemia, New York, United States) at 280 *x g* for 2 minutes, followed by incubation for 2 minutes at 25 °C. The plate was mounted on a magnetic stand until the liquid became clear. Finally, 50 µl of the supernatant which contained the eluted purified DNA was transferred from each well to the corresponding wells of a new skirted 96-well PCR plate (NEST® Biotechnology, Jiangsu, China).

3.3.10.5. Library quality control and quantification

Prior to sequencing, it was necessary to validate and assess the library. To achieve this, a quality assessment step was performed on a 2100 Bioanalyzer instrument (Agilent, Santa Clara, California, United States) which provided both library concentration and fragment size information. Bioanalyzer performs microfluidic electrophoretic separation on microfabricated chips and gives a visual representation of the range of DNA fragment sizes that make up the library, hence makes it easy to detect potential issues such as a high percentage of short DNA fragments. An Agilent HS DNA Kit was used.

First, HS dye concentrate and HS DNA gel matrix were allowed to equilibrate at room temperature for 30 minutes and vortexed (Lasec®, Cape Town, South Africa) for 10 seconds. The gel-dye mix was then prepared by adding 15 µl of HS DNA dye concentrate into the entire volume of the HS DNA gel matrix. The mix was vortexed for 10 seconds, transferred to a spin filter tube (Invitek Molecular, Berlin, Germany) and centrifuged on the prism microcentrifuge (Labnet International, Edison, New Jersey, United States) at 2,240 *x g* for 10 minutes at room temperature. Following this, HS DNA chip was placed on the chip priming station (Agilent, Santa Clara, California, United States) and 9 µl of the gel-dye mix was added to one of the wells marked **G**. The priming chip station was closed, and pressure was applied to the plunger (set at 1 ml) for 60 seconds to evenly distribute the gel-dye mix across the chip. The pressure was then released by slowly pulling back the plunger to the 1 ml position and the chip priming station was opened. Gel-dye mix (9 µl) was then added to the rest of the wells marked 'G'. A HS marker (5 µl) was added to the sample and ladder wells, and subsequently 1 µl of HS ladder was added to the ladder well. The libraries (1 µl) were added into the sample wells, and the chip was vortexed on a IKA MS3 vortex (IKA, Staufen, Germany) at 100 *x g* for 60 seconds. Lastly, the chip was carefully inserted into the 2100 Bioanalyzer and the run was started. Bioanalyzer detected DNA in the samples by their fluorescence and the output was presented in form of a gel-like view (**Figure 3.4**) and an electropherogram (**Figure 3.5**). Quantification of the DNA library

was also performed on the Qubit™ 3.0 fluorometer (Life Technologies by Thermo Fisher Scientific, Waltham, Massachusetts, United States) as described previously to determine concentration of the libraries.

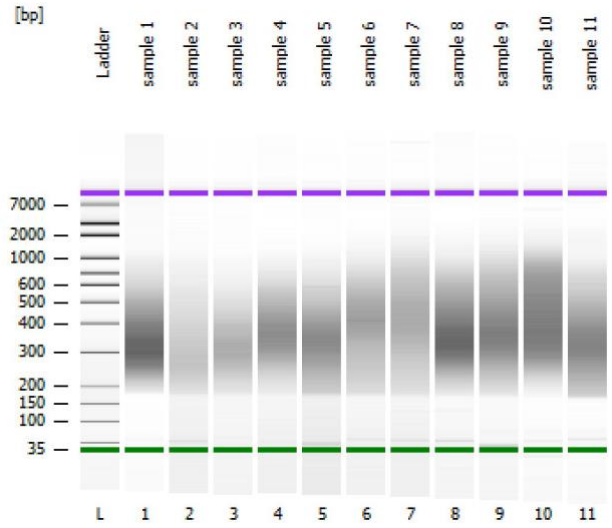


Figure 3.4. Gel-like image representation of the DNA library size distribution as presented on the Bioanalyzer. The samples produced a smear that averaged from around 200 bp to around 600 bp.

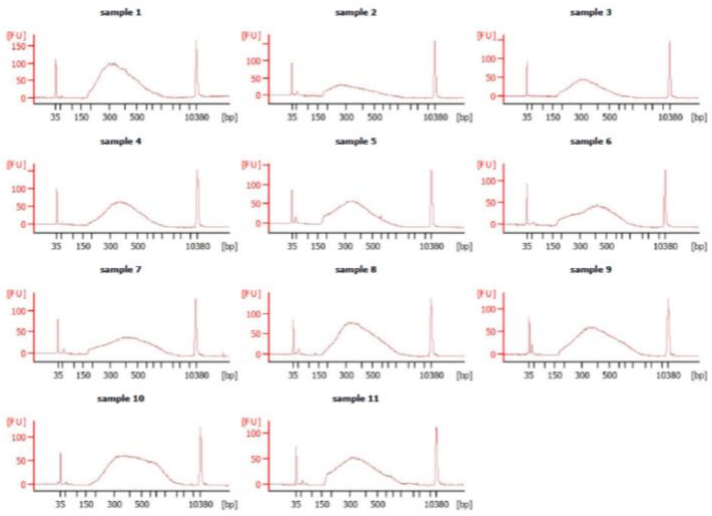


Figure 3.5. Bioanalyzer electropherogram representation of the library size distribution. FU stands for fluorescent unit. Samples 1-5 are the study samples. The average fragment sizes for sample 1 to 5 are 447 bp, 432 bp, 436 bp, 460 bp, and 467 bp, respectively.

3.3.10.6. Library normalisation to 4 nM and pooling of the libraries

Prior to sequencing, the barcoded libraries were normalised to equimolar concentrations and pooled into a single tube. The formula (shown below) took into consideration the concentrations of DNA obtained from Qubit™ and the average library size in base pairs as determined on Bioanalyzer. Dilution of the concentrated libraries to 4 nM was carried out using elution buffer (Qiagen, Hilden, Germany).

$$\frac{\boldsymbol{Concentration\ in\ ng/\mu l}}{(\boldsymbol{660 g/mol\ x\ Average\ library\ size})}\ \boldsymbol{X\ 10^6 = Concentration\ in\ nM}$$

All the libraries of equal concentration (5 µl of each library) were then pooled into a single 1.5 ml microcentrifuge tube (Molecular Bioproducts, San Diego, California, United States) and mixed by pipetting up and down.

3.3.10.7. Library denaturation and dilution to 8 pM using sodium hydroxide

Freshly diluted 0.2 N sodium hydroxide (NaOH) (Sigma-Aldrich, Saint Louis, Missouri, United States) was prepared from which 5 µl was added into the 1.5 ml microcentrifuge tube (Molecular Bioproducts, San Diego, California, United States) that contained 5 µl 4 nM of the pooled amounting to a total volume of 10 µl. The mixture was vortexed (Labnet International, Edison, New Jersey, United States) briefly, centrifuged on the prism microcentrifuge (Labnet International, Edison, New Jersey, United States) at 280 *x g* for 1 minute, which was followed by incubation for 5 minutes at room temperature to denature dsDNA into single strands. After this, 990 µl of pre-chilled Hybridisation buffer (HT1) (Illumina®, San Diego, California, United States) was added to the 10 µl of 4 nM denatured DNA library to dilute it further to 20 pM. To get to the desired final concentration of 8 pM, 360 µl of HT1 (Illumina®, San Diego, California, United States) was combined with 240 µl of the 20 pM library and mixed by inverting several times.

3.3.10.8. Denaturation and dilution of PhiX control to 20 pM

PhiX control (Illumina®, San Diego, California, United States) is derived from the bacteriophage genome, PhiX, which was the first DNA genome to be sequenced by Fred Sanger. It serves as a calibration for the overall performance of Illumina® sequencing platforms and for run quality monitoring such as cluster generation (Mukherjee *et al*., 2015; Sanger *et al*., 1977b). First, 2 µl of 10 nM PhiX was added to a microcentrifuge tube containing 3 µl of 10 mM Tris-Cl, pH 8.5 with 0.1 % Tween 20 (Merck KGaA, Darmstadt, Germany). This resulted in 5 µl of 4 nM PhiX that was then added to a tube containing 5 µl of

0.2 N NaOH (Sigma-Aldrich, Saint Louis, Missouri, United States). The mixture was vortexed briefly before being incubated for 5 minutes at 25 °C to allow denaturation of the PhiX library. Further dilution to 20 pM of the denatured PhiX was achieved by adding 990 µl of HT1 to the 10 µl denatured PhiX.

3.3.10.9. Combining PhiX control and the library.

A PhiX control spike-in of 5 % was used by combining 30 µl of the 20 pM PhiX library and 570 µl of 8 pM libraries resulting in a final volume of 600 µl. This final library was set aside on ice until ready for final heat denaturation.

3.3.11. Illumina® MiSeq sequencing

A V3 Illumina® MiSeq reagent cartridge was thawed in double-distilled water for around 90 minutes to defrost the reagents and was then dried with a paper towel. The V3 cartridge can generate up to 25 million reads. A new flow cell cleaned with double-distilled water, a waste bottle and PR2 incorporation buffer were loaded into their respective compartments in the MiSeq following prompts from the MiSeq Control Software. The MiSeq reagent cartridge consists of pre-filled clustering and sequencing reagents in foil-sealed reservoirs, while the flow cell is a glass-based substrate on which clusters generation and sequencing occurs. After this, the final library was denatured for 2 minutes at 96 °C on a heating block (Accublock™ Digital Dry Bath, Labnet International, Edison, New Jersey, United States) and immediately placed on ice. The reservoir called 'Load samples' was cleaned, pierced with a 1000 µl pipette and 600 µl of the denatured pooled library with 5 % PhiX control was added, after which the cartridge was inserted into the Illumina® MiSeq (Illumina®, San Diego, California, United States). The run was started after confirming all parameters for 600 cycles to generate 300 bp *x* 2 paired end reads.

3.3.12. Data analysis performed on the G5P[6] strain

3.3.12.1. Genome assembly

The raw reads obtained in FASTQ format were assembled using Geneious Prime® 2019.2.1 (**https://www.geneious.com/**; Kearse *et al*., 2012). Briefly, the paired-end reads were merged into single reads and trimmed to remove low quality and short reads. The reads were mapped to reference sequences obtained from GenBank. Consensus sequences covering the complete open reading frame (ORF) were submitted to the National Centre for Biotechnology Information (NCBI) GenBank and assigned accession numbers MT271025–MT271035. The ORF lengths were 3267 (VP1), 2673 (VP2), 2508 (VP3), 2328 (VP4), 1194 (VP6), 981 (VP7), 1482 (NSP1), 954 (NSP2), 942 (NSP3), 528 (NSP4), and 594 (NSP5).

3.3.12.2. Assignment of genotypes

The genotypes of each of the 11 rotavirus genome segments were determined using the online Virus Pathogen Resource (ViPR) (Pickett *et al*., 2012).

3.3.12.3. Phylogenetic analysis

Gene-specific multiple sequence alignments were made using the MAFFT plugin implemented in Geneious® Prime 2019.2.1 (VP7, VP4, VP1, VP3, NSP2-NSP5) and the MUSCLE algorithm embedded in MEGA 6.06 (for the VP2 and NSP1 segments) (Edgar, 2004; Katoh and Standley, 2013). Once aligned, the DNA Model Test program in MEGA 6.06 was used to identify the optimal evolutionary model for each genome segment (Tamura *et al*., 2013). Using an Akaike information criterion (corrected) (AICc), the following models were found to best fit the data: HKY+G+I (VP1), GTR+G+I (VP2, VP3, and VP4), T92+G (VP6, NSP1, NSP2, NSP3, NSP4, and NSP5), and T92+G+I (VP7). Maximum likelihood trees were constructed using the optimal models in MEGA version 6.06 (Guindon and Gascuel, 2003; Tamura *et al*., 2013) with 1000 bootstrap replicates to estimate branch support (Felsenstein, 1985). The shared nucleotide and amino acid sequence identities among strains were calculated for each gene using the *p*-distance algorithm in MEGA 6.06.

3.3.12.4. Reassortment analysis

Analysis and visualization of the aligned concatenated whole genomes was performed on the mVISTA online platform (Frazer *et al*., 2004; mVISTA instructions (lbl.gov)). The concatenated sequences were uploaded on the mVISTA online website in FASTA format. The LAGAN alignment program present on the platform was utilised. This program detects multiple sequence alignments, calculates and displays the

conservation between sequences. The results were then submitted via email after a few minutes as a PDF file.

3.4. Results

3.4.1. Nucleotide sequencing and identity of the strain

Illumina® MiSeq sequencing exhibited a phred score of Q30 and collectively yielded 98.8 Mbs of data for this specific sample. The whole genome of RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] was 18272 bps in size. The length and ORF of the 11 gene segments as determined by nucleotide sequencing are shown in **Table 3.2**. A BLASTn search was performed, and the strain exhibited maximum sequence identities of 95.7% - 98.0% with porcine and human porcine-like strains (**Table 3.2**). Based on the whole genome classification system, RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] exhibited a G5-P[6]-I1-R1-C1-M1-A8-N1-T1-E1-H1 genotype constellation (**Table 3.3**). The genetic constellation of the study strain was compared to those of other G5 and non-G5 strains retrieved from the GenBank (**Table 3.3**).

Table 3.2. The segment and ORF lengths of strain UFS-NGS-MRC-DPRU4723 and the highest sequence identities obtained using the Basic Local Alignment Search Tool (BLAST).

Genome segment Encoding	GenBank accession no.	Segment length	ORF length	Results of blast search			
				Most similar strain	GenBank accession no.	Similarity (%)	References
VP1	MT271025	3302	3267	GX54	KF041441	96.7	(Dong *et al*., 2013)
VP2	MT271026	2673	2673	R1207	LC389886	96.5	(Yahiro *et al*., 2018)
VP3	MT271027	2591	2508	R946	KF726060	95.7	(Zhou *et al*., 2015)
VP4	MT271028	2359	2328	KisB332	KJ870903	98.0	(Heylen *et al*., 2014)
NSP1	MT271029	1512	1482	NT0042	LC095894	98.1	(Kaneko *et al*., 2018)
VP6	MT271030	1356	1194	KYE-14-A048	KX988279	98.7	(Bwogi *et al*., 2017)
NSP3	MT271031	1076	942	12070-4	KX363287	97.1	(Phan *et al*., 2016)
NSP2	MT271032	954	954	YN	KJ466987	96.8	Https://www.ncbi.nlm.nih.gov/nuccore/kj466987
VP7	MT271033	1054	981	JN-2	KT820777	98.0	Https://www.ncbi.nlm.nih.gov/nuccore/kt820777
NSP4	MT271034	751	528	14150-54	KX363354	97.7	(Phan *et al*., 2016)
NSP5	MT271035	644	594	R479	GU189559	97.6	(Wang *et al*., 2007)

Table 3.3. Genotype natures of the 11 gene segments of Zambian strain UFS-NGS-MRC-DPRU4723 compared with those of selected human and porcine strains.

Strain	Genotype										
	VP7	**VP4**	**VP6**	**VP1**	**VP2**	**VP3**	**NSP1**	**NSP2**	**NSP3**	**NSP4**	**NSP5**
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6]	**G5**	**P[6]**	I1	R1	C1	M1	**A8**	N1	T1	E1	H1
RVA/Human-wt/BGR/BG260/2008/G5P[6]*	**G5**	**P[6]**	I1	R1	C1	M1	**A8**	N1	T1	E1	H1
RVA/Human-wt/JPN/Ryukyu-1120/2011/G5P[6]	**G5**	**P[6]**	**I5**	R1	C1	M1	**A8**	N1	T1	E1	H1
RVA/Human-wt/CHN/LL3354/2000/G5P[6]	**G5**	**P[6]**	**I5**	-	-	-	-	-	-	E1	-
RVA/Human-wt/CHN/LL4260/2001/G5P[6]	**G5**	**P[6]**	-	-	-	-	-	-	-	E1	-
RVA/Human-wt/CHN/LL36755/2003/G5P[6]	**G5**	**P[6]**	-	-	-	-	-	-	-	E1	-
RVA/Human-wt/VNM/KH210/2004/G5P[6]	**G5**	**P[6]**	-	-	-	-	-	-	-	E1	-
RVA/Human-wt/TWN/03-98P50/2009/G5P[6]*	**G5**	**P[6]**	**I5**	-	-	-	-	-	-	E1	-
RVA/Human-wt/CMR/6784/ARN/2000/G5P[7]	**G5**	**P[7]**	**I5**	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-tc/BRA/IAL28/1992/G5P[8]	**G5**	P[8]	**I5**	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Pig-tc/USA/OSU/1975/G5P[7]	**G5**	**P[7]**	**I5**	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Pig-wt/BEL/12R002/2012/G5P[7]	**G5**	**P[7]**	**I5**	R1	C1	M1	**A8**	N1	**T7**	E1	H1
RVA/Pig-wt/JPN/BU2/2014/G5P[7]	**G5**	**P[7]**	**I5**	R1	C1	M1	**A8**	N1	T1	E1	H1
RVA/Human-tc/USA/Wa/1974/G1P[8]	G1	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-tc/USA/DS-1/1976/G2P[4]	G2	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-tc/JPN/AU-1/1982/G3P[9]	**G3**	P[9]	I3	R3	C3	M3	A3	N3	T3	E3	H3
RVA/Pig-wt/BEL/12R006/2012/G3P[6]	**G3**	**P[6]**	**I5**	R1	C1	M1	**A8**	N1	T1	E1	H1
RVA/Human-tc/GBR/ST3/1974/G4P[6]	**G4**	**P[6]**	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Pig-tc/USA/Gottfried/1975/G4P[6]	**G4**	**P[6]**	I1	R1	C1	M1	**A8**	N1	T1	E1	H1
RVA/Human-tc/CHN/R479/2004/G4P[6]	**G4**	**P[6]**	**I5**	R1	C1	M1	A1	N1	**T7**	E1	H1
RVA/Human-wt/CHN/E931/2008/G4P[6]	**G4**	**P[6]**	I1	R1	C1	M1	**A8**	N1	T1	E1	H1
RVA/Human-wt/COD/KisB332/2008/G4P[6]	**G4**	**P[6]**	I1	R1	C1	M1	A1	N1	**T7**	E1	H1
RVA/Human-wt/CHN/GX54/2010/G4P[6]	**G4**	**P[6]**	I1	R1	C1	M1	**A8**	N1	T1	E1	H1
RVA/Pig-wt/BEL/12R005/2012/G4P[7]	**G4**	**P[7]**	**I5**	R1	C1	M1	**A8**	N1	**T7**	E1	H1

RVA/Human-wt/BEL/BE2001/2009/G9P[6]	**G9**	**P[6]**	**I5**	R1	C1	M1	**A8**	N1	**T7**	E1	H1
RVA/Human-tc/USA/WI61/1983/G9P[8]	**G9**	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/BEL/B3458/2003/G9P[8]	**G9**	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-tc/IND/mani-97/2006/G9P[19]	**G9**	**P[19]**	**I5**	R1	C1	M1	**A8**	N1	T1	E1	H1
RVA/Human-wt/BGD/Dhaka6/2001/G11P[25]	**G11**	**P[25]**	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/VNM/30378/2009/G26P[19]	**G26**	**P[19]**	**I5**	R1	C1	M1	**A8**	N1	T1	E1	H1
RVA/Human-wt/BRA/rj24598/2015/G26P[19]	**G26**	**P[19]**	**I5**	R1	C1	M1	**A8**	N1	T1	E1	H1

Blue shading indicates the gene segments with genotypes identical to those of UFS-NGS-MRC-DPRU4723. Bold font indicates genotypes associated with porcine strains. "–" indicates that no sequence data were available in GenBank/EMBL/DDBJ data banks. * Genotype assignment based on reports by (Hwang *et al*., 2012) (strain 03-98sP50) and (strain BG260) (Mladenova *et al*., 2012). To date, the nucleotide accession numbers for the 11 gene segments of strains 03-98sP50 and BG260 are not available in the GenBank, EMBL, or DDBJ data banks.

3.4.2. Sequence and phylogenetic analysis

To investigate the potential origin of RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6], phylogenetic trees were constructed for each of the 11 gene segments along with cognate gene sequences of RVA strains obtained from the GenBank.

3.4.2.1. Sequence and phylogenetic analysis of the VP7 gene

Phylogenetically, there are three known VP7 G5 lineages (I-III) (da Silva *et al*., 2011). The VP7 genes of RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] clustered into lineage II, which consisted only of porcine G5 strains from mainly Asia and the Americas (**Figure 3.6**). The VP7 gene showed the highest nucleotide (nt) and amino acid (aa) identities with the Chinese porcine strains RVA/Pig-wt/CHN/DZ-2/2013/G5P[X] nt (aa), 98.6% (99.0%), and RVA/Pig-wt/CHN/JN-2/2014/G5P[X] 98.5% (99.0%) and was distantly related to the strains within lineage III with lower sequence identities (nt, 83.4%–86.5%; aa, 90.4%–94.5%) (**Figure 3.6; Appendix 7a**). Overall, strains within lineage II exhibited sequence identities that were in the range nt, 89.6%–98.6%; aa, 92.4%–99.0% (**Appendix 7a**).

The comparison of the amino acid sequence of RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] to reference G5 strains e.g., RVA/Pig-wt/THA/CMP-001-12/2012/G5P[13] (lineage I), RVA/Pig-wt/BRA/ROTA24/2013/G5P [6] (lineage II) and RVA/Human-wt/JPN/Ryukyu-1120/2011/G5P[6] (lineage III) within each of the three lineages revealed a high identity (range 90.0%–94.9% (**Figure 3.7; Appendix 7a**). Numerous substitutions were identified in the nine VP7 variable regions, VR-1 to VR-9 (Green *et al*., 1989): VR-1 (I9V and I19V), VR-2 (V27T and V29T), VR-3 M/F39L, I40V, V41I, L/I43V, I/L/V47F, R49K, and A50T), VR-4 (K/A65T, V/M68A, M/A72T, and M/Q75T), VR-5/antigenic site A (N/S/D/T96A), VR-6 (I129V and D130E), VR-7/antigenic site B (N145D and A/V/E146G), VR-8/antigenic site C (L/S208T, A210T, T/V212I, S/A213I, I/M217T, V218I, and S220N), and VR-9/antigenic site F (A/M241T and S242N).

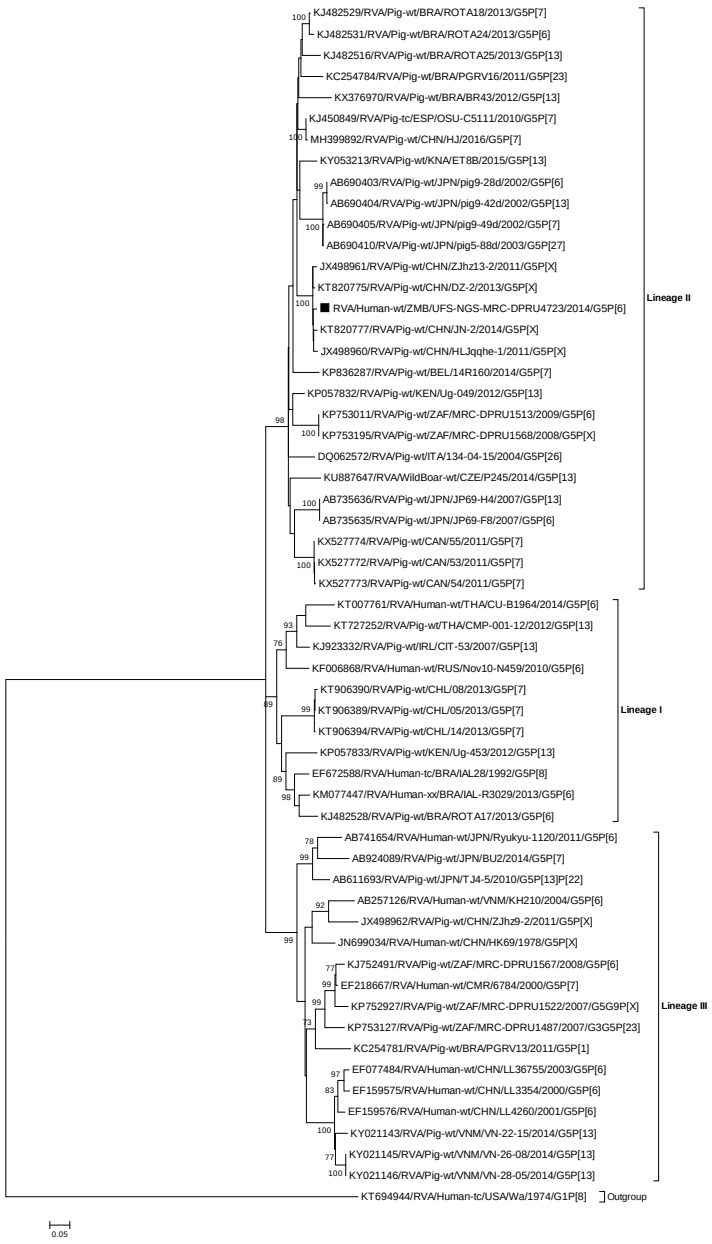
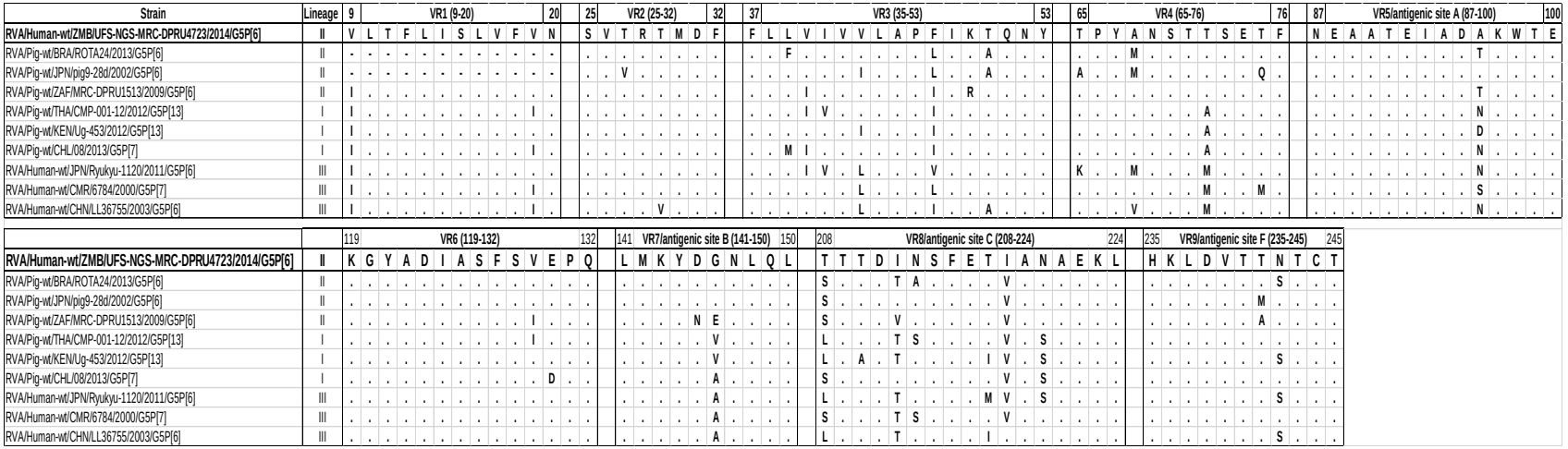


Figure 3.6. Phylogenetic tree constructed from the nucleotide sequences of the VP7 genes of strain RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] and representative strains. The position of strain RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] is shown by the black square (▪). Reference strains obtained from GenBank are represented by Accession number, Strain name, Country and year of isolation. The three closest strains as identified by BLASTn are also included. Bootstrap values ≥70% are shown adjacent to each branch node. Scale bar: 0.05 substitutions per nucleotide.



Strain	Lineage	9	VR1 (9-20)										20	25	VR2 (25-32)							32	37	VR3 (35-53)																53	65	VR4 (65-76)										76	87	VR5/antigenic site A (87-100)												100
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6]	II	V	L	T	F	L	I	S	L	V	F	V	N	S	V	T	R	T	M	D	F	F	L	L	V	I	V	V	L	A	P	F	I	K	T	Q	N	Y	T	P	Y	A	N	S	T	T	S	E	T	F	N	E	A	A	T	E	I	A	D	A	K	W	T	E
RVA/Pig-wt/BRA/ROTA24/2013/G5P[6]	II	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	.	F	.	.	.	.	.	.	L	.	.	A	.	.	.	.	.	.	M	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.
RVA/Pig-wt/JPN/pig9-28d/2002/G5P[6]	II	-	-	-	-	-	-	-	-	-	-	-	-	.	.	V	.	.	.	.	.	.	.	.	.	.	I	.	.	.	L	.	.	A	.	.	.	A	.	.	M	.	.	.	.	.	.	Q	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
RVA/Pig-wt/ZAF/MRC-DPRU1513/2009/G5P[6]	II	I	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	I	.	.	.	.	.	I	.	R	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.
RVA/Pig-wt/THA/CMP-001-12/2012/G5P[13]	I	I	.	.	.	.	.	.	.	.	.	I	.	.	.	.	.	.	.	.	.	.	.	I	V	.	.	.	.	I	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	N	.	.	.	.
RVA/Pig-wt/KEN/Ug-453/2012/G5P[13]	I	I	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	I	.	.	.	I	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	D	.	.	.	.
RVA/Pig-wt/CHL/08/2013/G5P[7]	I	I	.	.	.	.	.	.	.	.	.	I	.	.	.	.	.	.	.	.	.	.	.	.	.	M	I	.	.	.	I	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	N	.	.	.	.
RVA/Human-wt/JPN/Ryukyu-1120/2011/G5P[6]	III	I	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	I	V	.	L	.	.	.	V	.	.	.	.	.	.	K	.	.	M	.	.	.	M	.	.	.	.	.	.	.	.	.	.	N	.	.	.	.
RVA/Human-wt/CMR/6784/2000/G5P[7]	III	I	.	.	.	.	.	.	.	.	.	I	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	L	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	.	.	M	.	.	M	.	.	.	.	.	.	.	S	.	.	.	.
RVA/Human-wt/CHN/LL36755/2003/G5P[6]	III	I	.	.	.	.	.	.	.	.	.	I	.	.	.	.	.	V	.	.	.	.	.	.	.	.	.	.	.	L	.	.	.	I	.	.	A	.	.	.	.	.	.	V	.	.	.	M	.	.	.	.	.	.	.	.	.	.	N	.	.	.	.

		119	VR6 (119-132)												132	141	VR7/antigenic site B (141-150)									150	208	VR8/antigenic site C (208-224)																									224	235	VR9/antigenic site F (235-245)										245
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6]	II	K	G	Y	A	D	I	A	S	F	S	V	E	P	Q	L	M	K	Y	D	G	N	L	Q	L	T	T	T	D	I	N	S	F	E	T	I	A	N	A	E	K	L	H	K	L	D	V	T	T	N	T	C	T
RVA/Pig-wt/BRA/ROTA24/2013/G5P[6]	II	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.	T	A	.	.	.	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.
RVA/Pig-wt/JPN/pig9-28d/2002/G5P[6]	II	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.	.	.	.	.	.	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	M	.	.	.	.
RVA/Pig-wt/ZAF/MRC-DPRU1513/2009/G5P[6]	II	.	.	.	.	.	.	.	.	.	I	.	.	.	.	.	.	.	.	N	E	.	.	.	.	S	.	.	.	V	.	.	.	.	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.
RVA/Pig-wt/THA/CMP-001-12/2012/G5P[13]	I	.	.	.	.	.	.	.	.	.	I	.	.	.	.	.	.	.	.	.	V	.	.	.	.	L	.	.	.	T	S	.	.	.	.	V	.	S	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
RVA/Pig-wt/KEN/Ug-453/2012/G5P[13]	I	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	V	.	.	.	.	L	.	A	.	T	.	.	.	.	I	V	.	S	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.
RVA/Pig-wt/CHL/08/2013/G5P[7]	I	.	.	.	.	.	.	.	.	.	.	.	D	.	.	.	.	.	.	.	A	.	.	.	.	S	.	.	.	.	.	.	.	.	.	V	.	S	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
RVA/Human-wt/JPN/Ryukyu-1120/2011/G5P[6]	III	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	L	.	.	.	T	.	.	.	.	M	V	.	S	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.
RVA/Human-wt/CMR/6784/2000/G5P[7]	III	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	S	.	.	.	T	S	.	.	.	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
RVA/Human-wt/CHN/LL36755/2003/G5P[6]	III	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	L	.	.	.	T	.	.	.	.	I	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.

Figure 3.7. Comparison of the deduced amino acid sequence of the VP7 of strain RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] to a selection of human and animal G5 sequences obtained from the GenBank. Only amino acids which differ are shown. Variable regions designated VR-1 to VR-9 are shown. The dots (•) represents conserved amino acids relative to the study strain. The dashes (-) indicate the absence of amino acid residue in that location.

3.4.2.2. Sequence and phylogenetic analysis of the VP4 gene

The VP4 gene of RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] was phylogenetically compared to the already established five lineages (I-V) of genotype P[6] (Martella *et al*., 2006b). The P[6] gene of the study strain clustered into lineage V (**Figure 3.8**), which consisted of porcine and putative human porcine-like strains detected in parts of Europe and one African strain. A similarity analysis of the P[6] gene of the study strain with strains obtained from GenBank showed that the Zambian G5P[6] exhibited the highest sequence identity of 98.1% (98.3%) with a porcine-like human strain RVA/Human-wt/COD/KisB332/2008/G4P[6] from the Democratic Republic of Congo (**Appendix 7b**). All the African strains clustered into a separate lineage, lineage I, with sequence identities of 85.7%–86.8% (92.5%–93.9%).

The deduced amino acid sequences of the VP4 of RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] along with the reference P[6] strain from each of the five lineages was compared (**Figure 3.9**). The reference strains shared high amino acid identities ranging from 91.0% to 98.3% (**Appendix 7b**). Several amino acid changes were identified throughout the VP4 protein, and most of the substitutions were concentrated in the hypervariable region (amino acid 71-208) which houses the VR-3 (92–192) and includes a neutralization site at amino acid 135 (Burke *et al*., 1994; Mackow *et al*., 1988). Several amino acid substitutions were observed among the P[6] lineage I strains (Martella *et al*., 2006b) at the VR-3 (L105I, V108I and T134S) and VR-8 (D602N) variable regions. Other amino acid substitutions were identified among the P[6] lineages at VR-1 (S30N), VR-2 (I61V), VR-3 (V112I, N114S, V130I, H182N and T189S), VR-4 (I280V), and VR-9 (E698K). The potential trypsin cleavage sites at residues 241 and 247 (Arias *et al*., 1996) were highly conserved in all the strains with three substitutions at positions 242 (I to V), 243 (A to T), and 244 (H to Y).

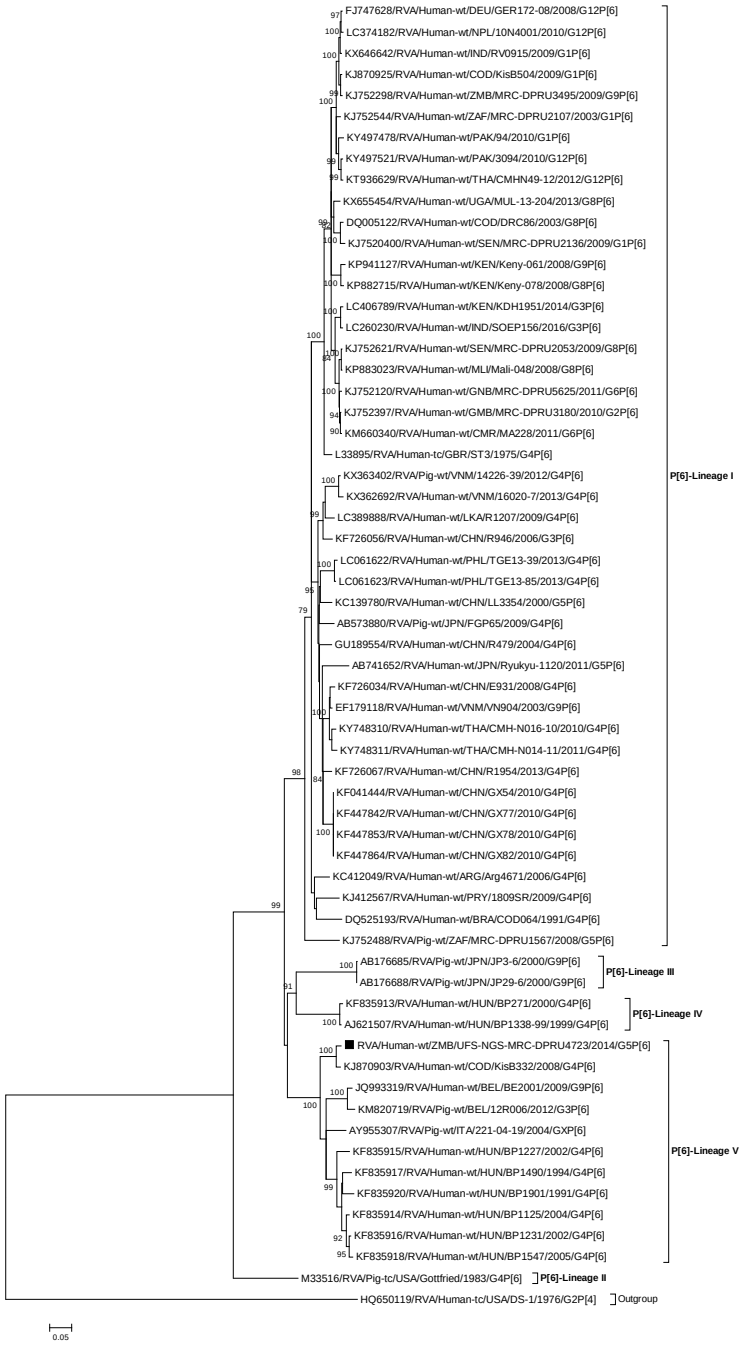


Figure 3.8. Phylogenetic tree constructed from the nucleotide sequences of the VP4 genes of strain RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] and representative strains. The position of strain RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] is shown by the black square (▪). Reference strains obtained from GenBank are represented by Accession number, Strain name, Country and year of isolation. The three closest strains as identified by BLASTn are also included. Bootstrap values ≥70% are shown adjacent to each branch node. Scale bar: 0.05 substitutions per nucleotide.

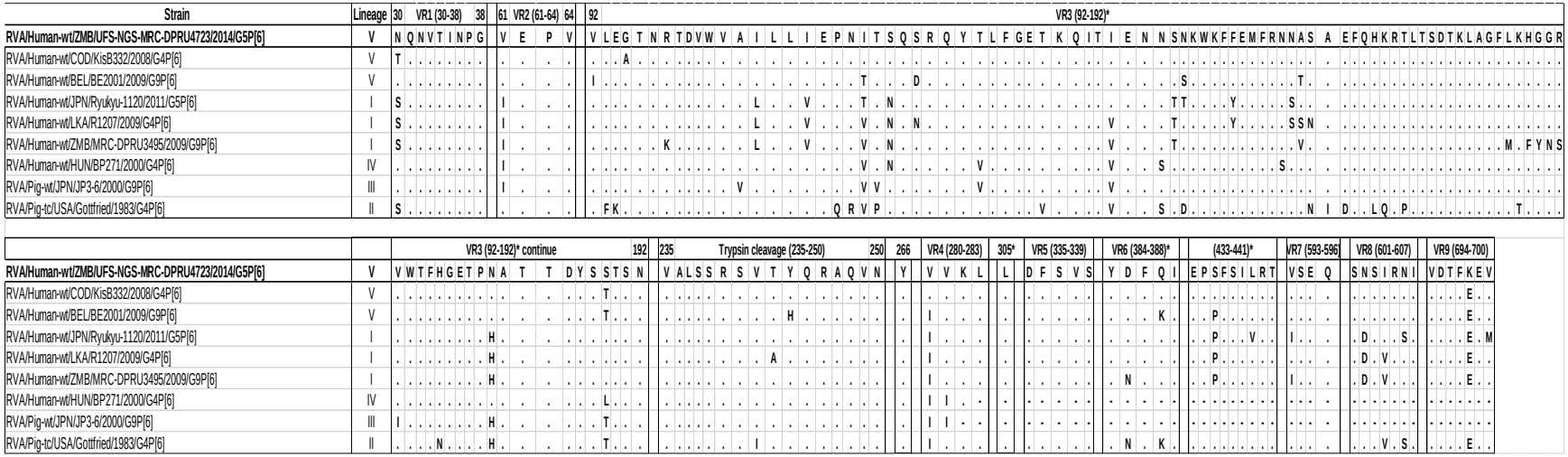


Figure 3.9. Comparison of the deduced amino acid sequence of the VP4 of strain RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] to a selection of older and contemporary P[6] sequences obtained from the GenBank. Only amino acids which differ are shown. Variable regions designated VR-1 to VR-9 are shown. * are regions in which amino acid substitution has been found in mutants selected with NMAbs. The dots (•) represent conserved amino acids relative to the study strain. The dashes (-) indicate the absence of amino acid residue in that location.

3.4.2.3. Phylogenetic analysis of the VP6 gene

The VP6 gene of RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] clustered closely with divergent African porcine strains from Uganda (RVA/Pig-wt/UGA/BUW-14-A003/2014/G3P[13], RVA/Pig-wt/UGA/KYE-14-A048/2014/G3P[13], and RVA/Pig-wt/UGA/KYE-14-A047/2014/G3P[13]) and a human porcine-like strain from the Democratic Republic of Congo (RVA/Human-wt/COD/KisB332/2008/G4P[6]) which displayed nt (aa) sequence identities ranging from 98.6% to 98.9% (98.9%–99.7 (**Figure 3.10; Appendix 7c**). Porcine-like Asian strains such as RVA/Human-wt/CHN/GX54/2010/G4P[6] and RVA/Human-wt/CHN/E931/2008/G4P[6] clustered separately, displaying identities of 88.7%–90.2% (97.5%–98.7%).

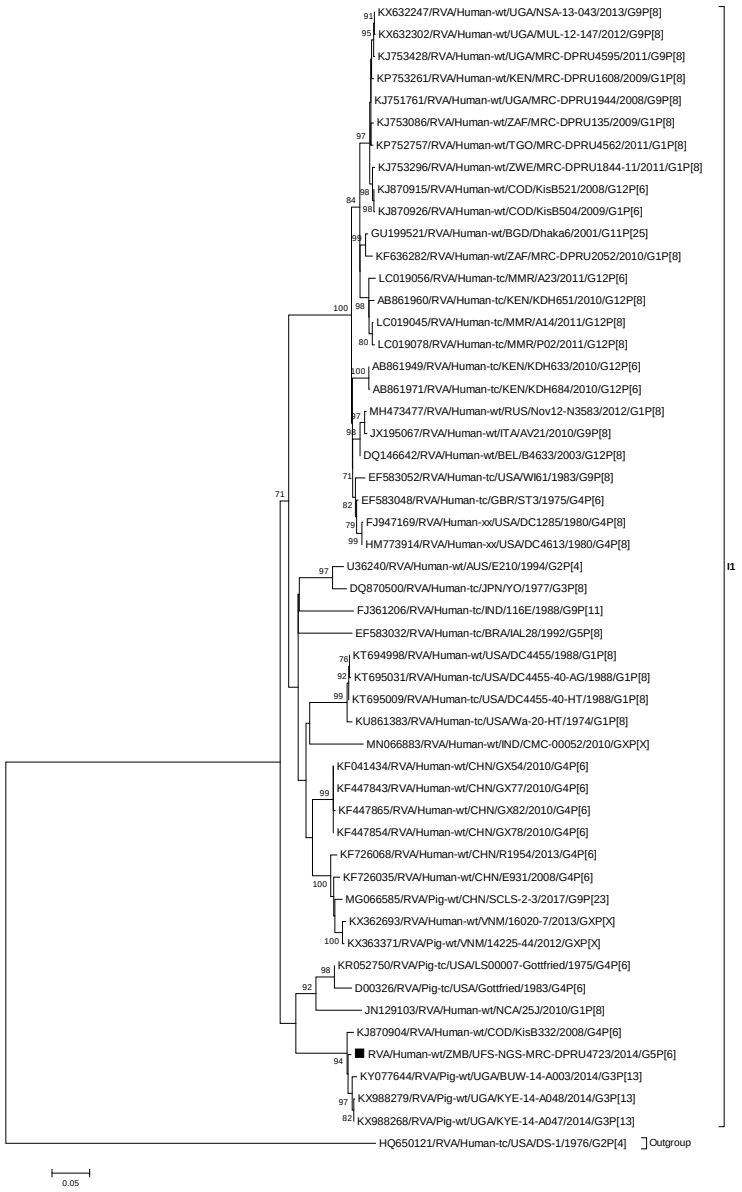


Figure 3.10. Phylogenetic tree constructed from the nucleotide sequences of the VP6 genes of strain RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] and representative strains. The position of strain RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] is shown by the black square (▪). Reference strains obtained from GenBank are represented by accession number, strain name, country, and year of isolation. The three closest strains, as identified by BLASTn, are also included. Bootstrap values ≥70% are shown adjacent to each branch node. Scale bar: 0.05 substitutions per nucleotide.

3.4.2.4. Phylogenetic analysis of the VP1 gene

The VP1 gene of RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] clustered only with porcine and porcine-like human strains from Asia (China and Vietnam) (**Appendix 8**). The VP1 gene exhibited a maximum nt (aa) sequence identity of 96.8% (98.9%) with the Chinese human porcine-like reassortant strains RVA/Human-wt/CHN/GX82/2010/G4P[6], RVA/Human-wt/CHN/GX78/2010/G4P[6], RVA/Human-wt/CHN/GX77/2010/G4P[6], and RVA/Human-wt/CHN/GX54/2010/G4P[6] (**Appendix 7d**). Overall, the Asian strains within the cluster showed sequence identities of 94.1%–96.8% (97.9%–98.9%). Human non-porcine African strains clustered separately, with lower identities of 88.2%–88.8% (96.3%–97.3%) (**Appendix 7d; Appendix 8**).

3.4.2.5. Phylogenetic analysis of the VP2 gene

The VP2 gene of strain RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] fell into a distinct cluster predominantly composed of porcine and porcine-like human strains from Asia (China, India, Vietnam, South Korea, and Sri Lanka) (**Appendix 9**). The VP2 gene of the study strain showed a maximum nt (aa) sequence identity of 96.6% (90.9%) with a Sri Lankan porcine-like human strain RVA/Human-wt/LKA/R1207/2009/G4P[6] (**Appendix 7e**).

3.4.2.6. Phylogenetic analysis of the VP3 gene

The VP3 gene of strain RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] clustered in a lineage composed mainly of Asian (Asia and Thailand) porcine and porcine-like human strains (**Appendix 10**), and exhibited the highest nt (aa) sequence identity with the Chinese porcine-like human strains—RVA/Human-wt/CHN/R946/2006/G3P[6], 95.8% (97.8%) and RVA/Human-wt/CHN/E931/2008/G4P[6], 95.7% (98.0%) (**Appendix 7f**). The overall similarities of the Asian strains within the lineage ranged from 84.8% to 95.8% (92.7%–97.8%). Non-porcine African strains clustered separately and showed lower sequence identities of 84.1%–84.5% (92.1%–92.7%).

3.4.2.7. Phylogenetic analysis of the NSP1 gene

The NSP1 gene of strain RVA/Human-wt/ZMB/UFS-NSG-MRC-DPRU4723/2014/G5P[6] was assigned to a porcine genotype A8 and clustered among Asian (Vietnam, China, and Bangladesh) porcine and porcine-like human strains and an African (Ghana) porcine strain (**Figure 3.11**). The NSP1 gene of the study strain was closest to strain RVA/Human-tc/VNM/NT0042/2007/G4P[6] displaying a nt (aa) sequence identity of 98.2% (97.9%) (**Appendix 7g**). The porcine and porcine-like human strains from Europe and the Americas

clustered separately showing sequence identities of 84.2%–85.9% (85.4%–88.2%) and 84.1%–85.9% (83.7%–88.3%), respectively.

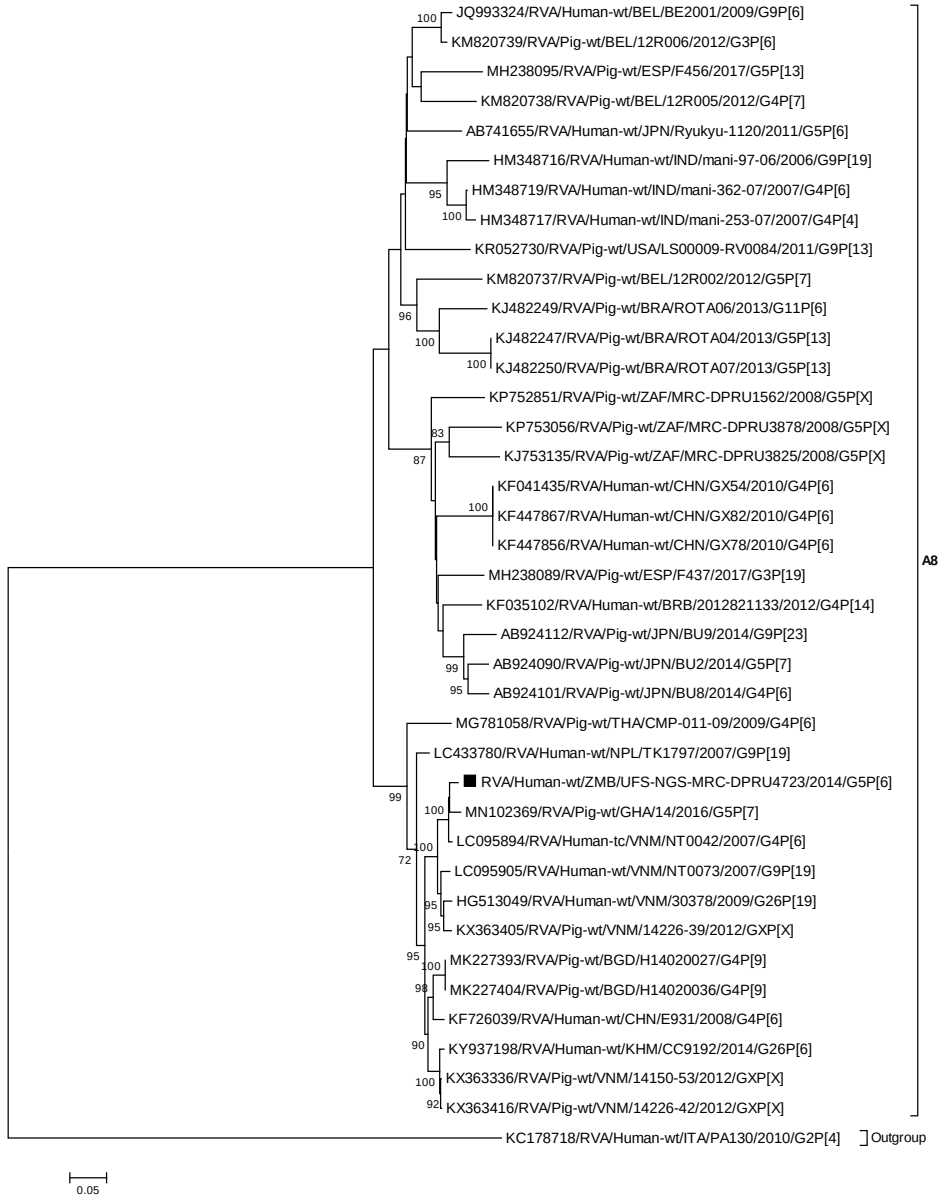


Figure 3.11. Phylogenetic tree constructed from the nucleotide sequences of the NSP1 genes of strain RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] and representative strains. The position of strain RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] is shown by the black square (▪). Reference strains obtained from GenBank are represented by Accession number, Strain name, Country and year of isolation. The three closest strains as identified by BLASTn are also included. Bootstrap values ≥70% are shown adjacent to each branch node. Scale bar: 0.05 substitutions per nucleotide.

3.4.2.8. Phylogenetic analysis of the NSP2 gene

The NSP2 gene of strain RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] clustered with Asian and European porcine and porcine-like human strains (**Appendix 11**). The nt (aa) similarity analysis showed that the NSP2 gene of the study strain was most similar to the Chinese porcine strains RVA/Pig-wt/CHN/YN/2012/GXP[X] and RVA/Pig-tc/CHN/SCMY-A3/2017/G9P[23]—96.8% (97.8%). Two African porcine strains, RVA/Pig-wt/ZAF/MRC-DPRU1487/2007/G3G5P[23] and RVA/Pig-wt/ZAF/MRC-DPRU1557/2008/G4G5P[23], were seen to cluster within the same lineage with sequence identities of 93.6%–93.7% (97.5%–97.8%) (**Appendix 7h**).

3.4.2.9. Phylogenetic analysis of the NSP3 gene

The NSP3 gene of strain RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] clustered closely with porcine and porcine-like human strains mainly from Asia (Thailand and Vietnam) and exhibited a maximum nt (aa) sequence identities of 96.5%–97.0% (98.4%–98.7%) with the strains RVA/Human-wt/VNM/30378/2009/G26P[19], RVA/Pig-wt/VNM/12070-4/2012/GXP[X], RVA/Human-wt/VNM/NT0205/2007/G4P[6], and RVA/Human-wt/VNM/NT0621/2008/G4P[6] (**Appendix 7i; Appendix 12**).

3.4.2.10. Phylogenetic analysis of the NSP4 gene

The NSP4 gene of strain RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] clustered with porcine and porcine-like human strains identified in Asia (China and Vietnam) and a porcine-like human strain from the Americas (Brazil) (**Appendix 13**). In this cluster, the closest strains to UFS-NGS-MRC-DPRU4723 were the wild pig strains (RVA/WildBoar-wt/CZE/P828/2015/G9P[23] and RVA/WildBoar-wt/CZE/P830/2015/G9P[23]) from the Czech Republic, with nt (aa) sequence identities of 97.5% (98.3%). The Asian strains within the cluster showed nt (aa) similarities of 96.2%–97.3% (97.7%–98.9%). Porcine and porcine-like human strains from the Americas clustered separately and exhibited identities of 87.2%–96.4% (94.3%–98.9%) (**Appendix 7j**).

3.4.2.11. Phylogenetic analysis of the NSP5 gene

The NSP5 gene of strain RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] clustered with porcine strains from Asia and showed the highest nt (aa) sequence identity of 98.6% (100%) with the porcine strains RVA/Pig-wt/CHN/TM-a/2009/G3P[8] and RVA/Pig-tc/CHN/TM-a-P20/2018/G9P[23] identified in China (**Appendix 7k**; **Appendix 14**). Overall, the porcine and porcine-like human strains from

Asia and the Americas displayed nt (aa) identities of in the range 94.8%–98.6% (98.0%–100%) and 93.9%–96.1% (95.9%–99.0%), respectively.

3.4.3. Reassortment analysis

The concatenated whole genome alignment of RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6], together with the Japanese G5P[6] strain and selected Chinese porcine-like human P[6] strains, was visualised (**Figure 3.12**). The whole genome of the Zambian G5P[6] strain demonstrated a relatively high degree of conservation with the Japanese G5P[6] strain and the two Chinese G4P[6] strains. With the exception of VP7 and VP4, the genome of the Chinese strain E931 exhibited the overall highest genomic conservation to the study strain. With the exception of VP7, VP3, and NSP1 genes, the Chinese strain GX54 shared a highly conserved genome with the study strain. The Japanese strain Ryukyu-1120 demonstrated a highly similar genome to the study strain for seven of the 11 genes, the exceptions being VP1, VP3, VP6, and VP7. The results of this analysis confirmed the genetic similarity between RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] and Asian (Chinese) porcine-like human strains, hence suggesting that the Zambian G5P[6] strain may have been derived via reassortment events.

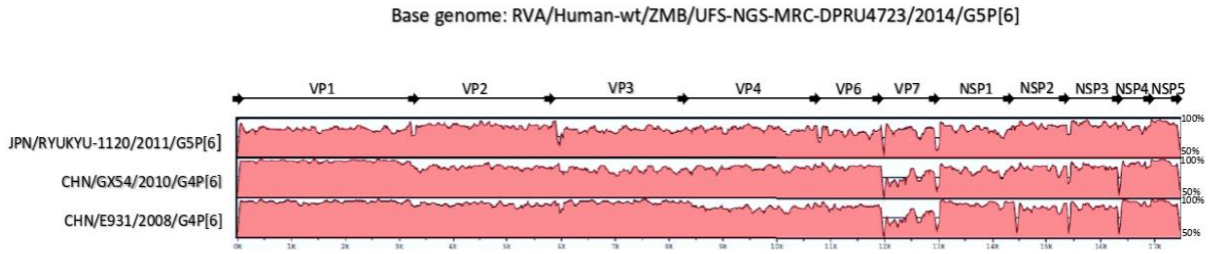


Figure 3.12. mVISTA whole genome nucleotide alignment comparing the Zambian G5P[6] strain (RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014G5P[6]) with the G5P[6] strain from Japan (Ryukyu-1120), whose whole genome sequence had been determined, and with selected porcine-like human P[6] strains from China (GX54 and E931). Strain names are shown on the left, and the proteins VP1-VP4, VP6-VP7, and NSP1-NSP5 are indicated on the top. The bottom scale indicates distance in kb. Percentile values on the right indicate sequence-based similarity between the study strain and the respective reference strains. Shading indicates the level of conservation.

3.5. Discussion

The detection of genotype G5 in humans, which is typical for pigs, is possibly due to interspecies transmission (Esona *et al*., 2009; Komoto *et al*., 2013). In Zambia, as with many countries in Africa, humans and farm animals live in proximity. The interaction between humans and animals could be the primary cause for zoonotic transmission, which could result in genetic reassortments and perhaps other mechanisms of genetic diversity, ultimately leading to the introduction and spread of animal genotypes into human populations (Steyer *et al*., 2008).

In this study, an analysis was conducted on a sample collected from a child admitted to a paediatric ward presenting with clinical symptoms (vomiting, diarrhoea, and fever) that are usually present during typical rotavirus infection. This raises the question whether such animal-derived strains are capable of mutating and effectively spreading within/across human populations as in the case of established typical Wa-like and DS-1-like genotype constellations, with the same magnitude of rotavirus disease severity. Furthermore, taking into consideration that the G5 and P[6] genotypes are not included in the currently available vaccines, the probability for such strains to have the potential to spread more swiftly from human to human may have implications for the effectiveness of current rotavirus vaccine candidates that are in use in African countries.

This study identified the complete genome of a reassortant porcine-like human strain, G5P[6], that showed the genotype constellation G5-P[6]-I1-R1-C1-M1-A8-N1-T1-E1-H1, which is commonly found in porcine and porcine-like human rotavirus strains (Silva *et al*., 2016). RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] was found to share the same constellation (I1-R1-C1-M1-A8-N1-T1-E1-H1) with the archival porcine strain, Gottfried, and porcine-like human strains—BG260, E931, and GX54 (Dong *et al*., 2013; Matthijnssens *et al*., 2008a; Mladenova *et al*., 2012; Zhou *et al*., 2015). In addition, porcine strains 12R002, 12R005, and 12R006, as well as porcine-like human strains Ryukyu-1120, mani-97, 30378, rj24598, and BE2001 shared the same constellation with strain RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] with the exception of VP6 (I5 instead of I1) and NSP3 (T7 instead of T1 gene segments) (Komoto *et al*., 2013; Mukherjee *et al*., 2011; My *et al*., 2014; Theuns *et al*., 2015; Zeller *et al*., 2012a).

A phylogenetic analysis of RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] showed that this strain was a possible reassortant, as it was closely related to both porcine and porcine-like human strains, predominantly from Asia, than to typical human RVA strains. The VP6, VP7, NSP2, NSP4, and NSP5 segments of this strain showed a close similarity to porcine strains. Although the remaining gene segments

(VP1, VP3, VP4, and NSP3) were closely related to human strains, all of these were porcine-like human strains (Dong *et al*., 2013; Heylen *et al*., 2014; Kaneko *et al*., 2018; My *et al*., 2014; Zeller *et al*., 2012a; Zhou *et al*., 2015). With a genotype 1 (Wa-like) backbone, this finding is consistent with the hypothesis that human Wa-like strains and porcine strains have a common ancestor (Matthijnssens *et al*., 2008a). However, the origin of the VP2 gene of the study strain was not very definitive, as it was not only close to porcine and porcine-like human strains but also to three human strains (DC1476, DC582, and DC1127). Phylogenetically, the clusters of these three strains were shown to be distinctive from the genes of contemporary, wild-type human strains (Zhang *et al*., 2014). Notably, the VP7 gene of RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] was located in lineage II, which comprised only porcine strains, hence implying the possibility of porcine-to-human interspecies transmission (da Silva *et al*., 2011). Phylogenetic analysis of porcine and human P[6] strains indicated that both porcine and human P[6] strains were present in P[6] lineages I, III, and V, hence showing that human P[6] strains might have separately emerged from at least three porcine-to-human transmissions (Martella *et al*., 2006b). This finding supports the Zambian G5P[6] strain, as the VP4 gene clustered and shared high nucleotide and amino acid identities with lineage V of P[6] porcine and porcine-like human strains. The NSP1 gene was most similar to porcine-like human strains. However, it was revealed to have the porcine genotype A8. Taking this together, it is likely that RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] originated by zoonotic transmission, coupled with reassortment events.

Several amino acid changes were identified in the nine variable regions when the VP7 gene of the study strain was compared to other G5 strains within each of the three lineages (Green *et al*., 1989). Additionally, the previously described conserved N-glycosylation site at residues 69–71 within the variable region 4 (VR-4) was found to be conserved in all the G5 strains used in this analysis (Ciarlet *et al*., 1995; Green *et al*., 1989). Four major antigenic regions have been described for the VP7 protein in rotaviruses (A, B, C and F) (Dyall-Smith *et al*., 1986; Kobayashi *et al*., 1991). Marked differences in the antigenic regions of RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] were seen when it was compared to other globally circulating G5 strains. Usually, antigenic regions A and C are said to be conserved within serotypes (Green *et al*., 1988). However, multiple substitutions were observed in these regions when comparing the Zambian G5 strain to other G5 strains globally.

The amino acid sequence for the VP4 gene was 775 amino acids long and displayed amino acid identity values ranging from 91.0% to 98.3% with the reference P[6] strains. Considering it has been established that strains with amino acid identities greater than 89% belong to the same P genotype (Gorziglia *et al*.,

1990), our findings show that RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] belongs to the genotype P[6]. The analysis of the amino acid sequences showed that the hypervariable region (amino acid 71-208) which houses the variable region 3 (VR-3) contained most of the substitutions. Furthermore, the potential trypsin cleavage sites (Arias *et al*., 1996) were conserved in all the P[6] strains. Several amino acid substitutions were observed among the lineage I P[6] strains. The presence of several amino acid changes in the VP4 gene of this strain compared to other circulating P[6] strains globally is in agreement with the hypothesis that the P[6] gene has been introduced to humans via independent reassortment events (Bányai *et al*., 2004; Martella *et al*., 2006b; Nyaga *et al*., 2018).

Rotaviruses are genetically diverse in nature and are host-species specific, suggesting that host species barriers and restrictions exist. However, rotaviruses of animal origin may cross the host species barrier and may acquire human rotavirus gene segments, which enables the viruses to efficiently spread across human populations (Martella *et al*., 2010). In this regard, G5 rotavirus strains have sporadically been documented in Latin America, Asia, Europe, and Africa (Bok *et al*., 2001; Esona *et al*., 2004, 2009; Gouvêa *et al*., 1994; Komoto *et al*., 2013; Li *et al*., 2008; Mladenova *et al*., 2012). Porcine P[6] strains seem to pose a lesser species barrier to humans (Theuns *et al*., 2015). Even though the relationship between porcine and human rotaviruses has already been established (Matthijnssens *et al*., 2008a), whole genome analysis in this study presented the possible occurrence of interspecies transmission and reassortment between human and porcine rotaviruses.

3.6. Conclusion

In summary, RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] was a reassortant possessing gene segment of porcine and porcine-like human origin, and was closest to Asian strains. It is presumed that pigs play a crucial part as a source for new or newly-evolved emerging human rotaviruses. This highlights the need for continuous large-scale surveillance and whole genome analysis of circulating porcine and human rotaviruses. Furthermore, it was imperative to examine the prevalence of G5P[6] strains in Zambia. Eventually, this should result in a greater understanding of the genes that determine the transmission between hosts successfully as well as to gain insights on complex reassortment patterns between porcine and human rotaviruses.

Chapter four: Four intergenogroup reassortants

4.1. Preamble

This chapter is presented as a publishable manuscript titled '***Whole genome analysis of human rotaviruses reveals single gene reassortant rotavirus strains in Zambia***' that has already undergone peer-review from journal reviewers. Following an invitation to contribute to the journal, the manuscript was submitted to the special issue on 'Gastroenteritis Viruses 2021' of the journal Viruses (impact factor 5.048) and the submission number **viruses-1264641** was obtained (**Appendix 15**). The manuscript addresses both objectives in an overlapping manner and demonstrates the genome constellation and phylogenetic attributes of four reassortant RVA that were identified in the post-vaccine period. Additionally, one reassortant strain was seen to be divergent in two gene segments after sequence and phylogenetic analysis.

The main body of the manuscript has been adapted here in its entirety, with the exception of the methodology section, which only includes the clinical information of the samples and data analysis aspects, as the methodology section of the previous chapter was expanded to accommodate both chapters. The abstract page is provided in **Appendix 16**.

Martin Nyaga and Jason Mwenda conceptualised the main project. Martin Nyaga, Julia Simwaka, Evans Mpabalwani, Mphahlele Jeffrey, Mapaseka Seheri, and Jason Mwenda facilitated obtaining of the samples. **Wairimu Maringa**, Peter Mwangi, and Martin Nyaga performed the laboratory experiments, formal analysis and bioinformatic analysis. **Wairimu Maringa** prepared the draft manuscript. Martin Nyaga supervised the project and sourced for funding.

4.2. Introduction

Group A rotavirus (RVA), a widespread and infectious pathogen that causes dehydrating diarrhoea, particularly in children under five years of age, was estimated to have caused approximately 128,000 deaths in 2016. A greater percentage of these deaths (approximately 105,000) occurred in sub-Saharan Africa (Troeger *et al*., 2018). The significance of RVA burden of disease led to the development of prophylactic vaccines. In that regard, the World Health Organization (WHO) recommended the use of rotavirus vaccines globally (WHO, 2013). Four WHO-prequalified rotavirus vaccines (Rotarix®, RotaTeq®, ROTAVAC® and ROTASIIL®) are currently in use in 110 countries worldwide as of 5th April 2021 (IVAC, 2021). The two-dose monovalent vaccine Rotarix® (RV1; GlaxoSmithKline Biologicals, Belgium) consists of a single human G1P[8] strain (WHO, 2013). In sub-Saharan Africa, Rotarix® is used in countries such as Kenya, Mauritania, Namibia, Niger, and Zimbabwe (IVAC, 2021). This vaccine was introduced in Lusaka,

Zambia in 2012 as a pilot project and then rolled out nation-wide in 2013 (Chilengi *et al*., 2015; Mpabalwani *et al*., 2016). Vaccine coverage in Zambia in 2019 was at 90% (WHO, 2021c).

Rotaviruses contain 11 segments of double-stranded RNA (dsRNA) that encodes six structural viral proteins (VP1-VP4, VP6-VP7) and five and/or six non-structural proteins (NSP1-NSP5/6) (Estes and Greenberg, 2013). A mature RVA particle comprises an inner core (VP2), which is surrounded by VP1 and VP3, a middle layer (VP6) and an outer layer (VP7) with spikes of the VP4 protruding from the outer layer (Estes and Greenberg, 2013). The antigenicity of the outer proteins, VP7 and VP4, is used to classify RVA into G-types (glycoprotein) and P-types (protease-sensitive), respectively (Estes and Greenberg, 2013). Because they are targets of neutralising antibodies that may provide serotype-specific and/or cross-protective immunity, these two proteins are considered critical for vaccine development (Hoshino and Kapikian, 2000). Further, a whole genome based genotyping system was established by the Rotavirus Classification Working Group (RCWG), whereby specific genotypes are assigned to the 11 segments of RVA. This system established three human RVA genogroups exhibiting the Wa-like (G1-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1), DS-1-like (G2-P[4]-I2-R2-C2-M2-A2-N2-T2-E2-H2), or the AU-1-like (G3-P[9]-I3-R3-C3-M3-A3-N3-T3-E3-H3) constellations (Matthijnssens *et al*., 2008b, 2011).

Due to the segmented genome of RVA, it is common for reassortment events to occur, which play a key role in generating the genetic diversity of the virus (Ghosh and Kobayashi, 2011). It is crucial to understand genetic exchange through reassortment, particularly those belonging to the two major genogroups, as well as various evolutionary mechanisms that contribute to genetic diversity. RVA genomes have high rates of mutation and are subject to frequent reassortment events, which are primarily responsible for rotavirus evolution (Donker and Kirkwood, 2012; Ghosh and Kobayashi, 2011; Hoxie and Dennehy, 2020; Kirkwood, 2010; Matthijnssens *et al*., 2010; Ramig, 1997). RVA with unusual G-P combinations such as G1P[4], G2P[6], G2P[8], G3P[4] and G8P[4] are known to circulate in human populations as a result of intergenogroup reassortment between co-circulating strains. The G1P[4] and G2P[8] have been shown to circulate among G1P[8] and G2P[4] strains (Banerjee *et al*., 2018; Dóró *et al*., 2015; Ghosh *et al*., 2012; Nyaga *et al*., 2014; Ramig, 1997; Seheri *et al*., 2018). Most human RVA strains possess either a typical Wa-like or DS-1-like constellation and are thought to have an evolutionary fitness advantage that allows them to spread widely and persist in human populations (Heiman *et al*., 2008; McDonald *et al*., 2009). Nevertheless, after the isolation of two naturally-occurring intergenogroup reassortants between Wa-like and DS-1-like in Bangladesh in 1985-1986 (Ward *et al*., 1990). RVA strains possessing mixed gene constellations of human and/or animal origin have been documented in various parts of the world (Cowley

et al., 2013; Heylen *et al*., 2014; Hoa-Tran *et al*., 2020; Jere *et al*., 2018; Katz *et al*., 2019; Komoto *et al*., 2016; Luchs *et al*., 2019; Nyaga *et al*., 2015, 2018).

RVA strain surveillance based on conventional genotyping of VP7 and VP4 has been conducted in Zambia (Simwaka *et al*., 2018). Unusual G- and P- combinations such as G1P[6] and G9P[6] were reported in 2011 before Rotarix® was implemented. On the contrary, only the G2P[6] was reported post-vaccine implementation (Simwaka *et al*., 2018). However, there is a dearth of Zambian whole genome sequence data. Here we report the whole genomes of four intergenogroup reassortant strains identified between 2014 and 2016 during the ongoing RVA surveillance in Zambia, to understand the mechanisms that result in genetic diversity among Zambian RVA post-Rotarix® introduction.

4.3. Methodology

4.3.1. Study samples

Stool samples were collected in the post-vaccine period from children who presented with acute gastroenteritis. The demographics and clinical profiles of the children from whom the study samples were taken at Arthur Davidson Children's Hospital (ACDH) and University Teaching Hospital (UTH) are shown **Table 4.1**. The four Zambian strains analysed in this study were collected from one female and three male children aged between 5-20 months, as part of the ongoing rotavirus surveillance by the WHO/AFRO. The strains demonstrated a sporadic transmission pattern, devoid of any sign of an outbreak infection, as they were identified in different parts of Ndola and Lusaka. Further, clinical information indicated that all the children had diarrhoea that lasted between a day and four days, with varying frequencies. Similarly, three children presented with two to three days of intermittent vomiting, and two children presented with fever. There were two cases of severe dehydration and one case of moderate dehydration because of diarrhoea and vomiting. Two of the four children had been vaccinated, while the other two were not vaccinated. However, all the strains were detected post-Rotarix® vaccine implementation (2014-2016). No mortality resulted due to illness, as all children fully recovered.

Table 4.1. Table showing the demographics and clinical profiles of the children from which the study samples were obtained.

Sample ID and year	Hospital	The child's place of residence	Sex	Age	Presenting illness symptoms	Dehydration status and treatment administered	Vaccination status	Outcome of illness
UFS-NGS-MRC-DPRU4749/2014	ACDH Ndola	Chifubu	Female	5 months	Diarrhoea for 4 days (4 episodes in 24 hours), no vomiting, temperature of 39°C	Moderate dehydration, treated with ORS	Not vaccinated	Alive
UFS-NGS-MRC-DPRU13232/2016	ACDH Ndola	Kawama	Male	7 months	Diarrhoea for 3 days (6 episodes in 24 hours), vomiting for 2 days (4 episodes in 24 hours), temperature of 38.2°C	Severe dehydration, treated with IV fluids	Vaccinated (1 dose)	Alive
UFS-NGS-MRC-DPRU13541/2016	ACDH Ndola	Mwange A	Male	8 months	Diarrhoea for 3 days (8 episodes in 24 hours), vomiting for 3 days (3 episodes in 24 hours), no fever	Severe dehydration, treated with IV fluids	Not vaccinated	Alive
UFS-NGS-MRC-DPRU13327/2016	UTH Lusaka	Kapata	Male	20 months	Diarrhoea for 1 day (3 episodes in 24 hours), vomiting for 3 days, no fever	No dehydration, treated with ORS	Vaccinated (2 doses)	Alive

4.3.2. Genome assembly

Sequence reads obtained from the Illumina® MiSeq platform in FASTQ format were first trimmed and subsequently assembled using Geneious® Prime 2019.2.1 (https://www.geneious.com/; Kearse *et al*., 2012). Genome assembly comprised both reference mapping as well as *de novo* assembly. The sequences were deposited into the GenBank under accession numbers MZ027412-MZ027455.

4.3.3. Identification of genotype constellations

The genotype of each of the 11 genome segments of the four Zambian RVA strains was identified on the Virus Pathogen Resource (ViPR), an online bioinformatics database and analysis resource for virological research (Pickett *et al*., 2012). The Basic Local Alignment Search Tool (BLAST) was also utilised as a complementary tool for genotype identification (Sayers *et al*., 2021).

4.3.4. Phylogenetic analysis

Reference sequences were compiled using BLAST as well as the Virus Variation Resource hosted by the National Centre for Biotechnology Information (NCBI) (Hatcher *et al*., 2017; Sayers *et al*., 2021). Multiple alignments were made for each gene using the MAFFT plugin in Geneious® Prime version 2019.2.1 (https://www.geneious.com/) and MUSCLE algorithm that is present in MEGA 6 (Katoh and Standley, 2013; Kearse *et al*., 2012; Tamura *et al*., 2013). Pairwise nucleotide and amino acid sequence identity matrices were calculated using the *p*-distance algorithm in MEGA 6 (Tamura *et al*., 2013). A maximum likelihood tree was constructed for each genome segment. Substitution models that best fit the data were selected based on corrected Akaike Information Criterion (AICc) in MEGA 6 (Guindon and Gascuel, 2003). The models used in this study were: GTR+G+I (VP1), TN93+G (VP2), GTR+I (VP3 and NSP1), T92+G+I (VP4 and VP7), T92+G (VP6, NSP2, NSP4, and NSP5), and TN93+I (NSP3). Branch support was estimated with 1000 bootstrap replicates (Felsenstein, 1985).

4.3.5. Protein modelling

Protein modelling was performed using the SWISS MODEL online server (SWISS-MODEL (expasy.org); Bienert *et al*., 2017; Waterhouse *et al*., 2018). Briefly, the amino acid fasta sequence was programmed to perform a template search, after a which a protein template with the 2dwr.1 structure and an X-ray diffraction resolution value of 2.50Å was selected from the SWISS MODEL template library. Modelling was then performed. The stereochemical quality of the protein structure was assessed using the Structure Assessment feature in SWISS MODEL. Superposition of the structures was then performed on PyMol

(http://www.pymol.org/; DeLano, 2002) to assess the structural conformation of the two protein structures, and the alignment value was generated in Root Mean Square Deviation (RMSD).

4.4. Results

4.4.1. Genotyping based on whole genome constellations

Following Illumina® MiSeq sequencing, complete or nearly complete nucleotide sequences for each of the 11 genes of the four study strains were obtained. The contig lengths and number of reads after assembly are shown in **Table 4.2**. The strains were named as RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13232/2016/G1P[8], RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13541/2016/G1P[8], RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13327/2016/G2P[4], and RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4749/2014/G2P[8] according to the guidelines for the uniformity of RVA by the RCWG, henceforth referred to as UFS-NGS-MRC-DPRU13232, UFS-NGS-MRC-DPRU13541, UFS-NGS-MRC-DPRU13327, and UFS-NGS-MRC-DPRU4749, respectively.

The genotype constellations demonstrated that the strains were generated through reassortment between Wa-like and DS-1-like strains. Applying the whole genome-based genotyping system (Matthijnssens *et al*., 2008b, 2011), UFS-NGS-MRC-DPRU13232, UFS-NGS-MRC-DPRU13541, UFS-NGS-MRC-DPRU13327, and UFS-NGS-MRC-DPRU4749 had the following constellations: G1-P[8]-I1-R1-C1-M1-A1-**N2**-T1-E1-H1, G1-P[8]-I1-R1-C1-M1-A1-**N2**-T1-E1-H1, G2-P[4]-I2-R2-C2-M2-A2-**N1**-T2-E2-H2 and G2-**P[8]**-I2-R2-C2-M2-A2-N2-T2-E-H2, respectively (**Table 4.2**) and were therefore considered mono-reassortants, as shown on the bolded genotypes. Strain UFS-NGS-MRC-DPRU13232 and UFS-NGS-MRC-DPRU13541 possessed Wa-like constellations except for the N2 NSP2 genotype. Strain UFS-NGS-MRC-DPRU13327 and UFS-NGS-MRC-DPRU4749 possessed DS-1-like constellations with the exception of N1 NSP2 genotype and P[8] VP4 genotype, respectively.

The 11 genes of the two Wa-like Zambian strains (UFS-NGS-MRC-DPRU13232 and UFS-NGS-MRC-DPRU13541) exhibited a high level of sequence conservation with >99% nucleotide sequence identity to each other. On the other hand, the two DS-1-like Zambian strains (UFS-NGS-MRC-DPRU13327 and UFS-NGS-MRC-DPRU4749) exhibited high nucleotide sequence identity (>97%) in the VP7, VP6, VP2, NSP1, NSP3, and NSP5 genes, whereas lower identities were observed in the VP4, VP1, VP3, NSP2, and NSP4 genes (82.7%, 91.0%, 87.9%, 82.7%, and 90.9%, respectively).

Table 4.2. The whole genome constellation of the four reassortant study strains detected between 2014 and 2016 (post-vaccine period) in Zambia along with the contig length and the number of reads mapped to each contig.

Strain		VP7	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5
UFS-NGS-MRC-DPRU13232	Genotype	G1	P[8]	I1	R1	C1	M1	A1	**N2**	T1	E1	H1
	Contig length	1062	2359	1356	3301	2717	2591	1567	1059	1074	750	644
	Reads mapped to contig	21238	4523	14997	87349	52209	52222	26784	53976	30125	25306	21366
UFS-NGS-MRC-DPRU13541	Genotype	G1	P[8]	I1	R1	C1	M1	A1	**N2**	T1	E1	H1
	Contig length	1063	2359	1352	3301	2729	2591	1567	1059	1074	750	663
	Reads mapped to contig	33485	10936	62838	108961	79014	134489	80109	33007	36184	34457	12638
UFS-NGS-MRC-DPRU4749	Genotype	G2	**P[8]**	I2	R2	C2	M2	A2	N2	T2	E2	H2
	Contig length	1062	2360	1356	3302	2684	2591	1569	1059	1066	750	815
	Reads mapped to contig	1445	4513	2302	6738	4388	5214	2315	1063	1268	916	471
UFS-NGS-MRC-DPRU13327	Genotype	G2	P[4]	I2	R2	C2	M2	A2	**N1**	T2	E2	H2
	Contig length	1062	2359	1354	3298	2684	2591	1566	1059	1066	751	798
	Reads mapped to contig	24446	51762	23311	67839	53795	60905	25147	11048	20338	13618	13618

The Wa-like genogroup is presented in green, while the DS-1-like genogroup is presented in red.

4.4.2. Phylogenetic and sequence analysis

To understand the genetic relationship of the four Zambian strains with global stains, a phylogenetic tree was resolved for each of the 11 gene segments. For the designation of lineages in the VP7, VP4, and VP1 trees, closely related strains as well as strains on the respective lineages, were selected from the GenBank using previously published articles as reference (Agbemabiese *et al*., 2019; Aida *et al*., 2016; Arista *et al*., 2006; Doan *et al*., 2012, 2015; Gouvêa *et al*., 1999).

4.4.2.1. Phylogenetic analysis of the VP7 genes (G1 and G2)

Reference RVA strains utilised in this analysis segregated into the known seven G1 lineages and five G2 lineages (Arista *et al*., 2006; Doan *et al*., 2015). A multiple sequence alignment and phylogenetic analysis of the VP7 genes of the four study strains showed that the Zambian G1 strains (UFS-NGS-MRC-DPRU13232 and UFS-NGS-MRC-DPRU13541) clustered with other reference strains in lineage G1 I (**Figure 4.1**). Lineage G1 I was comprised of African and Asian strains identified between 2003-2017 with maximum nucleotide (nt) and amino acid (aa) identities ranging from 96.8% - 99.1% and 97.5% - 99.7% with the two Zambian G1 strains (**Figure 4.1; Appendix 17 a,b**). Among the two Zambian G1 strains, the nt and aa identity was 100%. On the other hand, the Zambian G2 strains (UFS-NGS-MRC-DPRU4749 and UFS-NGS-MRC-DPRU13327 clustered in lineage G2 IV along with strains from Asia and Africa with nt (aa) identities of 93.8% - 99.6% (92.6% - 100%) (**Figure 4.1; Appendix 17 a,b**). A nt and aa similarity of 97.8% and 98.5% was shared between the two Zambian G2 strains.

4.4.2.2. Phylogenetic analysis of the VP4 genes (P[4] and P[8])

The VP4 P[8] and P[4] Zambian strains were compared to global selected reference strains that belong to the already established four P[4] and four P[8] lineages (Arista *et al*., 2006; Doan *et al*., 2012). Based on the VP4 phylogenetic tree, two of the Zambian P[8] strains (UFS-NGS-MRC-DPRU13232, and UFS-NGS-MRC-DPRU13541) clustered together in lineage P[8] III and shared a nt and aa identity of 99.8% and 99.9%, respectively (**Figure 4.2; Appendix 17 c,d**). Lineage P[8] III consisted of predominantly African strains (Cameroon, Togo, South Africa, and Zimbabwe) that showed nt (aa) identities of 97.6% - 99.0% (98.7% - 99.2%) to the two Zambian P[8] strains. The vaccine strain, RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P[8], clustered in lineage P[8] I with nt (aa) identities of 90.3% - 90.4% (93.9% - 94.1%) to the two aforementioned Zambian P[8] strains (**Appendix 17 c,d**). Interestingly, UFS-NGS-MRC-DPRU4749 clustered separately from the other lineages, including that containing Rotarix® (Lineage P[8] I; nt 84.9%), as well as the most common lineage globally (Lineage P[8] III) (Zeller *et al*., 2012b) that contained the other Zambian P[8] strains (**Figure 4.2**). This strain was closest to a South African strain that

clustered in lineage P[8] III, RVA/Human-wt/ZAF/MRC-DPRU2035/2010/G1P[8] with nt (aa) identity of 90.2% (92.8%).

For the P[4] Zambian strain, UFS-NGS-MRC-DPRU13327 clustered with lineage P[4] IV strains and exhibited maximum nt (aa) similarity of 99.6% (99.2%) and 99.4% (99.1%) to a Mozambican strain and an Indian strain, respectively (**Figure 4.2; Appendix 17 c,d**).

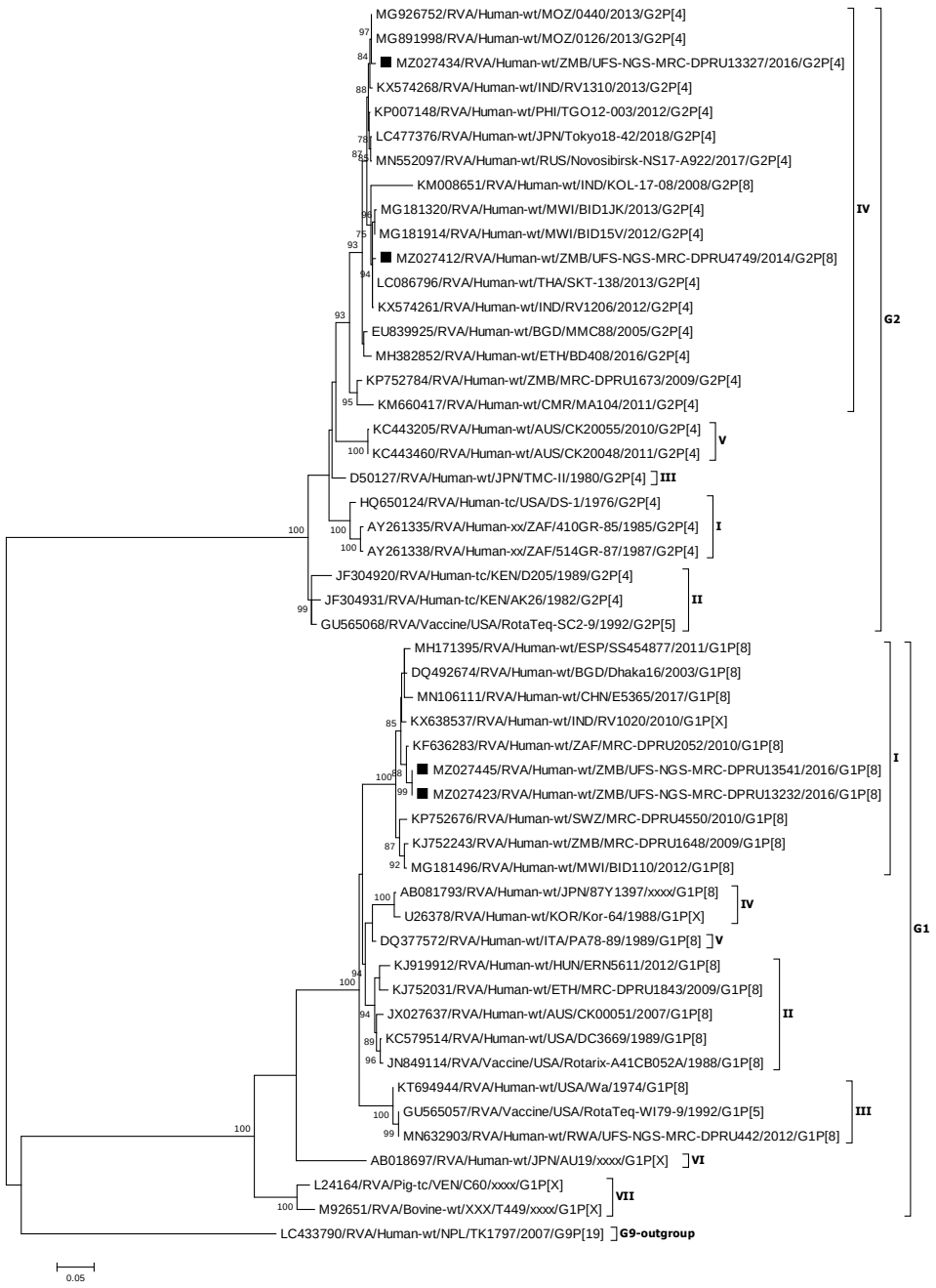


Figure 4.1. VP7 phylogenetic tree of the Zambian G1 and G2 strains indicated by black squares along with representative strains. Phylogenetic analysis was conducted using the maximum likelihood method with bootstrap values of 1000 replicates. The scale at the bottom indicates the number of nucleotide substitutions per site. Percent values of bootstrap values greater than or equal to 70 is indicated on the branch nodes.

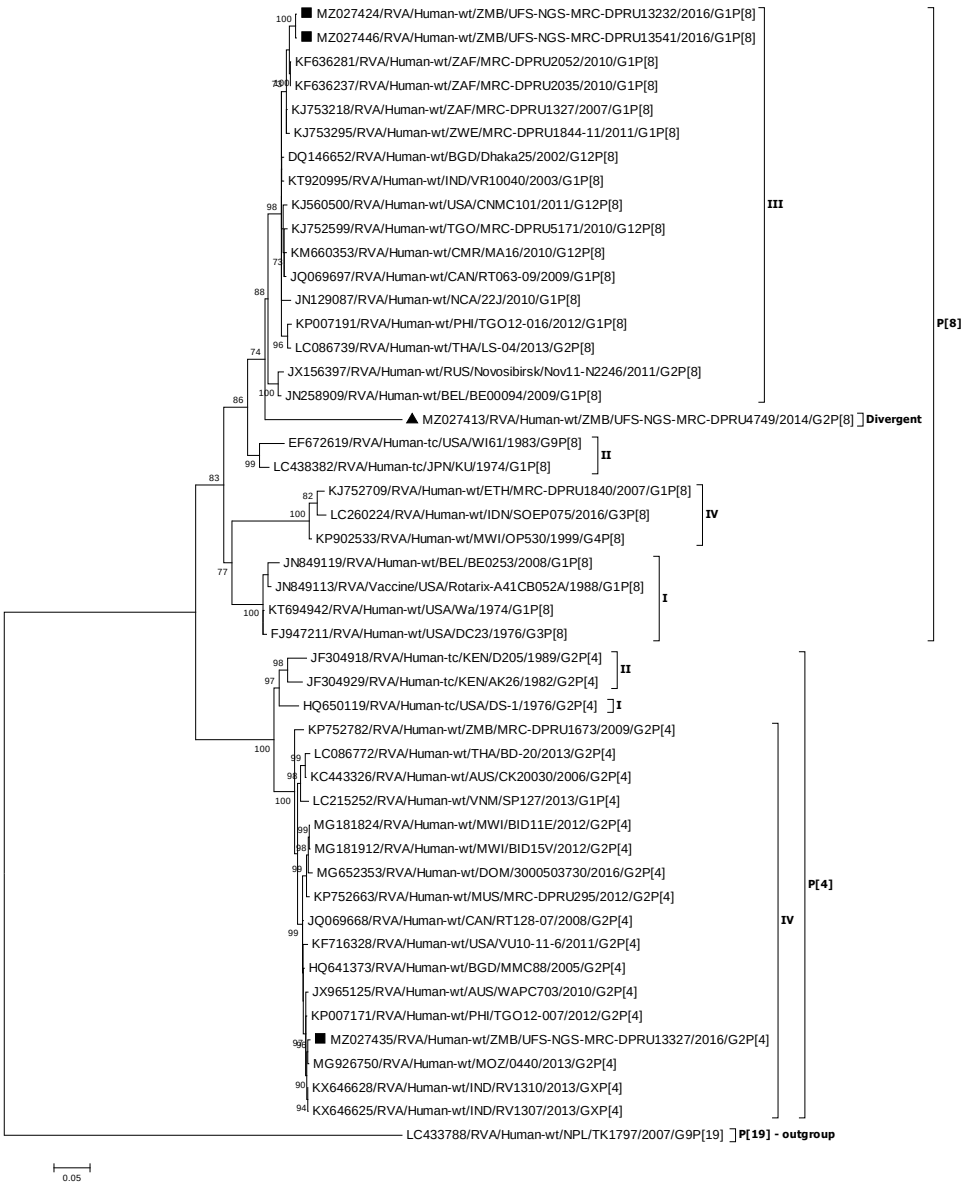


Figure 4.2. VP4 phylogenetic tree of the Zambian P[4] and P[8] strains indicated by black squares along with representative strains. Strain UFS-NGS-MRC-DPRU4749, indicated by a black triangle, is a divergent strain. Phylogenetic analysis was conducted using the maximum likelihood method with bootstrap values of 1000 replicates. The scale at the bottom indicates the number of nucleotide substitutions per site. Percent values of bootstrap values greater than or equal to 70 is indicated on the branch nodes.

4.4.2.3. Comparison of the VP4 antigenic epitopes of Zambian G2P[8] to Rotarix®

The nature of aa substitution occurring in P[8] strains in each lineage, including the phylogenetically distinct Zambian strain UFS-NGS-MRC-DPRU4749 that was seen to be phylogenetically distinct, was analysed relative to that of Rotarix®. It was observed that there were 26 fully conserved aa residues. Overall, most of the aa changes in the P[8] strains relative to Rotarix® were displayed in the VP8* (8-1 and 8-3) region (**Figure 4.3**). Lineage I P[8] strains possessed the same aa at all positions. While lineage II P[8] strains had five aa substitutions from Rotarix®(N195D, S125N, S131R, N135D, and I388L), the I388L substitution occurred only in one of the lineage II P[8] strains. Lineage III P[8] strains had six aa substitutions (E150D, N194G, N195G, S125N, S131R, and N135D) relative to Rotarix®. However, the substitution N195G was present in only one of the two lineage III P[8] strains. Lineage IV P[8] strains also known as *OP354-like* (Cunliffe *et al*., 2001; Nagashima *et al*., 2009; Zeller *et al*., 2015) had seven aa changes (N192D, N194T, N195S, N113D, S131R, I388L, and E459D).

Comparison of the divergent Zambian P[8] strain against Rotarix® showed 30 identical aa residues spanning the VP4 antigenic epitopes (**Figure 4.3**). Seven aa changes, E150D, N195G, N113D, V115A, S125N, S131R, and N135D, were seen in the study strain relative to Rotarix® (**Figure 4.3**). These changes were located on the surface of the protein structure (**Figure 4.4**). Analysis of the Zambian P[8] strain relative to two selected strains of the most common lineage, lineage P[8] III [53], identified two aa differences (D113N and A115V).

| | | Neutralisation epitopes | | |

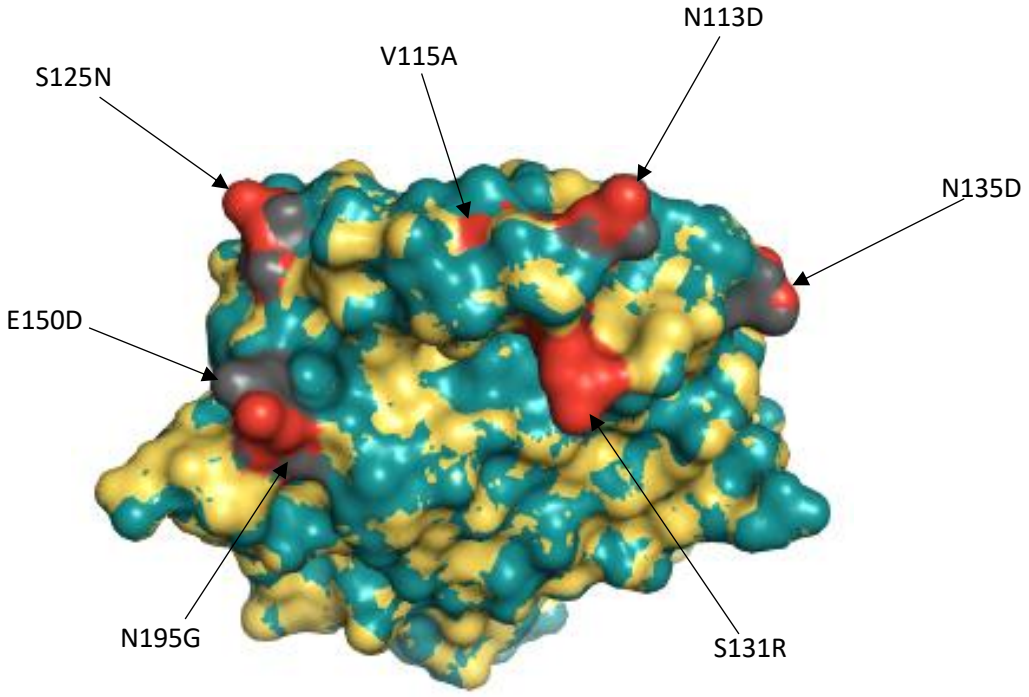


Figure 4.4. Surface representation of the VP8* protein of Rotarix® and the divergent study strain UFS-NGS-MRC-DPRU4749. The superposition of the two structures has the root square mean deviation of 0.048 Å. Rotarix® structure is represented by the teal colour whereas the Zambian P[8] strain is indicated in yellow. The red colour represents the amino acid changes observed on the Zambian study strain as compared to Rotarix® vaccine strain in grey.

4.4.2.4. Phylogenetic analysis of the VP1 gene

The two Zambian Wa-like strains (UFS-NGS-MRC-DPRU13232 and UFS-NGS-MRC-DPRU13541) clustered among R1 African strains. The two strains shared highest nt (aa) similarity of 99.4% (99.4% - 99.7%) with South African strains RVA/Human-wt/ZAF/MRC-DPRU2030/2010/G1P[8] and RVA/Human-wt/ZAF/MRC-DPRU2052/2010/G1P[8] (**Figure 4.5; Appendix 17 e,f**).

Doan *et al*. (2015) established five lineages for global R2 strains. More recently, Agbemabiese *et al*. (2019) proposed 14 lineages for R2 strains which included human and animal RVA strains. Based on this, one of the two DS-1-like Zambian strains, UFS-NGS-MRC-DPRU13327, clustered in lineage R2 V that mainly comprised of African strains (**Figure 4.5**). This strain displayed maximum nt (aa) identities of 99.4% (99.7%) with strains from Zimbabwe and Mozambique (**Appendix 17 e,f**). In the VP1 phylogenetic tree, a cluster of strains within the R2 genotype could not be classified under any lineage according to the established designations (Agbemabiese *et al*., 2019; Doan *et al*., 2015) and were therefore named "undefined".

Strain UFS-NGS-MRC-DPRU4749 clustered independently (**Figure 4.5**) and shared the highest similarity to RVA/Human-wt/IND/NIV1416591/2014/G9P[4] that clustered in Lineage R2 V, with nt (aa) identities of 93% (96.6%) (**Appendix 17 e,f**).

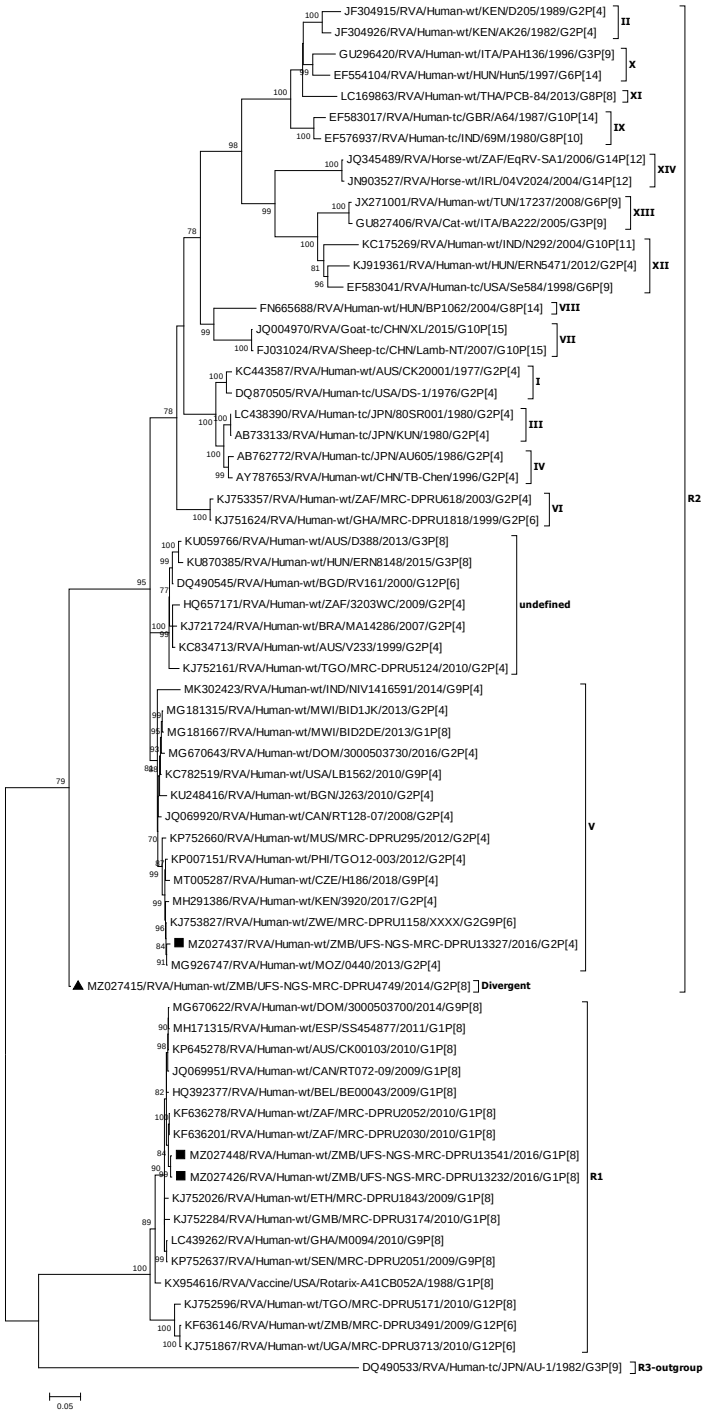


Figure 4.5. VP1 phylogenetic tree of the Zambian R1 and R2 strains indicated by black squares along with representative strains. Strain UFS-NGS-MRC-DPRU4749, indicated by a black triangle is a divergent strain. Phylogenetic analysis was conducted using the maximum likelihood method with bootstrap values of 1000 replicates. The scale at the bottom indicates the number of nucleotide substitutions per site. Percent values of bootstrap values greater than or equal to 70 is indicated on the branch nodes.

4.4.2.5. Phylogenetic analysis of the VP6, VP2, and VP3 genes

The VP6, VP2, and VP3 genes of the four Zambian strains clustered among African strains. The VP6 genes of Wa-like strains UFS-NGS-MRC-DPRU13232 and UFS-NGS-MRC-DPRU13541 displayed maximum nt identities (97.7% - 98.1%) with the VP6 genes of the South African strain RVA/Human-wt/ZAF/MRC-DPRU2052/2010/G1P[8]. Phylogenetically, the two Wa-like Zambian strains clustered together in lineage I1 (Appendix 16). On the other hand, the DS-1-like Zambian strains (UFS-NGS-MRC-DPRU4749 and UFS-NGS-MRC-DPRU13327) clustered separately under lineage I2 among strains identified in Malawi and Mozambique with nt and aa identities of 99.7% - 99.9% and 99.7% - 100% (**Appendix 17 g,h; Appendix 18**).

The VP2 genes of the two Wa-like Zambian strains clustered together in lineage C1 and exhibited highest nt identity of 98.7% with the South African strain RVA/Human-wt/ZAF/MRC-DPRU2052/2010/G1P[8] and Zimbabwean strain RVA/Human-wt/ZWE/MRC-DPRU1844-11/2011/G1P[8], whereas the two Zambian DS-1-like strains clustered in lineage C2, exhibiting maximum nt (aa) identities of 98.8% - 99.5% (99.4% - 100%) with VP2 genes of Malawian and Mozambican strains (**Appendix 17 i,j; Appendix 19**). Similar to the VP2 gene, the VP3 genes of the two Wa-like Zambian strains clustered together in lineage M1 that consisted predominantly of African strains. Highest nt (aa) identities of 99.1% - 99.2% (98.8% - 99.0%) was observed to the Zimbabwean strain RVA/Human-wt/ZWE/MRC-DPRU1844-11/2011/G1P[8] and the South African strain RVA/Human-wt/ZAF/MRC-DPRU2052/2010/G1P[8]. The VP3 genes of the two Zambian DS-1-like strains were in two different clusters within the M2 lineage. UFS-NGS-MRC-DPRU13327 showed highest nt similarity (99.4%) with Mozambican strain RVA/Human-wt/MOZ/0440/2013/G2P[4] whereas the other DS-1-like Zambian strain, UFS-NGS-MRC-DPRU4749, was closest to Malawian strains with nt (aa) identities of 99.3% - 99.6% (99.0% - 99.4%) (**Appendix 17 k,l; Appendix 20**).

4.4.2.6. Phylogenetic analysis of the NSP1-NSP5 genes

Phylogenetically, the NSP1, NSP3, NSP4, NSP4, and NSP5 genes of the two Zambian Wa-like strains (UFS-NGS-MRC-DPRU13232 and UFS-NGS-MRC-DPRU13541) clustered together in lineages A1, T1, E1 and H1, respectively, whereas the two DS-1-like Zambian strains (UFS-NGS-MRC-DPRU4749 and UFS-NGS-MRC-DPRU13327) clustered distant from each other in lineages A2, T2, E2 and H2 (**Appendices 21, 23-25**). For the NSP2 gene, UFS-NGS-MRC-DPRU13327 clustered in N1, while UFS-NGS-MRC-DPRU4749, UFS-NGS-MRC-DPRU13232 and UFS-NGS-MRC-DPRU13541 clustered together in lineage N2 (**Appendix 22**).

The NSP1 and NSP4 genes of UFS-NGS-MRC-DPRU13232 and UFS-NGS-MRC-DPRU13541 fell into clusters predominantly comprised of African strains, and were closest to the strains, RVA/Human-wt/ZWE/MRC-

DPRU1844-11/2011/G1P[8] and RVA/Human-wt/MRC-DPRU1544/2010/G1P[8], with nt (aa) identities of 98.0% - 98.5% (97.1% - 98.6%) (**Appendix 17 m,n,s,t; Appendix 21 and 24**). In contrast, the NSP3 and NSP5 genes of the two Zambian G1P[8] strains displayed the highest nt (99.0% - 99.1%) and aa (98.5% - 99.7%) identities to Brazilian strains (**Appendix 17 q,r,u,v; Appendix 23 and 25**).

For the DS-1-like Zambian strains, UFS-NGS-MRC-DPRU4749 clustered closely with Malawian strains in the NSP1, NSP3, NSP4 and NSP5 genes, displaying nt (aa) identities of 99.2% - 99.3% (98.6% - 98.85), 99.4% - 99.7% (99.7% - 100%), 98.9% (99.4%) and, 98.6% - 99.2% (99.0% - 99.5%), respectively (**Appendix 17 m,n,q-v; Appendices 21, 23-25**). UFS-NGS-MRC-DPRU13327, on the other hand, was closest related to Mozambican strain RVA/Human-wt/MOZ/0440/2013/G2P[4] in the NSP1, NSP3, and NSP5 genes with maximum nt (aa) identities of 99.5% (99.4%), 99.6% (99.7%), and 99.7% (99.5%) in those respective genes (**Appendix 17 m,n,q,r,u,v**; **Appendices 21, 23, and 25**). For the NSP4 gene, UFS-NGS-MRC-DPRU13327 clustered among Asian strains and exhibited the highest nt (aa) identity of 97.7% (98.9%) to strain RVA/Human-wt/IND/RV1206/2012/G2P[4] (**Appendix 17 s,t**; **Appendix 24**).

Based on the NSP2 gene, UFS-NGS-MRC-DPRU13327, UFS-NGS-MRC-DPRU13232 and UFS-NGS-MRC-DPRU13541 were seen to be reassortants. The DS-1-like Zambian strain, UFS-NGS-MRC-DPRU13327, belonged to genotype N1 and clustered among strains from Asia, Europe, and Oceania with maximum nt (aa) identities of 99.4% (100%) and 99.5% (99.7%) to a Russian and Indian strain, respectively (**Appendix 17 o,p**; **Appendix 22**). The two Wa-like Zambian strains, UFS-NGS-MRC-DPRU13232 and UFS-NGS-MRC-DPRU13541, along with the DS-1-like strain UFS-NGS-MRC-DPRU4749 belonged to genotype N2 and displayed highest nt (aa) similarity of 99.4% - 99.8% (98.7% - 99.7%) to Malawian strains (**Appendix 17 o,p**; **Appendix 22**).

4.5. Discussion

The present study reported on four intergenogroup reassortant strains in Zambia. Whole genome sequencing and analyses demonstrated that the four study strains possessed mixed genotypes in at least one gene segment within the constellation between Wa-like and DS-1-like genogroups, hence were considered intergenogroup reassortant strains. Such reassortant strains have been sporadically detected in countries such as Germany, Japan, Lebanon, Malawi, Rwanda, Senegal, South Africa, and Zimbabwe (Giammanco *et al*., 2014; Jere *et al*., 2018; Komoto *et al*., 2016; Mishra *et al*., 2020; Nyaga *et al*., 2015; Pietsch and Liebert, 2018; Rasebotsa *et al*., 2021; Thanh *et al*., 2018).

A key observation was made regarding strain UFS-NGS-MRC-DPRU4749. This strain was seen to be phylogenetically distinct in the VP4 gene, as it did not cluster into any of the already defined P[8] lineages (Arista *et al*., 2006). The same observation was made in the VP1 gene, whereby the Zambian strain clustered distinctly from other established R2 lineages proposed by Doan *et al*. 2015 and Agbemabiese *et al*. 2019, with a nucleotide variance of 7% to the closest strain. Further, the divergent Zambian strain was supported by bootstrap values of 88% and 79% at the branching node in the VP4 and VP1 phylogenetic trees, respectively. The large genetic distance to other global strains on both nt and aa level concurred with the distinct clustering seen in the VP4 and VP1 phylogenetic trees, thus strain UFS-NGS-MRC-DPRU4749 can be considered as a divergent strain.

The VP4 spike protein is proteolytically cleaved into VP8* and VP5* subunits by trypsin-like proteases present in the gastrointestinal tract of a host, which in the process activates the rotavirus particle (Dormitzer *et al*., 2004; Graham and Estes, 1980). The VP5* enables the penetration of the virus by permeabilising lipid vesicles during infection, while the VP8* is thought to mediate attachment to the host (Denisova *et al*., 1999; Dowling *et al*., 2000; Fiore *et al*., 1991). Four (8-1 to 8-4) and five (5-1 to 5-5) epitopes are contained in the VP8* and VP5* subunits, respectively, which are targets for neutralising monoclonal antibodies (Zeller *et al*., 2012b). Neutralising antibodies that target the VP8* neutralise infectivity of the virus by inhibiting attachment, while those directed against VP5* are thought to block membrane penetration (Ruggeri and Greenberg, 1991; Trask *et al*., 2012). The VP4 is involved in several important structural and functional roles such as attachment, penetration, and particle maturation. Due to this, the genetic variability is more restricted in humans as compared to the VP7 (Estes and Greenberg, 2013; Trask *et al*., 2012; Zeller *et al*., 2015). This characteristic is exploited by the current vaccines, Rotarix® which contains a single human G1P[8] and RotaTeq® that contains G1-G4 and a P[8] genotype (Rota Council, 2020b). Therefore, while the higher genetic variability in the VP7 may compromise immunity induced by vaccines, the VP4 component of vaccines may compensate when a human is infected with a P[8] strain. In agreement with the observation of low genetic variability in VP4, around 70% (26/37) of the aa residues belonging to the global human P[8] RVA strains, including Zambian strain UFS-NGS-MRC-DPRU4749, were fully conserved when compared to the Rotarix® vaccine strain.

Accumulation of point mutations, along with reassortment and other mechanisms of rotavirus evolution, is a key mechanism that generates genetic diversity in RVA over time (Donker and Kirkwood, 2012; Ghosh and Kobayashi, 2011; Hoxie and Dennehy, 2020; Kirkwood, 2010; Matthijnssens *et al*., 2010). Seven aa substitutions were identified in the VP8* (8-1 and 8-3) region when the study strain UFS-NGS-MRC-

DPRU4749 was compared against Rotarix®. Of the seven, four were seen to be radical in nature (N195G, N113D, S131R, and N135D). With respect to the nature of aa, the N195G substitution resulted in a change in polarity (polar to non-polar) whereas N113D, S131R, and N135D resulted in a change in charge (polar neutral to acidic polar negative, polar neutral to basic polar positive, and polar neutral to acidic polar negative, respectively) (Betts and Russell, 2007). One peculiar aa difference was the V115A which occurred only in the study strain. This mutation is considered conservative because the charge and polarity of the aa remained unchanged. It is therefore unlikely that this change would affect protein structure and hydrophobicity (Betts and Russell, 2007; Garnier *et al*., 1987). The impact of such a change on rotavirus transmission and vaccine effectiveness remains to be determined.

4.6. Conclusion

This study lends credence to reassortment being a major evolutionary mechanism in RVA. Because the other three Zambian strains were also collected during the post-vaccine period, the discovery of the phylogenetically and genetically divergent Zambian G2P[8] strain was unexpected. Given that this strain was identified in an unvaccinated child, it remains unclear whether the aa mutations present in the VP4 gene would have a negative impact on the effectiveness of the vaccine. Continuous surveillance of circulating RVA, along with whole genome sequencing and analysis is therefore critical in monitoring the impact of such reassortant strains on children, as well as their impact on effectiveness of current vaccine products.

Chapter five: Dissertation summary

5.1. Preamble

This chapter presents a summary and conclusions of the main findings of the study and elaborates on how the objectives were achieved.

5.2. General discussion and conclusions

This study was conducted as a pilot WHO/AFRO RVA surveillance project with samples from Zambia. The purpose of the work presented in this research was to investigate strains identified in Zambia at a whole genome level following the implementation of Rotarix® rotavirus vaccine.

Rotavirus detection has progressed over the years from conventional methods of characterisation such as electron microscopy and serological approaches, to immunoassays, and G- and P- genotyping through RT-PCR and Sanger sequencing (Amar *et al*., 2007; Bishop *et al*., 1974; Brandt *et al*., 1981; Gentsch *et al*., 1992; Gouvêa *et al*., 1990; Rubenstein and Miller, 1982). With the advent of NGS sequencing platforms, as well as the development of the genotype-based classification system, rotavirus characterisation studies at a whole genome level have become more common (Jere *et al*., 2018; Mokoena *et al*., 2020; Mwangi *et al*., 2020; Nyaga *et al*., 2018; Rasebotsa *et al*., 2021; Strydom et al., 2019). Based on data generated from NGS (Illumina® MiSeq platform), we demonstrated the importance of whole genome characterisation of rotavirus strains. Additionally, we addressed the terms of reference between the WHO and UFS-NGS (Appendix 1), which were interlinked to the study objectives starting with cDNA synthesis, DNA library preparation, whole genome sequencing, RVA phylogenetic analysis, and linking the data obtained to clinical and epidemiological information.

Rotaviruses are predisposed to genetic mutations and reassortment events due to their segmented genome and error prone RNA-dependent RNA polymerase (Ghosh and Kobayashi, 2011; Kirkwood, 2010). We identified five reassortant strains after successfully conducting whole genome sequencing. One strain, RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6], was the first porcine-like human rare reassortant strain to be identified in the African region as well as in Zambia. The remaining four strains (RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4749/2014/G2P[8], RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13327/2016/G2P[4], RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13232/2016/G1P[8], and RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13541/2016/G1P[8]) were intergenogroup reassortants, possessing both Wa-like and DS-1-like constellations (manuscript submitted to the journal Viruses with the manuscript number ‘viruses-1264641’ that is currently under review).

In chapter three, we presented a published article on a rare G5P[6] strain which addressed the two study objectives in an overlapping manner by illustrating the attributes of the strain at whole genome level. According to the clinical data from which the sample was obtained, the participant had gastroenteritis (intermittent vomiting and diarrhoea, accompanied by fever) for a period of four days, which led to the patient being admitted at the ACDH paediatric ward. The G5, P[6], and A8 genotypes are typically found in porcine RVA. Similarly, porcine RVA also exhibit the genotype 1 constellation (Kim *et al*., 2012; Martella *et al*., 2010; Monini *et al*., 2014; Silva *et al*., 2016). This human study strain was seen to have the G5-P[6]-I1-R1-C1-M1-A8-N1-T1-E1-H1 constellation. Interestingly, the Zambian G5P[6] strain clustered in a lineage made up entirely of porcine strains, according to phylogeny analysis of the VP7 encoding gene, and the same observation was seen in the VP6 encoding gene. The remaining genes clustered with porcine and porcine-like human strains. The pairwise similarity nt and aa identities of the rare G5P[6] strain with reference strains was consistent with the observations made in the phylogeny analysis. Further, the VP4 gene belonged to lineage V and contained several amino acid disparities compared to reference global P[6] strains. This finding was supported by multiple studies that elucidated the close relationship between porcine and human Wa-like P[6] strains (Bányai *et al*., 2004; Martella *et al*., 2006a; Nyaga *et al*., 2018). We also compared the genome of the G5P[6] to the genomes of reference porcine-like human strains by performing a reassortment analysis. The genome of the Zambian G5P[6] was seen to be highly similar to the genomes of the reference strains, suggesting that the strain was derived through reassortment.

The objectives were also addressed in an overlapping manner in chapter four, where we investigated four strains that were intergenogroup reassortants. We first linked the clinical information of the children from whom the strains in the samples were identified. All the children presented with symptoms typically associated with RVA-related disease. Diarrhoea with varying frequency ranging from 1-4 days was present in all the participants, while intermittent vomiting for 2-3 days was present in three of the four participants. Similarly, fever was observed in two participants. Moderate to severe dehydration due to loss of water and electrolytes from vomiting and diarrhoea was also recorded. Further, two of the four participants were vaccinated. The participants resided in different areas of Ndola and Lusaka, indicating a sporadic pattern rather than an outbreak. Two strains had the constellation, G1-P[8]-I1-R1-C1-M1-A1-N2-T1-E1-H1, whereas the other two had G2-P[8]-I2-R2-C2-M2-A2-N2-T2-E2-H2, and G2-P[4]-I2-R2-C2-M2-A2-N1-T2-E2-H2. G2P[8] strains usually circulate among G1P[8] and G2P[4] strains and are thought to arise via intergenogroup reassortment (Dóró *et al*., 2015). The Zambian G2P[8] strain (RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4749/2014/G2P[8]) that was identified in this study therefore likely arose through reassortment, and this is supported by the fact that G1P[8] and G2P[4] strains were most

prevalent in Zambia in 2014 (Simwaka *et al*., 2018). Interestingly, the G2P[8] strain identified in this study was genetically and phylogenetically divergent in the VP4 and VP1 genes. This divergence can be explained by the accumulation of point mutations and reassortment events that occur in tandem. As already established, the strain had a DS-1-like constellation with a Wa-like VP4 segment (P[8]) due to intergenogroup reassortment. Further, when compared to the vaccine strain and reference strains belonging to different lineages, aa substitutions that resulted in either a change in a polarity or a change in charge were found in the VP4 gene of this strain.

In conclusion, genome reassortment was the key mechanism that influenced genetic diversity of strains that circulated in Zambia post-vaccine implementation. Through whole genome sequencing and analysis, the genetic heterogeneity of atypical Zambian Wa-like and DS-1-like RVA as a result of animal-human and intergenogroup reassortment was demonstrated. Noteworthily, both the rare reassortant G5P[6] strain and the divergent G2P[8] reassortant strain were identified in 2014, a short time period after RVA vaccine introduction in November 2013. Because reassortment contributes directly to RVA diversification and adaptation, the findings of this study could aid in establishing the genetic diversity of RVA in Zambia at whole genome level, as well as developing new control and diagnostic strategies, taking into account the introduction of Rotarix® in the country.

5.3. Limitations and recommendations

The child from whom the rare reassortant G5P[6] strain was identified (in 2014) was unvaccinated. Given that Rotarix® was implemented countrywide in November 2013 (Mpabalwani *et al*., 2016, 2018), the emergence of this strain most likely coincided with the vaccine rollout. Given the short post-vaccine period, thus low vaccine coverage, it was difficult to ascertain whether vaccine implementation contributed to the emergence of this rare reassortant strain. Additionally, the study was limited to a sample size of five reassortant strains. However, rare and/or reassortant strains usually have a sporadic pattern globally. Unlike rare strains, only unusual strains such as the G2P[6] have been documented in Zambia (Simwaka *et al*., 2018), making this a novel report of a rare reassortant G5P[6] Zambian strain. Bányai *et al*. (2012) defined unusual strains such as G1P[4], G2P[6], and G8P[6] as those with a prevalence of 0.2-2.0%, while rare strains such as the G5P[6] have a prevalence of less than 0.2%.

Rotavirus can evolve in nature rapidly. Novel strains often emerge sporadically in human populations, with G- and P- combinations that are not incorporated in the current vaccine products. The G5P[6] strains, for example, have been able to cause human infections in various regions globally (Ahmed *et al*., 2007; Chieochansin *et al*., 2016; Komoto *et al*., 2013; Mladenova *et al*., 2012). Rotavirus vaccination is bound to

increase globally, and whether RVA evolution is attributable to RVA vaccines or is purely random remains enigmatic. Future studies could assess whether reassortment events and/or aa mutations present in the genes of RVA have an impact on current rotavirus vaccine candidates and whether they contribute to severe disease. Distinctive continuous surveillance of RVA strains and whole genome analysis is thus required.

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Appendices

Appendix 1: Terms of Reference between WHO and UFS-NGS.

WCCPRD6200921 2017/757922

Terms of Reference (ToRs) for TSA with the University of the Free State (UFS), South Africa to perform whole genome sequencing of rotavirus strains from the WHO/AFRO Rotavirus surveillance network

WHO/AFRO continues to support Member States to conduct surveillance for rotavirus diarrhea in under five children in the African region. The Rotavirus surveillance network countries monitor hospitalizations due to severe diarrhea in < 5 children and also the trends of the circulating rotavirus strains and report data to WHO and partners. Results show approximately 40% all diarrheal hospitalizations in< 5 children are due to rotavirus infections. Also, as part of surveillance network monitor and document rotavirus strains circulating in Africa countries pre and post rotavirus vaccine introduction. It is important to continue efforts to monitor rotavirus circulating strains every year in different African countries to ensure countries have high quality data to be able to monitor the possible impact of vaccines on circulating strains after introduction of these new rotavirus vaccines.

Thirty one (31) countries have been supported to introduce rotavirus vaccines into national immunization programs and 12 countries are using the established surveillance system to evaluate the impact and effectiveness of these vaccines including monitoring circulating strains for any possible change in epidemiology in countries that have currently introduced either of the two vaccines (Rotarix or RotaTeq). Rwanda introduced the pentavalent (RotaTeq) in 2012, Ethiopia and Zambia the monovalent (Rotarix) in 2013. Wide-ranging rotavirus strain diversity has been reported from data obtained from African countries as part of strain rotavirus surveillance. Some common circulating strains like the G1P[8]s (prototype Wa-like strain) and the G2P[4]s (prototype DS-1-like strain) have been detected in every rotavirus season since the initiation of the network.

The University of the Free State (UFS) has the required specialized molecular diagnostics capacity for whole genome sequencing of rotavirus strains and also high level management commitment and willingness to collaborate and contribute to implementation of agreed whole genome sequencing activities. In the past AFRO has signed TSAs with The Sefako Makgatho Health Sciences University, MEDUNSA Campus have fulfilled obligations and agreed TORs. UFS and MEDUNSA will collaborate extend the molecular characterization to include whole genome sequencing

The Terms of Reference (ToRs) will include:

a) Using cDNA synthesis and library preparation by NEBNext Ultra RNA Library Prep Kit for Illumina), conduct whole genome sequencing of rotaviruses strains on 600 specimens from 3 (Ethiopia, Rwanda and Zambia) [1] participating in the rotavirus surveillance and strain characterization

b) Conduct phylogenetic analysis of rotaviruses strains which determine the circulation of rotavirus vaccine strains.

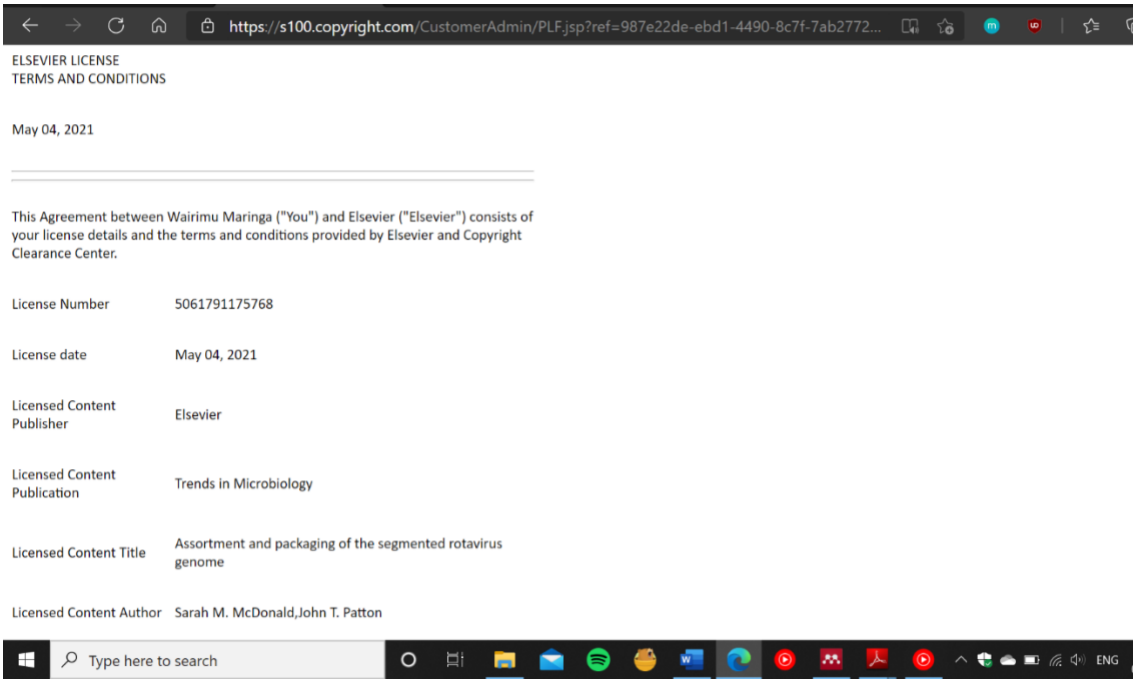
c) Link all the rotavirus whole sequence data to the cases and epidemiological/clinical information

d) Assess the data to determine whether there is evidence of vaccine pressure to guide further analysis

e) Based on the whole sequence data support development of a strategic plan for future characterization of rotavirus vaccine strains.

Ethiopia, Rwanda and Zambia

Appendix 2: Permission to use figure 2.1 (rotavirus architecture and morphology) and figure 2.4 (replication cycle).



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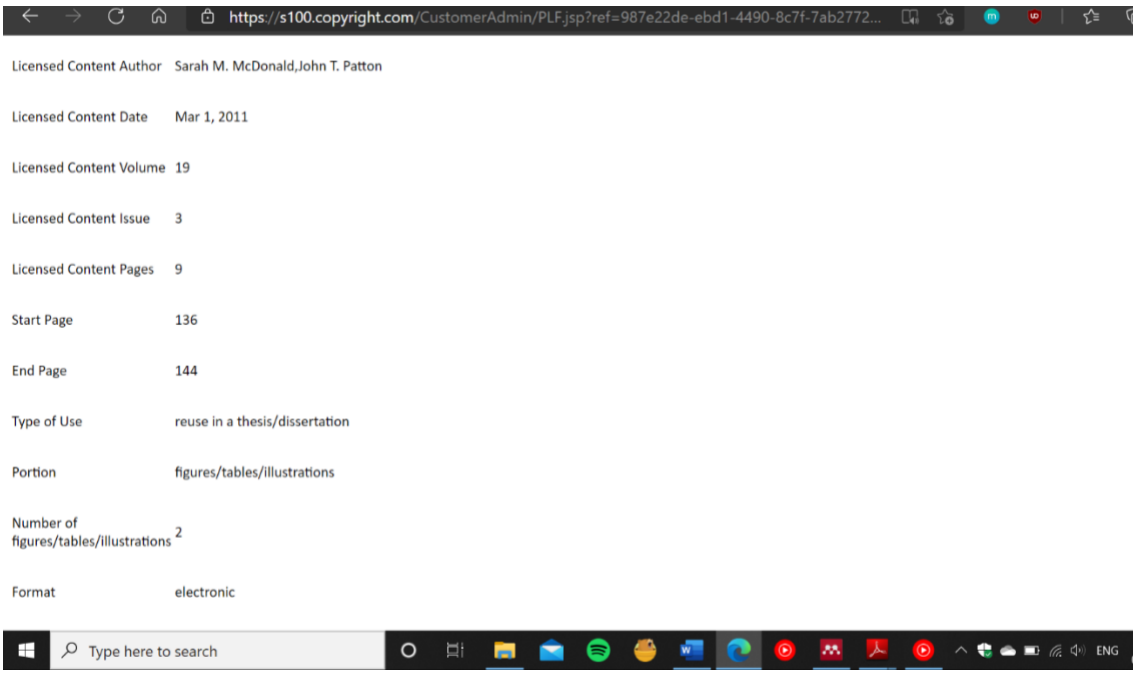

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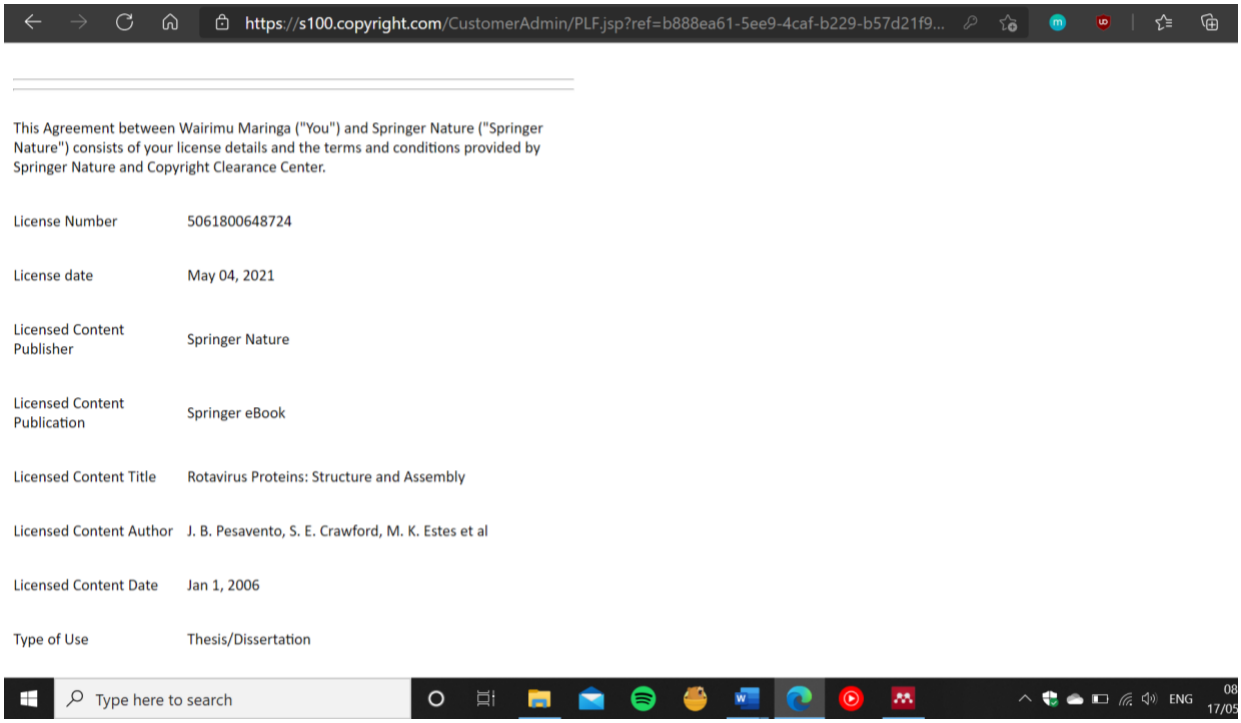
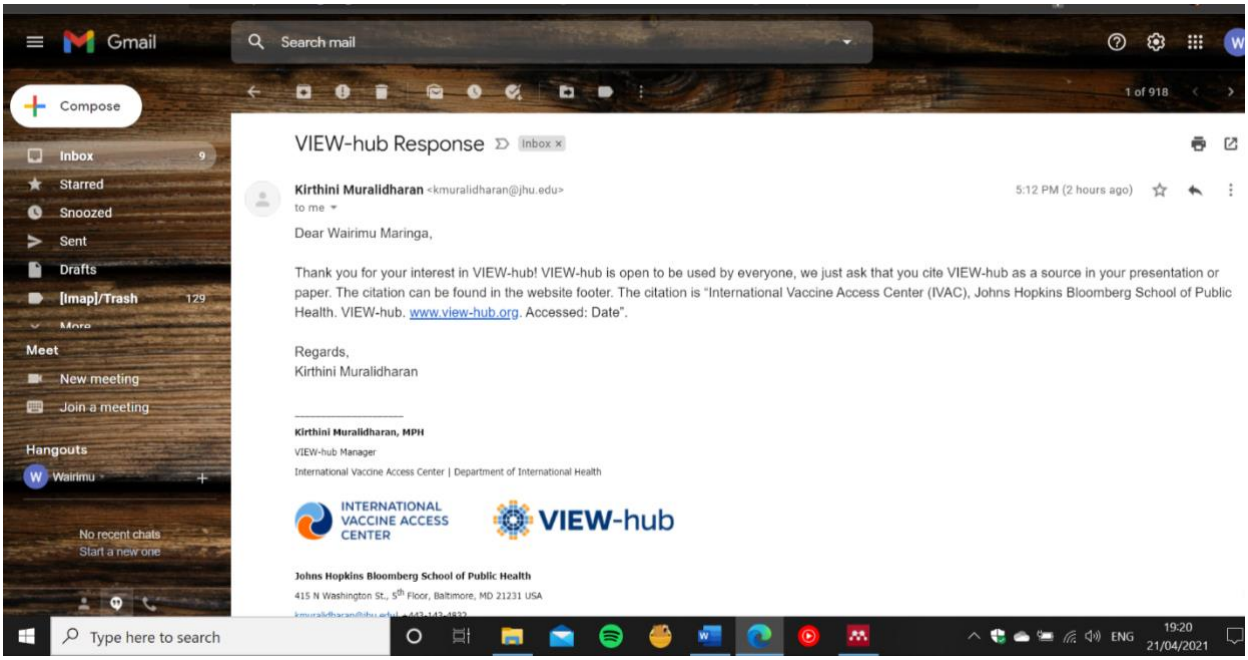
Appendix 3: Permission to use figure 2.3 (PAGE visualisation showing migration patterns of rotavirus segments).



Appendix 4: Permission to use figure 2.5 (map showing the global use of WHO-prequalified vaccines) and 2.6 (map showing vaccine introduction globally).



Appendix 5: Abstract page of the published Zambian G5P[6] article presented in chapter three.

pathogens

MDPI

Article

Molecular Characterisation of a Rare Reassortant Porcine-Like G5P[6] Rotavirus Strain Detected in an Unvaccinated Child in Kasama, Zambia

Wairimu M. Maringa [1], Peter N. Mwangi [1], Julia Simwaka [2], Evans M. Mpabalwani [3], Jason M. Mwenda [4], Ina Peenze [5], Mathew D. Esona [5], M. Jeffrey Mphahlele [5,6], Mapaseka L. Seheri [5] and Martin M. Nyaga [1,*]

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6 South African Medical Research Council, 1 Soutpansberg Road, Pretoria 0001, South Africa
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Received: 24 July 2020; Accepted: 14 August 2020; Published: 17 August 2020

check for updates

Abstract: A human-porcine reassortant strain, RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6], was identified in a sample collected in 2014 from an unvaccinated 12 month old male hospitalised for gastroenteritis in Zambia. We sequenced and characterised the complete genome of this strain which presented the constellation: G5-P[6]-I1-R1-C1-M1-A8-N1-T1-E1-H1. The genotype A8 is often observed in porcine strains. Phylogenetic analyses showed that VP6, VP7, NSP2, NSP4, and NSP5 genes were closely related to cognate gene sequences of porcine strains (e.g., RVA/Pig-wt/CHN/DZ-2/2013/G5P[X] for VP7) from the NCBI database, while VP1, VP3, VP4, and NSP3 were closely related to porcine-like human strains (e.g., RVA/Human-wt/CHN/E931/2008/G4P[6] for VP1, and VP3). On the other hand, the origin of the VP2 was not clear from our analyses, as it was not only close to both porcine (e.g., RVA/Pig-tc/CHN/SWU-1C/2018/G9P[13]) and porcine-like human strains (e.g., RVA/Human-wt/LKA/R1207/2009/G4P[6]) but also to three human strains (e.g., RVA/Human-wt/USA/1476/1974/G1P[8]). The VP7 gene was located in lineage II that comprised only porcine strains, which suggests the occurrence of independent porcine-to-human reassortment events. The study strain may have collectively been derived through interspecies transmission, or through reassortment event(s) involving strains of porcine and porcine-like human origin. The results of this study underline the importance of whole-genome characterisation of rotavirus strains and provide insights into interspecies transmissions from porcine to humans.

Keywords: whole-genome; genotype constellation; interspecies transmission; reassortment; porcine; porcine-like human

Appendix 6: Ethical approval from the HSREC to conduct this research.

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Health Sciences Research Ethics Committee

15-Apr-2020

Dear **Miss Wairimu Maringa**

Ethics Clearance: **Whole genome analysis of rare and/or novel rotavirus strains post-Rotarix introduction in Zambia**
Principal Investigator: **Miss Wairimu Maringa**
Department: **Medical Microbiology Department (Bloemfontein Campus)**

APPLICATION APPROVED

Please ensure that you read the whole document

With reference to your application for ethical clearance with the Faculty of Health Sciences, I am pleased to inform you on behalf of the Health Sciences Research Ethics Committee that you have been granted ethical clearance for your project.

Your ethical clearance number, to be used in all correspondence is:**UFS-HSD2020/0277/2104**

The ethical clearance number is valid for research conducted for one year from issuance. Should you require more time to complete this research, please apply for an extension.

We request that any changes that may take place during the course of your research project be submitted to the HSREC for approval to ensure we are kept up to date with your progress and any ethical implications that may arise. This includes any serious adverse events and/or termination of the study.

A progress report should be submitted within one year of approval, and annually for long term studies. A final report should be submitted at the completion of the study.

The HSREC functions in compliance with, but not limited to, the following documents and guidelines: The SA National Health Act. No. 61 of 2003; Ethics in Health Research: Principles, Structures and Processes (2015); SA GCP(2006); Declaration of Helsinki; The Belmont Report; The US Office of Human Research Protections 45 CFR 461 (for non-exempt research with human participants conducted or supported by the US Department of Health and Human Services- (HHS), 21 CFR 50, 21 CFR 56; CIOMS; ICH-GCP-E6 Sections 1-4; The International Conference on Harmonization and Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH Tripartite), Guidelines of the SA Medicines Control Council as well as Laws and Regulations with regard to the Control of Medicines, Constitution of the HSREC of the Faculty of Health Sciences.

For any questions or concerns, please feel free to contact HSREC Administration: 051-4017794/5 or email EthicsFHS@ufs.ac.za.

Thank you for submitting this proposal for ethical clearance and we wish you every success with your research.

Yours Sincerely

Dr. SM Le Grange
Chair : Health Sciences Research Ethics Committee

Health Sciences Research Ethics Committee
Office of the Dean: Health Sciences
T: +27 (0)51 401 7795/7794 | E: ethicsfhs@ufs.ac.za
IRB 00011992; REC 230408-011; IORG 0010096; FWA 00027947
Block D, Dean's Division, Room D104 | P.O. Box/Posbus 339 (Internal Post Box G40) | Bloemfontein 9300 | South Africa
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Appendix 7 a-k: Nucleotide and amino acid identities for the VP7, VP4, VP6, VP1-VP3, NSP1-NSP5 (G5P[6] article)

a.

VP7 nucleotide and amino acid identities among strains calculated using the *p* -distance algorithm in MEGA 6.06 (Tamura et al., 2013)								
Strain			**NT**		**AA**			**Location (Continent)**
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6]	Lineage II							
KT820775/RVA/Pig-wt/CHN/DZ-2/2013/G5P[X]	Lineage II		98.6		99.0			Asia
KT820777/RVA/Pig-wt/CHN/JN-2/2014/G5P[X]	Lineage II		98.5		99.0			Asia
JX498961/RVA/Pig-wt/CHN/ZJhz13-2/2011/G5P[X]	Lineage II		98.2		98.6			Asia
JX498960/RVA/Pig-wt/CHN/HLJqqhe-1/2011/G5P[X]	Lineage II		98.2		98.6			Asia
MH399892/RVA/Pig-wt/CHN/HJ/2016/G5P[7]	Lineage II		93.2		95.9			Asia
AB690405/RVA/Pig-wt/JPN/pig9-49d/2002/G5P[7]	Lineage II		91.8		94.9			Asia
AB690410/RVA/Pig-wt/JPN/pig5-88d/2003/G5P[27]	Lineage II		91.7		94.9			Asia
AB690403/RVA/Pig-wt/JPN/pig9-28d/2002/G5P[6]	Lineage II		91.1		94.9			Asia
AB690404/RVA/Pig-wt/JPN/pig9-42d/2002/G5P[13]	Lineage II		91.0		94.9			Asia
AB735636/RVA/Pig-wt/JPN/JP69-H4/2007/G5P[13]	Lineage II		89.6		92.4			Asia
AB735635/RVA/Pig-wt/JPN/JP69-F8/2007/G5P[6]	Lineage II		89.6		92.4			Asia
KT727252/RVA/Pig-wt/THA/CMP-001-12/2012/G5P[13]	Lineage I		86.7		93.5			Asia
KT007761/RVA/Human-wt/THA/CU-B1964/2014/G5P[6]	Lineage I		86.6		93.1			Asia
EF159575/RVA/Human-wt/CHN/LL3354/2000/G5P[6]	Lineage III		86.2		92.8			Asia
AB611693/RVA/Pig-wt/JPN/TJ4-5/2010/G5P[13]P[22]	Lineage III		86.0		93.5			Asia
JN699034/RVA/Human-wt/CHN/HK69/1978/G5P[X]	Lineage III		85.8		93.1			Asia
EF077484/RVA/Human-wt/CHN/LL36755/2003/G5P[6]	Lineage III		85.7		93.8			Asia
EF159576/RVA/Human-wt/CHN/LL4260/2001/G5P[6]	Lineage III		85.2		93.1			Asia
KY021145/RVA/Pig-wt/VNM/VN-26-08/2014/G5P[13]	Lineage III		85.1		92.8			Asia
KY021146/RVA/Pig-wt/VNM/VN-28-05/2014/G5P[13]	Lineage III		85.1		92.8			Asia
KY021143/RVA/Pig-wt/VNM/VN-22-15/2014/G5P[13]	Lineage III		85.0		92.8			Asia
AB741654/RVA/Human-wt/JPN/Ryukyu-1120/2011/G5P[6]	Lineage III		84.9		91.8			Asia
JX498962/RVA/Pig-xx/CHN/ZJhz9-2/2011/G5P[X]	Lineage III		84.6		93.5			Asia
AB924089/RVA/Pig-wt/JPN/BU2/2014/G5P[7]	Lineage III		84.3		90.4			Asia
AB257126/RVA/Human-wt/VNM/KH210/2004/G5P[6]	Lineage III		83.4		92.1			Asia
KP057832/RVA/Pig-wt/KEN/Ug-049/2012/G5P[13]	Lineage II		92.2		94.8			Africa
KP753011/RVA/Pig-wt/ZAF/MRC-DPRU1513/2009/G5P[6]	Lineage II		89.9		95.5			Africa
KP753195/RVA/Pig-wt/ZAF/MRC-DPRU1568/2008/G5P[X]	Lineage II		89.9		95.5			Africa
KP057833/RVA/Pig-wt/KEN/Ug-453/2012/G5P[13]	Lineage I		86.5		90.0			Africa
KJ752491/RVA/Pig-wt/ZAF/MRC-DPRU1567/2008/G5P[6]	Lineage III		86.5		94.5			Africa
EF218667/RVA/Human-wt/CMR/6784/2000/G5P[7]	Lineage III		86.3		94.3			Africa
KP752927/RVA/Pig-wt/ZAF/MRC-DPRU1522/2007/G5G9P[X]	Lineage III		85.4		93.5			Africa
KP753127/RVA/Pig-wt/ZAF/MRC-DPRU1487/2007/G3G5P[23]	Lineage III		85.2		92.4			Africa
KY053213/RVA/Pig-wt/KNA/ET8B/2015/G5P[13]	Lineage II		92.9		96.2			The Americas
KJ482529/RVA/Pig-wt/BRA/ROTA18/2013/G5P[7]	Lineage II		92.9		95.9			The Americas
KJ482531/RVA/Pig-wt/BRA/ROTA24/2013/G5P[6]	Lineage II		91.9		94.6			The Americas
KJ482516/RVA/Pig-wt/BRA/ROTA25/2013/G5P[13]	Lineage II		91.4		96.2			The Americas
KC254784/RVA/Pig-wt/BRA/PGRV16/2011/G5P[23]	Lineage II		91.2		93.7			The Americas
KX527774/RVA/Pig-wt/CAN/55/2011/G5P[7]	Lineage II		90.7		92.8			The Americas
KX527773/RVA/Pig-wt/CAN/54/2011/G5P[7]	Lineage II		90.6		93.1			The Americas
KX376970/RVA/Pig-wt/BRA/BR43/2012/G5P[13]	Lineage II		89.8		93.5			The Americas
KM077447/RVA/Human-xx/BRA/IAL-R3029/2013/G5P[6]	Lineage I		88.7		94.2			The Americas
EF672588/RVA/Human-tc/BRA/IAL28/1992/G5P[8]	Lineage I		87.6		91.8			The Americas
KT906389/RVA/Pig-wt/CHL/05/2013/G5P[7]	Lineage I		87.3		94.5			The Americas
KT906390/RVA/Pig-wt/CHL/08/2013/G5P[7]	Lineage I		87.0		93.9			The Americas
KJ482528/RVA/Pig-wt/BRA/ROTA17/2013/G5P[6]	Lineage I		86.9		93.3			The Americas
KC254781/RVA/Pig-wt/BRA/PGRV13/2011/G5P[1]	Lineage III		84.7		92.6			The Americas
KJ450849/RVA/Pig-tc/ESP/OSU-C5111/2010/G5P[7]	Lineage II		93.6		96.9			Europe
DQ062572/RVA/Pig-wt/ITA/134-04-15/2004/G5P[26]	Lineage II		92.0		96.2			Europe
KP836287/RVA/Pig-wt/BEL/14R160/2014/G5P[7]	Lineage II		91.3		94.8			Europe
KU887647/RVA/WildBoar-wt/CZE/P245/2014/G5P[13]	Lineage II		90.2		95.2			Europe
KF006868/RVA/Human-wt/RUS/Nov10-N459/2010/G5P[6]	Lineage I		88.7		94.8			Europe
KJ923332/RVA/Pig-wt/CIT-53/IRL/2007/G5P[13]	Lineage I		87.9		93.9			Europe

b.

VP4 nucleotide and amino acid identities among strains calculated using the *p* -distance algorithm in MEGA 6.06 (Tamura et al., 2013)							
Strain			**NT**		**AA**		**Location (Continent)**
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6]	Lineage V						
KX363402/RVA/Pig-wt/VNM/14226-39/2012/G4P[6]	Lineage I		87.4		95.2		Asia
KF041444/RVA/Human-wt/CHN/GX54/2010/G4P[6]	Lineage I		87.3		94.8		Asia
KF447842/RVA/Human-wt/CHN/GX77/2010/G4P[6]	Lineage I		87.3		94.8		Asia
KF447853/RVA/Human-wt/CHN/GX78/2010/G4P[6]	Lineage I		87.3		94.8		Asia
KF447864/RVA/Human-wt/CHN/GX82/2010/G4P[6]	Lineage I		87.3		94.8		Asia
KF726056/RVA/Human-wt/CHN/R946/2006/G3P6	Lineage I		87.2		95.3		Asia
KX362692/RVA/Human-wt/VNM/16020-7/2013/G4P[6]	Lineage I		87.1		94.9		Asia
LC389888/RVA/Human-wt/LKA/R1207/2009/G4P[6]	Lineage I		87.0		94.6		Asia
KF726034/RVA/Human-wt/CHN/E931/2008/G4P[6]	Lineage I		87.0		94.4		Asia
KF726067/RVA/Human-wt/CHN/R1954/2013/G4P[6]	Lineage I		87.0		94.8		Asia
GU189554/RVA/Human-wt/CHN/R479/2004/G4P[6]	Lineage I		86.9		94.5		Asia
KC139780/RVA/Human-wt/CHN/LL3354/2000/G5P[6]	Lineage I		86.8		94.4		Asia
AB573880/RVA/Pig-wt/JPN/FGP65/2009/G4P[6]	Lineage I		86.8		93.3		Asia
LC061623/RVA/Human-wt/PHL/TGE13-85/2013/G4P[6]	Lineage I		86.5		94.6		Asia
KY748310/RVA/Human-wt/THA/CMH-N016-10/2010/G4P[6]	Lineage I		86.5		94.8		Asia
LC061622/RVA/Human-wt/PHL/TGE13-39/2013/G4P[6]	Lineage I		86.5		94.2		Asia
KY748311/RVA/Human-wt/THA/CMH-N014-11/2011/G4P[6]	Lineage I		86.4		94.1		Asia
KX646642/RVA/Human-wt/IND/RV0915/2009/G1P[6]	Lineage I		86.3		93.6		Asia
EF179118/RVA/Human-wt/VNM/VN904/2003/G9P[6]	Lineage I		86.3		90.6		Asia
LC374182/RVA/Human-wt/NPL/10N4001/2010/G12P[6]	Lineage I		86.2		93.9		Asia
AB741652/RVA/Human-wt/JPN/Ryukyu-1120/2011/G5P[6]	Lineage I		86.1		93.2		Asia
LC260230/RVA/Human-wt/IND/SOEP156/2016/G3P[6]	Lineage I		86.1		92.9		Asia
KY497521/RVA/Human-wt/PAK/3094/2010/G12P[6]	Lineage I		85.9		92.8		Asia
KT936629/RVA/Human-wt/THA/CMHN49-12/2012/G12P[6]	Lineage I		85.9		92.7		Asia
KY497478/RVA/Human-wt/PAK/94/2010/G1P[6]	Lineage I		85.9		92.8		Asia
AB176688/RVA/Pig-wt/JPN/JP29-6/2000/G9P[6]	Lineage III		83.5		91.4		Asia
AB176685/RVA/Pig-wt/JPN/JP3-6/2000/G9P[6]	Lineage III		83.4		91.0		Asia
KJ870903/RVA/Human-wt/COD/KisB332/2008/G4P[6]	Lineage V		98.1		98.3		Africa
KJ752488/RVA/Pig-wt/ZAF/MRC-DPRU1567/2008/G5P[6]	Lineage I		86.8		93.1		Africa
KJ752298/RVA/Human-wt/ZMB/MRC-DPRU3495/2009/G9P[6]	Lineage I		86.6		93.9		Africa
KJ870925/RVA/Human-wt/COD/KisB504/2009/G1P[6]	Lineage I		86.3		93.5		Africa
KP883023/RVA/Human-wt/MLI/Mali-048/2008/G8P[6]	Lineage I		86.1		93.2		Africa
KJ752544/RVA/Human-wt/ZAF/MRC-DPRU2107/2003/G1P[6]	Lineage I		86.1		93.6		Africa
KJ752621/RVA/Human-wt/SEN/MRC-DPRU2053/2009/G8P[6]	Lineage I		86.1		93.3		Africa
KP941127/RVA/Human-wt/KEN/Keny-061/2008/G9P[6]	Lineage I		86.1		93.1		Africa
KJ752397/RVA/Human-wt/GMB/MRC-DPRU3180/2010/G2P[6]	Lineage I		86.1		93.6		Africa
KJ752120/RVA/Human-wt/GNB/MRC-DPRU5625/2011/G6P[6]	Lineage I		86.0		93.5		Africa
KM660340/RVA/Human-wt/CMR/MA228/2011/G6P[6]	Lineage I		86.0		93.3		Africa
KJ7520400/RVA/Human-wt/SEN/MRC-DPRU2136/2009/G1P[6]	Lineage I		85.9		92.7		Africa
KP882715/RVA/Human-wt/KEN/Keny-078/2008/G8P[6]	Lineage I		85.9		93.0		Africa
DQ005122/RVA/Human-wt/COD/DRC86/2003/G8P[6]	Lineage I		85.9		92.7		Africa
LC406789/RVA/Human-wt/KEN/KDH1951/2014/G3P[6]	Lineage I		85.9		92.8		Africa
KX655454/RVA/Human-wt/UGA/MUL-13-204/2013/G8P[6]	Lineage I		85.9		92.5		Africa
KJ412567/RVA/Human-wt/PRY/1809SR/2009/G4P[6]	Lineage I		87.5		95.3		The Americas
KC412049/RVA/Human-wt/ARG/Arg4671/2006/G4P[6]	Lineage I		87.2		94.6		The Americas
DQ525193/RVA/Human-wt/BRA/COD064/1991/G4P[6]	Lineage I		86.3		93.0		The Americas
M33516/RVA/Pig-tc/USA/Gottfried/1983/G4P[6]	Lineage II		83.5		91.9		The Americas
AY955307/RVA/Pig-wt/ITA/221-04-19/2004/GXP[6]	Lineage V		92.7		94.0		Europe
KF835914/RVA/Human-wt/HUN/BP1125/2004/G4P[6]	Lineage V		91.9		95.8		Europe
KF835916/RVA/Human-wt/HUN/BP1231/2002/G4P[6]	Lineage V		91.9		96.5		Europe
KF835917/RVA/Human-wt/HUN/BP1490/1994/G4P[6]	Lineage V		91.5		96.1		Europe
JQ993319/RVA/Human-wt/BEL/BE2001/2009/G9P[6]	Lineage V		91.4		95.9		Europe
KF835920/RVA/Human-wt/HUN/BP1901/1991/G4P[6]	Lineage V		91.4		96.1		Europe
KF835918/RVA/Human-wt/HUN/BP1547/2005/G4P[6]	Lineage V		91.3		95.9		Europe
KM820719/RVA/Pig-wt/BEL/12R006/2012/G3P[6]	Lineage V		91.0		95.5		Europe
KF835915/RVA/Human-wt/HUN/BP1227/2002/G4P[6]	Lineage V		90.9		93.7		Europe
L33895/RVA/Human-tc/GBR/ST3/1975/G4P[6]	Lineage I		86.5		92.3		Europe
FJ747628/RVA/Human-wt/DEU/GER172-08/2008/G12P[6]	Lineage I		86.3		94.0		Europe
KF835913/RVA/Human-wt/HUN/BP271/2000/G4P[6]	Lineage IV		86.0		91.9		Europe
AJ621507/RVA/Human-wt/HUN/BP1338-99/1999/G4P[6]	Lineage IV		85.6		92.2		Europe

c.

VP6 nucleotide and amino acid identities among strains calculated using the *p* -distance algorithm in MEGA 6.06 (Tamura et al., 2013)			
Strain	**NT**	**AA**	**Location (Continent)**
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6]			
KF726035/RVA/Human-wt/CHN/E931/2008/G4P[6]	90.2	98.7	Asia
KF726068/RVA/Human-wt/CHN/R1954/2013/G4P[6]	90.2	98.7	Asia
KX363371/RVA/Pig-wt/VNM/14225-44/2012/GXP[X]	90.2	98.0	Asia
KX362693/RVA/Human-wt/VNM/16020-7/2013/GXP[X]	89.9	98.2	Asia
MN066883/RVA/Human-wt/IND/CMC-00052/2010/GXP[X]	89.9	98.2	Asia
MG066585/RVA/Pig-wt/CHN/SCLS-2-3/2017/G9P[23]	89.8	98.7	Asia
KF041434/RVA/Human-wt/CHN/GX54/2010/G4P[6]	89.8	98.5	Asia
KF447843/RVA/Human-wt/CHN/GX77/2010/G4P[6]	89.8	98.5	Asia
KF447854/RVA/Human-wt/CHN/GX78/2010/G4P[6]	89.8	98.5	Asia
LC019078/RVA/Human-tc/MMR/P02/2011/G12P[8]	89.5	98.7	Asia
KF447865/RVA/Human-wt/CHN/GX82/2010/G4P[6]	89.5	98.2	Asia
DQ870500/RVA/Human-tc/JPN/YO/1977/G3P[8]	89.4	98.7	Asia
LC019045/RVA/Human-tc/MMR/A14/2011/G12P[8]	89.3	97.7	Asia
LC019056/RVA/Human-tc/MMR/A23/2011/G12P[6]	89.0	98.7	Asia
FJ361206/RVA/Human-tc/IND/116E/1988/G9P[11]	88.8	97.5	Asia
GU199521/RVA/Human-wt/BGD/Dhaka6/2001/G11P[25]	88.7	98.7	Asia
KX988268/RVA/Pig-wt/UGA/KYE-14-A047/2014/G3P[13]	98.9	99.7	Africa
KX988279/RVA/Pig-wt/UGA/KYE-14-A048/2014/G3P[13]	98.8	99.7	Africa
KJ870904/RVA/Human-wt/COD/KisB332/2008/G4P[6]	98.7	99.7	Africa
KY077644/RVA/Pig-wt/UGA/BUW-14-A003/2014/G3P[13]	98.6	98.9	Africa
KJ870926/RVA/Human-wt/COD/KisB504/2009/G1P[6]	89.3	98.7	Africa
KJ753086/RVA/Human-wt/ZAF/MRC-DPRU135/2009/G1P[8]	89.2	98.7	Africa
KJ870915/RVA/Human-wt/COD/KisB521/2008/G12P[6]	89.2	98.7	Africa
AB861949/RVA/Human-tc/KEN/KDH633/2010/G12P[6]	88.9	98.5	Africa
AB861960/RVA/Human-tc/KEN/KDH651/2010/G12P[8]	88.9	98.7	Africa
KJ751761/RVA/Human-wt/UGA/MRC-DPRU1944/2008/G9P[8]	88.9	98.7	Africa
KP753261/RVA/Human-wt/KEN/MRC-DPRU1608/2009/G1P[8]	88.9	99.0	Africa
KJ753296/RVA/Human-wt/ZWE/MRC-DPRU1844-11/2011/G1P[8]	88.9	98.7	Africa
AB861971/RVA/Human-tc/KEN/KDH684/2010/G12P[6]	88.8	98.2	Africa
KP752757/RVA/Human-wt/TGO/MRC-DPRU4562/2011/G1P[8]	88.8	98.7	Africa
KJ753428/RVA/Human-wt/UGA/MRC-DPRU4595/2011/G9P[8]	88.8	98.7	Africa
KF636282/RVA/Human-wt/ZAF/MRC-DPRU2052/2010/G1P[8]	88.7	98.5	Africa
KX632302/RVA/Human-wt/UGA/MUL-12-147/2012/G9P[8]	88.6	98.5	Africa
KR052750/RVA/Pig-tc/USA/LS00007-Gottfried/1975/G4P[6]	91.4	99.2	The Americas
JN129103/RVA/Human-wt/NCA/25J/2010/G1P[8]	90.5	98.2	The Americas
D00326/RVA/Pig-tc/USA/Gottfried/1983/G4P[6]	90.3	98.7	The Americas
EF583032/RVA/Human-tc/BRA/IAL28/1992/G5P[8]	90.2	98.0	The Americas
KT695009/RVA/Human-tc/USA/DC4455-40-HT/1988/G1P[8]	90.2	98.0	The Americas
KT694998/RVA/Human-wt/USA/DC4455/1988/G1P[8]	90.1	98.0	The Americas
HM773914/RVA/Human-xx/USA/DC4613/1980/G4[P8]	90.0	98.5	The Americas
KU861383/RVA/Human-tc/USA/Wa-20-HT/1974/G1P[8]	90.0	97.5	The Americas
FJ947169/RVA/Human-xx/USA/DC1285/1980/G4P[8]	89.9	98.5	The Americas
KT695031/RVA/Human-tc/USA/DC4455-40-AG/1988/G1P[8]	89.9	97.7	The Americas
EF583052/RVA/Human-tc/USA/WI61/1983/G9P[8]	89.6	98.0	The Americas
EF583048/RVA/Human-tc/GBR/ST3/1975/G4P[6]	89.8	98.5	Europe
DQ146642/RVA/Human-wt/BEL/B4633/2003/G12P[8]	89.2	98.5	Europe
MH473477/RVA/Human-wt/RUS/Nov12-N3583/2012/G1P[8]	89.1	98.5	Europe
JX195067/RVA/Human-wt/ITA/AV21/2010/G9P[8]	89.0	98.5	Europe
U36240/RVA/Human-wt/AUS/E210/1994/G2P[4]	89.8	98.0	Oceania

d.

VP1 nucleotide and amino acid identities among strains calculated using the *p* -distance algorithm in MEGA 6.06 (Tamura et al., 2013)			
Strain	**NT**	**AA**	**Location (Continent)**
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6]			
KF447861/RVA/Human-wt/CHN/GX82/2010/G4P[6]	96.8	98.9	Asia
KF447850/RVA/Human-wt/CHN/GX78/2010/G4P[6]	96.8	98.9	Asia
KF447839/RVA/Human-wt/CHN/GX77/2010/G4P[6]	96.8	98.9	Asia
KF041441/RVA/Human-wt/CHN/GX54/2010/G4P[6]	96.8	98.9	Asia
KF726036/RVA/Human-wt/CHN/E931/2008/G4P[6]	96.2	98.7	Asia
MK410286/RVA/Pig-tc/CHN/SWU-1C/2018/G9P[13]	96.0	98.3	Asia
KF726058/RVA/Human-wt/CHN/R946/2006/G3P[6]	95.2	98.3	Asia
LC095880/RVA/Human-tc/VNM/NT0001/2007/G3P[6]	95.1	98.6	Asia
MH624173/RVA/Pig-wt/CHN/SC11/2017/G9P[23]	94.7	98.3	Asia
KF726069/RVA/Human-wt/CHN/R1954/2013/G4P[6]	94.6	97.9	Asia
MH898987/RVA/Pig-tc/CHN/SCJY-5/2017/G9P[23]	94.5	97.9	Asia
MH137269/RVA/Pig-wt/CHN/SCLSHL-2-3/2017/G9P[23]	94.3	98.2	Asia
LC095902/RVA/Human-wt/VNM/NT0073/2007/G9P[19]	94.2	98.3	Asia
MH697624/RVA/Pig-tc/CHN/TM-a-P20/2018/G9P[23]	94.1	98.4	Asia
LC019074/RVA/Human-tc/MMR/P02/2011/G12P[8]	88.8	97.1	Asia
KF371992/RVA/Human-wt/CHN/Y106/2004/G3P[8]	88.7	97.2	Asia
LC019041/RVA/Human-tc/MMR/A14/2011/G12P[8]	88.7	97.1	Asia
DQ146649/RVA/Human-wt/BGD/Dhaka25/2002/G12P[8]	88.7	97.0	Asia
MF580875/RVA/Human-wt/CHN/JS/2010/GXP[X]	88.5	97.2	Asia
FJ361201/RVA/Human-tc/IND/116E/1988/G9P[11]	88.4	97.1	Asia
EF560705/RVA/Human-wt/BGD/Dhaka6/2001/G11P[25]	88.4	97.1	Asia
HQ609553/RVA/Human-wt/IND/613158/2006/G1P[8]	88.4	97.0	Asia
LC374184/RVA/Human-wt/NPL/10N4001/2010/G12P[6]	88.2	97.2	Asia
AB741649/RVA/Human-wt/JPN/Ryukyu-1120|2011/G5P[6]	85.7	96.9	Asia
KJ752004/RVA/Human-wt/ETH/MRC-DPRU5002/2010/G12P[8]	88.8	97.1	Africa
KP752790/RVA/Human-wt/ETH/MRC-DPRU4970/2010/G12P[8]	88.8	97.1	Africa
KJ753423/RVA/Human-wt/UGA/MRC-DPRU4595/2011/G9P[8]	88.7	97.2	Africa
KP752753/RVA/Human-wt/TGO/MRC-DPRU4562/2011/G1P[8]	88.6	97.2	Africa
MG181480/RVA/Human-wt/MWI/0P5-001/2008/G1P[8]	88.6	97.1	Africa
KX632298/RVA/Human-wt/UGA/MUL-12-147/2012/G9P[8]	88.6	97.2	Africa
KJ870922/RVA/Human-wt/COD/KisB504/2009/G1P[6]	88.5	97.1	Africa
KP753257/RVA/Human-wt/KEN/MRC-DPRU1608/2009/G1P[8]	88.5	97.2	Africa
KP882728/RVA/Human-wt/KEN/Keny-110/2009/G1P[8]	88.5	97.3	Africa
KJ751757/RVA/Human-wt/UGA/MRC-DPRU1944/2008/G9P[8]	88.5	97.3	Africa
KJ870911/RVA/Human-wt/COD/KisB521/2008/G12P[6]	88.5	96.8	Africa
KF636146/RVA/Human-wt/ZMB/MRC-DPRU3491/2009/G12P[6]	88.4	96.7	Africa
AB861945/RVA/Human-tc/KEN/KDH633/2010/G12P[6]	88.4	96.3	Africa
KF636278/RVA/Human-wt/ZAF/MRC-DPRU2052/2010/G1P[8]	88.2	97.0	Africa
JQ069904/RVA/Human-wt/CAN/RT092-07/2007/G1P[8]	88.7	97.2	The Americas
EF583029/RVA/Human-tc/BRA/IAL28/1992/G5P[8]	88.6	96.8	The Americas
KT919569/RVA/Human-wt/USA/VU12-13-21/2013/G12P[8]	88.6	97.0	The Americas
JQ069922/RVA/Human-wt/CAN/RT133-07/2008/G1P[8]	88.5	97.2	The Americas
HM773744/RVA/Human-wt/USA/2007719825/2007/G1P[8]	88.4	96.8	The Americas
JN129047/RVA/Human/NCA/25J/2010/G1P[8]	88.4	96.9	The Americas
JQ069930/RVA/Human-wt/CAN/RT186-07/2008/G1P[8]	88.4	97.1	The Americas
JQ069950/RVA/Human-wt/CAN/RT070-09/2009/G1P[8]	88.3	96.5	The Americas
GU199514/RVA/Pig-tc/USA/OSU/1975/G5P[7]	86.0	97.5	The Americas
M32805/RVA/Pig-tc/USA/Gottfried/1983/G4P[6]	85.2	96.3	The Americas
DQ870501/RVA/Human-wt/BEL/B3458/2003/G9P[8]	88.7	97.2	Europe
HQ392349/RVA/Human-wt/BEL/BE00039/2008/G1P[8]	88.6	97.2	Europe
DQ146638/RVA/Human-wt/BEL/B4633/2003/G12P[8]	88.5	97.1	Europe
JX195085/RVA/Human-wt/ITA/JES11/2010/G9P[8]	88.5	97.0	Europe
KJ919385/RVA/Human-wt/HUN/ERN5611/2012/G1P[8]	88.0	97.1	Europe
JQ309138/RVA/Horse-tc/GBR/H-1/1975/G5P[7]	86.9	97.1	Europe
JX027813/RVA/Human-wt/AUS/CK00083/2008/G1P[8]	88.6	97.2	Oceania

e.

VP2 nucleotide and amino acid identities among strains calculated using the *p* -distance algorithm in MEGA 6.06 (Tamura et al., 2013)			
Strain	**NT**	**AA**	**Location (Continent)**
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6]			
LC389886/RVA/Human-wt/LKA/R1207/2009/G4P[6]	96.6	90.9	Asia
MK410287/RVA/Pig-tc/CHN/SWU-1C/2018/G9P[13]	93.4	83.5	Asia
KF726037/RVA/Human-wt/CHN/E931/2008/G4P[6]	92.5	79.4	Asia
MN066812/RVA/Human-wt/IND/CMC-00038/2011/G4P[X]	92.5	81.1	Asia
MF940424/RVA/Pig-tc/KOR/K71/2006/G5P[7]	92.3	79.3	Asia
HG513046/RVA/Human-wt/VNM/30378/2009/G26P[19]	91.4	76.8	Asia
GU199519/RVA/Human-wt/BGD/Dhaka6/2001/G11P[25]	89.1	79.0	Asia
LC019053/RVA/Human-tc/MMR/A23/2011/G12P[6]	88.3	69.0	Asia
LC086748/RVA/Human-wt/THA/PCB-118/2013/G1P[8]	88.3	68.9	Asia
GU189552/RVA/Human-tc/CHN/R479/G4P[6]	88.2	68.6	Asia
DQ492670/RVA/Human-wt/BGD/Dhaka16/2003/G1P[8]	88.2	69.2	Asia
HQ609555/RVA/Human-wt/IND/6361/2006/G1P[8]	88.2	68.7	Asia
KY857561/RVA/Human-wt/IND/RV1305/2013/G1P[8]	88.1	68.9	Asia
HQ609557/RVA/Human-wt/IND/61060/2006/G1P[8]	88.1	69.0	Asia
LC374130/RVA/Human-wt/NPL/09N3140/2009/G12P[6]	88.1	68.7	Asia
LC019042/RVA/Human-tc/MMR/A14/2011/G12P[8]	88.0	69.0	Asia
LC019064/RVA/Human-tc/MMR/A25/2011/G12P[8]	88.0	69.0	Asia
KF041442/RVA/Human-wt/CHN/GX54/2010/G4P[6]	88.0	67.6	Asia
KF447840/RVA/Human-wt/CHN/GX77/2010/G4P[6]	88.0	67.6	Asia
KF447851/RVA/Human-wt/CHN/GX78/2010/G4P[6]	88.0	67.6	Asia
KF447862/RVA/Human-wt/CHN/GX82/2010/G4P[6]	88.0	67.6	Asia
DQ146661/RVA/Human-wt/BGD/Dhaka12/2003/G12P[6]	88.0	68.5	Asia
LC019086/RVA/Human-tc/MMR/P39/2011/G12P[8]	87.9	68.7	Asia
HQ641294/RVA/Giantpanda-wt/CHN/CH-1/2008/G1P[7]	87.7	68.3	Asia
KF636279/RVA/Human-wt/ZAF/2052/2010/G1P[8]	88.3	69.6	Africa
KP753202/RVA/Human-wt/ZMB/MRC-DPRU3506/2009/G12P[6]	88.3	69.0	Africa
KJ751758/RVA/Human-wt/UGA/MRC-DPRU1944/2008/G9P[8]	88.2	68.7	Africa
KP752754/RVA/Human-wt/TGO/MRC-DPRU4562/2011/G1P[8]	88.2	68.6	Africa
KJ752815/RVA/Human-wt/ZAF/MRC-DPRU4090/2011/G12P[6]	88.1	68.6	Africa
AB861957/RVA/Human-tc/KEN/KDH651/2010/G12P[8]	88.1	68.6	Africa
AB861968/RVA/Human-tc/KEN/KDH684/2010/G12P[6]	88.1	68.3	Africa
AB861946/RVA/Human-tc/KEN/KDH633/2010/G12P[6]	88.0	68.3	Africa
KC579499/RVA/Human-wt/USA/DC582/1979/G1P[8]	92.4	79.3	The Americas
KC579565/RVA/Human-wt/USA/DC1476/1974/G1P[8]	92.4	79.1	The Americas
KC580283/RVA/Human-wt/USA/DC1127/1977/G1P[8]	92.4	78.8	The Americas
GU199515/RVA/Pig-tc/USA/OSU/1975/G5P[7]	92.4	79.3	The Americas
GU199487/RVA/Pig-tc/USA/Gottfried/1975/G4P[6]	91.5	77.5	The Americas
KU861380/RVA/Human-tc/USA/Wa-20-HT/1974/G1P[8]	89.4	72.2	The Americas
FJ947319/RVA/Human-xx/USA/DC1730/1979/G3P[8]	88.6	69.3	The Americas
EF583030/RVA/Human-tc/BRA/IAL28/1992/G5P[8]	88.6	69.3	The Americas
JQ069838/RVA/Human-wt/CAN/RT133-07/2008/G1P[8]	88.3	69.2	The Americas
JQ069866/RVA/Human-wt/CAN/RT070-09/2009/G1P[8]	88.3	69.7	The Americas
HM773745/RVA/Human-wt/USA/2007719825/2007/G1P[8]	88.1	68.7	The Americas
EF583050/RVA/Human-tc/USA/WI61/1983/G9P[8]	88.1	68.7	The Americas
JQ309139/RVA/Horse-tc/GBR/H-1/1975/G5P[7]	92.2	78.4	Europe
EF583046/RVA/Human-tc/GBR/ST3/1975/G4P[6]	88.5	70.4	Europe
JN651885/RVA/Human-wt/BEL/BE00108/2010/G1P[8]	88.3	69.3	Europe
HQ392364/RVA/Human-wt/BEL/BE00040/2008/G1P[8]	88.2	69.2	Europe
HQ392265/RVA/Human-wt/BEL/BE00031/2008/G1P[8]	88.1	68.6	Europe
DQ870502/RVA/Human-wt/BEL/B3458/2003/G9P[8]	87.9	68.3	Europe
JF490445/RVA/Human-wt/AUS/CK00037/2006/G1P[8]	88.4	69.4	Oceania
JF490213/RVA/Human-wt/AUS/CK00014/2004/G1P[8]	88.3	68.9	Oceania
JX027823/RVA/Human-wt/AUS/CK00083/2008/G1P[8]	88.1	68.7	Oceania

f.

VP3 nucleotide and amino acid identities among strains calculated using the *p* -distance algorithm in MEGA 6.06 (Tamura et al., 2013)						
Strain		**NT**		**AA**		**Location (Continent)**
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6]						
KF726060/RVA/Human-wt/CHN/R946/2006/G3P[6]		95.8		97.8		Asia
KF726038/RVA/Human-wt/CHN/E931/2008/G4P[6]		95.7		98.0		Asia
MK597962/RVA/Pig-tc/CHN/SCLS-X1/2018/G3P[13]		88.1		94.7		Asia
MK597973/RVA/Pig-tc/CHN/SCLS-3/2018/G3P[13]		88.1		94.9		Asia
MK597984/RVA/Human-tc/CHN/SCLS-R3/2018/G3P[13]		87.9		94.6		Asia
AB741651/RVA/Human-wt/JPN/Ryukyu-1120|2011/G5P[6]		84.9		93.9		Asia
KF726071/RVA/Human-wt/CHN/R1954/2013/G4P[6]		84.9		94.4		Asia
KX363380/RVA/Pig-wt/VNM/14225-45/2012/GXP[X]		84.8		93.7		Asia
GU189553/RVA/Human-tc/CHN/R479/2004/G4P[6]		84.8		94.4		Asia
GU199494/RVA/Human-wt/NPL/KTM368/2004/G11P[25]		84.7		92.8		Asia
LC433798/RVA/Human-wt/NPL/TK2615/2008/G11P[25]		84.7		92.8		Asia
LC433809/RVA/Human-wt/NPL/TK2620/2008/G11P[25]		84.7		92.8		Asia
EF560706/RVA/Human-wt/BGD/Dhaka6/2001/G11P[25]		84.6		92.5		Asia
KC140592/RVA/Human-wt/KOR/CAU12-2/2012/G11P[25]		84.6		92.8		Asia
HQ641295/RVA/Giantpanda-wt/CHN/CH-1/2008/G1P[7]		84.6		93.8		Asia
KF041443/RVA/Human-wt/CHN/GX54/2010/G4P[6]		84.5		93.9		Asia
KF447841/RVA/Human-wt/CHN/GX77/2010/G4P[6]		84.5		93.9		Asia
KF447852/RVA/Human-wt/CHN/GX78/2010/G4P[6]		84.5		93.9		Asia
KF447863/RVA/Human-wt/CHN/GX82/2010/G4P[6]		84.5		93.9		Asia
HG513047/RVA/Human-wt/VNM/30378/2009/G26P[19]		84.3		93.8		Asia
LC019065/RVA/Human-tc/MMR/A25/2011/G12P[8]		84.3		92.6		Asia
LC019043/RVA/Human-tc/MMR/A14/2011/G12P[8]		84.3		92.6		Asia
KY497520/RVA/Human-wt/PAK/3094/2010/G12P[6]		84.3		92.8		Asia
LC086760/RVA/Human-wt/THA/SKT-98/2013/G1P[8]		84.3		92.5		Asia
MF580867/RVA/Human-wt/CHN/JS/2016/G9P[8]		84.3		92.6		Asia
AB779631/RVA/Pig-wt/THA/CMP40-08/2008/G3P[23]		84.2		92.7		Asia
FJ361203/RVA/Human-tc/IND/116E/1988/G9P[11]		84.2		91.6		Asia
MF580866/RVA/Human-wt/CHN/JS/2015/G9P[8]		84.1		92.7		Asia
LC019054/RVA/Human-tc/MMR/A23/2011/G12P[6]		84.1		92.3		Asia
LC374131/RVA/Human-wt/NPL/09N3140/2009/G12P[6]		84.1		92.5		Asia
KP752792/RVA/Human-wt/ETH/MRC-DPRU4970/2010/G12P[8]		84.5		92.6		Africa
MG181460/RVA/Human-wt/MWI/MW2-1254/2005/G1P[8]		84.4		92.6		Africa
KP752755/RVA/Human-wt/TGO/MRC-DPRU4562/2011/G1P[8]		84.4		92.3		Africa
KJ751759/RVA/Human-wt/UGA/MRC-DPRU1944/2008/G9P[8]		84.4		92.1		Africa
AB861947/RVA/Human-tc/KEN/KDH633/2010/G12P[6]		84.3		92.5		Africa
AB861969/RVA/Human-tc/KEN/KDH684/2010/G12P[6]		84.3		92.5		Africa
KJ753021/RVA/Human-wt/ZAF/MRC-DPRU138/2009/G12P[8]		84.2		92.7		Africa
KJ870924/RVA/Human-wt/COD/KisB504/2009/G1P[6]		84.1		92.3		Africa
JN129083/RVA/Human-wt/NCA/OL/2010/G4P[6]		88.3		95.1		The Americas
MG407647/RVA/Human-wt/BRA/rj24598/2015/G26P[19]		84.8		94.0		The Americas
GU199488/RVA/Pig-tc/USA/Gottfried/1975/G4P[6]		84.5		93.4		The Americas
FJ947166/RVA/Human-xx/USA/DC1285/1980/G4P[8]		84.5		92.5		The Americas
HM773911/RVA/Human-xx/USA/DC4613/1980/G4P[8]		84.5		92.5		The Americas
KU861392/RVA/Human-tc/USA/Wa-20-AG/1974/G1P[8]		84.3		92.8		The Americas
JN129075/RVA/Human-wt/NCA/25J/2010/G1P[8]		84.3		92.5		The Americas
JQ069782/RVA/Human-wt/CAN/RT070-09/2009/G1P[8]		84.3		92.8		The Americas
EF583031/RVA/Human-tc/BRA/IAL28/1992/G5P[8]		83.9		91.7		The Americas
EF583051/RVA/Human-tc/USA/WI61/1983/G9P[8]		83.7		91.3		The Americas
JX416206/RVA/Human-tc/VEN/M37/1982/G1P[6]		83.7		91.4		The Americas
JQ993323/RVA/Human-wt/BEL/BE2001/2009/G9P[6]		85.1		92.9		Europe
JQ309140/RVA/Horse-tc/GBR/H-1/1975/G5P[7]		84.9		93.2		Europe
MH171338/RVA/Human-wt/ESP/SS257451/2012/G12P[8]		84.5		92.7		Europe
EF583047/RVA/Human-tc/GBR/ST3/1975/G4P[6]		84.4		91.7		Europe
JX195076/RVA/Human-wt/ITA/AV28/2010/G9P[8]		84.2		92.2		Europe
HQ392264/RVA/Human-wt/BEL/BE00031/2008/G1P[8]		84.1		92.6		Europe
KJ919597/RVA/Human-wt/HUN/ERN5611/2012/G1P[8]		84.0		92.5		Europe
JF490168/RVA/Human-wt/AUS/CK00008/2004/G1P[8]		84.2		92.7		Oceania

g.

NSP1 nucleotide and amino acid identities among strains calculated using the *p* -distance algorithm in MEGA 6.06 (Tamura et al., 2013)			
Strain	**NT**	**AA**	**Location (Continent)**
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6]			
LC095894/RVA/Human-tc/VNM/NT0042/2007/G4P[6]	98.2	97.9	Asia
KX363405/RVA/Pig-wt/VNM/14226-39/2012/GXP[X]	96.2	96.5	Asia
LC095905/RVA/Human-wt/VNM/NT0073/2007/G9P[19]	96.1	96.9	Asia
HG513049/RVA/Human-wt/VNM/30378/2009/G26P[19]	95.8	96.3	Asia
KX363336/RVA/Pig-wt/VNM/14150-53/2012/GXP[X]	94.7	95.3	Asia
KX363416/RVA/Pig-wt/VNM/14226-42/2012/GXP[X]	94.6	95.1	Asia
KY937198/RVA/Human-wt/KHM/CC9192/2014/G26P[6]	94.3	94.4	Asia
KF726039/RVA/Human-wt/CHN/E931/2008/G4P[6]	94.0	94.7	Asia
MK227393/RVA/Pig-wt/BGD/H14020027/G4P[9]	93.9	94.9	Asia
MK227404/RVA/Pig-wt/BGD/H14020036/G4P[9]	93.9	94.9	Asia
LC433780/RVA/Human-wt/NPL/TK1797/2007/G9P[19]	93.9	94.0	Asia
MG781058/RVA/Pig-wt/THA/CMP-011-09/2009/G4P[6]	90.7	91.2	Asia
AB741655/RVA/Human-wt/JPN/Ryukyu-1120/2011/G5P[6]	86.4	88.3	Asia
HM348719/RVA/Human-wt/IND/mani-362-07/2007/G4P[6]	86.1	86.8	Asia
HM348717/RVA/Human-wt/IND/mani-253-07/2007/G4P[4]	85.7	85.6	Asia
AB924090/RVA/Pig-wt/JPN/BU2/2014/G5P[7]	85.4	87.9	Asia
HM348716/RVA/Human-wt/IND/mani-97-06/2006/G9P[19]	85.1	86.6	Asia
AB924101/RVA/Pig-wt/JPN/BU8/2014/G4P[6]	85.0	87.2	Asia
AB924112/RVA/Pig-wt/JPN/BU9/2014/G9P[23]	84.7	87.9	Asia
KF041435/RVA/Human-wt/CHN/GX54/2010/G4P[6]	84.7	86.2	Asia
KF447856/RVA/Human-wt/CHN/GX78/2010/G4P[6]	84.7	86.2	Asia
KF447867/RVA/Human-wt/CHN/GX82/2010/G4P[6]	84.7	86.2	Asia
MN102369/RVA/Pig-wt/GHA/14/2016/G5P[7]	97.4	96.9	Africa
KP752851/RVA/Pig-wt/ZAF/MRC-DPRU1562/2008/G5P[X]	85.9	86.4	Africa
KP753056/RVA/Pig-wt/ZAF/MRC-DPRU3878/2008/G5P[X]	85.7	88.7	Africa
KJ753135/RVA/Pig-wt/ZAF/MRC-DPRU3825/2008/G5P[X]	84.6	88.3	Africa
KR052730/RVA/Pig-wt/USA/LS00009-RV0084/2011/G9P[13]	85.9	88.3	The Americas
KF035102/RVA/Human-wt/BRB/2012821133/2012/G4P[14]	85.5	87.2	The Americas
KJ482249/RVA/Pig-wt/BRA/ROTA06/2013/G11P[6]	84.8	85.4	The Americas
KJ482247/RVA/Pig-wt/BRA/ROTA04/2013/G5P[13]	84.1	83.7	The Americas
KJ482250/RVA/Pig-wt/BRA/ROTA07/2013/G5P[13]	84.1	83.7	The Americas
KM820739/RVA/Pig-wt/BEL/12R006/2012/G3P[6]	85.9	87.4	Europe
KM820738/RVA/Pig-wt/BEL/12R005/2012/G4P[7]	85.7	88.2	Europe
MH238089/RVA/Pig-wt/ESP/F437/2017/G3P[19]	85.5	87.4	Europe
JQ993324/RVA/Human-wt/BEL/BE2001/2009/G9P[6]	85.0	87.2	Europe
MH238095/RVA/Pig-wt/ESP/F456/2017/G5P[13]	84.8	85.4	Europe
KM820737/RVA/Pig-wt/BEL/12R002/2012/G5P[7]	84.2	86.0	Europe

h.

NSP2 nucleotide and amino acid identities among strains calculated using the *p* -distance algorithm in MEGA 6.06 (Tamura et al., 2013)			
Strain	**NT**	**AA**	**Location (Continent)**
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6]			
KJ466987/RVA/Pig-wt/CHN/YN/2012/GXP[X]	96.8	97.8	Asia
MH910070/RVA/Dog-tc/CHN/SCCD-A/2017/G9P[23]	96.8	97.8	Asia
MK026442/RVA/Pig-tc/CHN/SCMY-A3/2017/G9P[23]	96.6	97.8	Asia
GU189556/RVA/Human-tc/CHN/R479/2004/G4P[6]	92.1	96.8	Asia
AB741656/RVA/Human-wt/JPN/Ryukyu-1120/2011/G5P[6]	91.7	96.2	Asia
HG513052/RVA/Human-wt/VNM/30378/2009/G26P[19]	88.1	91.8	Asia
KF041436/RVA/Human-wt/CHN/GX54/2010/G4P[6]	88.0	92.4	Asia
KF447846/RVA/Human-wt/CHN/GX77/2010/G4P[6]	88.0	92.4	Asia
KF447868/RVA/Human-wt/CHN/GX82/2010/G4P[6]	88.0	92.4	Asia
KF447857/RVA/Human-wt/CHN/GX78/2010/G4P[6]	87.9	91.8	Asia
MF580909/RVA/Human-wt/CHN/JS2016/2016/G9P[8]	87.8	91.5	Asia
MF580903/RVA/Human-wt/CHN/JS2010/2010/G9P[8]	87.6	91.8	Asia
MF580908/RVA/Human-wt/CHN/JS2015/2015/G9P[8]	87.6	91.8	Asia
DQ146696/RVA/Human-tc/PHL/L26/1987/G12P[4]	87.6	93.4	Asia
KX674708/RVA/Human-wt/IND/RV1302/2013/G1P[8]	87.5	92.4	Asia
EF560709/RVA/Human-wt/BGD/Dhaka6/2001/G11P[25]	87.4	91.8	Asia
KX674709/RVA/Human-wt/IND/RV1305/2013/G1P[8]	87.3	92.1	Asia
LC227889/RVA/Human-wt/IND/Kol-018/2011/G9P[4]	87.2	91.8	Asia
HM348720/RVA/Human-tc/IND/mani-97/2006/G9P[19]	87.2	91.8	Asia
LC374199/RVA/Human-wt/NPL/10N4155/2010/G12P[6]	86.9	91.8	Asia
KP753117/RVA/Pig-wt/ZAF/MRC-DPRU1487/2007/G3G5P[23]	93.7	97.8	Africa
KP752954/RVA/Pig-wt/ZAF/MRC-DPRU1557/2008/G4G5P[23]	93.6	97.5	Africa
KP752760/RVA/Pig-wt/ZAF/MRC-DPRU1576/2007/G5P[X]	93.2	97.2	Africa
JX271008/RVA/Human-wt/TUN/17237/2008/G6P[9]	91.0	93.4	Africa
MG181454/RVA/Human-wt/MWI/MW2-1253/2005/G1P[8]	87.4	91.8	Africa
KX632305/RVA/Human-wt/UGA/MUL-12-147/2012/G9P[8]	87.4	92.4	Africa
KP752749/RVA/Human-wt/TGO/MRC-DPRU4562/2011/G1P[8]	87.3	92.4	Africa
JN605411/RVA/Human-wt/CMR/MRC-DPRU1424/2009/G9P[8]	87.2	91.8	Africa
AB861952/RVA/Human-tc/KEN/KDH633/2010/G12P[6]	87.1	91.8	Africa
KJ870929/RVA/Human-wt/COD/KisB504/2009/G1P[6]	87.1	91.8	Africa
JN605455/RVA/Human-wt/KEN/MRC-DPRU2427/2010/G9P[8]	87.0	91.8	Africa
JN605422/RVA/Human-wt/ZWE/MRC-DPRU1723/2009/G9P[8]	86.9	91.5	Africa
JN605433/RVA/Human-wt/ZAF/MRC-DPRU4677/2010/G9P[8]	86.9	91.5	Africa
EF672587/RVA/Human-tc/BRA/IAL28/1992/G5P[8]	92.5	96.5	The Americas
KJ820876/RVA/Human-tc/BRA/R70/1997/G1P[9]	91.8	95.6	The Americas
GU199489/RVA/Pig-tc/USA/Gottfried/1983/G4P[6]	88.4	93.7	The Americas
HM773619/RVA/Human-wt/USA/2009727047/2009/G9P[8]	87.6	91.8	The Americas
HM467966/RVA/Human-wt/USA/LB2771/1975/G1P[8]	87.6	90.9	The Americas
EF672622/RVA/Human-tc/USA/WI61/1983/G9P[8]	87.4	91.5	The Americas
JQ069368/RVA/Human-wt/CAN/RT070-09/2009/G1P[8]	87.1	91.5	The Americas
JQ309142/RVA/Horse-tc/GBR/H-1/1975/G5P[7]	92.6	96.2	Europe
KC020034/RVA/Human-wt/RUS/O1154/2011/G3P[9]	91.5	94.0	Europe
KC020027/RVA/Human-wt/RUS/O202/2007/G3P[9]	91.1	94.6	Europe
JQ993325/RVA/Human-wt/BEL/BE2001/2009/G9P[6]	88.5	93.1	Europe
KC155685/RVA/Human-wt/RUS/Nov11-N1936/2011/G2P[8]	88.1	92.4	Europe
EF672615/RVA/Human-tc/GBR/ST3/1975/G4P[6]	87.8	92.7	Europe
KU048700/RVA/Human-wt/ITA/PA525-14/2014/G12P[8]	87.7	91.8	Europe
JX683000/RVA/Human-wt/RUS/Nov12-N4285/2012/G3P[8]	87.5	91.8	Europe
DQ146645/RVA/Human-wt/BEL/B4633/2003/G12P[8]	87.5	92.4	Europe
JX683001/RVA/Human-wt/RUS/Nov12-N3835/2012/G2G3P[8]	87.4	91.8	Europe
KU048694/RVA/Human-wt/ITA/ME864-12/2012/G12P[8]	87.3	92.1	Europe
KJ918873/RVA/Human-wt/HUN/ERN5014/2012/G1P[8]	87.1	92.1	Europe
EF990710/RVA/Human-wt/BEL/B3458/2003/G9P[8]	87.1	91.8	Europe
KU048702/RVA/Human-wt/ITA/PA417-14/2014/G12P[8]	87.0	92.1	Europe

i.

NSP3 nucleotide and amino acid identities among strains calculated using the *p* -distance algorithm in MEGA 6.06 (Tamura et al., 2013)			
Strain	**NT**	**AA**	**Location (Continent)**
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6]			
KX363287/RVA/Pig-wt/VNM/12070-4/2012/GXP[X]	97.0	98.4	Asia
LC095885/RVA/Human-tc/VNM/NT0001/2007/G3P[6]	97.0	98.4	Asia
LC095951/RVA/Human-wt/VNM/NT0621/2008/G4P[6]	97.0	98.7	Asia
LC095929/RVA/Human-wt/VNM/NT0205/2007/G4P[6]	96.8	98.7	Asia
KX363418/RVA/Pig-wt/VNM/14226-42/2012/GXP[X]	96.5	98.7	Asia
HG513051/RVA/Human-wt/VNM/30378/2009/G26P[19]	96.5	98.7	Asia
KY937200/RVA/Human-wt/KHM/CC9192/2014/G26P[6]	96.3	98.4	Asia
MG781049/RVA/Human-wt/THA/CMH-N014-11/2011/G4P[6]	95.5	97.7	Asia
AB779642/RVA/Pig-wt/THA/CMP29/08/2008/G3P[13]	95.3	97.1	Asia
LC208016/RVA/Human-wt/NPL/07N1760/2007/G26P[19]	95.2	96.5	Asia
KU363139/RVA/Human-wt/THA/CMHS-070-13/2013/G9P[19]	95.1	97.7	Asia
LC190494/RVA/Human-wt/THA/KKL-117/2014/G9P[23]	94.9	97.4	Asia
AB779643/RVA/Pig-wt/THA/CMP40/08/2008/G3P[23]	94.4	95.8	Asia
KU363140/RVA/Pig-wt/THA/CMP-015-12/2012/G9P[19]	94.4	97.4	Asia
MG781039/RVA/Human-wt/THA/CMH-N016-10/2010/G4P[6]	94.3	97.4	Asia
MG781060/RVA/Pig-wt/THA/CMP-011-09/2009/G4P[6]	94.3	97.7	Asia
AB741657/RVA/Human-wt/JPN/Ryukyu-1120/2011/G5P[6]	93.3	98.4	Asia
HQ609571/RVA/Human-wt/IND/613158/2006/G1P[8]	88.0	92.6	Asia
MF580901/RVA/Human-wt/CHN/JS2015/2015/G9P[8]	87.4	92.6	Asia
KF371859/RVA/Human-wt/CHN/E2461/2011/G3P[8]	87.3	91.6	Asia
MF580900/RVA/Human-wt/CHN/JS2014/2014/G9P[8]	87.0	92.3	Asia
MF580902/RVA/Human-wt/CHN/JS2016/2016/G9P[8]	86.9	92.3	Asia
LC374134/RVA/Human-wt/NPL/09N3140/2009/G12P[6]	86.8	91.9	Asia
DQ492677/RVA/Human-wt/BGD/Dhaka16/2003/G1P[8]	86.7	92.3	Asia
LC086755/RVA/Human-wt/THA/PCB-118/2013/G1P[8]	86.6	91.9	Asia
GU199523/RVA/Human-wt/BGD/Dhaka6/2001/G11P[25]	86.3	91.6	Asia
LC086766/RVA/Human-wt/THA/SKT-98/2013/G1P[8]	86.3	91.6	Asia
KX632251/RVA/Human-wt/UGA/NSA-13-043/2013/G9P[8]	87.8	92.9	Africa
KX632306/RVA/Human-wt/UGA/MUL-12-147/2012/G9P[8]	87.6	92.6	Africa
KP752750/RVA/Human-wt/TGO/MRC-DPRU4562/2011/G1P[8]	87.5	92.6	Africa
KJ753079/RVA/Human-wt/ZAF/MRC-DPRU135/2009/G1P[8]	87.4	92.3	Africa
KJ870919/RVA/Human-wt/COD/KisB521/2008/G12P[6]	87.2	91.9	Africa
MG181268/RVA/Human-wt/MWI/MW2-191/2000/G1P[8]	87.1	91.6	Africa
AB861964/RVA/Human-tc/KEN/KDH651/2010/G12P[8]	86.8	91.9	Africa
AB861953/RVA/Human-tc/KEN/KDH633/2010/G12P[6]	86.6	91.9	Africa
MG181510/RVA/Human-wt/MWI/BID111/2012/G1P[8]	86.6	91.6	Africa
MG181554/RVA/Human-wt/MWI/BID1AC/2012/G1P[8]	86.6	91.3	Africa
MG407653/RVA/Human-wt/BRA/rj24598/2015/G26P[19]	96.1	98.4	The Americas
EF672586/RVA/Human-tc/BRA/IAL28/1992/G5P[8]	89.1	95.5	The Americas
JQ069264/RVA/Human-wt/CAN/RT186-07/2008/G1P[8]	88.1	92.6	The Americas
JQ069284/RVA/Human-wt/CAN/RT070-09/2009/G1P[8]	88.0	92.9	The Americas
JQ069261/RVA/Human-wt/CAN/RT178-07/2008/G1P[8]	87.8	92.3	The Americas
JQ069259/RVA/Human-wt/CAN/RT172-07/2008/G1P[8]	87.3	92.3	The Americas
JN129005/RVA/Human/NCA/25J/2010/G1P[8]	86.8	91.6	The Americas
MF161607/RVA/Human-wt/BRA/1A2703/2011/G1P[8]	86.6	91.9	The Americas
JX195071/RVA/Human-wt/ITA/AV21/2010/G9P[8]	86.9	91.9	Europe
DQ146646/RVA/Human-wt/BEL/B4633/2003/G12P[8]	86.8	92.3	Europe
KU048712/RVA/Human-wt/ITA/RG179-13/2013/G12P[8]	86.6	91.6	Europe
EF990711/RVA/Human-wt/BEL/B3458/2003/G9P[8]	86.3	91.6	Europe
JX416224/RVA/Human-tc/AUS/McN13/1980/G3P2A[6]	88.5	92.9	Oceania

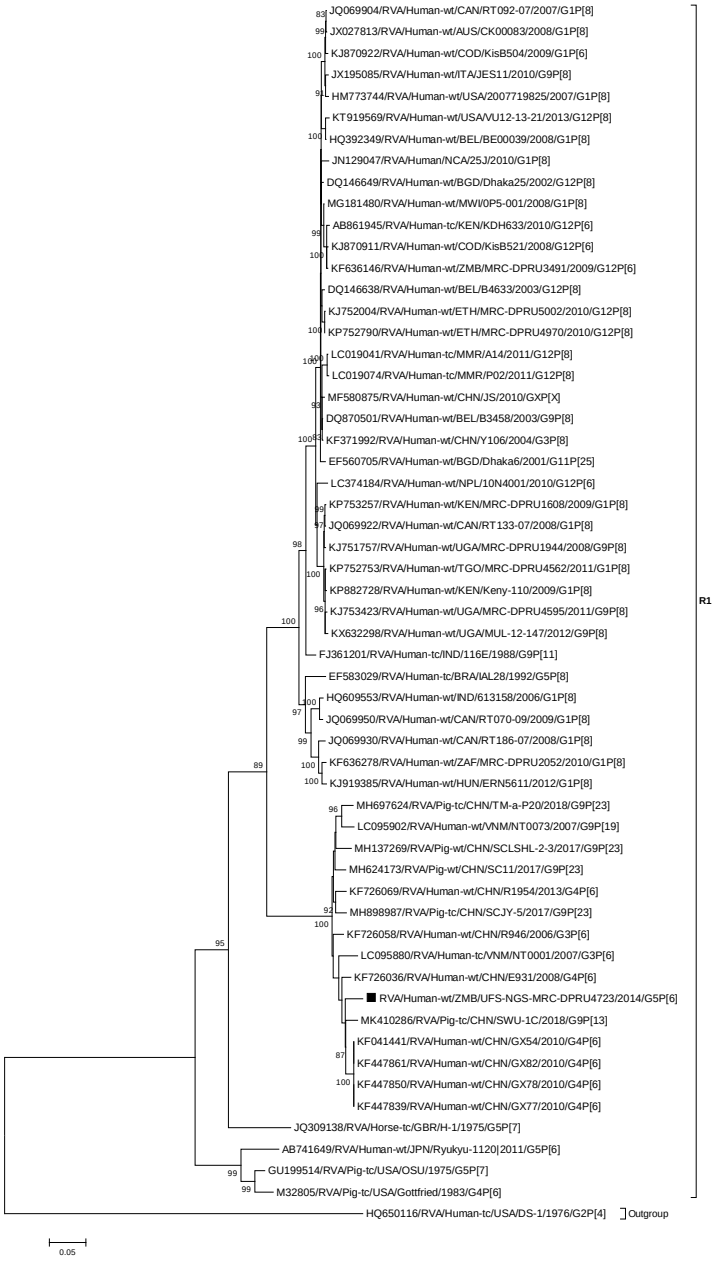
j.

NSP4 nucleotide and amino acid identities among strains calculated using the *p* -distance algorithm in MEGA 6.06 (Tamura et al., 2013)			
Strain	**NT**	**AA**	**Location (Continent)**
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6]			
KX363354/RVA/Pig-wt/VNM/14150-54/2012/GXP[X]	97.3	97.7	Asia
LC095952/RVA/Human-wt/VNM/NT0621/2008/G4P[6]	97.3	98.9	Asia
KF726042/RVA/Human-wt/CHN/E931/2008/G4P[6]	96.9	98.9	Asia
LC095930/RVA/Human-wt/VNM/NT0205/2007/G4P[6]	96.7	98.3	Asia
HG513054/RVA/Human-wt/VNM/30378/2009/G26P[19]	96.2	96.6	Asia
EF159574/RVA/Human-wt/CHN/LL36755/2003/G5P[6]	94.6	98.3	Asia
EF159572/RVA/Human-wt/CHN/LL3354/2000/G5P[6]	94.4	98.3	Asia
KF726075/RVA/Human-wt/CHN/R1954/2013/G4P[6]	94.1	97.7	Asia
MH137271/RVA/Pig-wt/CHN/SCLSHL-2-3/2017/G9P[23]	94.1	96.6	Asia
KF041438/RVA/Human-wt/CHN/GX54/2010/G4P[6]	93.5	98.3	Asia
KF447848/RVA/Human-wt/CHN/GX77/2010/G4P[6]	93.5	98.3	Asia
KF447859/RVA/Human-wt/CHN/GX78/2010/G4P[6]	93.5	98.3	Asia
KF447870/RVA/Human-wt/CHN/GX82/2010/G4P[6]	93.5	98.3	Asia
HQ609574/RVA/Human-wt/IND/613158/2006/G1P[8]	93.1	97.7	Asia
JQ863318/RVA/Human-tc/IND/57M/1980/G4P[10]	92.7	94.3	Asia
U78558/RVA/Human-wt/IND/116E/1988/G9P[11]	92.7	95.4	Asia
AB008237/RVA/Human-tc/JPN/ITO/1981/G3P[8]	92.5	96.0	Asia
EF159573/RVA/Human-wt/CHN/LL4260/2001/G5P[6]	92.3	96.6	Asia
EF560711/RVA/Human-wt/BGD/Dhaka6/2001/G11P[25]	91.0	94.8	Asia
MF580893/RVA/Human-wt/CHN/JS2014/2014/G9P[8]	91.0	94.8	Asia
MF580894/RVA/Human-wt/CHN/JS2015/2015/G9P[8]	91.0	94.8	Asia
DQ490543/RVA/Human-wt/BGD/RV161/2000/G12P[6]	91.0	94.3	Asia
MF580889/RVA/Human-wt/CHN/JS2010/2010/G9P[8]	90.6	94.3	Asia
MG781040/RVA/Human-wt/THA/CMH-N016-10/2010/G4P[6]	90.6	97.1	Asia
MF580895/RVA/Human-wt/CHN/JS2016/2016/G9P[8]	90.4	93.7	Asia
MG781050/RVA/Human-wt/THA/CMH-N014-11/2011/G4P[6]	89.8	96.6	Asia
AB741658/RVA/Human-wt/JPN/Ryukyu-1120|2011/G5P[6]	88.9	94.8	Asia
GU189558/RVA/Human-tc/CHN/R479/2004/G4P[6]	88.7	96.0	Asia
KY937201/RVA/Human-wt/KHM/CC9192/2014/G26P[6]	88.1	95.4	Asia
GQ240623/RVA/Human-tc/IND/mani-97/2006/G9P[19]	87.2	94.8	Asia
KX632307/RVA/Human-wt/UGA/MUL-12-147/2012/G9P[8]	90.4	94.3	Africa
KX632252/RVA/Human-wt/UGA/NSA-13-043/2013/G9P[8]	90.2	94.3	Africa
KP752943/RVA/Human-wt/TGO/MRC-DPRU4578/2010/G12P[6]	88.7	94.3	Africa
KJ751865/RVA/Human-wt/UGA/MRC-DPRU3713/2010/G12P[6]	88.5	94.3	Africa
KJ870931/RVA/Human-wt/COD/KisB504/2009/G1P[6]	88.5	94.3	Africa
KJ870909/RVA/Human-wt/COD/KisB332/2008/G4P[6]	88.5	94.3	Africa
AB861976/RVA/Human-tc/KEN/KDH684/2010/G12P[6]	88.3	94.3	Africa
KX655493/RVA/Human-wt/UGA/KTV-13-023/2013/G12P[6]	88.3	93.7	Africa
MG407654/RVA/Human-wt/BRA/rj24598/2015/G26P[19]	96.4	98.9	The Americas
KT695058/RVA/Human-wt/USA/DC3695/1989/G1P[8]	93.5	96.6	The Americas
EF672589/RVA/Human-tc/BRA/IAL28/1992/G5P[8]	92.9	94.3	The Americas
AB361284/RVA/Human-tc/USA/D/1974/G1P[8]	92.5	96.0	The Americas
JN129019/RVA/Human-wt/NCA/25J/2010/G1P[8]	90.8	94.3	The Americas
GU199490/RVA/Pig-tc/USA/Gottfried/1975/G4P[6]	87.2	95.4	The Americas
D88831/RVA/Pig-tc/USA/OSU/1976/G5P[7]	87.2	96.0	The Americas
MK283698/RVA/WildBoar-wt/CZE/P828/2015/G9P[23]	97.5	98.3	Europe
MK283699/RVA/WildBoar-wt/CZE/P830/2015/G9P[23]	97.5	98.3	Europe
GQ465005/RVA/Human-wt/RUS/RUS-Nov04-H390/2004/G1P[4]	92.7	95.4	Europe
DQ146647/RVA/Human-wt/BEL/B4633/2003/G12P[8]	91.4	95.4	Europe
EF990712/RVA/Human-wt/BEL/B3458/2003/G9P[8]	91.4	94.8	Europe
EF672617/RVA/Human-tc/GBR/ST3/1975/G4P[6]	91.4	96.0	Europe
KP013455/RVA/Human-wt/DEN/W21578/2010/G9P[8]	91.2	94.3	Europe
GQ465012/RVA/Human-wt/RUS/Nov05-701/2005/G1G3P[8]	91.2	94.8	Europe
GQ465026/RVA/Human-wt/RUS/Nov09-B34/2009/G3P[8]	91.2	94.3	Europe
JQ993327/RVA/Human-wt/BEL/BE2001/2009/G9P[6]	88.5	93.7	Europe
JX416225/RVA/Human-tc/AUS/McN13/1980/G3P[6]	92.9	95.4	Oceania

k.

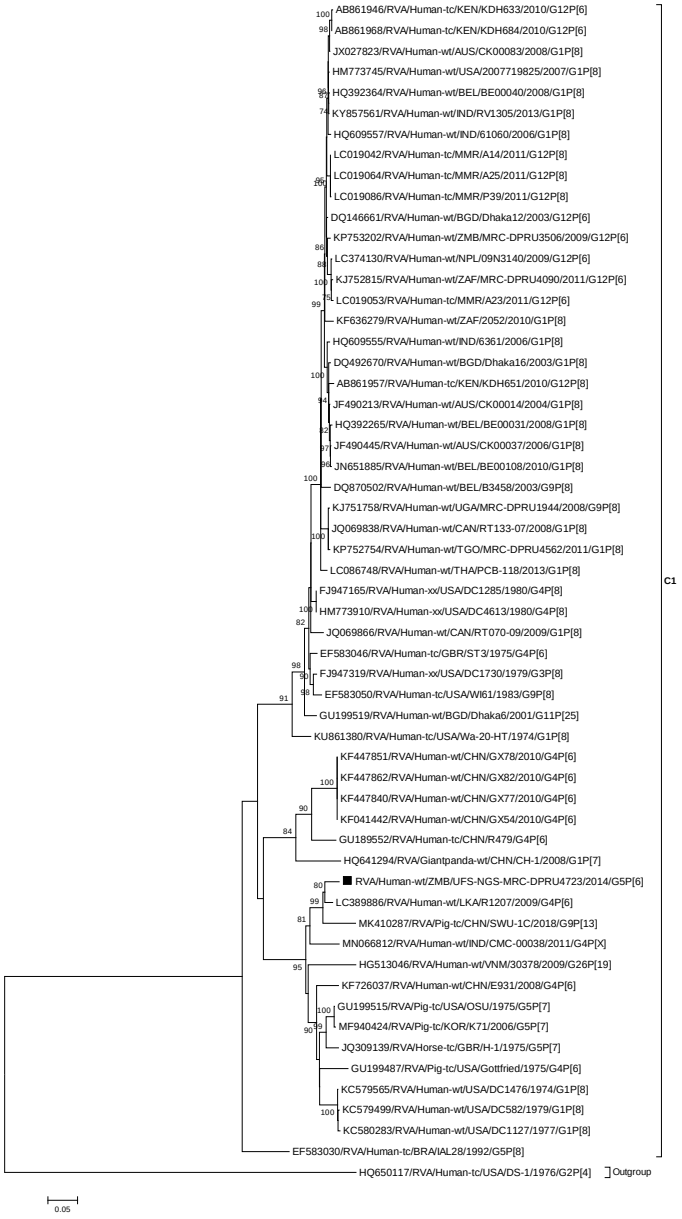
NSP5 nucleotide and amino acid identities among strains calculated using the *p* -distance algorithm in MEGA 6.06 (Tamura et al., 2013)			
Strain	**NT**	**AA**	**Location (Continent)**
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6]			
KC113254/RVA/Pig-wt/CHN/TM-a/2009/G3P[8]	98.6	100	Asia
MH697634/RVA/Pig-tc/CHN/TM-a-P20/2018/G9P[23]	98.6	100	Asia
GU189559/RVA/Human-tc/CHN/R479/2004/G4P[6]	97.6	99.0	Asia
LC433784/RVA/Human-wt/NPL/TK1797/2007/G9P[19]	97.5	99.5	Asia
MK227397/RVA/Pig-wt/BGD/H14020027/G4P[49]	97.3	99.0	Asia
MH137270/RVA/Pig-wt/CHN/SCLSHL-2-3/2017/G9P[23]	97.3	99.5	Asia
KU886312/RVA/Pig-wt/CHN/HLJ-15-1/2015/GXP[X]	97.3	99.5	Asia
LC095920/RVA/Human-wt/VNM/NT0077/2007/G4P[6]	97.3	99.5	Asia
KF726043/RVA/Human-wt/CHN/E931/2008/G4P[6]	97.1	100	Asia
KF041439/RVA/Human-wt/CHN/GX54/2010/G4P[6]	97.1	100	Asia
KF447849/RVA/Human-wt/CHN/GX77/2010/G4P[6]	97.1	100	Asia
KF447860/RVA/Human-wt/CHN/GX78/2010/G4P[6]	97.1	100	Asia
KF447871/RVA/Human-wt/CHN/GX82/2010/G4P[6]	97.1	100	Asia
KX363314/RVA/Pig-wt/VNM/12129-48/2012/GXP[X]	97.1	100	Asia
HM348728/RVA/Human-tc/IND/mani-97/2006/G9P[19]	96.3	98.0	Asia
AB741659/RVA/Human-wt/JPN/Ryukyu-1120|2011/G5P[6]	96.3	98.5	Asia
MG781051/RVA/Human-wt/THA/CMH-N014-11/2011/G4P[6]	96.3	99.0	Asia
MG781041/RVA/Human-wt/THA/CMH-N016-10/2010/G4P[6]	96.3	98.5	Asia
LC208018/RVA/Human-wt/NPL/07N1760/2007/G26P[19]	96.3	98.0	Asia
KF726065/RVA/Human-wt/CHN/R946/2006/G3P[6]	96.1	97.5	Asia
MN066810/RVA/Human-wt/IND/CMC-00038/2011/G4P[X]	96.1	98.5	Asia
KU363144/RVA/Pig-wt/THA/CMP-015-12/2012/G9P[19]	96.1	99.0	Asia
FJ361211/RVA/Human-tc/IND/116E/1988/G9P[11]	95.9	99.0	Asia
EF560712/RVA/Human-wt/BGD/Dhaka6/2001/G11P[25]	95.3	98.0	Asia
DQ146681/RVA/Human-wt/BGD/Matlab13/2003/G12P[6]	95.3	98.0	Asia
LC019073/RVA/Human-tc/MMR/A25/2011/G12P[8]	95.1	97.0	Asia
LC368117/RVA/Human-wt/NPL/06N0359/2006/G12P[6]	94.9	98.0	Asia
LC372857/RVA/Human-wt/NPL/07N0900/2007/G12P[6]	94.9	98.0	Asia
HG513055/RVA/Human-wt/VNM/30378/2009/G26P[19]	94.8	97.0	Asia
LC019062/RVA/Human-tc/MMR/A23/2011/G12P[6]	94.6	97.5	Asia
KF371687/RVA/Human-tc/CHN/R709/2005/G3P[8]	94.1	97.5	Asia
HQ657148/RVA/Human-wt/ZAF/3133WC/2009/G12P[4]	95.3	98.0	Africa
HQ657159/RVA/Human-wt/ZAF/3176WC/2009/G12P[6]	95.3	98.0	Africa
KJ870932/RVA/Human-wt/COD/KisB504/2009/G1P[6]	94.8	98.0	Africa
AB861955/RVA/Human-tc/KEN/KDH633/2010/G12P[6]	94.8	97.5	Africa
AB861977/RVA/Human-tc/KEN/KDH684/2010/G12P[6]	94.8	97.5	Africa
AB938310/RVA/Human-tc/MWI/MAL38/2007/G1P[8]	94.8	98.0	Africa
MG181479/RVA/Human-wt/MWI/MW2-1274/2005/G1P[8]	94.8	98.0	Africa
KJ870921/RVA/Human-wt/COD/KisB521/2008/G12P[6]	94.8	98.0	Africa
KP752752/RVA/Human-wt/TGO/MRC-DPRU4562/2011/G1P[8]	94.6	97.0	Africa
KX632319/RVA/Human-wt/UGA/MUL-12-093/2012/G9P[8]	94.6	97.0	Africa
KX632253/RVA/Human-wt/UGA/NSA-13-043/2013/G9P[8]	94.4	96.4	Africa
KX632330/RVA/Human-wt/UGA/MUL-13-285/2013/G9P[8]	94.4	96.4	Africa
KX632308/RVA/Human-wt/UGA/MUL-12-147/2012/G9P[8]	94.4	97.0	Africa
KJ659441/RVA/Pig-tc/USA/LS00008/1975/G4P[6]	96.1	99.0	The Americas
GU199491/RVA/Pig-tc/USA/Gottfried/1975/G4P[6]	95.9	99.0	The Americas
KU361045/RVA/Human-wt/BRA/QUI-152-F1/2010/G1P[8]	95.4	98.0	The Americas
MF161837/RVA/Human-wt/BRA/1A2703/2011/G1P[8]	95.4	98.0	The Americas
FJ794017/RVA/Human-wt/BRA/rj1528-98/1998/G9P[8]	94.9	98.0	The Americas
EF672590/RVA/Human-tc/BRA/IAL28/1992/G5P[8]	93.9	95.9	The Americas
MK167200/RVA/Human-wt/RUS/S12-40/2012/G4P[6]P[8]	97.0	99.5	Europe
JQ993328/RVA/Human-wt/BEL/BE2001/2009/G9P[6]	95.1	98.0	Europe
KU048765/RVA/Human-wt/ITA/PA417-14/2014/G12P[8]	94.8	97.0	Europe
KU048768/RVA/Human-wt/ITA/ME659-14/2014/G12P[8]	94.6	97.0	Europe
EF672618/RVA/Human-tc/GBR/ST3/1975/G4P[6]	94.6	97.0	Europe
KJ919283/RVA/Human-wt/HUN/ERN5611/2012/G1P[8]	94.4	96.4	Europe
EF990713/RVA/Human-wt/BEL/B3458/2003/G9P[8]	94.4	98.0	Europe

Appendix 8: VP1 phylogenetic tree of Zambian G5P[6] and reference strains.



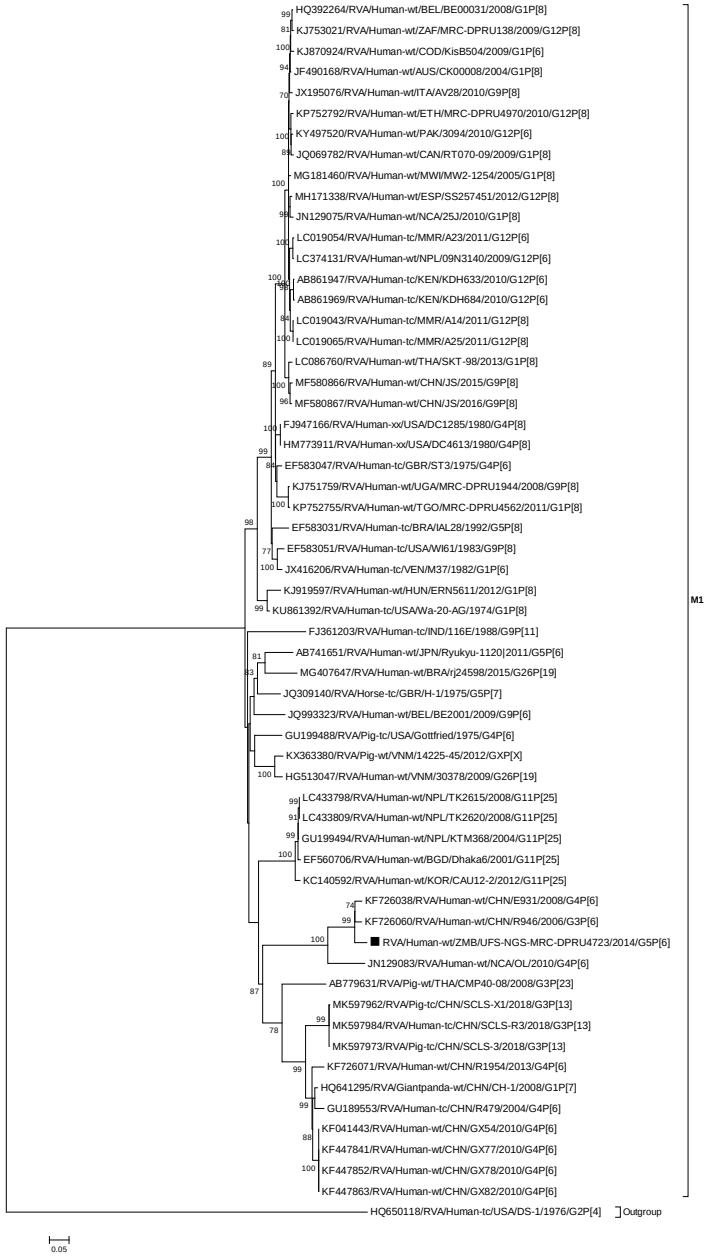
Phylogenetic tree constructed from the nucleotide sequences of the VP1 genes of strain RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] and representative strains. The position of strain RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] is shown by the black square (▪). Reference strains obtained from GenBank are represented by Accession number, Strain name, Country and year of isolation. The three closest strains as identified by BLASTn are also included. Bootstrap values ≥70% are shown adjacent to each branch node. Scale bar: 0.05 substitutions per nucleotide.

Appendix 9: VP2 phylogenetic tree of Zambian G5P[6] and reference strains.



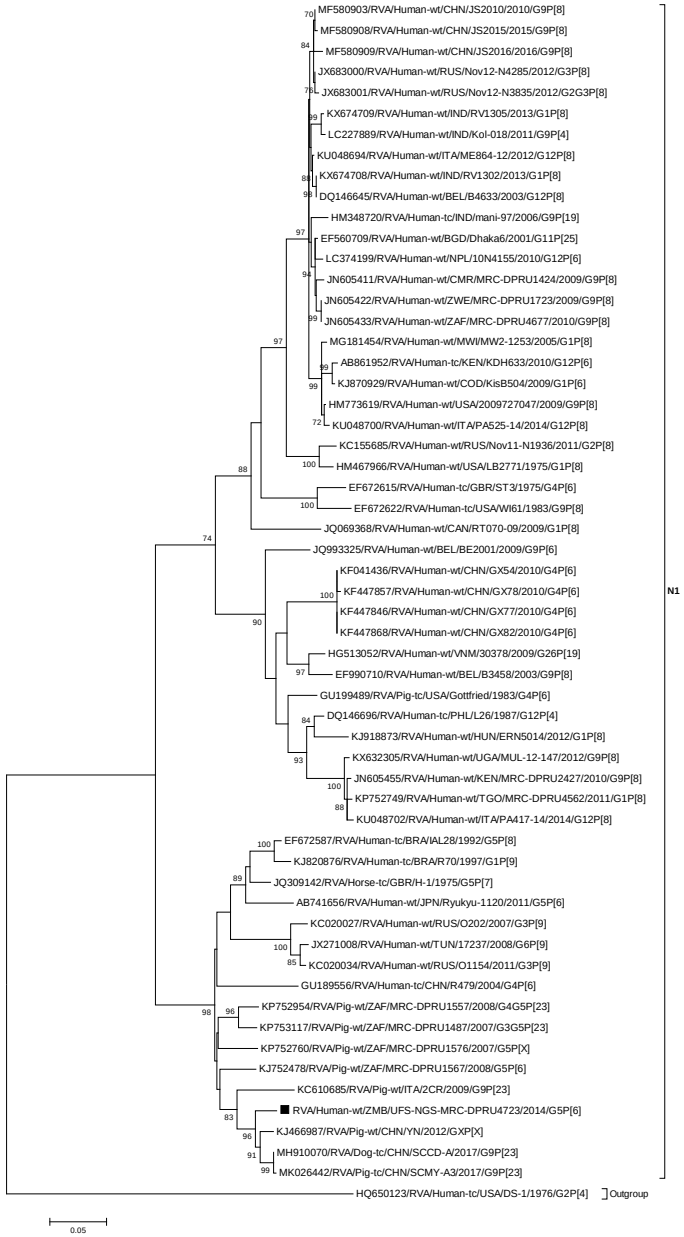
Phylogenetic tree constructed from the nucleotide sequences of the VP2 genes of strain RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] and representative strains. The position of strain RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] is shown by the black square (▪). Reference strains obtained from GenBank are represented by Accession number, Strain name, Country and year of isolation. The three closest strains as identified by BLASTn are also included. Bootstrap values ≥70% are shown adjacent to each branch node. Scale bar: 0.05 substitutions per nucleotide.

Appendix 10: VP3 phylogenetic tree of Zambian G5P[6] and reference strains.



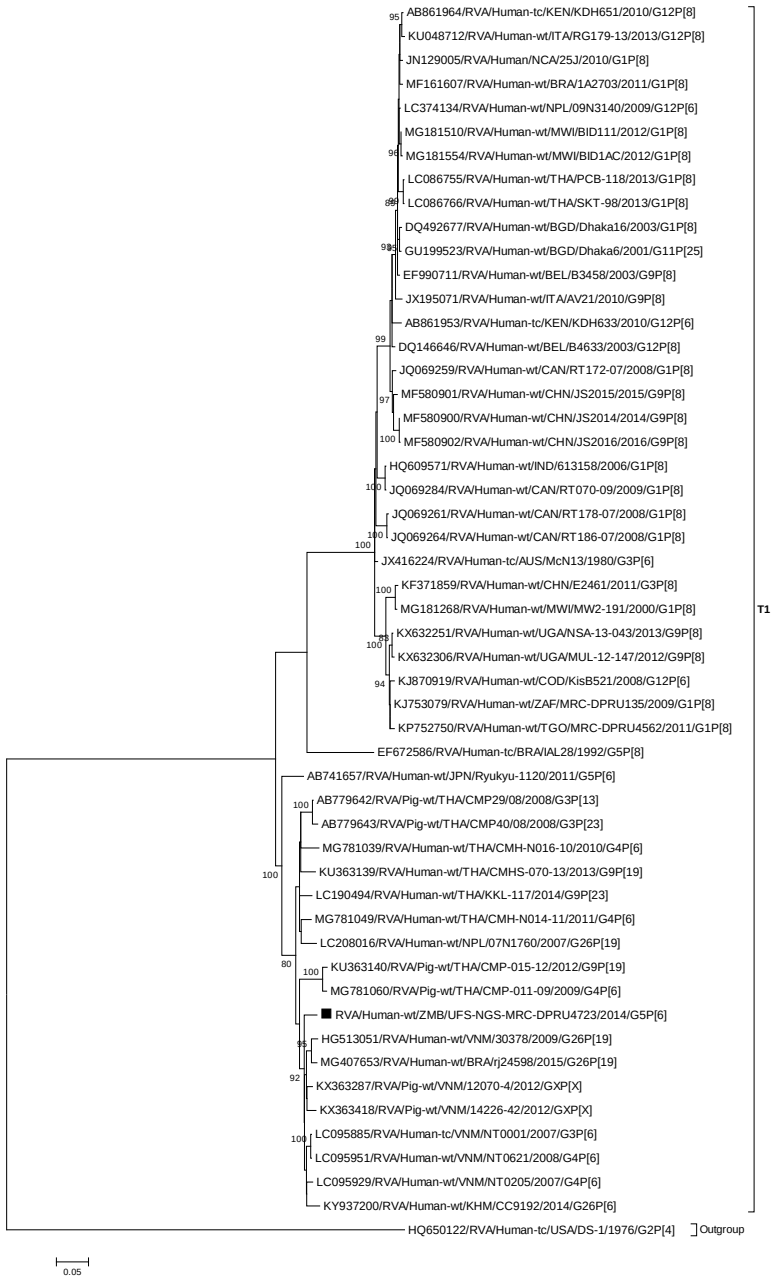
Phylogenetic tree constructed from the nucleotide sequences of the VP3 genes of strain RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] and representative strains. The position of strain RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] is shown by the black square (▪). Reference strains obtained from GenBank are represented by Accession number, Strain name, Country and year of isolation. The three closest strains as identified by BLASTn are also included. Bootstrap values ≥70% are shown adjacent to each branch node. Scale bar: 0.05 substitutions per nucleotide.

Appendix 11: NSP2 phylogenetic tree of Zambian G5P[6] and reference strains.



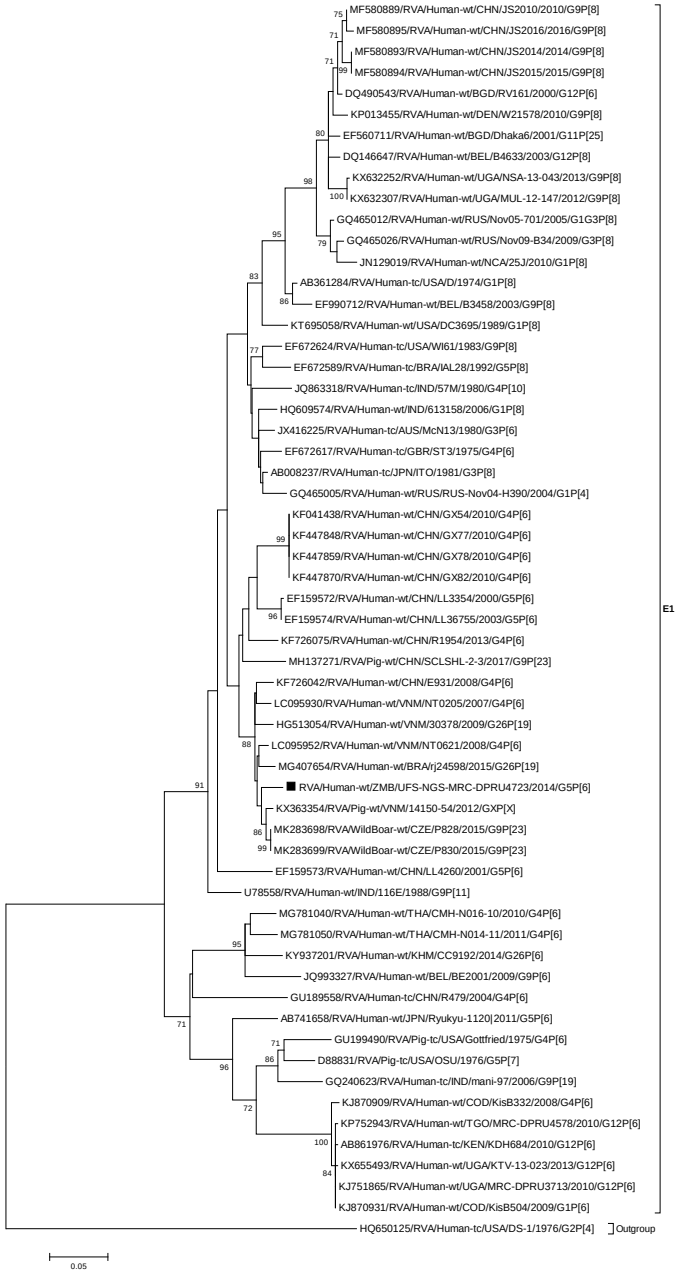
Phylogenetic tree constructed from the nucleotide sequences of the NSP2 genes of strain RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] and representative strains. The position of strain RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] is shown by the black square (▪). Reference strains obtained from GenBank are represented by Accession number, Strain name, Country and year of isolation. The three closest strains as identified by BLASTn are also included. Bootstrap values ≥70% are shown adjacent to each branch node. Scale bar: 0.05 substitutions per nucleotide.

Appendix 12: NSP3 phylogenetic tree of Zambian G5P[6] and reference strains.



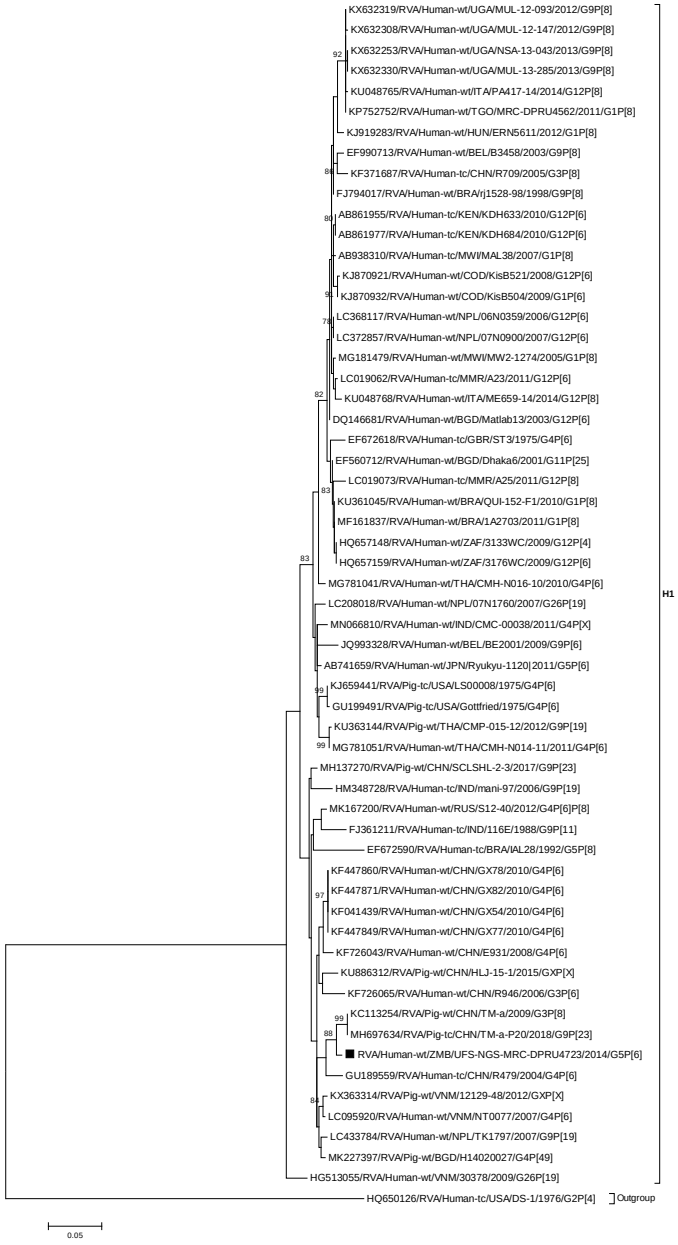
Phylogenetic tree constructed from the nucleotide sequences of the NSP3 genes of strain RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] and representative strains. The position of strain RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] is shown by the black square (▪). Reference strains obtained from GenBank are represented by Accession number, Strain name, Country and year of isolation. The three closest strains as identified by BLASTn are also included. Bootstrap values ≥70% are shown adjacent to each branch node. Scale bar: 0.05 substitutions per nucleotide.

Appendix 13: NSP4 phylogenetic tree of Zambian G5P[6] and reference strains.



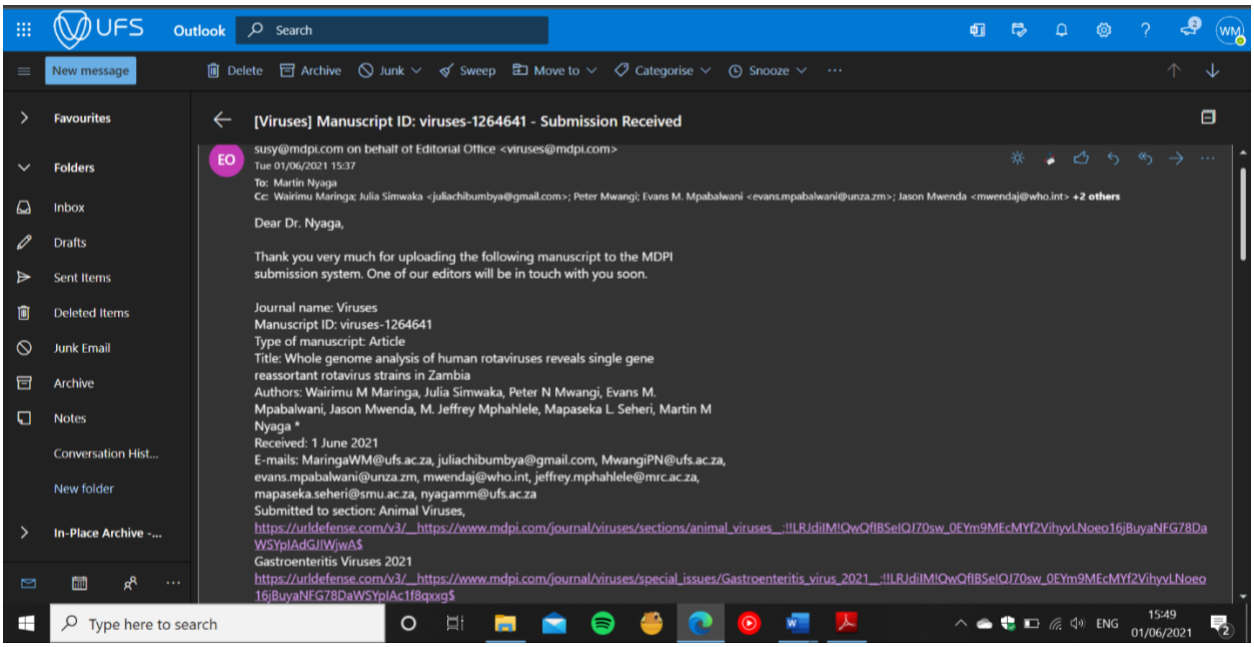
Phylogenetic tree constructed from the nucleotide sequences of the NSP4 genes of strain RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] and representative strains. The position of strain RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] is shown by the black square (▪). Reference strains obtained from GenBank are represented by Accession number, Strain name, Country and year of isolation. The three closest strains as identified by BLASTn are also included. Bootstrap values ≥70% are shown adjacent to each branch node. Scale bar: 0.05 substitutions per nucleotide.

Appendix 14: NSP5 phylogenetic tree of Zambian G5P[6] and reference strains.



Phylogenetic tree constructed from the nucleotide sequences of the NSP5 genes of strain RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] and representative strains. The position of strain RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4723/2014/G5P[6] is shown by the black square (▪). Reference strains obtained from GenBank are represented by Accession number, Strain name, Country and year of isolation. The three closest strains as identified by BLASTn are also included. Bootstrap values ≥70% are shown adjacent to each branch node. Scale bar: 0.05 substitutions per nucleotide.

Appendix 15: Submission number (viruses-1264641) of the manuscript '***Whole genome analysis of human rotaviruses reveals single gene reassortant rotavirus strains in Zambia***' that was submitted to the special issue on Gastroenteritis Viruses 2021 of the journal Viruses.



Appendix 16: Abstract page of the manuscript '***Whole genome analysis of human rotaviruses reveals single gene reassortant rotavirus strains in Zambia***' presented in chapter four submitted to the journal Viruses and currently under review.

viruses

MDPI

Article

Whole genome analysis of human rotaviruses reveals single gene reassortant rotavirus strains in Zambia

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Abstract: Zambia implemented Rotarix® vaccine into her national immunisation program in 2013. In this study, four unusual strains collected in the post-vaccine period were subjected to whole genome sequencing and analysis. The four strains possessed atypical genotype constellations, with at least one reassortant genome segment within the constellation. In one of the study strains that was genetically and phylogenetically divergent, two additional lineages within the VP4 (lineage P[8] V) and VP1 (lineage R2 XV) are proposed as new lineages. Pairwise analyses demonstrated several amino acid disparities in the VP4 antigenic sites of this strain compared to that of Rotarix®. Considering that all study participants experienced varying clinically significant symptoms associated with RVA disease, the link between clinical and genomic data for the study strains revealed that not only typical RVA strains, but also these atypical Zambian study strains, are associated with disease.

Keywords: rotavirus; whole genome; genotype constellation; intergenogroup reassortment; amino acid; lineage; Rotarix®

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1. Introduction

Group A rotavirus (RVA), a widespread and infectious pathogen that causes dehydrating diarrhoea in children under five years of age, was estimated to have caused approximately 128,000 deaths in 2016. A greater percentage of these deaths (~105,000) occurred in sub-Saharan Africa [1,2]. The significance of RVA burden of disease led to the development of prophylactic vaccines. In that regard, the World Health Organization (WHO) recommended the use of rotavirus vaccines globally [3]. Four WHO-prequalified rotavirus vaccines (Rotarix®, RotaTeq®, ROTAVAC® and ROTASIIL®) are currently in use in 110 countries worldwide as of 5th April 2021 [4]. The two-dose monovalent vaccine Rotarix® (RV1; GlaxoSmithKline Biologicals, Belgium) consists of a single human G1P[8] strain [3]. In sub-Saharan Africa, RV1 is used in countries such as Kenya, Mauritania, Namibia, Niger, and Zimbabwe [4]. This vaccine was introduced in Lusaka, Zambia in

Appendix 17a-b: Nucleotide and amino acid identities for the VP7 of the four Zambian reassortants

a.

VP7 nucleotide identities among strains calculated using the p-distance algorithm in MEGA 6 (Tamura et al., 2013)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4749/2014/G2P[8] - Lineage G2 IV																																																			
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13327/2016/G2P[4] - Lineage G2 IV	97.8																																																	
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13232/2016/G1P[8] - Lineage G1 I	73.2	73.5																																																
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13541/2016/G1P[8] - Lineage G1 I	73.2	73.5	100.0																																														
LC086796/RVA/Human-wt/THA/SKT-138/2013/G2P[4] - Lineage G2 IV	99.6	98.2	73.4	73.4																																													
MG181320/RVA/Human-wt/MWI/BID1JK/2013/G2P[4] - Lineage G2 IV	99.1	97.7	73.3	73.3	99.5																																												
MG181914/RVA/Human-wt/MWI/BID15V/2012/G2P[4] - Lineage G2 IV	99.3	97.9	73.3	73.3	99.7	99.8																																											
LC477376/RVA/Human-wt/JPN/Tokyo18-42/2018/G2P[4] - Lineage G2 IV	98.5	98.7	73.5	73.5	98.9	98.4	98.6																																										
MN552097/RVA/Human-wt/RUS/Novosibirsk-NS17-A922/2017/G2P[4] - Lineage G2 IV	98.3	98.7	73.3	73.3	98.7	98.2	98.4	99.8																																									
KP007148/RVA/Human-wt/PHI/TGO12-003/2012/G2P[4] - Lineage G2 IV	98.5	98.9	73.6	73.6	98.9	98.4	98.6	99.6	99.6																																								
EU839925/RVA/Human-wt/BGD/MMC88/2005/G2P[4] - Lineage G2 IV	97.8	97.8	73.5	73.5	98.2	97.7	97.9	98.3	98.3	98.5																																							
MH382852/RVA/Human-wt/ETH/BD408/2016/G2P[4] - Lineage G2 IV	97.2	97.2	73.7	73.7	97.7	97.6	97.6	97.8	97.8	98.0	98.7																																						
MG926752/RVA/Human-wt/MOZ/0440/2013/G2P[4] - Lineage G2 IV	98.3	99.5	73.7	73.7	98.7	98.2	98.4	99.2	99.2	99.4	98.3	97.8																																					
MG891998/RVA/Human-wt/MOZ/0126/2013/G2P[4] - Lineage G2 IV	98.3	99.5	73.7	73.7	98.7	98.2	98.4	99.2	99.2	99.4	98.3	97.8	100.0																																				
KP752784/RVA/Human-wt/ZMB/MRC-DPRU1673/2009/G2P[4] - Lineage G2 IV	95.5	95.7	73.7	73.7	95.9	95.9	95.8	96.0	96.0	96.2	96.5	96.8	96.2																																				
KM660417/RVA/Human-wt/CMR/MA104/2011/G2P[4] - Lineage G2 IV	94.6	94.4	73.2	73.2	95.0	94.9	94.9	94.7	94.7	94.9	95.6	95.2	94.9	94.9	97.6																																				
KM008651/RVA/Human-wt/IND/KOL-17-08/2008/G2P[8] - Lineage G2 IV	94.9	93.8	73.4	73.4	95.2	94.8	95.0	94.5	94.3	94.5	94.2	93.8	94.3	94.3	92.1	91.2																																			
KF636283/RVA/Human-wt/ZAF/MRC-DPRU2052/2010/G1P[8] - Lineage G1 I	73.3	73.4	99.1	99.1	73.5	73.4	73.4	73.4	73.2	73.5	73.6	73.8	73.6	73.6	73.6	73.4	73.1	73.5																																		
KX638537/RVA/Human-wt/IND/RV1020/2010/G1P[X] - Lineage G1 I	73.5	73.4	98.3	98.3	73.7	73.6	73.6	73.6	73.4	73.7	73.8	74.0	73.6	73.6	73.4	73.1	73.7	98.6																																		
KX574268/RVA/Human-wt/IND/RV1310/2013/G2P[4] - Lineage G2 IV	98.2	99.0	73.7	73.7	98.6	98.3	98.5	99.1	99.1	99.3	98.2	97.9	99.5	99.5	96.5	95.0	94.4	73.6	73.8																																		
KX574261/RVA/Human-wt/IND/RV1206/2012/G2P[4] - Lineage G2 IV	99.5	98.1	73.5	73.5	99.9	99.4	99.6	98.8	98.6	98.8	98.1	97.6	98.6	98.6	95.8	94.9	95.1	73.6	73.8	98.5																																
DQ492674/RVA/Human-wt/BGD/Dhaka16/2003/G1P[8] - Lineage G1 I	73.2	73.1	97.9	97.9	73.4	73.3	73.3	73.3	73.1	73.4	73.5	73.7	73.3	73.3	73.5	73.2	73.4	98.6	98.8	73.5	73.5																																
MH171395/RVA/Human-wt/ESP/S5454877/2011/G1P[8] - Lineage G1 I	73.0	72.9	97.7	97.7	73.2	73.1	73.1	73.1	72.9	73.2	73.3	73.5	73.1	73.1	73.1	72.8	73.2	98.2	98.4	73.3	73.3	99.0																																
MN106111/RVA/Human-wt/CHN/ES365/2017/G1P[8] - Lineage G1 I	73.5	73.2	97.6	97.6	73.5	73.4	73.4	73.4	73.2	73.5	73.6	73.8	73.4	73.4	73.6	73.3	73.6	98.1	98.3	98.3																																
MG181496/RVA/Human-wt/MWI/BID11D/2012/G1P[8] - Lineage G1 I	73.7	73.2	96.9	96.9	73.9	73.6	73.8	73.6	73.4	73.7	73.8	73.8	73.6	73.6	73.4	73.0	74.0	97.2	97.7	73.8	74.0	97.4	97.4	97.0	97.1																														
KJ752243/RVA/Human-wt/ZMB/MRC-DPRU1648/2009/G1P[8] - Lineage G1 I	74.0	73.5	96.8	96.8	74.2	73.9	74.1	73.9	73.7	73.8	74.1	74.1	73.9	73.9	73.7	73.3	74.3	97.1	97.3	74.1	74.3	97.3	96.9	97.0	99.3																														
KP752676/RVA/Human-wt/SWZ/MRC-DPRU4550/2010/G1P[8] - Lineage G1 I	73.1	73.0	97.0	97.0	73.3	73.2	73.2	73.2	73.0	73.3	73.4	73.6	73.2	73.2	73.2	72.9	73.4	97.3	97.6	73.4	73.4	97.8	97.3	97.2	98.3	98.2																														
KJ752031/RVA/Human-wt/ETH/MRC-DPRU1843/2009/G1P[8] - Lineage G1 II	72.9	73.0	93.2	93.2	73.1	73.0	73.0	73.2	73.0	73.3	73.4	73.6	73.3	73.3	73.4	73.2	73.4	93.1	93.8	73.5	73.2	93.6	93.4	93.1	94.1	93.8	93.6																														
KJ752243/RVA/Human-wt/ZMB/MRC-DPRU1648/2009/G1P[8] - Lineage G1 I	74.0	73.5	96.8	96.8	74.2	73.9	74.1	73.9	73.7	73.8	74.1	74.1	73.9	73.9	73.7	73.3	74.3	97.1	97.3	74.1	74.3	97.3	96.9	97.0	99.3																														
KP752676/RVA/Human-wt/SWZ/MRC-DPRU4550/2010/G1P[8] - Lineage G1 I	73.1	73.0	97.0	97.0	73.3	73.2	73.2	73.2	73.0	73.3	73.4	73.6	73.2	73.2	73.2	72.9	73.4	97.3	97.6	73.4	73.4	97.8	97.3	97.2	98.3	98.2																														
MN632903/RVA/Human-wt/RWA/UFS-NGS-MRC-DPRU442/2012/G1P[8] - Lineage G1 I	72.8	73.1	91.1	91.1	73.0	72.9	72.9	73.1	73.0	73.3	73.4	73.6	73.3	73.3	73.2	73.3	73.6	91.2	91.5	73.5	73.1	91.3	91.1	91.4	91.0	90.9	90.7	92.6	93.0	93.4	93.6	92.3	99.3																														
GU565057/RVA/Vaccine/USA/RotaTeq-W179-9/1992/G1P[5] - Lineage G1 III	72.8	73.1	91.1	91.1	73.0	72.9	72.9	73.1	73.0	73.3	73.4	73.6	73.3	73.3	73.2	73.3	73.6	91.2	91.5	73.5	73.1	91.3	91.1	91.4	91.0	90.9	90.7	92.6	93.0	93.4	93.6	92.3	99.3	100.0																													
U26378/RVA/Human-wt/KOR/Kor-64/1988/G1P[X] - Lineage G1 IV	73.5	73.6	92.3	92.3	73.7	73.6	73.6	73.6	73.6	73.7	74.0	74.3	73.9	73.9	73.4	73.1	74.0	92.2	93.0	74.1	73.8	92.1	91.9	91.8	92.4	92.3	92.1	94.3	94.5	94.7	94.9	93.9	93.0	92.7	92.7																													
AB081793/RVA/Human-wt/JPN/87Y1397/xxxx/G1P[8] - Lineage G1 IV	72.9	73.0	92.7	92.7	73.1	73.0	73.0	73.0	73.0	73.1	73.4	73.7	73.3	73.3	73.0	72.7	73.3	92.6	93.3	73.5	73.2	92.4	92.2	92.1	92.8	92.7	92.4	94.6	94.6	95.0	95.2	94.2	93.2	92.7	92.7	99.1																													
DQ377572/RVA/Human-wt/ITA/PA78-89/1989/G1P[8] - Lineage G1 V	73.8	74.1	93.6	93.6	74.1	73.9	73.9	73.9	73.9	74.1	74.3	74.5	74.3	74.3	74.3	73.9	74.5	94.4	94.5	74.2	94.0	93.8	93.6	94.2	93.9	93.7	96.4	96.4	97.0	97.3	96.0	94.6	94.2	94.2	96.4	96.8																													
AB018697/RVA/Human-wt/JPN/AU19/xxxx/G1P[X] - Lineage G1 VI	72.2	72.3	85.6	85.6	72.4	72.4	72.3	72.6	72.3	72.4	72.8	73.4	72.7	72.7	73.5	73.0	73.0	85.8	86.2	72.9	72.6	86.0	85.6	85.9	85.5	85.3	85.1	87.3	87.1	87.1	87.0	86.8	86.7	86.7	87.1	87.0	87.1																													
M92651/RVA/Bovine-wt/XXX/T449/xxxx/G1P[X] - Lineage G1 VII	71.8	71.8	84.2	84.2	71.7	71.8	71.8	71.6	71.6	71.7	72.0	72.1	71.9	71.9	72.2	72.4	71.7	84.2	84.8	72.2	71.8	84.7	84.5	84.4	84.7	85.0	84.6	85.1	84.8	84.8	85.0	85.1	84.4	84.1	84.6	84.9	84.9	85.3																												
L24164/RVA/Pig-tc/VEN/C60/xxxx/G1P[X] - Lineage G1 VII	72.2	72.2	84.3	84.3	72.1	72.3	72.2	72.0	72.0	72.1	72.4	72.6	72.3	72.3	73.0	73.1	72.2	84.3	84.9	72.7	72.2	84.8	84.4	84.5	85.0	85.3	84.9	85.6	85.1	85.2	85.4	85.4	84.2	83.9	83.9	84.8	85.1	85.3	85.5	96.4																												
JF304920/RVA/Human-tc/KEN/D205/1989/G2P[4] - Lineage G2 II	91.0	91.4	75.0	75.0	91.4	91.1	91.3	91.5	91.3	91.3	91.8	91.9	91.5	91.5	92.0	91.1	89.1	74.7	74.8	91.8	91.3	74.5	74.3	74.8	74.4	74.7	74.8	74.4	74.1	74.2	74.3	74.6	74.1	74.0	74.0	74.3	74.1	74.4	74.1	73.3	73.2																													
JF304931/RVA/Human-tc/KEN/AK26/1982/G2P[4] - Lineage G2 II	92.1	92.6	74.0	74.0	92.6	92.4	92.7	92.4	92.2	92.4	93.0	92.7	92.7	93.0	92.7	89.5	74.1	74.0	93.0	92.4	73.9	73.7	74.2	73.6	73.9	74.0	73.8	73.7	73.9	73.9	74.2	74.1	74.0	74.0	73.9	73.7	74.4	74.3	74.0	72.9	96.5																												
GU565068/RVA/Vaccine/USA/RotaTeq-SC2-9/1992/G2P[5] - Lineage G2 II	92.4	92.9	74.2	74.2	92.9	92.6	92.8	92.8	92.6	92.8	92.9	93.0	93.0	93.0	93.3	92.7	89.7	74.3	74.2	93.3	92.8	74.1	73.9	74.4	73.8	74.1	74.2	74.1	73.8	74.2	74.2	74.3	74.0	73.9	73.9	74.2	74.0	74.5	73.9	73.1	73.1	97.0	98.3																												
HQ650124/RVA/Human-tc/USA/DS-1/1976/G2P[4] - Lineage G2 I	92.7	93.2	73.1	73.1	93.1	93.1	93.2	93.0	93.0	93.2	93.7	93.0	93.4	93.4	93.7	93.4	89.6	73.0	73.0	93.7	93.2	72.9	72.4	73.0	72.6	72.9	72.8	73.3	73.0	73.0	73.2	73.4	74.0	73.7	73.7	73.3	72.9	73.7	73.5	73.6	73.8	92.7	94.3	94.0																											
AY261335/RVA/Human-tc/ZAF/410GR-85/1985/G2P[4] - Lineage G2 I	92.0	92.4	72.9	72.9	92.4	92.1	92.3	92.3	92.3	92.6	93.1	92.3	92.8	92.8	92.9	92.6	89.0	72.9	72.9	93.1	92.6	72.8	72.3	73.1	72.4	72.8	72.6	73.4	73.1	73.1	73.3	73.5	73.7	73.6	73.6	73.2	72.8	73.7	73.8	73.8	74.0	91.9	93.1	93.3	97.8																											
AY261338/RVA/Human-wt/ZAF/141GR-87/1987/G2P[4] - Lineage G2 I	92.0	92.4	73.0	73.0	92.4	92.1	92.3	92.3	92.3	92.6	93.1	92.3	92.8	92.8	92.9	92.6	89.0	72.9	72.9	93.1	92.6	72.8	72.3	73.1	72.4	72.8	72.6	73.4	73.1	73.1	73.3	73.5	73.7	73.6	73.6	73.2	72.8	73.7	73.8	73.8	74.0	91.9	93.1	93.3	97.8	99.4																											
DS0127/RVA/Human-wt/JPN/TMC-IV/1980/G2P[4] - Lineage G2 III	93.6	94.0	73.5	73.5	94.0	93.7	93.9	94.1	94.1	94.1	94.8	94.5	94.3	94.3	95.4	94.9	90.4	73.7	73.4	94.4	93.9	73.3	72.9	73.4	73.0	73.3	73.0	73.2	73.4	74.0	73.6	73.3	73.3	73.7	73.5	73.9	73.4	72.8	73.2	93.8	94.7	94.9	94.8	94.4	94.4																											
KC443205/RVA/Human-wt/AUS/CK20055/2010/G2P[4] - Lineage G2 V	92.6	92.7	73.3	73.3	92.8	92.3	92.6	92.4	92.4	92.7	93.0	92.8	93.0	93.0	93.3	92.8	89.1	73.4	73.0	92.9	92.9	73.1	72.7	73.2	73.0	73.1	72.8	73.3	73.2	73.0	73.2	74.0	73.6	73.5	73.5	72.7	72.3	73.5	73.1	72.6	72.9	92.0	93.2	93.1	93.3	92.4	92.4	94.2	
KC443460/RVA/Human-wt/AUS/CK20048/2011/G2P[4] - Lineage G2 V	92.7	92.8	73.4	73.4	92.9	92.4	92.7	92.6	92.6	92.8	93.1	92.9	93.1	93.1	93.4	92.9	89.2	73.5	73.1	93.0	93.0	73.2	72.8	73.3	73.1	73.2	72.9	73.4	73.3	73.1	73.3	74.1	73.7	73.6	73.6	72.8	72.4	73.5	73.2	72.7	73.0	92.1	93.3	93.2	93.4	92.6	92.6	94.3	99.9	
LC433790/RVA/Human-wt/NPL/TK1797/2007/G9P[19] - outgroup	74.1	73.6	76.1	76.1	74.1	73.9	74.0	73.9	73.7	73.7	74.3	74.2	73.8	73.8	75.2	75.2	74.1	76.0	76.2	74.1	74.1	76.5	76.4	76.7	75.9	76.0	76.1	77.0	76.3	76.5	76.8	76.8	75.9	75.9	75.9	76.4	76.3	75.9	75.6	74.0	74.7	75.2	74.8	75.5	73.9	73.8	73.7	74.1	73.8	73.9	

b.

VP7 amino acid identities among strains calculated using the p-distance algorithm in MEGA 6 (Tamura et al., 2013)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4749/2014/G2P[8] - Lineage G2 IV																																																		
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13327/2016/G2P[4] - Lineage G2 IV	98.5																																																	
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13232/2016/G1P[8] - Lineage G1 I	74.5	74.8																																																
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13541/2016/G1P[8] - Lineage G1 I	74.5	74.8	100.0																																														
LC086796/RVA/Human-wt/THA/SKT-138/2013/G2P[4] - Lineage G2 IV	100.0	98.5	74.5	74.5																																													
MG181320/RVA/Human-wt/MWI/BID1JK/2013/G2P[4] - Lineage G2 IV	99.4	97.9	73.9	73.9	99.4																																												
MG181914/RVA/Human-wt/MWI/BID15V/2012/G2P[4] - Lineage G2 IV	99.7	98.2	74.2	74.2	99.7	99.7																																											
LC477376/RVA/Human-wt/JPN/Tokyo18-42/2018/G2P[4] - Lineage G2 IV	99.4	99.1	74.8	74.8	99.4	98.8	99.1																																										
MN552097/RVA/Human-wt/RUS/Novosibirsk-NS17-A922/2017/G2P[4] - Lineage G2 IV	99.1	98.8	74.5	74.5	99.1	98.5	98.8	99.7																																									
KP007148/RVA/Human-wt/PHI/TGO12-003/2012/G2P[4] - Lineage G2 IV	99.4	99.1	74.8	74.8	99.4	98.8	99.1	100.0	99.7																																								
EU839925/RVA/Human-wt/BGD/MMC88/2005/G2P[4] - Lineage G2 IV	99.1	98.2	74.8	74.8	99.1	98.5	98.8	99.7	99.4	99.7																																							
MH382852/RVA/Human-wt/ETH/BD408/2016/G2P[4] - Lineage G2 IV	98.2	97.2	75.2	75.2	98.2	97.5	97.9	98.2	97.9	98.2	98.5																																						
MG926752/RVA/Human-wt/MOZ/0440/2013/G2P[4] - Lineage G2 IV	99.1	99.4	75.2	75.2	99.1	98.5	98.8	99.7	99.4	99.7	98.8	97.9																																						
MG891998/RVA/Human-wt/MOZ/0126/2013/G2P[4] - Lineage G2 IV	99.1	99.4	75.2	75.2	99.1	98.5	98.8	99.7	99.4	99.7	98.8	97.9	100.0																																						
KP752784/RVA/Human-wt/ZMB/MRC-DPRU1673/2009/G2P[4] - Lineage G2 IV	98.8	97.9	74.2	74.2	98.8	98.2	98.5	98.8	98.5	98.8	98.5	97.5	98.5																																						
KM660417/RVA/Human-wt/CMR/MA104/2011/G2P[4] - Lineage G2 IV	97.2	96.3	73.9	73.9	97.2	96.9	96.9	97.2	96.9	97.2	96.9	96.0	96.9	96.9	97.9																																						
KM008651/RVA/Human-wt/IND/KOL-17-08/2008/G2P[8] - Lineage G2 IV	94.2	92.6	75.8	75.8	94.2	93.6	93.9	93.6	93.3	93.6	93.3	92.6	93.3	93.3	92.9	91.4																																					
KF636283/RVA/Human-wt/ZAF/MRC-DPRU2052/2010/G1P[8] - Lineage G1 I	74.5	74.8	99.7	99.7	74.5	73.9	74.2	74.8	74.5	74.8	74.8	75.2	75.2	75.2	74.2	73.9	75.8																																					
KX638537/RVA/Human-wt/IND/RV1020/2010/G1P[X] - Lineage G1 I	74.5	74.2	99.1	99.1	74.5	73.9	74.2	74.8	74.5	74.8	74.8	75.2	74.5	74.5	74.2	73.9	75.8	99.4																																				
KX574268/RVA/Human-wt/IND/RV1310/2013/G2P[4] - Lineage G2 IV	99.4	99.1	74.8	74.8	99.4	98.8	99.1	100.0	99.7	100.0	99.1	98.2	99.7	99.7	98.8	97.2	93.6	74.8	74.8																																				
KX574261/RVA/Human-wt/IND/RV1206/2012/G2P[4] - Lineage G2 IV	100.0	98.5	74.5	74.5	100.0	99.4	99.7	99.4	99.1	99.4	99.1	98.2	99.1	99.1	98.8	97.2	94.2	74.5	74.5	99.4																																				
DQ492674/RVA/Human-wt/BGD/Dhaka16/2003/G1P[8] - Lineage G1 I	74.8	74.5	98.8	98.8	74.8	74.2	74.5	75.2	74.8	75.2	75.2	75.5	74.8	74.8	74.5	74.2	76.1	99.1	99.1	75.2	74.8																																				
MH171395/RVA/Human-wt/ESP/S5454877/2011/G1P[8] - Lineage G1 I	73.9	73.6	98.5	98.5	73.9	73.3	73.6	74.2	73.9	74.2	74.2	74.5	73.9	73.9	73.6	73.3	75.2	98.8	98.8	74.2	73.9	98.5																																				
MN106111/RVA/Human-wt/CHN/ES365/2017/G1P[8] - Lineage G1 I	74.5	74.2	98.8	98.8	74.5	73.9	74.2	74.8	74.5	74.8	74.8	75.2	74.5	74.5	74.2	73.9	75.8	98.5	98.5	74.8	74.5	98.2	97.9																																				
MG181496/RVA/Human-wt/MWI/BID11D/2012/G1P[8] - Lineage G1 I	74.5	73.6	97.9	97.9	74.5	73.9	74.2	74.2	73.9	74.2	74.2	74.5	73.9	73.9	73.6	73.3	75.8	98.2	98.2	74.2	74.5	97.9	97.5	97.9																																				
KJ752243/RVA/Human-wt/ZMB/MRC-DPRU1648/2009/G1P[8] - Lineage G1 I	74.8	73.9	97.5	97.5	74.8	74.2	74.5	74.5	74.2	74.5	74.5	74.8	74.2	74.2	73.9	73.6	76.1	97.9	97.9	74.5	74.8	97.5	97.2	97.5	99.7																																				
KP752676/RVA/Human-wt/SWZ/MRC-DPRU4550/2010/G1P[8] - Lineage G1 I	74.5	74.2	98.2	98.2	74.5

Appendix 17c-d: Nucleotide and amino acid identities for the VP4 of the four Zambian reassortants

c.

VP4 nucleotide identites among strains calculated using the p-distance algorithm in MEGA 6 (Tamura et al., 2013)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4749/2014/G2P[8] - Divergent																																																
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13327/2016/G2P[4] - Lineage P[4] IV	82.7																																														
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13232/2016/G1P[8] - Lineage P[8] III	90.3	86.9																																												
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13541/2016/G1P[8] - Lineage P[8] III	90.2	86.9	99.8																																											
KF636281/RVA/Human-wt/ZAF/MRC-DPRU2052/2010/G1P[8] - Lineage P[8] III	90.1	86.8	99.0	99.0																																									
KF636237/RVA/Human-wt/ZAF/MRC-DPRU2035/2010/G1P[8] - Lineage P[8] III	90.1	86.8	99.0	99.0	100.0																																								
KJ753218/RVA/Human-wt/ZAF/MRC-DPRU1327/2007/G1P[8] - Lineage P[8] III	89.8	86.8	98.8	98.8	99.4	99.4																																								
KJ753295/RVA/Human-wt/ZWE/MRC-DPRU1844-11/2011/G1P[8] - Lineage P[8] III	89.8	86.9	98.7	98.7	99.3	99.3	99.5																																							
KM660353/RVA/Human-wt/CMR/MA16/2010/G1P[8] - Lineage P[8] III	89.6	86.8	97.6	97.6	98.3	98.3	98.8	98.6																																							
KJ753299/RVA/Human-wt/TGO/MRC-DPRU5171/2010/G12P[8] - Lineage P[8] III	89.6	86.9	97.6	97.6	98.2	98.2	98.7	98.5	99.5																																					
DQ146652/RVA/Human-wt/BGD/Dhaka25/2002/G12P[8] - Lineage P[8] III	89.7	87.0	98.3	98.3	99.0	99.0	99.5	99.2	99.1	99.0																																				
JQ069697/RVA/Human-wt/CAN/RT063-09/2009/G1P[8] - Lineage P[8] III	89.6	86.8	97.7	97.7	98.4	98.4	98.9	98.7	99.5	99.4	99.2																																			
MG926750/RVA/Human-wt/MOZ/0440/2013/G2P[4] - Lineage P[4] IV	82.6	99.6	86.7	86.8	86.8	86.8	86.8	86.9	86.8	86.9	87.0	86.8																																		
KX646628/RVA/Human-wt/IND/RV1310/2013/G9[P[4] - Lineage P[4] IV	82.7	99.4	86.8	86.9	86.9	86.9	86.9	87.1	86.9	87.0	87.2	87.0	99.6																																	
KX646625/RVA/Human-wt/IND/RV1307/2013/G9[P[4] - Lineage P[4] IV	82.7	99.4	86.8	86.9	86.9	87.0	86.9	87.1	86.9	87.1	87.2	87.1	99.6	100.0																																	
KP007171/RVA/Human-wt/PHI/TGO12-007/2012/G2P[4] - Lineage P[4] IV	82.5	99.3	86.7	86.8	86.8	86.9	86.8	87.0	86.9	87.0	87.0	86.9	99.5	99.6	99.6																																	
JX965125/RVA/Human-wt/AUS/WAPC703/2010/G2P[4] - Lineage P[4] IV	82.6	99.1	86.6	86.7	86.7	86.8	86.7	86.9	86.8	86.9	86.9	86.9	99.3	99.4	99.4	99.6																																	
HQ641373/RVA/Human-wt/BGD/MMCB8/2005/G2P[4] - Lineage P[4] IV	82.8	99.0	86.7	86.8	86.9	87.0	86.9	87.1	87.0	87.2	87.2	87.1	99.2	99.3	99.3	99.4	99.3																																
MG181824/RVA/Human-wt/MWI/BID11E/2012/G2P[4] - Lineage P[4] IV	82.6	98.2	86.6	86.7	86.8	86.9	86.8	87.1	87.1	87.2	87.0	87.0	98.3	98.5	98.4	98.6	98.5	99.0																																
MG181912/RVA/Human-wt/MWI/BID15V/2012/G2P[4] - Lineage P[4] IV	82.6	98.1	86.6	86.7	86.9	86.9	86.9	87.1	87.1	87.2	87.1	87.1	98.3	98.4	98.4	98.5	98.5	98.9	99.9																																
MG652353/RVA/Human-wt/DOM/3000503730/2016/G2P[4] - Lineage P[4] IV	82.8	97.8	86.5	86.6	86.7	86.8	86.7	87.0	87.0	87.1	86.9	86.9	98.0	98.1	98.1	98.2	98.2	98.6	99.3	99.3																																
KP752663/RVA/Human-wt/MUS/MRC-DPRU295/2012/G2P[4] - Lineage P[4] IV	82.7	98.2	86.9	86.9	87.1	87.1	87.1	87.3	87.3	87.5	87.3	87.3	98.4	98.5	98.5	98.7	98.5	98.9	99.3	99.2	98.9																															
JN849119/RVA/Human-wt/BEL/BE0253/2008/G1P[8] - Lineage P[8] I	84.7	86.7	89.9	90.0	90.1	90.2	90.4	90.3	89.9	90.1	90.3	90.0	86.8	86.9	87.0	86.9	86.7	87.0	86.9	86.9	86.9	87.0																														
KJ752795/RVA/Human-wt/ETH/MRC-DPRU1840/2007/G1P[8] - Lineage P[8] IV	83.4	85.0	88.0	88.1	88.1	88.1	88.3	88.3	88.1	88.4	88.4	88.2	85.1	85.1	85.2	85.0	84.8	85.2	85.3	85.3	85.3	85.1	85.3	88.7																								
JX156397/RVA/Human-wt/RUS/Novosibirsk/Nov11-N2246/2011/G2P[8] - Lineage P[8] III	87.8	86.7	95.0	95.0	95.4	95.5	96.0	95.8	96.0	96.0	96.3	96.1	86.7	86.9	87.0	86.8	86.8	86.9	86.8	86.8	86.9	87.1	90.6	88.4																								
KX646625/RVA/Human-wt/IND/RV1307/2013/G9[P[4] - Lineage P[4] IV	82.7	99.4	86.8	86.9	86.9	86.9	86.9	87.1	86.9	87.1	87.2	87.1	99.6	100.0																																	
JN258909/RVA/Human-wt/PHI/TGO12-016/2012/G1P[8] - Lineage P[8] III	89.1	87.1	97.1	97.1	97.8	97.8	98.2	97.9	98.3	98.5	98.4	97.1	87.3	87.4	87.2	87.1	87.3	87.2	87.3	87.2	87.3	87.2	87.6	89.8	88.4	95.9	96.1																				
KJ560500/RVA/Human-wt/USA/CNMC101/2011/G12P[8] - Lineage P[8] III	89.4	86.9	97.6	97.6	98.2	98.3	98.7	98.5	99.1	99.0	99.0	99.3	86.9	87.1	87.1	87.0	86.9	87.1	87.1	87.1	87.0	87.3	89.9	88.3	96.0	96.4	98.2																				
LC260224/RVA/Human-wt/IDN/SOEP075/2016/G3P[8] - Lineage P[8] IV	83.6	85.3	88.0	88.1	88.2	88.2	88.4	88.4	88.3	88.6	88.5	88.5	85.3	85.3	85.4	85.3	85.0	85.4	85.3	85.3	85.2	85.3	89.0	98.1	88.7	88.9	88.5	88.6																				
JN129087/RVA/Human-wt/NCA/22/2010/G1P[8] - Lineage P[8] III	89.0	86.8	97.2	97.2	97.8	97.8	98.2	98.1	98.3	98.3	98.5	98.5	86.8	87.0	87.0	86.8	86.7	86.9	86.7	86.8	86.7	87.2	89.8	87.9	95.8	96.1	97.9	98.2	88.3																			
KT920995/RVA/Human-wt/IND/VRL0040/2013/G1P[8] - Lineage P[8] III	89.5	86.8	98.0	98.0	98.6	98.7	99.1	99.0	99.1	99.1	99.5	99.3	86.8	87.0	87.1	86.9	86.8	87.0	86.9	87.0	86.9	87.2	90.2	88.2	96.1	96.6	98.5	99.1	88.5	98.7																		
LC086739/RVA/Human-wt/THA/LS-04/2013/G2P[8] - Lineage P[8] III	89.2	86.7	97.3	97.3	97.9	97.9	98.3	98.1	98.4	98.4	98.5	98.5	86.7	86.9	86.9	86.7	86.6	86.8	86.9	86.9	86.7	87.1	89.8	88.2	95.8	96.0	99.1	98.4	88.4	97.9	98.6																	
KF716328/RVA/Human-wt/USA/VU10-11-6/2011/G2P[4] - Lineage P[4] IV	82.7	98.5	86.7	86.8	86.9	87.0	86.9	87.1	86.9	87.1	87.2	87.0	98.7	98.8	98.8	99.0	98.8	99.3	98.6	98.5	98.2	98.6	87.1	85.5	86.8	87.0	87.2	87.0	85.5	86.9	87.0	86.7																	
LC086772/RVA/Human-wt/THA/BD-20/2013/G2P[4] - Lineage P[4] IV	82.8	97.1	87.2	87.2	87.2	87.2	87.2	87.3	87.2	87.3	87.3	87.2	97.2	97.4	97.3	97.5	97.3	97.9	97.3	97.3	97.3	97.0	97.3	86.7	85.6	86.9	87.1	87.5	87.3	85.6	87.1	87.2	87.0	97.6																
LC215252/RVA/Human-wt/VNM/SP127/2013/G1P[4] - Lineage P[4] IV	82.9	97.2	86.9	86.9	87.1	87.0	87.0	87.1	86.8	86.9	87.0	86.9	97.4	97.5	97.5	97.6	97.4	98.0	97.4	97.3	97.1	97.3	86.9	85.2	86.5	86.8	87.2	86.9	85.3	86.7	86.8	86.6	97.5	98.0															
KP752782/RVA/Human-wt/ZMB/MRC-DPRU1673/2009/G2P[4] - Lineage P[4] IV	82.7	97.1	87.0	87.1	87.1	87.2	87.0	87.2	86.9	87.1	87.1	87.0	97.3	97.4	97.4	97.6	97.4	97.9	97.2	97.2	97.1	97.2	86.8	84.7	86.6	86.9	87.1	87.0	84.8	86.9	86.9	86.8	97.5	97.2	97.2														
KC443326/RVA/Human-wt/AUS/CK20030/2006/G2P[4] - Lineage P[4] IV	82.8	97.5	87.0	87.1	87.2	87.2	87.2	87.2	87.2	87.3	87.3	87.2	97.7	97.8	97.8	97.9	97.8	98.3	97.8	97.7	97.4	97.7	86.6	85.4	86.8	87.0	87.5	87.2	85.5	87.1	87.1	87.0	97.8	99.3	98.3	97.5														
JQ069668/RVA/Human-wt/CAN/RT128-07/2008/G2P[4] - Lineage P[4] IV	82.8	98.9	86.8	86.9	87.0	87.0	87.0	87.2	87.1	87.2	87.2	87.1	99.1	99.2	99.2	99.4	99.2	99.7	99.1	99.0	98.7	99.1	87.0	85.1	87.0	87.3	87.3	87.2	85.3	86.9	87.0	86.8	99.3	97.9	98.0	97.9	98.3												
HQ650119/RVA/Human-tc/USA/DS-1/1976/G2P[4] - Lineage P[4] I	82.8	93.8	86.9	87.0	87.0	87.1	87.3	87.2	87.2	87.2	87.2	87.2	94.0	94.3	94.2	94.1	93.9	94.3	93.8	93.8	93.6	93.9	87.1	85.5	87.2	87.4	87.3	87.3	85.7	87.3	87.2	86.9	94.4	94.1	93.9	94.3	94.2	94.3											
JF304918/RVA/Human-tc/KEN/D205/1989/G2P[4] - Lineage P[4] II	82.6	93.2	86.4	86.5	86.5	86.5	86.6	86.7	86.5	86.7	86.7	86.6	93.4	93.6	93.6	93.5	93.3	93.9	93.5	93.5	93.1	93.3	86.8	85.3	86.6	86.7	86.6	86.8	85.4	86.5	86.7	86.5	93.7	93.8	93.3	93.6	93.7	93.7	95.0										
JF304929/RVA/Human-tc/KEN/AK26/1982/G2P[4] - Lineage P[4] II	82.9	93.9	87.0	87.1	87.2	87.1	87.2	87.3	87.2	87.4	87.4	87.3	94.1	94.4	94.3	94.1	94.0	94.5	94.0	94.0	93.8	94.1	87.2	85.5	87.6	87.7	87.3	87.2	85.6	87.2	87.4	87.0	94.4	94.1	94.1	94.0	94.0	94.4	95.3	96.2							
KT694942/RVA/Human-wt/USA/Wa/1974/G1P[8] - Lineage P[8] I	85.3	87.1	90.5	90.6	90.7	90.8	91.0	90.9	90.5	90.8	90.9	90.7	87.2	87.3	87.3	87.2	86.9	87.3	87.2	87.2	87.2	87.3	97.9	89.3	91.5	91.4	90.5	90.6	89.5	90.5	90.9	90.5	87.5	86.9	87.0	87.2	86.9	87.3	87.7	87.4	87.7						
EF672619/RVA/Human-tc/USA/WI61/1983/G9P[8] - Lineage P[8] II	86.5	87.2	92.1	92.2	92.6	92.6	92.7	92.8	92.6	92.7	92.9	92.7	87.2	87.4	87.5	87.2	87.1	87.4	87.3	87.3	87.4	87.5	90.4	89.0	93.5	93.4	92.7	92.9	89.1	92.7	93.1	92.8	87.5	87.3	87.1	87.1	87.2	87.4	87.4	86.4	87.2	91.2						
LC438382/RVA/Human-tc/JPN/KU/1974/G1P[8] - Lineage P[8] II	87.2	87.3	93.3	93.3	93.7	93.7	94.0	94.0	93.9	93.9	94.1	93.9	87.3	87.4	87.5	87.3	87.1	87.3	87.1	87.1	87.1	87.3	91.3	88.8	94.2	94.4	93.6	93.9	89.0	93.7	94.2	93.8	87.4	87.4	87.3	87.1	87.2	87.3	87.2	86.7	87.6	92.2	96.2					
KP902533/RVA/Human-wt/MWI/OPS30/1999/G4P[8] - Lineage P[8] IV	83.6	85.4	88.1	88.2	88.3	88.2	88.4	88.4	88.2	88.5	88.5	88.4	85.6	85.6	85.6	85.5	85.3	85.7	85.6	85.6	85.5	85.7	89.3	97.9	88.5	88.7	88.4	88.3	97.7	88.2	88.4	88.2	85.8	85.8	85.8	85.1	85.6	85.6	86.1	85.9	86.1	90.2	89.3	88.9				
FJ947211/RVA/Human-wt/USA/DC23/1976/G3P[8] - Lineage P[8] I	85.3	86.9	90.2	90.3	90.4	90.5	90.7	90.6	90.2	90.5	90.6	90.4	86.9	87.1	87.1	87.0	86.7	87.1	87.0	87.0	87.0	87.1	97.8	88.9	91.2	91.1	90.2	90.3	89.2	90.2	90.6	90.2	87.3	86.7	86.8	86.9	86.7	87.1	87.4	87.2	87.4	99.4	91.0	92.0	89.9				
JN849113/RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P[8] - Lineage P[8] I	84.9	86.9	90.3	90.4	90.5	90.5	90.7	90.6	90.3	90.5	90.7	90.4	87.0	87.1	87.1	87.0	86.8	87.1	86.9	87.0	87.0	87.1	98.3	89.0	91.1	91.0	90.2	90.4	89.1	90.3	90.6	90.2	87.3	86.8	87.0	87.0	86.8	87.1	87.5	87.1	87.4	98.9	90.8	91.7	89.8	98.6			
LC433788/RVA/Human-wt/NPL/TK1797/2007/G9P[19] - outgroup	76.9	77.0	77.1	77.2	77.4	77.4	77.7	77.4	77.6	77.6	77.5	77.6	77.1	77.1	77.1	77.0	76.8	77.3	76.9	76.9	77.1	77.0	77.5	76.6	77.3	77.4	77.8	77.6	77.1	77.7	77.7	77.6	77.1	77.0	77.1	77.1	76.9	77.1	76.8	77.0	76.8	77.9	77.4	77.8	76.8	77.8	77.8	77.8

d.

VP4 amino acid identities among strains calculated using the p-distance algorithm in MEGA 6 (Tamura et al., 2013)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4749/2014/G2P[8] - Divergent																																															
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13327/2016/G2P[4] - Lineage P[4] IV	85.9																																														
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13232/2016/G1P[8] - Lineage P[8] III	93.3	90.5																																												
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13541/2016/G1P[8] - Lineage P[8] III	93.3	90.6	99.9																																											
KF636281/RVA/Human-wt/ZAF/MRC-DPRU2052/2010/G1P[8] - Lineage P[8] III	92.8	90.7	99.1	99.2																																										
KF636237/RVA/Human-wt/ZAF/MRC-DPRU2035/2010/G1P[8] - Lineage P[8] III	92.8	90.7	99.1	99.2	100.0																																									
KJ753218/RVA/Human-wt/ZAF/MRC-DPRU1327/2007/G1P[8] - Lineage P[8] III	92.8	90.8	99.1	99.2	99.7	99.7																																								
KJ753295/RVA/Human-wt/ZWE/MRC-DPRU1844-11/2011/G1P[8] - Lineage P[8] III	92.6	90.7	99.0	99.1	99.6	99.6	99.9																																							
KM660353/RVA/Human-wt/CMR/MA16/2010/G1P[8] - Lineage P[8] III	92.2	91.0	98.6	98.7	99.2	99.2	99.5	99.4																																						
KJ753299/RVA/Human-wt/TGO/MRC-DPRU5171/2010/G12P[8] - Lineage P[8] III	92.4	91.1	98.7	98.8	99.4	99.4	99.6	99.5	99.6																																					
DQ146652/RVA/Human-wt/BGD/Dhaka25/2002/G12P[8] - Lineage P[8] III	92.4	91.0	98.7	98.8	99.4	99.4	99.6	99.5	99.6	99.7																																				
JQ069697/RVA/Human-wt/CAN/RT063-09/2009/G1P[8] - Lineage P[8] III	92.2	91.1	98.6	98.7	99.2	99.2	99.5	99.4	99.5	99.6	99.6																																			
MG926750/RVA/Human-wt/MOZ/0440/2013/G2P[4] - Lineage P[4] IV	85.9	99.2	90.6	90.7	91.0	91.0	91.1	91.0	91.2	91.4	91.2	91.4																																		
KX646628/RVA/Human-wt/IND/RV1310/2013/G9[P[4] - Lineage P[4] IV	85.8	99.1	90.6	90.7	91.2	91.2	91.4	91.2	91.5	91.6	91.5	91.6	99.4																																		
KX646625/RVA/Human-wt/IND/RV1307/2013/G9[P[4] - Lineage P[4] IV	85.6	99.0	90.6	90.7	91.2	91.2	91.4	91.2	91.5	91.6	91.5	91.6	99.2	99.9																																		
KP007171/RVA/Human-wt/PHI/TGO12-007/2012/G2P[4] - Lineage P[4] IV	85.8	99.2	90.6	90.7	91.2	91.2	91.4	91.2	91.5	91.6	91.5	91.6	99.5	99.5																																		
JX965125/RVA/Human-wt/AUS/WAPC703/2010/G2P[4] - Lineage P[4] IV	85.6	99.1	90.5	90.6	91.1	91.1	91.2	91.1	91.4	91.5	91.4	91.5	99.4	99.5	99.4	99.6																																		
HQ641373/RVA/Human-wt/BGD/MMCB8/2005/G2P[4] - Lineage P[4] IV	86.0	99.2	90.8	91.0	91.5	91.5	91.6	91.5	91.7	91.9	91.7	91.9	99.5	99.6	99.5	99.7	99.6																																		
MG181824/RVA/Human-wt/MWI/BID11E/2012/G2P[4] - Lineage P[4] IV	85.8	98.8	90.6	90.7	91.2	91.2	91.4	91.2	91.5	91.6	91.5	91.6	99.1	99.2	99.1	99.4	99.2	99.6																																		
MG181912/RVA/Human-wt/MWI/BID15V/2012/G2P[4] - Lineage P[4] IV	85.8	98.6	90.6	90.7	91.2	91.2	91.4	91.2	91.5	91.6	91.5	91.6	98.8	99.0	98.8	99.1	99.0	99.4	99.7																																		
MG652353/RVA/Human-wt/DOM/3000503730/2016/G2P[4] - Lineage P[4] IV	86.2	98.7	90.5	90.6	91.1	91.1	91.2	91.1	91.4	91.5	91.4	91.5	99.0	99.1	99.0	99.2	99.1	99.5	99.6	99.4																																		
KP752663/RVA/Human-wt/MUS/MRC-DPRU295/2012/G2P[4] - Lineage P[4] IV	85.9	99.0	90.7	90.8	91.4	91.4	91.5	91.4	91.6	91.7	91.6	91.7	99.2	99.4	99.2	99.5	99.4	99.7	99.9	99.6	99.7																																		
JN849119/RVA/Human-wt/BEL/BE0253/2008/G1P[8] - Lineage P[8] I	89.0	89.2	93.9	94.1	94.6	94.6	94.7	94.6	94.6	94.7	94.6	94.5	89.4	89.7	89.7	89.7	89.5	89.9	89.7	89.7	90.1	89.8																																		
KJ752795/RVA/Human-wt/ETH/MRC-DPRU1840/2007/G1P[8] - Lineage P[8] IV	87.1	88.5	91.6	91.7	92.3	92.3	92.3	92.1	92.4	92.6	92.4	92.3	88.9	89.0	89.0	89.0	89.0	89.0	88.8	88.8	88.6	88.9	92.6																																		
JX156397/RVA/Human-wt/RUS/Novosibirsk/Nov11-N2246/2011/G2P[8] - Lineage P[8] III	91.5	90.7	97.2	97.3	97.8	97.8	98.1	97.9	98.1	98.2	98.2	98.1	90.8	91.1	91.1	91.1	91.2	91.4	91.1	91.1	91.0	91.2	94.1	91.9																																		
JN258909/RVA/Human-wt/PHI/TGO12-016/2012/G1P[8] - Lineage P[8] III	91.8	90.8	97.5	97.7	98.2	98.2	98.5	98.3	98.5	98.6	98.6	98.5	91.0	91.2	91.2	91.2	91.1	91.5	91.2	91.2	91.1	91.4	94.3	92.0	99.1																																		
KP007191/RVA/Human-wt/PHI/TGO12-012/2012/G1P[8] - Lineage P[8] III	92.2	91.0	98.6	98.7	99.2	99.2	99.2	99.1	99.2	99.4	99.4	99.2	91.2	91.5	91.5	91.5	91.4	91.7	91.5	91.5	91.4	91.6	94.5	92.5	97.8	98.2																																		
KJ560500/RVA/Human-wt/USA/CNMC101/2011/G12P[8] - Lineage P[8] III	92.1	91.0	98.5	98.6	99.1	99.1	99.4	99.2	99.4	99.5	99.5	99.4	91.2	91.5	91.5	91.5	91.4	91.7	91.5	91.5	91.4	91.6	94.3	92.4	97.9	98.3	99.1																																		
LC260224/RVA/Human-wt/IDN/SOEP075/2016/G3P[8] - Lineage P[8] IV	87.6	89.0	92.1	92.3	92.8	92.8	92.8	92.6	92.9	93.2	92.9	92.8	89.4	89.5	89.5	89.5	89.5	89.3	89.3	89.2	89.4	93.0	98.5	92.4	92.5	93.3	92.9																																		
JN129087/RVA/Human-wt/NCA/22/2010/G1P[8] - Lineage P[8]

Appendix 17e-f: Nucleotide and amino acid identities for the VP1 of the four Zambian reassortants

e.

VP1 nucleotide identities among strains calculated using the p-distance algorithm in MEGA 6 (Tamura et al., 2013)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4749/2014/G2P[8] - Divergent																																																																										
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU1327/2016/G2P[4] - Lineage V	91.0																																																																							
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU1223/2016/G3P[8]	87.2	80.1																																																																					
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU1354/2016/G1P[8]	87.3	80.2	99.7																																																																			
MK302423/RVA/Human-wt/IND/NIV1416591/2014/G9P[4] - Lineage V	93.0	95.5	81.3	81.4																																																																		
MG181315/RVA/Human-wt/MWI/BID24/2013/G2P[4] - Lineage V	92.2	97.5	79.7	79.8	96.7																																																																	
MG181667/RVA/Human-wt/MWI/BID30/2013/G2P[8] - Lineage V	92.0	97.3	79.6	79.7	96.5	99.8																																																																
MG926747/RVA/Human-wt/MOZ/0440/2013/G2P[4] - Lineage V	91.1	99.4	79.9	80.0	95.8	97.9	97.7																																																																
KJ753827/RVA/Human-wt/ZWE/NMC-DPRU1158/XXXX/G2[6P[8] - Lineage V	91.2	99.4	80.0	80.1	95.8	98.0	97.8	99.8																																																																			
KP027151/RVA/Human-wt/PHL/TU12-2012/2012/G2P[4] - Lineage V	90.9	98.8	79.7	79.8	95.6	97.8	97.6	99.3	99.4																																																																	
KF636201/RVA/Human-wt/ZAF/NMC-DPRU2030/2010/G1P[8]	87.2	80.2	99.4	99.4	81.4	79.8	79.6	79.9	80.0	79.8																																																																	
KF636278/RVA/Human-wt/ZAF/NMC-DPRU2032/2010/G1P[8]	87.2	80.2	99.4	99.4	81.4	79.8	79.6	79.9	80.0	79.8	100.0																																																															
MH171315/RVA/Human-wt/ESP/SAS4877/2011/G1P[8]	86.9	79.9	98.9	98.9	81.4	79.6	79.4	79.7	79.8	79.5	99.3	99.2																																																															
KF752637/RVA/Human-wt/SEN/NMC-DPRU2051/2009/G9P[8]	87.0	80.2	98.5	98.5	81.6	79.7	79.6	79.9	79.9	79.7	98.9	98.8	99.0																																																															
LC439262/RVA/Human-wt/GHA/M0094/2010/G9P[8]	87.0	80.3	98.5	98.5	81.6	79.8	79.6	79.9	79.9	79.7	98.9	98.8	99.0	99.9																																																														
JQ609351/RVA/Human-wt/CAN/RT072-09/2009/G1P[8]	87.0	80.1	98.9	98.9	81.4	79.7	79.5	79.8	79.9	79.6	99.3	99.3	99.8	99.0	99.0																																																													
MG673822/RVA/Human-wt/DOM/300503700/2014/G9P[8]	86.9	80.0	98.8	98.9	81.4	79.6	79.5	79.7	79.8	79.5	99.2	99.2	99.8	98.9	99.7																																																														
KC752226/RVA/Human-wt/ETH/NMC-DPRU1843/2009/G2P[8]	87.0	80.2	98.4	98.4	81.6	79.7	79.6	79.9	80.0	79.7	98.7	98.7	98.8	99.0	99.0	98.9	98.8																																																													
KJ752284/RVA/Human-wt/GMB/NMC-DPRU3174/2013/G1P[8]	86.8	80.1	98.2	98.2	81.5	79.6	79.4	79.8	79.8	79.6	98.6	98.5	98.6	98.8	98.8	98.7	98.6	98.7																																																											
JF304915/RVA/Human-wt/KEN/0205/1989/G2P[4] - Lineage II	83.8	86.9	79.2	79.3	85.5	86.9	86.7	86.8	86.9	86.7	79.4	79.4	79.3	79.4	79.4	79.5	79.3	79.5	79.2																																																											
JF304926/RVA/Human-wt/KEN/AA426/1980/G2P[4] - Lineage II	83.9	87.0	78.8	78.8	85.6	86.9	86.7	87.0	87.0	86.9	78.9	78.9	78.8	79.0	79.1	79.0	78.9	79.0	78.8	96.3																																																								
KC443587/RVA/Human-wt/UGA/NMC-DPRU2000/1977/G2P[4] - Lineage I	86.2	90.4	79.3	79.3	89.0	90.6	90.4	90.5	90.5	90.2	79.5	79.5	79.3	79.5	79.5	79.5	79.4	79.5	79.2	86.7																																																							
DQ870505/RVA/Human-tc/USA/DS-1/1976/G2P[4] - Lineage I	85.9	90.6	79.2	79.3	88.8	90.6	90.4	90.8	90.8	90.4	79.3	79.3	79.2	79.3	79.4	79.4	79.3	79.4	79.1	86.3	86.5	98.5																																																				
LC438290/RVA/Human-tc/PHL/NVRC-DPRU1980/G2P[4] - Lineage III	85.8	90.1	79.3	79.3	88.4	90.1	90.2	90.2	90.3	90.0	79.4	79.4	79.4	79.5	79.5	79.4	79.6	79.1	78.9	86.7	86.7	95.8	95.8																																																			
AB733133/RVA/Human-tc/PRI/WUR/1980/G2P[4] - Lineage III	85.7	90.1	79.3	79.3	88.4	90.2	90.2	90.2	90.2	89.9	79.3	79.3	79.3	79.5	79.5	79.5	79.3	79.5	79.1	86.6	86.6	95.8	95.8	100.0																																																	
AB762772/RVA/Human-tc/PRY/AUS65/1986/G2P[4] - Lineage IV	85.6	90.1	79.3	79.3	88.2	90.2	90.1	90.2	90.2	90.0	79.2	79.2	79.2	79.3	79.4	79.4	79.2	79.4	79.0	86.4	86.6	95.5	95.5	97.5	97.5																																																	
AY787653/RVA/Human-wt/CHN/TB-Chen/1996/G2P[4] - Lineage IV	85.6	90.2	79.2	79.2	88.4	90.1	90.0	90.2	90.3	90.0	79.2	79.2	79.2	79.3	79.3	79.4	79.2	79.4	79.1	86.4	86.6	95.6	95.6	97.7	97.7	98.7																																																	
KU284816/RVA/Human-wt/BGN/2163/2010/G2P[4] - Lineage V	93.1	96.9	79.0	79.1	96.0	98.4	98.3	97.3	97.4	98.7	79.4	79.5	79.3	79.5	79.5	79.5	79.2	79.5	79.1	86.3	86.5	95.9	95.9	97.2	97.2	98.2	98.4																																																	
KU284826/RVA/Human-wt/BGN/2203/2010/G2P[4] - Lineage V	93.1	96.9	79.0	79.1	96.0	98.4	98.3	97.3	97.4	98.7	79.4	79.5	79.3	79.5	79.5	79.5	79.2	79.5	79.1	86.3	86.5	95.9	95.9	97.2	97.2	98.2	98.4	100.0																																																
MH057171/RVA/Human-wt/ESP/SAS4877/2011/G1P[8]	86.9	79.9	98.9	98.9	81.4	79.6	79.4	79.7	79.8	79.5	99.3	99.2																																																													
KJ752637/RVA/Human-wt/SEN/NMC-DPRU2051/2009/G9P[8]	87.0	80.2	98.5	98.5	81.6	79.7	79.6	79.9	79.9	79.7	98.9	98.8	99.0	99.9	99.9	98.9	98.8	99.7	99.2	99.1																																																			
KF752637/RVA/Human-wt/SEN/NMC-DPRU2051/2009/G9P[8]	93.8	89.7	99.1	98.9	91.0	89.8	89.7	89.7	89.9	89.4	99.2	99.1	98.9																																																													
KF636278/RVA/Human-wt/ZAF/NMC-DPRU2052/2010/G1P[8]	93.8	89.7	99.6	99.4	90.8	89.6	89.5	89.7	89.9	89.5	99.9																																																											
MH171315/RVA/Human-wt/ESP/SAS4877/2011/G1P[8]	91.7	89.5	99.1	98.9	90.6	89.4	89.3	89.5	89.7	89.3	99.1	99.2																																																													
KF752637/RVA/Human-wt/SEN/NMC-DPRU2051/2009/G9P[8]	93.8	89.7	99.1	98.9	91.0	89.8	89.7	89.7	89.9	89.4	99.2	99.1	98.9																																																													
KJ639262/RVA/Human-wt/GHA/M0094/2010/G9P[8]	91.9	89.9	99.0	98.8	90.4	89.2	89.1	89.4	89.6	89.2	98.8	98.7																																																													
JQ609351/RVA/Human-wt/CAN/RT072-09/2009/G1P[8]	93.9	89.7	99.4	99.2	90.8	89.6	89.5	89.7	89.9	89.4	99.4	99.4	99.2	99.1																																																												
MG673822/RVA/Human-wt/DOM/300503700/2014/G9P[8]	93.9	89.7	99.3	99.1	90.4	89.4	89.5	89.7	89.9	89.4	99.4	99.3	99.4	99.1	99.0	99.9																																																												
KC752226/RVA/Human-wt/ETH/NMC-DPRU1843/2009/G2P[8]	94.1	90.1	99.1	98.9	91.2	90.0	89.9	90.1	90.3	89.8	99.2	99.1	98.9	99.3	99.2	99.2	99.1																																																												
KJ752284/RVA/Human-wt/GMB/NMC-DPRU3174/2013/G1P[8]	94.0	89.8	99.3	99.1	91.1	89.9	89.8	89.8	90.0	89.5	99.4	99.3	99.1	99.4	99.4	99.4	99.3	99.4																																																											
JF304915/RVA/Human-wt/KEN/0205/1989/G2P[4] - Lineage II	94.2	97.0	89.1	89.1	95.5	97.1	97.0	97.0	97.2	97.2	96.8	89.2	89.2	89.4	89.5	89.3	89.3	89.4	89.4	89.6	89.4																																																						
JF304926/RVA/Human-wt/KEN/AA426/1980/G2P[4] - Lineage II	94.5	97.3	89.4	89.4	95.8	97.3	97.2	97.3	97.5	97.2	89.5	89.5	89.3	89.8	89.9	89.5	89.5	89.8	89.7	98.1																																																						
KC443587/RVA/Human-wt/UGA/NMC-DPRU2000/1977/G2P[4] - Lineage I	94.0	97.3	89.4	89.4	95.9	97.5	97.4	97.3	97.5	97.2	89.7	89.7	89.5	89.9	90.0	89.7	89.7	90.0	89.8	89.5	89.5	98.6	98.6	97.2																																																				
DQ870505/RVA/Human-tc/USA/DS-1/1976/G2P[4] - Lineage I	94.0	97.3	89.6	89.6	95.9	97.6	97.4	97.3	97.5	97.2	89.7	89.7	89.5	89.9	90.0	89.7	89.7	90.1	90.0	96.7	97.2	99.6																																																					
LC438290/RVA/Human-tc/PHL/NVRC-DPRU1980/G2P[4] - Lineage III	94.2	97.5	89.6	89.6	96.0	97.7	97.6	97.5	97.7	97.3	89.7	89.7	89.4	90.0	90.1	89.7	89.6	90.0	89.8	89.5	89.5	98.4	98.4	97.0	99.4																																																			
AB733133/RVA/Human-tc/PRI/WUR/1980/G2P[4] - Lineage III	94.1	97.4	89.6	89.6	95.9	97.6	97.5	97.4	97.6	97.2	89.6	89.6	89.3	89.9	90.0	89.6	89.5	90.0	89.9	96.8	97.3	98.7	98.5	99.0																																																				
AB762772/RVA/Human-tc/PRY/AUS65/1986/G2P[4] - Lineage IV	94.0	97.3	89.8	89.8	95.9	97.5	97.4	97.3	97.5	97.1	89.7	89.7	89.6	90.0	90.1	89.7	89.7	90.1	90.0	96.5	97.1	98.4	98.2	99.3	99.2																																																			
AY787653/RVA/Human-wt/CHN/TB-Chen/1996/G2P[4] - Lineage IV	94.1	97.3	89.6	89.6	95.9	97.5	97.5	97.4	97.5	97.2	89.7	89.7	89.5	90.0	90.1	89.7	89.7	90.0	90.0	96.5	97.1	98.4	98.3	99.3	99.2	99.4																																																			
KU284816/RVA/Human-wt/BGN/2163/2010/G2P[4] - Lineage V	94.0	97.5	88.1	88.1	96.3	97.8	97.7	97.6	97.6	97.2	88.1	88.1	88.0	88.3	88.4	88.1	88.1	88.5	88.4	95.1	95.7	95.9	95.9	96.0	95.9	95.8	95.9																																																					
KU284826/RVA/Human-wt/BGN/2203/2010/G2P[4] - Lineage V	94.0	97.5	88.1	88.1	96.3	97.8	97.7	97.6	97.6	97.2	88.1	88.1	88.0	88.3	88.4	88.1	88.1	88.5	88.4	95.1	95.7	95.9	95.9	96.0	95.9	95.8	95.9	100.0																																																				
MH057171/RVA/Human-wt/ESP/SAS4877/2011/G1P[8]	95.1	98.7	89.7	89.7	97.2	98.7	98.6	98.8	98.8	98.6	89.8	89.8	89.6	90.1	90.2	89.8	89.8	90.2	90.1	97.2	97.7	97.5	97.5	97.7	97.6	97.6	97.5	97.2																																																				
KJ753827/RVA/Human-wt/ZWE/NMC-DPRU1158/XXXX/G2[6P[8] - Lineage V	95.2	98.8	89.5	89.5	97.1	98.7	98.6	98.9	98.9	98.7	89.6	89.6	89.4	89.8	89.9	89.6	89.6	89.8	89.7	96.8	97.2	97.2	97.3	97.4	97.3	97.2	97.2	98.9																																																				
KP027151/RVA/Human-wt/PHL/TU12-2012/2012/G2P[4] - Lineage V	95.1	99.4	89.3	89.3	97.2	98.9	98.8	99.4	99.6																																																																				
KF636201/RVA/Human-wt/ZAF/NMC-DPRU2030/2010/G1P[8]	93.9	89.7	99.7	99.5	90.8	89.6	89.5	89.7	89.9	89.5																																																																			
KF636278/RVA/Human-wt/ZAF/NMC-DPRU2052/2010/G1P[8]	93.8	89.7	99.6	99.4	90.8	89.6	89.5	89.7	89.9	89.5	99.9																																																																			
MH171315/RVA/Human-wt/ESP/SAS4877/2011/G1P[8]	91.7	89.5	99.1	98.9	90.6	89.4	89.3	89.5	89.7	89.3	99.1	99.2																																																																				
KP752637/RVA/Human-wt/SEN/NMC-DPRU2051/2009/G9P[8]	93.8	89.7	99.1	98.9	91.0	89.8	89.7	89.7	89.9	89.4	99.2	99.1	98.9																																																																					
KJ639262/RVA/Human-wt/GHA/M0094/2010/G9P[8]	91.9	89.9	99.0	98.8	90.4	89.2	89.1	89.4	89.6	89.2	98.8	98.7																																																																					
JQ609351/RVA/Human-wt/CAN/RT072-09/2009/G1P[8]	93.9	89.7	99.4	99.2	90.8	89.6	89.5	89.7	89.9	89.4	99.4	99.4	99.2	99.1																																																																					
MG673822/RVA/Human-wt/DOM/300503700/2014/G9P[8]	93.9	89.7	99.3	99.1	90.4	89.4	89.5	89.7	89.9	89.4	99.4	99.3	99.4	99.1	99.0	99.9																																																																					
KC752226/RVA/Human-wt/ETH/NMC-DPRU1843/2009/G2P[8]	94.1	90.1	99.1	98.9	91.2	90.0	89.9	90.1	90.3	89.8	99.2	99.1	98.9	99.3	99.2	99.2	99.1																																																																									
KJ752284/RVA/Human-wt/GMB/NMC-DPRU3174/2013/G1P[8]	94.0	89.8	99.3	99.1	91.1	89.9	89.8	89.8	90.0	89.5	99.4	99.3	99.1	99.4	99.4	99.4	99.3	99.4																																																																									
JF304915/RVA/Human-wt/KEN/0205/1989/G2P[4] - Lineage II	94.2	97.0	89.1	89.1	95.5	97.1	97.0	97.0	97.2	97.2	96.8	89.2	89.2	89.4	89.5	89.3	89.3	89.4	89.4	89.6	89.4	99.3																		

Appendix 17g-h: Nucleotide and amino acid identities for the VP6 of the four Zambian reassortants

g.

VP6 nucleotide identites among strains calculated using the p-distance algorithm in MEGA 6 (Tamura et al., 2013)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4749/2014/G2P[8]																																										
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13327/2016/G2P[4]	97.5																																									
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13541/2016/G1P[8]	79.3	79.6																																								
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13232/2016/G1P[8]	79.5	79.8	99.3																																							
MG181770/RVA/Human-wt/MWI/BID11S/2012/G2P[4]	99.9	97.4	79.4	79.5																																						
MG892019/RVA/Human-wt/MOZ/0257/2012/G8P[4]	99.9	97.4	79.3	79.5	99.8																																					
DQ490549/RVA/Human-wt/BGD/RV161/2000/G12P[6]	98.6	98.1	79.3	79.5	98.5	98.5																																				
HQ641367/RVA/Human-wt/BGD/MMC88/2005/G2P[4]	97.8	99.5	79.5	79.7	97.7	97.7	98.6																																			
MG181825/RVA/Human-wt/MWI/BID11E/2012/G2P[4]	97.7	99.0	79.6	79.8	97.6	97.6	98.2	99.5																																		
MG181913/RVA/Human-wt/MWI/BID15V/2012/G2P[4]	97.7	99.0	79.8	80.0	97.6	97.6	98.4	99.5	99.8																																	
EF560707/RVA/Human-wt/BGD/Dhaka6/2001/G11P[25]	79.5	79.7	97.7	98.0	79.6	79.5	79.7	79.8	79.9	80.1																																
GU199507/RVA/Human-wt/BGD/Matlab36/2002/G11P[8]	79.5	79.8	97.4	97.7	79.5	79.5	79.6	79.9	80.0	80.1	99.2																															
KF636282/RVA/Human-wt/ZAF/MRC-DPRU2052/2010/G1P[8]	79.6	79.8	97.7	98.1	79.7	79.6	79.8	79.9	80.0	80.1	98.9	98.7																														
EU556223/RVA/Human-wt/KOR/CAU-202/2005/G9P[8]	79.5	79.6	97.3	97.7	79.5	79.5	79.6	79.7	79.8	80.0	99.3	99.2	98.6																													
KJ412714/RVA/Human-wt/PRY/1638SR/2008/G1P[8]	79.6	79.8	97.7	98.0	79.7	79.6	79.8	79.9	80.0	80.1	99.4	99.3	98.9	99.4																														
MN106125/RVA/Human-wt/CHN/E5365/2017/G1P[8]	79.6	79.8	97.1	97.4	79.7	79.6	79.8	79.9	80.0	80.1	99.1	98.8	98.3	98.9	99.0																													
MG926751/RVA/Human-wt/MOZ/0440/2013/G2P[4]	97.3	99.7	79.7	79.9	97.2	97.2	97.9	99.3	98.8	98.8	79.8	79.9	79.9	79.7	79.9	79.9																												
MG891997/RVA/Human-wt/MOZ/0126/2013/G2P[4]	97.5	99.8	79.6	79.8	97.4	97.4	98.1	99.5	99.0	99.0	79.7	79.8	79.8	79.6	79.8	79.8	99.7																											
JX965142/RVA/Human-wt/AUS/WAPC703/2010/G2P[4]	97.6	99.7	79.7	79.9	97.5	97.5	98.3	99.7	99.2	99.2	79.8	79.9	79.9	79.7	79.9	79.9	99.6	99.7																										
KP007150/RVA/Human-wt/PHI/TGO12-003/2012/G2P[4]	97.3	99.5	79.6	79.8	97.2	97.2	98.1	99.5	99.0	99.0	79.7	79.8	79.8	79.6	79.8	79.8	99.3	99.5	99.7																								
MT767406/RVA/Human-wt/RUS/Moscow-714/2014/G2P[4]	97.1	99.2	79.7	80.1	97.0	97.0	97.7	99.1	98.6	98.6	80.0	80.1	80.1	79.9	80.1	80.1	99.2	99.2	99.3	99.1																						
KJ752299/RVA/Human-wt/ZMB/MRC-DPRU3495/2009/G9P[6]	79.7	80.1	95.9	96.2	79.8	79.7	80.1	80.1	80.2	80.4	97.2	97.1	96.6	97.2	97.6	96.9	80.1	80.1	80.1	80.1	80.4																					
KP[8]82749/RVA/Human-wt/MLI/Mali-021/2008/G1P[8]	79.5	79.9	96.6	97.0	79.6	79.5	79.7	79.8	79.9	80.1	98.6	98.7	97.8	98.6	98.7	98.2	79.8	79.7	79.8	79.7	80.0	96.9																				
KP753216/RVA/Human-wt/TGO/MRC-DPRU5153/2010/G1P[8]	79.1	79.5	95.5	95.8	79.2	79.1	79.5	79.5	79.8	79.8	97.1	97.0	96.6	97.1	97.5	96.8	79.5	79.5	79.5	79.5	79.7	98.4	96.6																			
KJ752589/RVA/Human-wt/ZAF/MRC-DPRU121/2011/G1P[8]	79.3	79.5	96.1	96.4	79.4	79.3	79.6	79.5	79.8	80.0	97.3	97.2	96.8	97.3	97.7	97.1	79.5	79.5	79.5	79.5	79.7	98.5	97.1	98.1																		
JX027820/RVA/Human-wt/AUS/CK00083/2008/G1P[8]	79.6	80.2	95.6	96.0	79.7	79.6	80.1	80.3	80.4	80.6	97.2	97.3	96.7	97.2	97.7	97.2	80.3	80.2	80.3	80.2	80.5	98.6	97.2	98.5	98.2																
JQ230073/RVA/Human-wt/RUS/Nov09-D189/G1P[8]	79.7	79.9	96.7	97.1	79.8	79.7	79.9	80.0	80.1	80.2	98.2	98.5	97.7	98.2	98.7	97.8	80.0	79.9	80.0	79.9	80.1	97.2	97.7	97.4	97.4	97.2															
KP752675/RVA/Human-wt/SWZ/MRC-DPRU4550/2010/G1P[8]	79.4	80.1	95.3	95.6	79.5	79.4	79.8	80.1	80.2	80.4	96.9	97.0	96.4	96.9	97.3	96.8	80.1	80.1	80.1	80.1	80.3	98.4	96.8	98.3	98.1	99.3	97.0															
KT921029/RVA/Human-wt/USA/CNMC9/2011/G1P[8]	79.5	79.9	96.5	96.8	79.6	79.5	79.9	80.0	79.9	80.1	97.8	98.1	97.3	97.8	98.2	97.4	80.0	79.9	80.0	79.9	80.1	97.0	97.2	97.1	97.2	96.9	98.9	96.7													
AB861960/RVA/Human-tc/KEN/KDH651/2010/G12P[8]	79.6	79.9	95.8	96.1	79.7	79.6	80.0	80.0	80.1	80.3	97.4	97.3	96.9	97.4	97.8	97.3	80.0	79.9	80.0	79.9	80.1	98.7	97.2	98.4	98.5	98.6	97.4	98.4	97.2												
JQ069614/RVA/Human-wt/CAN/RT063-09/2009/G1P[8]	79.5	80.2	95.5	95.8	79.6	79.5	80.0	80.3	80.4	80.6	97.1	97.2	96.6	97.1	97.5	97.0	80.3	80.2	80.3	80.2	80.5	98.4	97.0	98.3	98.1	99.5	97.0	99.5	96.7	98.4											
KJ752288/RVA/Human-wt/GMB/MRC-DPRU3174/2010/G1P[8]	79.4	79.7	96.3	96.6	79.5	79.4	79.7	79.8	79.9	80.1	97.9	97.8	97.4	97.9	98.5	97.5	79.8	79.7	79.8	79.7	80.0	96.9	97.3	97.1	97.2	97.0	98.0	96.8	97.6	97.1	96.6										
KJ752209/RVA/Human-wt/ZAF/MRC-DPRU82/2012/G2P[4]	97.2	96.7	79.5	79.9	97.1	97.1	98.2	97.1	96.7	96.9	80.0	79.9	80.1	79.9	80.1	80.1	96.6	96.7	96.8	96.6	96.1	80.1	80.0	79.7	79.9	80.1	80.5	80.1	80.0	80.2	80.1	80.0									
KP752783/RVA/Human-wt/ZMB/MRC-DPRU1673/2009/G2P[4]	97.9	97.4	79.6	79.8	97.8	97.8	99.0	97.7	97.4	97.6	80.1	80.0	80.1	80.0	80.1	80.1	97.2	97.4	97.5	97.2	96.8	80.2	80.1	79.8	80.0	80.2	80.4	80.1	80.1	80.3	80.1	80.1	98.9								
KP752564/RVA/Human-wt/ZAF/MRC-DPRU5594/2011/G2P[4]	97.6	97.2	79.0	79.2	97.5	97.5	98.7	97.7	97.4	97.4	79.5	79.4	79.5	79.4	79.5	79.5	97.1	97.2	97.5	97.4	96.9	79.6	79.3	79.0	79.2	79.6	79.6	79.4	79.5	79.5	79.5	79.3	97.1	97.8							
LC066643/RVA/Human-wt/THA/PCB-180/2013/G1P[8]	96.6	98.5	79.4	79.7	96.6	96.6	97.4	98.8	98.3	98.3	79.8	79.9	79.9	79.7	79.9	79.9	98.3	98.5	98.6	98.3	98.1	80.2	80.0	79.5	79.6	80.4	80.0	80.2	80.0	80.1	80.4	79.8	96.1	96.7	96.7						
KJ721700/RVA/Human-wt/BRA/ES16238/2009/G2P[4]	97.3	99.2	79.6	79.8	97.2	97.4	98.2	99.5	99.0	99.2	79.9	80.0	80.0	79.8	80.0	80.0	99.0	99.2	99.4	99.2	98.7	80.2	79.9	79.6	79.6	80.4	80.1	80.2	80.1	80.1	80.4	79.9	96.9	97.4	97.2	98.3					
KJ753609/RVA/Human-wt/ZAF/MRC-DPRU1362/2007/G2P[4]	98.0	97.5	79.5	79.9	97.9	97.9	99.1	97.8	97.5	97.7	80.0	79.9	80.1	79.9	80.1	80.1	97.3	97.5	97.6	97.3	96.9	80.1	80.0	79.7	79.9	80.1	80.3	80.1	80.0	80.2	80.1	80.0	99.0	99.7	97.9	96.8	97.5				
KP752697/RVA/Human-wt/THA/PCB-180/2013/G1P[8]	96.6	98.5	79.4	79.7	96.6	96.6	97.4	98.8	98.3	98.3	79.8	79.9	79.9	79.7	79.9	79.9	98.3	98.5	98.6	98.3	98.1	80.2	80.0	79.5	79.6	80.4	80.0	80.2	80.0	80.1	80.4	79.8	96.1	96.7	96.7						
KP752697/RVA/Human-wt/GMB/MRC-DPRU3199/2010/G2P[4]	97.5	97.2	79.2	79.5	97.4	97.4	98.6	97.7	97.3	97.3	79.7	79.6	79.8	79.6	79.8	79.8	97.0	97.2	97.4	97.3	96.8	79.7	79.5	79.3	79.5	79.9	79.9	79.6	79.7	79.8	79.8	79.5	97.0	97.7	97.7	99.7	96.6	97.2	97.8		
KM660383/RVA/Human-wt/CMR/BA368/2010/G2P[4]	97.6	97.2	79.1	79.4	97.5	97.5	98.7	97.7	97.4	97.4	79.6	79.5	79.7	79.5	79.7	79.7	97.1	97.2	97.5	97.4	96.9	79.8	79.5	79.2	79.4	79.8	79.8	79.5	79.6	79.7	79.7	79.5	97.1	97.8	99.5	96.7	97.2	97.9	99.4		
KX954619/RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P[8]	79.6	80.1	88.9	89.2	79.6	79.6	79.8	80.1	80.1	80.3	89.1	89.2	89.1	89.4	89.5	89.2	80.1	80.2	80.2	80.1	80.4	88.9	89.4	88.9	89.8	89.4	89.3	89.1	88.9	89.0	89.1	89.6	80.0	80.0	79.7	80.3	80.2	79.9	79.9	79.9	
DQ490538/RVA/Human-tc/JPN/AU-1/1982/G3P[9] - outgroup	81.8	81.6	78.8	79.1	81.9	81.9	81.6	81.5	81.7	81.6	79.4	79.2	79.3	79.0	79.2	79.3	81.5	81.5	81.5	81.4	81.5	79.1	79.0	79.2	78.9	79.4	79.1	79.2	78.7	79.0	79.3	79.5	81.3	81.4	81.6	81.3	81.2	81.3	81.6	81.2	78.7	

h.

VP6 amino acid identities among strains calculated using the p-distance algorithm in MEGA 6 (Tamura et al., 2013)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4749/2014/G2P[8]																																										
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13327/2016/G2P[4]	99.7																																									
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13541/2016/G1P[8]	91.7	91.9																																								
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13232/2016/G1P[8]	92.2	92.4	98.7																																							
MG181770/RVA/Human-wt/MWI/BID11S/2012/G2P[4]	100.0	99.7	91.7	92.2																																						
MG892019/RVA/Human-wt/MOZ/0257/2012/G8P[4]	100.0	99.7	91.7	92.2	100.0																																					
DQ490549/RVA/Human-wt/BGD/RV161/2000/G12P[6]	100.0	99.7	91.7	92.2	100.0	100.0																																				
HQ641367/RVA/Human-wt/BGD/MMC88/2005/G2P[4]	100.0	99.7	91.7	92.2	100.0	100.0	100.0																																			
MG181825/RVA/Human-wt/MWI/BID11E/2012/G2P[4]	100.0	99.7	91.7	92.2	100.0	100.0	100.0	100.0																																		
MG181913/RVA/Human-wt/MWI/BID15V/2012/G2P[4]	100.0	99.7	91.7	92.2	100.0	100.0	100.0	100.0	100.0																																	
EF560707/RVA/Human-wt/BGD/Dhaka6/2001/G11P[25]	92.7	92.4	98.5	99.2	92.7	92.7	92.7	92.7	92.7	92.7																																
GU199507/RVA/Human-wt/BGD/Matlab36/2002/G11P[8]	92.7	92.4	98.5	99.2	92.7	92.7	92.7	92.7	92.7	92.7	100.0																															
KF636282/RVA/Human-wt/ZAF/MRC-DPRU2052/2010/G1P[8]	92.4	92.2	98.2	99.0	92.4	92.4	92.4	92.4	92.4	92.4	99.7	99.7																														
EU556223/RVA/Human-wt/KOR/CAU-202/2005/G9P[8]	91.9	91.7	97.7	98.5	91.9	91.9	91.9	91.9	91.9	91.9	99.2	99.2	99.0																													
KJ412714/RVA/Human-wt/PRY/1638SR/2008/G1P[8]	92.7	92.4	98.5	99.2	92.7	92.7	92.7	92.7	92.7	92.7	100.0	100.0	99.7	99.2																														
MN106125/RVA/Human-wt/CHN/E5365/2017/G1P[8]	92.4	92.2	98.2	99.0	92.4	92.4	92.4	92.4	92.4	92.4	99.7	99.7	99.5	99.0	99.7																													
MG926751/RVA/Human-wt/MOZ/0440/2013/G2P[4]	99.5	99.7	91.7	92.2	99.5	99.5	99.5	99.5	99.5	99.5	92.2	92.2	91.9	91.4	92.2	91.9																												
MG891997/RVA/Human-wt/MOZ/0126/2013/G2P[4]	99.5	99.7	91.7	92.2	99.5	99.5	99.5	99.5	99.5	99.5	92.2	92.2	91.9	91.4	92.2	91.9	99.5																											
JX965142/RVA/Human-wt/AUS/WAPC703/2010/G2P[4]	99.7	100.0	91.9	92.4	99.7	99.7	99.7	99.7	99.7	99.7	92.4	92.4	92.2	91.7	92.4	92.2	99.7	99.7																										
KP007150/RVA/Human-wt/PHI/TGO12-003/2012/G2P[4]	99.5	99.7	91.7	92.2	99.5	99.5	99.5	99.5	99.5	99.5	92.2	92.2	91.9	91.4	92.2	91.9	99.5	99.5	99.7																								
MT767406/RVA/Human-wt/RUS/Moscow-714/2014/G2P[4]	99.5	99.7	91.7	92.2	99.5	99.5	99.5	99.5	99.5	99.5	92.2	92.2	91.9	91.4	92.2	91.9	99.5	99.5	99.7	99.5																						
KJ752299/RVA/Human-wt/ZMB/MRC-DPRU3495/2009/G9P[6]	92.7	92.4	98.5	99.2	92.7	92.7	92.7	92.7	92.7	92.7	100.0	100.0	99.7	99.2	100.0	99.7	92.2	92.2	92.4	92.2	92.2																					
KP[8]82749/RVA/Human-wt/MLI/Mali-021/2008/G1P[8]	92.7	92.4	98.5	99.2	92.7	92.7	92.7	92.7	92.7	92.7	100.0	100.0	99.7	99.2	100.0	99.7	92.2	92.2	92.4	92.2	92.2	100.0																				
KP753216/RVA/Human-wt/TGO/MRC-DPRU5153/2010/G1P[8]	92.7	92.4	98.5	99.2	92.7	92.7	92.7	92.7	92.7	92.7	100.0	100.0	99.7	99.2	100.0	99.7	92.2	92.2	92.4	92.2	92.2	100.0	100.0																			
KJ752589/RVA/Human-wt/ZAF/MRC-DPRU121/2011/G1P[8]	92.7	92.4	98.5	99.2	92.7	92.7	92.7	92.7	92.7	92.7	100.0	100.0	99.7	99.2	100.0	99.7	92.2	92.2	92.4	92.2	92.2	100.0	100.0	100.0																		
JX027820/RVA/Human-wt/AUS/CK00083/2008/G1P[8]	92.7	92.4	98.5	99.2	92.7	92.7	92.7	92.7	92.7	92.7	100.0	100.0	99.7	99.2	100.0	99.7	92.2	92.2	92.4	92.2	92.2	100.0	100.0	100.0	100.0																	
JQ230073/RVA/Human-wt/RUS/Nov09-D189/G1P[8]	92.7	92.4	98.5	99.2	92.7	92.7	92.7	92.7	92.7	92.7	100.0	100.0	99.7	99.2	100.0	99.7	92.2	92.2	92.4	92.2	92.2	100.0	100.0	100.0	100.0	100.0																
KP752675/RVA/Human-wt/SWZ/MRC-DPRU4550/2010/G1P[8]	92.7	92.4	98.5	99.2	92.7	92.7	92.7	92.7	92.7	92.7	100.0	100.0	99.7	99.2	100.0	99.7	92.2	92.2	92.4	92.2	92.2	100.0	100.0	100.0	100.0	100.0	100.0															
KT921029/RVA/Human-wt/USA/CNMC9/2011/G1P[8]	92.7	92.4	98.5	99.2	92.7	92.7	92.7	92.7	92.7	92.7	100.0	100.0	99.7	99.2	100.0	99.7	92.2	92.2	92.4	92.2	92.2	100.0	100.0	100.0	100.0	100.0	100.0	100.0														
AB861960/RVA/Human-tc/KEN/KDH651/2010/G12P[8]	92.7	92.4	98.5	99.2	92.7	92.7	92.7	92.7	92.7	92.7	100.0	100.0	99.7	99.2	100.0	99.7	92.2	92.2	92.4	92.2	92.2	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0													
JQ069614/RVA/Human-wt/CAN/RT063-09/2009/G1P[8]	92.9	92.7	98.2	99.0	92.9	92.9	92.9	92.9	92.9	92.9	99.7	99.7	99.5	99.0	99.7	99.5	92.4	92.4	92.7	92.4	92.4	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.7												
KJ752288/RVA/Human-wt/GMB/MRC-DPRU3174/2010/G1P[8]	92.7	92.4	98.5	99.2	92.7	92.7	92.7	92.7	92.7	92.7	100.0	100.0	99.7	99.2	100.0	99.7	92.2	92.2	92.4	92.2	92.2	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.7											
KJ752209/RVA/Human-wt/ZAF/MRC-DPRU82/2012/G2P[4]	100.0	99.7	91.7	92.2	100.0	100.0	100.0	100.0	100.0	100.0	92.7	92.7	92.4	91.9	92.7	92.4	99.5	99.5	99.7	99.5	99.5	92.7	92.7	92.7	92.7	92.7	92.7	92.7	92.7	92.7	92.9	92.7										
KP752783/RVA/Human-wt/ZMB/MRC-DPRU1673/2009/G2P[4]	100.0	99.7	91.7	92.2	100.0	100.0	100.0	100.0	100.0	100.0	92.7	92.7	92.4	91.9	92.7	92.4	99.5	99.5	99.7	99.5	99.5	92.7	92.7	92.7	92.7	92.7	92.7	92.7	92.7	92.7	92.9	92.7	100.0									
KP752564/RVA/Human-wt/ZAF/MRC-DPRU5594/2011/G2P[4]	99.2	99.0	90.9	91.4	99.2	99.2	99.2	99.2	99.2	99.2	91.9	91.9	91.7	91.2	91.9	91.7	98.7	98.7	99.0	98.7	98.7	91.9	91.9	91.9	91.9	91.9	91.9	91.9	91.9	92.2	91.9	99.2	99.2								
LC066643/RVA/Human-wt/THA/PCB-180/2013/G1P[8]	99.5	99.2	91.2	91.7	99.5	99.5	99.5	99.5	99.5	99.5	92.2	92.2	91.9	91.4	92.2	91.9	99.0	99.0	99.2	99.0	99.0	92.2	92.2	92.2	92.2	92.2	92.2	92.2	92.2	92.2	92.4	92.2	99.5	99.5	98.7						
KJ721700/RVA/Human-wt/BRA/ES16238/2009/G2P[4]	100.0	99.7	91.7	92.2	100.0	100.0	100.0	100.0	100.0	100.0	92.7	92.7	92.4	91.9	92.7	92.4	99.5	99.5	99.7	99.5	99.5	92.7	92.7	92.7	92.7	92.7	92.7	92.7	92.7	92.9	92.7	100.0	100.0	99.2	99.5						
KJ753609/RVA/Human-wt/ZAF/MRC-DPRU1362/2007/G2P[4]	99.7	99.5	91.4	92.4	99.7	99.7	99.7	99.7	99.7	99.7	92.4	92.4	92.2	91.7	92.4	92.2	99.2	99.2	99.5	99.2	99.2	92.4	92.4	92.4	92.4	92.4	92.4	92.4	92.4	92.7	92.4	99.7	99.7	99.0	99.2	99.7					
KP752697/RVA/Human-wt/GMB/MRC-DPRU3199/2010/G2P[4]	99.5	99.2	91.2	91.7	99.5	99.5	99.5	99.5	99.5	99.5	92.2	92.2	91.9	91.4	92.2	91.9	99.0	99.0	99.2	99.0	99.0	92.2	92.2	92.2	92.2	92.2	92.2	92.2	92.2	92.4	92.2	99.5	99.5	99.7	99.0	99.5	99.2				
KM660383/RVA/Human-wt/CMR/BA368/2010/G2P[4]	99.2	99.0	90.9	91.4	99.2	99.2	99.2	99.2	99.2	99.2	91.9	91.9	91.7	91.2	91.9	91.7	98.7	98.7	99.0	98.7	98.7	91.9	91.9	91.9	91.9	91.9	91.9	91.9	91.9	92.2	91.9	99.2	99.2	99.5	98.7	99.2	99.0	99.7			
KX954619/RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P[8]	92.2	92.4	97.0	97.7	92.2	92.2	92.2	92.2	92.2	92.2	98.0	98.0	97.7	97.7	98.0	97.7	92.2	92.7	92.4	92.2	92.2	98.0	98.0	98.0	98.0	98.0

Appendix 17i-j: Nucleotide and amino acid identities for the VP2 of the four Zambian reassortants

i.

VP2 nucleotide identities among strains calculated using the p-distance algorithm in MEGA 6 (Tamura et al., 2013)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
RVA/Human-wt/ZMB/UFS-NGS-MRC-DRPU4749/2014/G2P[8]																																								
RVA/Human-wt/ZMB/UFS-NGS-MRC-DRPU13327/2016/G2P[4]	98.6																																							
RVA/Human-wt/ZMB/UFS-NGS-MRC-DRPU13232/2016/G1P[8]	80.6	81.1																																						
RVA/Human-wt/ZMB/UFS-NGS-MRC-DRPU13541/2016/G1P[8]	80.5	81.0	99.9																																					
MG181833/RVA/Human-wt/MWI/BID19T/2012/G2P[4]	99.5	99.1	81.1	81.0																																				
MG926748/RVA/Human-wt/MOZ/0440/2013/G2P[4]	98.9	99.5	81.1	81.0	99.4																																			
KJ752239/RVA/Human-wt/ZMB/MRC-DPRU1648/2009/G1P[8]	81.0	81.4	97.5	97.4	81.4	81.4																																		
KP752867/RVA/Human-wt/ZMB/MRC-DPRU1660/2008/G12P[6]	80.8	81.2	97.3	97.3	81.3	81.3	98.9																																	
DQ492670/RVA/Human-wt/BGD/Dhaka16/2003/G1P[8]	80.9	81.4	97.1	97.0	81.4	81.4	98.0	97.8																																
KP753213/RVA/Human-wt/TGO/MRC-DPRU5153/2010/G1P[8]	80.7	81.2	96.5	96.4	81.2	81.2	97.3	97.2	98.9																															
KJ751558/RVA/Human-wt/SEN/MRC-DPRU2130-09/2009/G1P[8]	81.0	81.5	96.9	96.8	81.4	81.4	97.8	97.6	99.3	98.7																														
KJ752285/RVA/Human-wt/GMB/MRC-DPRU3174/2010/G1P[8]	80.9	81.4	96.9	96.8	81.4	81.4	97.8	97.6	99.2	98.7	99.9																													
LC086748/RVA/Human-wt/THA/PCB-118/2013/G1P[8]	80.7	81.1	96.5	96.5	81.1	81.1	97.5	97.5	97.5	96.8	97.3	97.2																												
KJ751890/RVA/Human-wt/ETH/MRC-DPRU2241/2009/G3P[6]	97.2	97.3	80.7	80.6	97.6	97.6	81.0	80.9	80.9	80.7	81.0	81.0	80.7																											
KJ753606/RVA/Human-wt/ZAF/MRC-DPRU1362/2007/G2P[4]	97.5	97.6	80.9	80.8	98.0	97.9	81.1	81.0	81.1	80.9	81.1	81.1	80.8	98.0																										
KP752780/RVA/Human-wt/ZMB/MRC-DPRU1673/2009/G2P[4]	97.5	97.5	80.8	80.7	97.9	97.8	80.9	80.8	80.8	80.7	80.9	80.9	80.7	97.8	99.5																									
LC086737/RVA/Human-wt/THA/LS-04/2013/G2P[8]	97.0	97.2	81.0	80.9	97.5	97.4	81.2	81.1	81.1	81.2	81.2	81.2	80.8	97.7	97.5																									
KJ753524/RVA/Human-wt/SEN/MRC-DPRU1915/2008/G2P[4]	97.2	97.3	80.8	80.8	97.7	97.6	81.2	81.1	81.1	81.0	81.2	81.2	80.8	99.5	98.0	97.9	97.7																							
JQ069805/RVA/Human-wt/CAN/RT036-07/2007/G2P[4]	97.7	97.7	80.9	80.8	98.1	98.0	81.1	81.0	81.1	81.0	81.1	81.1	80.8	98.1	99.6	99.4	97.8	98.1																						
KP007194/RVA/Human-wt/PHI/TGO12-016/2012/G1P[8]	98.9	99.1	81.2	81.1	99.4	99.4	81.5	81.3	81.4	81.2	81.5	81.4	81.2	97.5	97.8	97.6	97.3	97.5	97.9																					
MN066793/RVA/Human-wt/IND/CMC_00025/2012/G2P[8]	99.4	98.9	80.9	80.8	99.8	99.2	81.3	81.1	81.2	81.0	81.3	81.2	81.0	97.5	97.9	97.8	97.3	97.5	98.0	99.2																				
MG181657/RVA/Human-wt/MWI/BID28S/2013/G1P[8]	99.2	98.8	81.0	80.9	99.7	99.1	81.3	81.1	81.3	81.1	81.3	81.3	81.0	97.4	98.0	97.9	97.2	97.5	98.1	99.1	99.5																			
MG670673/RVA/Human-wt/DOM/3000503734/2016/G2P[8]	99.2	98.8	80.9	80.8	99.6	99.1	81.3	81.1	81.1	81.0	81.2	81.2	81.0	97.3	97.7	97.6	97.2	97.4	97.8	99.1	99.5	99.3																		
KC443785/RVA/Human-wt/AUS/CK20051/2010/G2P[4]	98.9	99.1	81.3	81.2	99.4	99.4	81.6	81.4	81.6	81.3	81.6	81.6	81.3	97.6	97.9	97.8	97.4	97.6	98.0	99.2	99.2	99.1	99.1																	
MK302426/RVA/Human-wt/IND/NIV1416591/2014/G9P[4]	98.5	99.0	81.3	81.2	98.9	99.4	81.6	81.4	81.6	81.4	81.6	81.6	81.3	97.2	97.5	97.5	97.0	97.2	97.6	99.0	98.8	98.6	98.6	99.0																
KF636279/RVA/Human-wt/ZAF/MRC-DPRU2052/2010/G1P[8]	80.8	81.3	98.7	98.7	81.3	81.3	98.0	98.0	97.8	97.3	97.6	97.6	97.2	81.1	81.1	81.0	81.2	81.3	81.1	81.4	81.1	81.2	81.1	81.5	81.5														
KJ753293/RVA/Human-wt/ZWE/MRC-DPRU1844-11/2011/G1P[8]	80.5	81.1	98.7	98.7	81.0	81.0	98.0	98.0	97.8	97.2	97.6	97.6	97.2	80.7	80.9	80.7	81.0	80.9	80.9	81.1	80.8	80.9	80.9	81.2	81.2	99.4													
KJ753007/RVA/Human-wt/ZAF/MRC-DPRU1491/2010/G2P[4]P[8]	80.8	81.3	98.7	98.6	81.3	81.3	97.9	98.0	97.8	97.2	97.6	97.5	97.1	81.0	81.1	81.0	81.2	81.3	81.1	81.4	81.1	81.2	81.1	81.4	81.4	100.0	99.4												
KC443489/RVA/Human-wt/AUS/CK20043/2010/G1P[8]	80.8	81.2	98.7	98.7	81.3	81.2	98.1	98.1	98.0	97.3	97.8	97.8	97.4	80.8	81.0	80.9	81.1	81.0	81.0	81.3	81.1	81.1	81.1	81.4	81.4	99.4	99.4	99.4											
KJ753347/RVA/Human-wt/ETH/MRC-DPRU850/2012/G12P[8]	80.9	81.4	98.5	98.4	81.4	81.3	97.8	97.9	97.8	97.2	97.6	97.5	97.1	81.0	81.2	81.0	81.3	81.1	81.2	81.4	81.2	81.3	81.3	81.6	81.4	99.1	99.1	99.0	99.5										
KT918788/RVA/Human-wt/USA/VU12-13-73/2012/G12P[8]	80.8	81.4	98.3	98.2	81.3	81.3	97.6	97.7	97.4	96.9	97.3	97.3	96.9	81.0	81.2	81.0	81.3	81.2	81.2	81.4	81.1	81.2	81.2	81.5	81.5	98.9	98.9	98.9	99.0	98.8									
KJ751934/RVA/Human-wt/SWZ/MRC-DPRU5119/2010/G1P[8]	81.0	81.4	98.0	97.9	81.5	81.4	98.1	97.9	97.5	97.0	97.4	97.4	97.1	81.0	81.3	81.2	81.3	81.2	81.3	81.5	81.3	81.4	81.3	81.6	81.6	98.6	98.6	98.6	98.6	98.3	98.4								
KJ627025/RVA/Human-wt/PRY/10SR/2002/G9P[4]	80.8	81.3	98.0	97.9	81.3	81.3	98.2	98.1	98.1	97.5	97.8	97.8	97.5	81.0	81.1	80.8	81.2	81.2	81.1	81.4	81.1	81.2	81.1	81.5	81.4	98.4	98.4	98.4	98.5	98.3	98.1	98.4							
HQ392405/RVA/Human-wt/BEL/BE00045/2009/G1P[8]	80.9	81.3	97.9	97.8	81.4	81.3	98.0	97.8	97.5	96.9	97.4	97.3	97.0	81.0	81.2	81.1	81.2	81.2	81.2	81.4	81.2	81.3	81.2	81.5	81.5	98.6	98.6	98.5	98.6	98.3	98.4	99.9	98.3						
MH291366/RVA/Human-wt/KEN/4019/2017/G2P[4]	98.6	99.1	81.3	81.2	98.9	99.4	81.6	81.4	81.5	81.3	81.6	81.5	81.3	97.2	97.5	97.3	97.0	97.2	97.6	99.0	98.9	98.6	98.6	99.0	99.3	81.4	81.1	81.4	81.3	81.4	81.4	81.4	81.3	81.4					
KJ940062/RVA/Human-wt/BRA/RJ17745/2010/G2P[4]	98.5	98.6	81.1	81.0	99.0	98.9	81.3	81.3	81.2	81.0	81.3	81.3	81.1	97.9	98.2	98.1	97.7	97.9	98.3	98.8	98.8	98.7	98.7	98.9	98.4	81.3	81.1	81.3	81.2	81.4	81.4	81.5	81.4	81.4	98.4				
DQ490546/RVA/Human-wt/BGD/RV161/2000/G12P[6]	97.8	97.9	81.0	81.0	98.3	98.2	81.2	81.1	81.2	81.0	81.3	81.2	81.0	98.2	98.5	98.3	98.0	98.3	98.6	98.1	98.1	98.1	98.0	98.2	97.9	81.3	81.0	81.2	81.1	81.3	81.3	81.4	81.3	81.3	97.8	98.4			
MN067081/RVA/Human-wt/IND/CMC_00033/2012/G1P[8]	80.7	81.2	98.4	98.3	81.2	81.1	97.7	97.8	97.5	96.9	97.4	97.3	97.1	80.8	81.1	80.9	81.1	81.1	81.0	81.3	81.0	81.1	81.0	81.3	81.3	99.0	99.1	99.0	99.1	98.8	99.1	98.5	98.2	98.4	81.3	81.2	81.1		
KX954617/RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P[8]	81.3	81.8	93.1	93.0	81.7	81.7	93.2	93.2	93.5	93.1	93.2	93.2	93.2	81.2	81.3	81.3	81.5	81.4	81.4	81.8	81.6	81.5	81.5	81.8	81.7	93.4	93.5	93.4	93.4	93.4	93.5	93.8	93.7	93.8	81.8	81.6	81.5	93.4	
DQ490536/RVA/Human-tc/JPN/AU-1/1982/G3P[9] - outgroup	81.1	81.4	80.3	80.3	81.4	81.4	80.8	80.4	80.5	80.4	80.5	80.5	80.4	81.4	81.5	81.3	81.4	81.4	81.4	81.4	81.4	81.4	81.4	81.6	81.6	80.3	80.3	80.3	80.3	80.3	80.4	80.4	80.3	80.5	81.6	81.4	81.5	80.2	80.0

j.

VP2 amino acid identities among strains calculated using the p-distance algorithm in MEGA 6 (Tamura et al., 2013)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
RVA/Human-wt/ZMB/UFS-NGS-MRC-DRPU4749/2014/G2P[8]																																								
RVA/Human-wt/ZMB/UFS-NGS-MRC-DRPU13327/2016/G2P[4]	99.4																																							
RVA/Human-wt/ZMB/UFS-NGS-MRC-DRPU13232/2016/G1P[8]	90.7	91.3																																						
RVA/Human-wt/ZMB/UFS-NGS-MRC-DRPU13541/2016/G1P[8]	90.6	91.2	99.8																																					
MG181833/RVA/Human-wt/MWI/BID19T/2012/G2P[4]	99.4	100.0	91.3	91.2																																				
MG926748/RVA/Human-wt/MOZ/0440/2013/G2P[4]	99.4	100.0	91.3	91.2	100.0																																			
KJ752239/RVA/Human-wt/ZMB/MRC-DPRU1648/2009/G1P[8]	90.8	91.4	99.4	99.2	91.4	91.4																																		
KP752867/RVA/Human-wt/ZMB/MRC-DPRU1660/2008/G12P[6]	90.8	91.3	99.2	99.0	91.3	91.3	99.5																																	
DQ492670/RVA/Human-wt/BGD/Dhaka16/2003/G1P[8]	91.0	91.5	99.5	99.3	91.5	91.5	99.7	99.4																																
KP753213/RVA/Human-wt/TGO/MRC-DPRU5153/2010/G1P[8]	90.6	91.2	99.1	98.9	91.2	91.2	99.2	99.0	99.5																															
KJ751558/RVA/Human-wt/SEN/MRC-DPRU2130-09/2009/G1P[8]	90.8	91.4	99.4	99.2	91.4	91.4	99.5	99.3	99.9	99.4																														
KJ752285/RVA/Human-wt/GMB/MRC-DPRU3174/2010/G1P[8]	90.8	91.4	99.4	99.2	91.4	91.4	99.5	99.3	99.9	99.4	100.0																													
LC086748/RVA/Human-wt/THA/PCB-118/2013/G1P[8]	91.0	91.5	99.3	99.1	91.5	91.5	99.4	99.2	99.5	99.2	99.4	99.4																												
KJ751890/RVA/Human-wt/ETH/MRC-DPRU2241/2009/G3P[6]	99.1	99.7	91.2	91.1	99.7	99.7	91.3	91.2	91.4	91.1	91.3	91.3	91.4																											
KJ753606/RVA/Human-wt/ZAF/MRC-DPRU1362/2007/G2P[4]	99.1	99.7	91.4	91.3	99.7	99.7	91.5	91.4	91.6	91.3	91.5	91.5	91.6	99.3																										
KP752780/RVA/Human-wt/ZMB/MRC-DPRU1673/2009/G2P[4]	99.1	99.7	91.4	91.3	99.7	99.7	91.5	91.4	91.6	91.3	91.5	91.5	91.6	99.3	99.8																										
LC086737/RVA/Human-wt/THA/LS-04/2013/G2P[8]	99.3	99.9	91.4	91.3	99.9	99.9	91.5	91.4	91.6	91.3	91.5	91.5	91.6	99.5	99.5	99.5																								
KJ753524/RVA/Human-wt/SEN/MRC-DPRU1915/2008/G2P[4]	98.9	99.4	91.4	91.3	99.4	99.4	91.5	91.4	91.6	91.3	91.5	91.5	91.6	99.5	99.1	99.1	99.3																							
JQ069805/RVA/Human-wt/CAN/RT036-07/2007/G2P[4]	99.0	99.5	91.5	91.4	99.5	99.5	91.6	91.5	91.8	91.4	91.6	91.6	91.8	99.2	99.7	99.7	99.4	99.0																						
KP007194/RVA/Human-wt/PHI/TGO12-016/2012/G1P[8]	99.2	99.8	91.4	91.3	99.8	99.8	91.5	91.4	91.6	91.3	91.5	91.5	91.6	99.4	99.4	99.4	99.7	99.2	99.3																					
MN066793/RVA/Human-wt/IND/CMC_00025/2012/G2P[8]	99.2	99.8	91.1	91.0	99.8	99.8	91.2	91.1	91.3	91.0	91.2	91.2	91.3	99.4	99.4	99.4	99.7	99.2	99.3	99.5																				
MG181657/RVA/Human-wt/MWI/BID28S/2013/G1P[8]	99.4	100.0	91.3	91.2	100.0	100.0	91.4	91.3	91.5	91.2	91.4	91.4	91.5	99.7	99.7	99.7	99.9	99.4	99.5	99.8	99.8																			
MG670673/RVA/Human-wt/DOM/3000503734/2016/G2P[8]	99.3	99.9	91.2	91.1	99.9	99.9	91.3	91.2	91.4	91.1	91.3	91.3	91.4	99.5	99.5	99.5	99.8	99.3	99.4	99.7	99.7	99.9																		
KC443785/RVA/Human-wt/AUS/CK20051/2010/G2P[4]	99.4	100.0	91.3	91.2	100.0	100.0	91.4	91.3	91.5	91.2	91.4	91.4	91.5	99.7	99.7	99.7	99.9	99.4	99.5	99.8	99.8	100.0	99.9																	
MK302426/RVA/Human-wt/IND/NIV1416591/2014/G9P[4]	99.2	99.8	91.1	91.0	99.8	99.8	91.2	91.1	91.3	91.0	91.2	91.2	91.3	99.4	99.4	99.4	99.7	99.2	99.3	99.5	99.5	99.8	99.8	99.8																
KF636279/RVA/Human-wt/ZAF/MRC-DPRU2052/2010/G1P[8]	91.0	91.5	99.8	99.5	91.5	91.5	99.7	99.4	99.8	99.3	99.7	99.7	99.5	91.4	91.6	91.6	91.6	91.6	91.8	91.6	91.3	91.5	91.4	91.5	91.3															
KJ753293/RVA/Human-wt/ZWE/MRC-DPRU1844-11/2011/G1P[8]	90.8	91.4	99.7	99.4	91.4	91.4	99.5	99.3	99.7	99.2	99.5	99.5	99.4	91.3	91.5	91.5	91.5	91.5	91.6	91.5	91.2	91.4	91.3	91.4	91.2	99.9														
KJ753007/RVA/Human-wt/ZAF/MRC-DPRU1491/2010/G2P[4]P[8]	91.0	91.5	99.8	99.5	91.5	91.5	99.7	99.4	99.8	99.3	99.7	99.7	99.5	91.4	91.6	91.6	91.6	91.6	91.8	91.6	91.3	91.5	91.4	91.5	91.3	100.0	99.9													
KC443489/RVA/Human-wt/AUS/CK20043/2010/G1P[8]	91.0	91.5	99.8	99.5	91.5	91.5	99.7	99.4	99.8	99.3	99.7	99.7	99.5	91.4	91.6	91.6	91.6	91.6	91.8	91.6	91.3	91.5	91.4	91.5	91.3	100.0	99.9	100.0												
KJ753347/RVA/Human-wt/ETH/MRC-DPRU850/2012/G12P[8]	91.0	91.5	99.8	99.5	91.5	91.5	99.7	99.4	99.8	99.3	99.7	99.7	99.5	91.4	91.6	91.6	91.6	91.6	91.8	91.6	91.3	91.5	91.4	91.5	91.3	100.0	99.9	100.0	100.0											
KT918788/RVA/Human-wt/USA/VU12-13-73/2012/G12P[8]	91.0	91.5	99.7	99.4	91.5	91.5	99.5	99.3	99.7	99.2	99.5	99.5	99.4	91.4	91.6	91.6	91.6	91.6	91.8	91.6	91.3	91.5	91.4	91.5	91.3	99.9	99.8	99.9	99.9	99.9										
KJ751934/RVA/Human-wt/SWZ/MRC-DPRU5119/2010/G1P[8]	91.0	91.5	99.5	99.3	91.5	91.5	99.4	99.2	99.5	99.1	99.4	99.4	99.3	91.4	91.6	91.6	91.4	91.6	91.8	91.6	91.3	91.5	91.4	91.5	91.3	99.8	99.7	99.8	99.8	99.8	99.7									
KJ627025/RVA/Human-wt/PRY/10SR/2002/G9P[4]	91.0	91.5	99.5	99.3	91.5	91.5	99.4	99.2	99.5	99.1	99.4	99.4	99.3	91.4	91.6	91.6	91.6	91.6	91.8	91.6	91.3	91.5	91.4	91.5	91.3	99.8	99.7	99.8	99.8	99.8	99.7	99.5								
HQ392405/RVA/Human-wt/BEL/BE00045/2009/G1P[8]	90.8	91.4	99.4	99.2	91.4	91.4	99.3	99.1	99.4	99.0	99.3	99.3	99.2	91.3	91.5	91.5	91.3	91.5	91.6	91.5	91.2	91.4	91.3	91.4	91.2	99.7	99.5	99.7	99.7	99.7	99.5	99.9	99.4							
MH291366/RVA/Human-wt/KEN/4019/2017/G2P[4]	99.3	99.9	91.2	91.1	99.9	99.9	91.3	91.2	91.4	91.1	91.3	91.3	91.4	99.5	99.5	99.5	99.8	99.3	99.4	99.7	99.9	99.9	99.8	99.9	99.7	91.4	91.3	91.4	91.4	91.4	91.4	91.4	91.3							
KJ940062/RVA/Human-wt/BRA/RJ17745/2010/G2P[4]	99.3	99.9	91.4	91.3	99.9	99.9	91.5	91.4	91.6	91.3	91.5	91.5	91.6	99.5	99.5	99.5	99.8	99.3	99.4	99.7	99.7	99.9	99.8	99.9	99.7	91.6	91.5	91.6	91.6	91.6	91.6	91.6	91.6	91.5	99.8					
DQ490546/RVA/Human-wt/BGD/RV161/2000/G12P[6]	99.4	100.0	91.3	91.2	100.0	100.0	91.4	91.3	91.5	91.2	91.4	91.4	91.5	99.7	99.7	99.7	99.9	99.4	99.5	99.8	99.8	100.0	99.9	100.0	99.8	91.5	91.4	91.5	91.5	91.5	91.5	91.5	91.4	99.9	99.9					
MN067081/RVA/Human-wt/IND/CMC_00033/2012/G1P[8]	90.8	91.4	99.7	99.4	91.4	91.4	99.5	99.3	99.7	99.2	99.5	99.5	99.4	91.3	91.5	91.5	91.5	91.5	91.6	91.5	91.2	91.4	91.3	91.4	91.2	99.9	99.8	99.9	99.9	99.9	99.8	99.7	99.7	99.5	91.3	91.5	91.4			
KX954617/RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P[8]	91.1	91.6	97.9	97.7	91.6	91.6	98.1	97.8	98.4	98.1	98.3	98.3	98.1	91.6	91.7	91.7	91.7	91.9	91.9	91.7	91.4	91.6	91.5	91.6	91.4	98.2	98.1	98.2	98.2	98.2	98.1	97.9	97.9	97.8	91.5	91.7	91.6	98.1		
DQ490536/RVA/Human-tc/JPN/AU-1/1982/G3P[9] - outgroup	94.7	95.3	93.5	93.3	95.3	95.3	93.6	93.3	93.7	93.3	93.7	93.7	93.6	95.2	95.3	95.3	95.4	94.9	95.2	95.3	95.3	95.3	95.2	95.3	95.1	93.7	93.6	93.7	93.7	93.7	93.6	93.5	93.5	93.3	95.2	95.4	95.3	93.6	93.7	

Appendix 17k-l: Nucleotide and amino acid identities for the VP3 of the four Zambian reassortants

k.

VP3 nucleotide identities among strains calculated using the p-distance algorithm in MEGA 6 (Tamura et al., 2013)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13541/2016/G1P[8]																																										
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13232/2016/G1P[8]	99.8																																									
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4749/2014/G2P[8]	77.0	76.9																																								
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13327/2016/G2P[4]	76.0	76.0	87.9																																							
MG181911/RVA/Human-wt/MWI/BID15V/2012/G2P[4]	77.0	77.0	99.5	87.7																																						
MG181614/RVA/Human-wt/MWI/BID1PU/2013/G1P[8]	77.1	77.0	99.3	87.6	99.6																																					
KC443786/RVA/Human-wt/AUS/CK20051/2010/G2P[4]	77.1	77.0	99.3	87.8	99.4	99.2																																				
MH291350/RVA/Human-wt/KEN/3920/2017/G2P[4]	77.2	77.1	99.2	87.7	99.3	99.1	99.1																																			
KX536658/RVA/Human-wt/IND/RV09/2009/G9P[4]	77.1	77.0	98.7	87.9	98.8	98.6	99.0	98.6																																		
KC442976/RVA/Human-wt/USA/VU08-09-38/2008/G2P[4]	77.2	77.1	98.7	87.9	98.8	98.6	99.0	98.6	99.9																																	
KJ753525/RVA/Human-wt/SEN/MRC-DPRU1915/2008/G2P[4]	77.2	77.0	98.6	88.0	98.8	98.6	98.9	98.5	99.0	99.1																																
KP753180/RVA/Human-wt/UGA/MRC-DPRU3710/2009/G2P[4]	77.1	76.9	98.5	88.0	98.6	98.5	98.7	98.3	98.9	99.0	99.8																															
LC086738/RVA/Human-wt/THA/LS-04/2013/G2P[8]	77.1	77.1	96.6	87.9	96.7	96.5	96.8	96.5	97.0	97.0	97.0	96.8																														
KJ721709/RVA/Human-wt/BRA/R17745/2010/G2P[4]	77.3	77.2	97.7	88.0	97.8	97.6	97.8	97.6	98.0	98.0	97.9	97.8	96.8																													
MG926749/RVA/Human-wt/MOZ/0440/2013/G2P[4]	76.2	76.2	87.8	99.4	87.7	87.5	87.8	87.6	87.9	87.9	87.9	87.9	87.8	88.1																												
KP007153/RVA/Human-wt/PHI/TGO12-003/2012/G2P[4]	76.2	76.2	87.7	99.0	87.7	87.5	87.8	87.6	87.8	87.8	87.8	87.8	87.7	88.0	99.3																												
KU199272/RVA/Human-wt/BGN/I306/2010/G2P[4]	76.0	76.1	87.7	98.8	87.6	87.5	87.7	87.6	87.8	87.8	87.8	87.8	87.7	88.0	99.1	99.3																											
MG670701/RVA/Human-wt/DOM/3000503734/2016/G2P[8]	76.2	76.3	87.3	98.5	87.3	87.1	87.4	87.2	87.5	87.5	87.5	87.5	87.4	87.7	98.8	99.3	98.8																										
MT005289/RVA/Human-wt/CZE/H186/2018/G9P[4]	76.2	76.3	87.5	98.4	87.5	87.3	87.6	87.4	87.7	87.7	87.7	87.7	87.6	87.9	98.8	99.0	98.7	98.5																									
LC477526/RVA/Human-wt/JPN/Tokyo18-42/2018/G2P[4]	76.2	76.2	87.6	98.7	87.5	87.4	87.6	87.5	87.7	87.7	87.7	87.7	87.6	87.9	99.0	99.6	99.0	99.1	98.8																							
MH170019/RVA/Human-wt/PAK585/2016/G1P[8]	76.3	76.4	87.7	97.8	87.6	87.5	87.7	87.6	87.8	87.9	87.8	87.7	87.7	87.9	98.1	98.3	98.6	97.8	97.8	98.0																					
JQ069768/RVA/Human-wt/CAN/RT008-09/2009/G2P[4]	76.3	76.4	87.7	98.2	87.7	87.5	87.8	87.7	88.0	88.0	87.9	88.0	87.7	88.2	98.4	98.6	98.8	98.1	97.9	98.4	97.8																				
KJ753294/RVA/Human-wt/ZWE/MRC-DPRU1844-11/2011/G1P[8]	99.2	99.2	76.9	76.0	77.0	77.0	77.0	77.1	77.0	77.1	77.1	77.0	77.2	77.3	76.2	76.2	76.1	76.3	76.3	76.2	76.4	76.4																			
KF636280/RVA/Human-wt/ZAF/MRC-DPRU2052/2010/G1P[8]	99.1	99.2	76.8	76.2	76.8	76.9	76.9	77.0	76.9	77.0	76.9	76.8	77.1	77.2	76.3	76.4	76.2	76.5	76.5	76.4	76.5	76.5	99.4																		
MG181526/RVA/Human-wt/MWI/BID14A/2012/G1P[8]	97.6	97.7	77.2	76.6	77.3	77.3	77.4	77.4	77.2	77.3	77.4	77.3	77.5	77.7	76.7	76.8	76.6	76.8	76.9	76.8	77.0	76.9	97.9	97.9																	
KJ752240/RVA/Human-wt/ZMB/MRC-DPRU1648/2009/G1P[8]	98.2	98.3	76.8	76.4	76.9	77.0	77.0	77.1	77.1	77.1	77.1	77.0	77.3	77.4	76.5	76.6	76.4	76.6	76.6	76.5	76.7	76.7	98.5	98.5	98.6																
KJ752341/RVA/Human-wt/ZAF/MRC-DPRU1191/2009/G12P[8]	98.2	98.4	77.1	76.7	77.2	77.2	77.2	77.3	77.2	77.3	77.2	77.1	77.4	77.5	76.8	76.8	76.7	76.8	76.9	76.8	77.0	77.0	98.5	98.4	98.1	98.4															
KM660325/RVA/Human-wt/CMR/MA127/2011/G12P[8]	98.1	98.2	77.2	76.2	77.3	77.3	77.3	77.4	77.4	77.2	77.3	77.2	77.5	77.4	76.3	76.4	76.2	76.5	76.5	76.4	76.6	76.4	98.4	98.4	97.6	98.2	98.5														
KJ751715/RVA/Human-wt/GMB/MRC-DPRU3176/2010/G1P[8]	98.1	98.2	77.0	76.5	77.1	77.2	77.2	77.3	77.1	77.2	77.1	77.0	77.4	77.4	76.6	76.7	76.5	76.8	76.8	76.6	76.8	76.7	98.3	98.3	97.7	98.3	98.6	98.9													
KP752650/RVA/Human-wt/TGO/MRC-DPRU2209/2009/G1P[8]	98.2	98.2	77.2	76.3	77.3	77.4	77.4	77.5	77.3	77.4	77.3	77.2	77.6	77.6	76.4	76.5	76.4	76.6	76.7	76.5	76.6	76.6	98.4	98.4	97.9	98.4	98.7	99.4	99.0												
KP752868/RVA/Human-wt/ZMB/MRC-DPRU1660/2008/G12P[6]	97.8	97.9	76.7	76.3	76.8	76.8	76.8	76.9	76.9	77.0	77.0	76.9	77.2	77.1	76.4	76.5	76.3	76.6	76.6	76.4	76.6	76.6	98.1	98.0	97.7	98.0	98.4	98.0	98.1	98.2											
MH171343/RVA/Human-wt/ESP/SS66209011/2013/G12P[8]	98.2	98.2	77.0	76.3	77.1	77.2	77.2	77.2	77.1	77.2	77.2	77.1	77.3	77.4	76.4	76.5	76.4	76.6	76.6	76.5	76.6	76.6	98.4	98.4	97.8	98.3	98.5	98.4	98.4	98.6	98.2										
JQ069706/RVA/Human-wt/CAN/RT005-07/2007/G1P[8]	97.6	97.6	76.8	76.2	76.9	77.0	77.0	77.1	77.0	77.0	77.1	77.0	77.1	77.2	76.3	76.4	76.3	76.4	76.5	76.3	76.6	76.6	97.9	97.7	97.4	97.7	98.0	97.8	97.8	97.8	97.5	97.9										
KJ752708/RVA/Human-wt/ETH/MRC-DPRU1840/2007/G1P[8]	97.6	97.7	76.8	76.2	76.9	77.0	77.0	77.1	77.0	77.0	77.1	77.0	77.1	77.2	76.3	76.4	76.3	76.4	76.5	76.3	76.6	76.6	98.0	97.8	97.4	97.8	98.2	97.9	97.9	98.0	97.6	98.0	99.2									
KP[6]45324/RVA/Human-wt/AUS/CK00108/2011/G1P[8]	97.9	98.0	77.3	76.7	77.4	77.4	77.4	77.5	77.6	77.5	77.6	77.6	77.4	77.6	77.7	76.8	76.8	76.7	77.0	76.9	76.8	77.0	77.0	98.3	98.1	97.7	98.2	98.4	98.0	98.1	98.2	97.9	98.2	97.9	98.0						
JN129072/RVA/Human-wt/NCA/18J/2010/G1P[8]	98.0	98.1	77.1	76.6	77.2	77.2	77.3	77.4	77.2	77.2	77.4	77.2	77.3	77.4	76.6	76.8	76.6	76.8	76.8	76.7	76.9	76.8	98.2	98.1	97.6	98.0	98.2	98.0	98.2	98.2	97.8	98.8	97.5	97.6	97.9						
KX954618/RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P[8]	91.6	91.6	76.5	75.9	76.6	76.5	76.7	76.6	76.8	76.8	76.9	76.8	76.8	77.1	75.8	75.9	76.0	76.0	76.0	75.8	76.1	76.1	91.5	91.7	91.1	91.3	91.7	91.5	91.5	91.7	91.4	91.6	91.3	91.3	91.7	91.6					
MG181460/RVA/Human-wt/MWI/MW2-1254/2005/G1P[8]	98.6	98.6	77.0	76.5	77.1	77.2	77.2	77.3	77.2	77.2	77.3	77.2	77.3	77.4	76.6	76.7	76.5	76.8	76.8	76.6	76.8	76.8	98.8	98.8	99.0	99.5	98.8	98.6	98.7	98.8	98.4	98.7	98.2	98.3	98.6	98.4	91.5				
DQ146662/RVA/Human-wt/BGD/Dhaka12/2003/G12P[6]	98.6	98.6	76.9	76.3	77.0	77.0	77.1	77.2	77.0	77.1	77.2	77.0	77.2	77.3	76.4	76.5	76.3	76.6	76.6	76.4	76.6	76.6	98.9	98.8	98.3	98.7	99.0	98.6	98.7	98.8	98.8	98.9	98.3	98.4	98.6	98.6	91.7	99.1			
MG181482/RVA/Human-wt/MWI/0P5-001/2008/G1P[8]	98.5	98.6	76.9	76.4	77.0	77.0	77.0	77.1	77.0	77.1	77.1	77.0	77.2	77.2	76.5	76.6	76.4	76.7	76.7	76.6	76.8	76.7	98.8	98.7	98.9	99.4	98.8	98.5	98.6	98.6	98.4	98.6	98.1	98.2	98.5	98.3	91.5	99.8	99.0		
MG181834/RVA/Human-wt/MWI/BID19T/2012/G2P[4]	77.1	77.0	99.6	87.8	99.9	99.7	99.5	99.4	98.9	98.9	98.8	98.7	96.8	97.9	87.7	87.7	87.7	87.3	87.5	87.6	87.7	87.7	77.0	76.9	77.4	77.0	77.2	77.4	77.2	77.4	76.8	77.2	77.0	77.0	77.5	77.3	76.7	77.2	77.1	77.0	
DQ490537/RVA/Human-tc/JPN/AU-1/1982/G3P[9] - outgroup	77.2	77.2	75.4	76.3	75.4	75.4	75.5	75.4	75.3	75.3	75.4	75.3	75.4	75.7	76.2	76.2	76.2	76.2	76.2	76.3	76.2	77.4	77.2	77.4	77.5	77.8	77.1	77.4	77.2	77.3	77.7	77.6	77.6	77.7	77.5	77.3	77.6	77.5	77.4	75.4	

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VP3 amino acid identites among strains calculated using the p-distance algorithm in MEGA 6 (Tamura et al., 2013)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13541/2016/G1P[8]																																										
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13232/2016/G1P[8]	99.5																																									
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4749/2014/G2P[8]	80.7	80.5																																								
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13327/2016/G2P[4]	80.7	80.8	93.2																																							
MG181911/RVA/Human-wt/MWI/BID15V/2012/G2P[4]	80.8	80.8	99.2	93.1																																						
MG181614/RVA/Human-wt/MWI/BID1PU/2013/G1P[8]	80.8	80.8	99.0	92.9	99.4																																					
KC443786/RVA/Human-wt/AUS/CK20051/2010/G2P[4]	80.8	80.8	99.2	93.4	99.3	99.2																																				
MH291350/RVA/Human-wt/KEN/3920/2017/G2P[4]	81.0	81.0	99.2	93.2	99.3	99.2	99.3																																			
KX536658/RVA/Human-wt/IND/RV09/2009/G9P[4]	80.6	80.6	98.2	93.1	98.3	98.2	98.8	98.3																																		
KC442976/RVA/Human-wt/USA/VU08-09-38/2008/G2P[4]	80.7	80.7	98.3	93.2	98.4	98.3	98.9	98.4	99.9																																	
KJ753525/RVA/Human-wt/SEN/MRC-DPRU1915/2008/G2P[4]	81.1	80.8	98.6	93.4	98.8	98.8	99.2	98.7	98.7	98.8																																
KP753180/RVA/Human-wt/UGA/MRC-DPRU3710/2009/G2P[4]	81.2	81.0	98.4	93.4	98.7	98.7	99.0	98.6	98.6	98.7	99.9																															
LC086738/RVA/Human-wt/THA/LS-04/2013/G2P[8]	80.6	80.8	96.8	93.5	96.9	96.8	97.4	97.0	96.9	97.0	97.2	97.2																														
KJ721709/RVA/Human-wt/BRA/R17745/2010/G2P[4]	81.3	81.3	98.0	94.0	98.1	98.0	98.3	98.2	97.8	98.0	98.2	98.2	98.0																													
MG926749/RVA/Human-wt/MOZ/0440/2013/G2P[4]	81.0	81.1	92.9	99.8	92.8	92.7	93.2	92.9	92.8	92.9	93.2	93.2	93.3	93.8																												
KP007153/RVA/Human-wt/PHI/TGO12-003/2012/G2P[4]	80.7	81.1	92.8	99.4	92.9	92.8	93.3	93.1	92.9	93.1	93.3	93.3	93.4	93.9	99.6																												
KU199272/RVA/Human-wt/BGN/I306/2010/G2P[4]	80.6	81.0	92.6	99.0	92.7	92.6	93.1	93.1	92.7	92.8	93.1	93.1	93.2	93.7	99.3	99.4																											
MG670701/RVA/Human-wt/DOM/3000503734/2016/G2P[8]	80.6	81.0	92.3	98.7	92.5	92.3	92.8	92.6	92.5	92.6	92.8	92.8	92.9	93.4	98.9	99.3	98.7																											
MT005289/RVA/Human-wt/CZE/H186/2018/G9P[4]	80.6	81.0	92.5	99.0	92.6	92.5	92.9	92.7	92.6	92.7	92.9	92.9	93.1	93.5	99.3	99.4	99.0	98.7																											
LC477526/RVA/Human-wt/JPN/Tokyo18-42/2018/G2P[4]	80.5	80.8	92.6	99.0	92.7	92.6	93.1	92.8	92.7	92.8	93.1	93.1	93.3	93.8	99.3	99.6	99.0	98.9	99.0																									
MH170019/RVA/Human-wt/PAK585/2016/G1P[8]	80.4	80.7	92.1	98.6	92.2	92.1	92.6	92.6	92.5	92.6	92.6	92.6	92.8	93.2	98.8	98.9	99.0	98.2	98.6	98.6																					
JQ069768/RVA/Human-wt/CAN/RT008-09/2009/G2P[4]	81.1	81.4	92.7	98.4	92.8	92.7	93.2	93.2	92.8	92.9	93.2	93.2	93.3	93.8	98.7	98.8	98.8	98.1	98.4	98.4	98.2																				
KJ753294/RVA/Human-wt/ZWE/MRC-DPRU1844-11/2011/G1P[8]	98.8	98.8	80.5	80.7	80.8	80.8	80.8	81.0	80.6	80.7	81.1	81.2	80.8	81.3	81.0	81.0	80.8	80.8	80.8	80.7	80.6	81.3																			
KF636280/RVA/Human-wt/ZAF/MRC-DPRU2052/2010/G1P[8]	99.0	99.0	80.4	80.6	80.7	80.7	80.7	80.8	80.5	80.6	81.0	81.1	80.7	81.2	80.8	80.8	80.7	80.7	80.7	80.6	80.5	81.2	99.3																		
MG181526/RVA/Human-wt/MWI/BID14A/2012/G1P[8]	97.8	97.8	80.6	80.7	81.0	81.0	81.0	81.1	80.7	80.8	81.2	81.3	81.0	81.4	81.0	81.0	80.8	80.8	80.8	80.8	80.6	81.3	98.1	98.3																	
KJ752240/RVA/Human-wt/ZMB/MRC-DPRU1648/2009/G1P[8]	98.0	98.0	80.2	80.6	80.6	80.6	80.6	80.7	80.5	80.5	80.8	81.0	80.6	81.1	80.8	80.8	80.7	80.7	80.7	80.7	80.5	81.2	98.2	98.4	99.2																
KJ752341/RVA/Human-wt/ZAF/MRC-DPRU1191/2009/G12P[8]	98.3	98.6	80.5	80.7	80.8	80.8	80.8	81.0	80.6	80.7	80.8	81.0	80.8	81.3	81.0	81.0	80.8	80.8	80.8	80.7	80.6	81.3	98.6	98.9	98.3	98.4															
KM660325/RVA/Human-wt/CMR/MA127/2011/G12P[8]	97.8	97.8	80.1	80.0	80.5	80.5	80.5	80.6	80.2	80.4	80.7	80.8	80.5	80.7	80.2	80.2	80.1	80.1	80.1	80.0	79.9	80.6	98.1	98.3	98.0	98.1	98.8														
KJ751715/RVA/Human-wt/GMB/MRC-DPRU3176/2010/G1P[8]	98.3	98.3	80.1	80.4	80.5	80.5	80.5	80.6	80.2	80.4	80.7	80.8	80.5	81.0	80.6	80.6	80.5	80.6	80.5	80.4	80.2	81.0	98.3	98.6	98.1	98.2	99.0	98.6													
KP752650/RVA/Human-wt/TGO/MRC-DPRU2209/2009/G1P[8]	98.3	98.3	80.4	80.4	80.7	80.7	80.7	80.8	80.5	80.6	81.0	81.1	80.7	81.2	80.6	80.6	80.5	80.5	80.5	80.4	80.2	81.0	98.6	98.8	98.3	98.4	99.3	99.3	99.0												
KP752868/RVA/Human-wt/ZMB/MRC-DPRU1660/2008/G12P[6]	98.1	98.1	79.9	80.1	80.2	80.2	80.2	80.4	80.0	80.1	80.5	80.6	80.2	80.7	80.4	80.4	80.2	80.2	80.2	80.1	80.0	80.7	98.3	98.6	98.3	98.2	98.8	98.3	98.6	98.8											
MH171343/RVA/Human-wt/ESP/SS66209011/2013/G12P[8]	98.3	98.3	80.1	80.5	80.5	80.5	80.5	80.6	80.2	80.4	80.7	80.8	80.6	81.0	80.7	80.7	80.6	80.6	80.6	80.5	80.4	81.1	98.6	99.0	98.3	98.4	99.2	98.6	98.8	99.0	98.8										
JQ069706/RVA/Human-wt/CAN/RT005-07/2007/G1P[8]	97.5	97.5	80.4	80.6	80.7	80.7	80.7	80.8	80.5	80.6	81.0	81.1	80.5	81.1	80.8	80.8	80.7	80.7	80.7	80.6	80.5	81.2	97.8	98.0	97.7	97.6	98.2	97.8	98.0	98.2	98.0	98.2									
KJ752708/RVA/Human-wt/ETH/MRC-DPRU1840/2007/G1P[8]	97.7	97.7	80.4	80.7	80.7	80.7	80.7	80.8	80.5	80.6	81.0	81.1	80.5	81.2	81.0	81.0	80.8	80.8	80.8	80.7	80.6	81.3	98.1	98.3	98.0	97.8	98.7	98.0	98.2	98.4	98.2	98.6	99.0								
KP[6]45324/RVA/Human-wt/AUS/CK00108/2011/G1P[8]	97.5	97.5	80.7	81.0	81.1	81.1	81.1	81.2	80.8	81.0	81.3	81.4	81.1	81.6	81.2	81.2	81.1	81.1	81.1	81.0	80.8	81.4	98.0	98.0	97.7	97.8	98.2	97.7	98.0	98.2	98.0	98.2	97.4	97.6							
JN129072/RVA/Human-wt/NCA/18J/2010/G1P[8]	98.1	98.1	80.2	80.4	80.6	80.6	80.6	80.7	80.4	80.5	80.8	81.0	80.7	81.1	80.6	80.8	80.7	80.7	80.7	80.6	80.5	81.2	98.3	98.6	98.3	98.2	98.8	98.3	98.6	98.8	98.6	99.3	98.0	98.2	98.0						
KX954618/RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P[8]	95.2	95.2	80.7	81.1	81.1	81.1	81.1	81.2	80.8	81.0	81.3	81.4	81.1	81.8	81.1	81.1	81.0	81.0	81.0	80.8	80.7	81.4	95.1	95.4	95.2	95.1	95.8	95.4	95.6	95.9	95.6	95.9	95.1	95.6	95.7	95.6					
MG181460/RVA/Human-wt/MWI/MW2-1254/2005/G1P[8]	98.3	98.3	80.5	80.8	80.8	80.8	80.8	81.0	80.6	80.7	81.1	81.2	80.8	81.3	81.1	81.1	81.0	81.0	81.0	80.7	80.7	81.4	98.6	98.8	99.5	99.6	98.8	98.4	98.6	98.8	98.6	98.8	98.0	98.2	98.2	98.6	95.4				
DQ146662/RVA/Human-wt/BGD/Dhaka12/2003/G12P[6]	98.7	98.7	80.5	80.7	80.8	80.8	80.8	81.0	80.6	80.7	81.1	81.2	80.8	81.3	81.0	81.0	80.8	80.8	80.8	80.7	80.6	81.3	99.2	99.2	98.7	98.8	99.4	98.9	99.2	99.4	99.2	99.4	98.7	98.9	98.6	99.2	95.9	99.2			
MG181482/RVA/Human-wt/MWI/0P5-001/2008/G1P[8]	98.1	98.1	80.4	80.7	80.7	80.7	80.7	80.8	80.5	80.6	81.0	81.1	80.7	81.2	81.0	81.0	80.8	80.8	80.8	80.8	80.6	81.3	98.3	98.6	99.3	99.4	98.6	98.2	98.3	98.6	98.3	98.6	97.7	98.0	98.0	98.3	95.2	99.8	98.9		
MG181834/RVA/Human-wt/MWI/BID19T/2012/G2P[4]	81.1	81.1	99.4	93.3	99.8	99.6	99.5	99.5	98.6	98.7	98.9	98.8	97.1	98.3	93.1	93.2	92.9	92.7	92.8	92.9	92.5	93.1	81.1	81.0	81.2	80.8	81.1	80.7	80.7	81.0	80.5	80.7	81.0	81.0	81.3	80.8	81.3	81.1	81.1	81.0	
DQ490537/RVA/Human-tc/JPN/AU-1/1982/G3P[9] - outgroup	83.1	83.2	82.9	83.8	83.1	83.0	83.2	83.4	83.2	83.4	83.4	83.5	83.1	84.0	83.7	83.6	83.6	83.6	83.2	83.8	83.4	83.7	83.6	83.4	83.4	83.1

Appendix 17m-n: Nucleotide and amino acid identities for the NSP1 of the four Zambian reassortants

m.

NSP1 nucleotide identities among strains calculated using the p-distance algorithm in MEGA 6 (Tamura et al., 2013)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4749/2014/G2P[8]																																										
RVA/Human-wt/ZMB/UFS-NGS-MRC-DRPU13327/2016/G2P[4]	98.2																																									
RVA/Human-wt/ZMB/UFS-NGS-MRC-DRPU13541/2016/G1P[8]	74.8	74.8																																								
RVA/Human-wt/ZMB/UFS-NGS-MRC-DRPU13232/2016/G1P[8]	74.8	74.8	100.0																																							
MG926742/RVA/Human-wt/MOZ/0440/2013/G2P[4]	98.6	99.5	74.7	74.7																																						
KJ753287/RVA/Human-wt/ZWE/MRC-DPRU1844-11/2011/G1P[8]	74.8	74.7	98.2	98.2	74.7																																					
KF636207/RVA/Human-wt/ZAF/MRC-DPRU1544/2010/G1P[8]	74.7	74.5	98.0	98.0	74.5	99.2																																				
KJ753645/RVA/Human-wt/MUS/MRC-DPRU293/XXXX/G2P[4]	99.6	98.5	74.8	74.8	98.9	74.8	74.7																																			
KJ753819/RVA/Human-wt/ZWE/MRC-DPRU1158/XXXX/G2G9P[6]	98.6	99.3	74.7	74.7	99.7	74.7	74.5	98.9																																		
KP007176/RVA/Human-wt/PHI/TGO12-007/2012/G2P[4]	98.8	98.7	74.7	74.7	99.1	74.7	74.5	99.1	99.1																																	
KP007154/RVA/Human-wt/PHI/TGO12-003/2012/G2P[4]	98.6	98.5	74.6	74.6	98.9	74.6	74.5	98.9	98.9	99.7																																
MG181607/RVA/Human-wt/MWI/BID11W/2013/G1P[8]	99.2	98.2	74.9	74.9	98.6	74.9	74.8	99.3	98.6	98.8	98.6																															
MG181915/RVA/Human-wt/MWI/BID15V/2012/G2P[4]	99.2	98.3	74.9	74.9	98.7	74.9	74.8	99.4	98.7	98.9	98.7	99.7																														
KX536670/RVA/Human-wt/IND/RV09/2009/G9P[4]	99.2	98.5	74.7	74.7	98.9	74.7	74.5	99.5	98.9	99.1	98.9	99.3	99.4																													
KJ753518/RVA/Human-wt/SEN/MRC-DPRU1915/2008/G2P[4]	99.0	98.2	74.6	74.6	98.6	74.6	74.5	99.2	98.6	98.8	98.6	99.1	99.2	99.4																												
KP753173/RVA/Human-wt/UGA/MRC-DPRU3710/2009/G2P[4]	98.7	97.9	74.6	74.6	98.3	74.6	74.6	99.0	98.3	98.5	98.3	98.8	98.9	99.1	99.6																												
KP752774/RVA/Human-wt/ZMB/MRC-DPRU1673/2009/G2P[4]	97.2	96.6	75.1	75.1	97.1	75.0	74.9	97.5	97.1	97.0	96.8	97.3	97.3	97.5	97.6	97.3																												
KJ751796/RVA/Human-wt/ZAF/MRC-DPRU1280-05/2005/G2P[8]	97.2	96.6	75.2	75.2	97.1	75.1	75.0	97.5	97.1	97.0	96.8	97.3	97.3	97.5	97.6	97.3	99.7																												
KC443604/RVA/Human-wt/AUS/CK20002/2000/G2P[4]	97.1	96.5	75.1	75.1	96.9	75.1	75.1	97.3	96.9	96.8	96.7	97.1	97.2	97.4	97.5	97.2	98.1	98.1																												
KP[8]82357/RVA/Human-wt/GHA/Ghan-008/2009/G2P[4]	97.2	96.6	75.1	75.1	96.9	75.3	75.0	97.5	96.9	97.0	96.8	97.1	97.2	97.4	97.5	97.2	97.8	97.9	97.8																												
KP752688/RVA/Human-wt/GMB/MRC-DPRU3199/2010/G2P[4]	97.1	96.4	74.9	74.9	96.6	75.0	74.7	97.3	96.6	96.8	96.6	97.0	97.1	97.3	97.3	97.1	97.5	97.7	97.5	99.6																										
DQ490540/RVA/Human-wt/BGD/RV161/2000/G12P[6]	97.3	96.6	74.7	74.7	97.1	74.8	74.7	97.6	97.1	97.1	96.9	97.4	97.5	97.5	97.7	97.5	98.2	98.2	98.2	97.7	97.4																										
JQ069378/RVA/Human-wt/CAN/RT008-07/2007/G2P[4]	97.1	96.2	75.1	75.1	96.6	75.2	75.1	97.3	96.6	96.7	96.5	97.0	97.1	97.3	97.3	97.1	97.7	97.8	97.9	97.4	97.1	97.7																										
KU360966/RVA/Human-wt/BRA/QUI-130-F2/2010/G12P[6]	97.7	97.3	74.9	74.9	97.7	74.9	74.8	98.1	97.5	97.6	97.4	97.7	97.8	98.0	98.1	97.8	97.2	97.2	97.1	96.9	96.6	97.2	96.9																										
LC433791/RVA/Human-wt/NPL/TK2615/2008/G11P25	74.6	74.5	97.8	97.8	74.5	98.6	98.4	74.6	74.6	74.5	74.4	74.7	74.7	74.5	74.4	74.4	74.8	74.9	74.9	74.9	74.7	74.7	74.9	74.7																										
MG181486/RVA/Human-wt/MWI/0P5-001/2008/G1P[8]	74.9	74.9	97.8	97.8	74.8	98.6	98.4	74.9	74.9	74.8	74.7	75.1	75.1	74.8	74.7	74.7	75.1	75.3	75.2	75.3	75.0	74.9	75.2	74.9	99.0																										
HQ025979/RVA/Human-wt/KOR/CAU-195/2006/G12P[6]	74.9	74.9	97.5	97.5	74.9	98.4	98.2	74.9	75.0	74.9	74.8	75.1	75.1	74.9	74.8	74.8	75.2	75.3	75.3	75.3	75.1	75.1	75.1	75.1	98.8	98.9																										
KJ753566/RVA/Human-wt/ZAF/MRC-DPRU4079-11/2011/G1P[8]	75.1	75.0	96.8	96.8	74.9	97.7	97.5	74.9	75.1	74.9	74.9	75.2	75.2	74.9	74.9	74.9	75.3	75.4	75.2	75.4	75.1	75.1	75.1	75.2	98.1	97.9	98.2																										
KJ751708/RVA/Human-wt/GMB/MRC-DPRU3176/2010/G1P[8]	75.0	75.1	97.3	97.3	75.0	98.1	97.9	75.1	75.1	75.0	74.9	75.3	75.3	75.0	74.9	74.9	75.3	75.5	75.4	75.5	75.2	75.1	75.4	75.1	98.6	98.9	98.2	97.5																										
KJ752233/RVA/Human-wt/ZMB/MRC-DPRU1648/2009/G1P[8]	74.7	74.6	97.5	97.5	74.5	98.2	97.9	74.7	74.7	74.5	74.5	74.8	74.8	74.5	74.5	74.5	74.9	75.0	75.1	75.1	74.9	74.7	74.9	74.8	98.6	99.4	98.4	97.5	98.3																										
KJ751928/RVA/Human-wt/SWZ/MRC-DPRU5119/2010/G1P[8]	74.7	74.8	97.4	97.4	74.7	98.2	98.0	74.7	74.9	74.7	74.7	75.0	75.0	74.7	74.7	74.7	75.1	75.2	75.1	75.2	74.9	74.9	75.0	75.0	98.8	98.6	98.9	98.1	98.1	98.2																										
KP[8]82753/RVA/Human-wt/MLI/Mali-021/2008/G1P[8]	74.9	74.9	97.6	97.6	74.9	98.4	98.2	75.0	74.9	74.9	74.8	75.1	75.1	74.9	74.8	74.8	75.2	75.3	75.3	75.3	75.1	75.0	75.3	75.1	98.7	99.0	98.4	97.6	98.7	98.5	98.3																										
KF636273/RVA/Human-wt/ZAF/MRC-DPRU2052/2010/G1P[8]	74.6	74.4	97.9	97.9	74.5	99.2	99.9	74.6	74.5	74.5	74.4	74.7	74.7	74.5	74.4	74.5	74.8	74.9	75.0	74.9	74.7	74.6	75.0	74.7	98.4	98.4	98.1	97.4	97.8	97.9	97.9	98.2																										
KC769377/RVA/Human-wt/AUS/CK00066/2007/G1P[8]	74.5	74.5	96.9	96.9	74.4	97.7	97.5	74.5	74.5	74.4	74.3	74.8	74.8	74.5	74.5	74.6	74.9	75.0	74.9	75.0	74.7	74.7	74.8	74.8	98.2	98.2	98.4	97.6	97.6	97.7	98.3	97.8	97.5																										
KJ753463/RVA/Human-wt/ZWE/MRC-DPRU1102/2012/G9P[8]	74.6	74.5	96.9	96.9	74.5	97.7	97.5	74.6	74.6	74.5	74.4	74.9	74.9	74.6	74.5	74.7	74.9	75.1	75.0	75.1	74.8	74.7	74.7	74.7	98.2	98.3	98.4	97.6	97.7	97.7	98.4	97.8	97.5	99.0																										
KX954620/RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P[8]	75.3	75.3	83.8	83.8	75.2	84.2	84.2	75.5	75.3	75.3	75.1	75.4	75.3	75.3	75.3	75.3	75.8	75.9	75.8	76.0	75.7	75.4	76.0	75.5	84.1	84.1	84.5	84.4	84.1	84.1	84.4	84.2	84.2	84.3	84.2																										
HQ392247/RVA/Human-wt/BEL/BE00030/2008/G1P[8]	74.4	74.6	83.5	83.5	74.5	83.8	83.8	74.7	74.7	74.5	74.3	74.6	74.5	74.5	74.4	74.4	75.2	75.3	75.0	75.1	74.8	74.7	75.1	74.6	83.8	83.7	84.0	83.8	83.7	83.7	84.0	83.8	83.8	84.0	83.8	97.5																										
JQ069436/RVA/Human-wt/CAN/RT004-09/2009/G3P[8]	75.6	75.7	83.4	83.4	75.6	83.6	83.4	75.9	75.8	75.6	75.5	75.8	75.7	75.8	75.8	75.8	76.2	76.4	76.2	76.1	75.8	75.8	76.2	75.7	83.7	83.6	83.9	83.5	83.7	83.6	84.0	83.7	83.4	84.0	83.8	95.6	95.2																										
KP752785/RVA/Human-wt/ETH/MRC-DPRU4970/2010/G12P[8]	75.4	75.5	84.2	84.2	75.5	84.1	84.0	75.5	75.7	75.8	75.5	75.5	75.6	75.4	75.6	75.8	76.0	76.1	75.8	75.8	75.5	75.7	75.9	75.6	84.3	84.2	84.8	84.5	84.3	84.0	84.5	84.5	84.0	84.7	84.5	91.6	90.6	90.3																										
MG181585/RVA/Human-wt/MWI/BID1KY/2013/G1P[8]	99.3	98.3	75.0	75.0	98.7	75.0	74.9	99.4	98.7	98.9	98.7	99.9	99.7	99.4	99.2	98.9	97.3	97.3	97.2	97.2	97.1	97.5	97.1	97.8	74.8	75.1	75.2	75.3	75.3	74.9	75.1	75.2	74.8	74.9	74.9	75.5	74.7	75.9	75.5																										
DQ146677/RVA/Human-wt/BGD/Matlab13/2003/G1P[6]	75.0	74.9	98.2	98.2	74.9	98.8	98.6	75.0	75.0	74.9	74.8	75.1	75.1	74.9	74.8	74.8	75.2	75.3	75.3	75.3	75.1	75.1	75.3	75.1	99.5	99.2	99.0	98.2	98.7	98.8	98.8	98.9	98.6	98.4	98.4	84.5	84.0	83.9	84.7	75.2																										
LC433780/RVA/Human-wt/NPL/TK1797/2007/G9P[19] - outgroup	74.5	74.3	79.1	79.1	74.6	79.1	79.1	74.6	74.6	74.6	74.4	74.7	74.7	74.6	74.3	74.3	74.6	74.7	74.2	74.5	74.2	74.2	74.4	74.3	78.8	78.8	79.4	79.0	78.8	78.8	78.9	79.0	79.2	79.0	79.1	77.7	77.2	77.6	78.0	74.7	79.2	

n.

NSP1 amino acid identities among strains calculated using the p-distance algorithm in MEGA 6 (Tamura et al., 2013)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4749/2014/G2P[8]																																										
RVA/Human-wt/ZMB/UFS-NGS-MRC-DRPU13327/2016/G2P[4]	98.1																																									
RVA/Human-wt/ZMB/UFS-NGS-MRC-DRPU13541/2016/G1P[8]	69.1	69.8																																								
RVA/Human-wt/ZMB/UFS-NGS-MRC-DRPU13232/2016/G1P[8]	69.1	69.8	100.0																																							
MG926742/RVA/Human-wt/MOZ/0440/2013/G2P[4]	98.4	99.4	69.5	69.5																																						
KJ753287/RVA/Human-wt/ZWE/MRC-DPRU1844-11/2011/G1P[8]	69.1	69.5	98.6	98.6	69.5																																					
KF636207/RVA/Human-wt/ZAF/MRC-DPRU1544/2010/G1P[8]	69.1	69.5	98.1	98.1	69.5	98.8																																				
KJ753645/RVA/Human-wt/MUS/MRC-DPRU293/XXXX/G2P[4]	99.2	98.6	69.5	69.5	98.8	69.5	69.5																																			
KJ753819/RVA/Human-wt/ZWE/MRC-DPRU1158/XXXX/G2G9P[6]	98.4	99.4	69.5	69.5	99.6	69.5	69.5	98.8																																		
KP007176/RVA/Human-wt/PHI/TGO12-007/2012/G2P[4]	99.0	98.4	69.3	69.3	98.6	69.3	69.3	99.4	98.6																																	
KP007154/RVA/Human-wt/PHI/TGO12-003/2012/G2P[4]	98.6	97.9	69.1	69.1	98.1	69.1	69.1	99.0	98.1	99.6																																
MG181607/RVA/Human-wt/MWI/BID11W/2013/G1P[8]	98.8	97.7	69.5	69.5	97.9	69.5	69.5	98.8	97.9	98.6	98.1																															
MG181915/RVA/Human-wt/MWI/BID15V/2012/G2P[4]	98.6	97.9	69.5	69.5	98.1	69.5	69.5	99.0	98.1	98.8	98.4	99.4																														
KX536670/RVA/Human-wt/IND/RV09/2009/G9P[4]	98.8	98.1	69.5	69.5	98.4	69.5	69.5	99.2	98.4	99.0	98.6	98.8	99.0																													
KJ753518/RVA/Human-wt/SEN/MRC-DPRU1915/2008/G2P[4]	98.8	98.1	69.1	69.1	98.4	69.1	69.1	99.2	98.4	99.0	98.6	98.8	99.0	99.2																												
KP753173/RVA/Human-wt/UGA/MRC-DPRU3710/2009/G2P[4]	98.6	97.9	69.3	69.3	98.1	69.3	69.3	99.0	98.1	98.8	98.4	98.6	98.8	99.0	99.4																												
KP752774/RVA/Human-wt/ZMB/MRC-DPRU1673/2009/G2P[4]	97.3	97.1	70.0	70.0	97.3	70.0	70.0	97.7	97.3	97.5	97.1	97.1	97.5	97.5	97.5	97.3																												
KJ751796/RVA/Human-wt/ZAF/MRC-DPRU1280-05/2005/G2P[8]	97.1	96.9	70.2	70.2	97.1	70.2	70.2	97.5	97.1	97.3	96.9	96.9	97.3	97.3	97.3	97.1	99.8																												
KC443604/RVA/Human-wt/AUS/CK20002/2000/G2P[4]	97.3	97.1	69.8	69.8	97.3	69.3	69.8	97.7	97.3	97.5	97.1	97.1	97.3	97.5	97.5	97.3	97.9	97.7																												
KP[8]82357/RVA/Human-wt/GHA/Ghan-008/2009/G2P[4]	96.9	96.7	70.4	70.4	96.9	70.4	70.4	97.3	96.9	97.5	97.3	96.7	96.9	97.1	97.1	96.9	97.9	97.7	97.9																												
KP752688/RVA/Human-wt/GMB/MRC-DPRU3199/2010/G2P[4]	96.9	96.3	70.0	70.0	96.5	70.0	70.0	97.3	96.5	97.5	97.1	96.7	96.9	97.1	97.1	96.9	97.5	97.3	97.5	99.2																												
DQ490540/RVA/Human-wt/BGD/RV161/2000/G12P[6]	97.7	97.5	69.8	69.8	97.7	69.8	69.8	98.1	97.7	97.9	97.5	97.5	97.7	97.9	97.9	97.7	98.6	98.4	98.8	97.9	97.5																												
JQ069378/RVA/Human-wt/CAN/RT008-07/2007/G2P[4]	97.3	97.1	70.2	70.2	97.3	70.2	70.2	97.7	97.3	97.5	97.1	97.1	97.3	97.5	97.5	97.3	98.1	98.1	97.9	97.5	97.1	98.4																												
KU360966/RVA/Human-wt/BRA/QUI-130-F2/2010/G12P[6]	97.3	97.1	69.8	69.8	97.3	69.8	69.8	97.9	97.3	97.5	97.1	97.1	97.3	97.5	97.5	97.3	97.1	96.9	97.1	96.7	96.3	97.5	97.1																												
LC433791/RVA/Human-wt/NPL/TK2615/2008/G11P25	68.5	68.9	98.4	98.4	68.9	99.0	98.6	68.9	69.3	68.7	68.5	68.9	68.9	68.9	68.5	68.7	69.3	69.5	69.1	69.8	69.3	69.1	69.5	69.1																												
MG181486/RVA/Human-wt/MWI/0P5-001/2008/G1P[8]	68.5	68.9	97.5	97.5	68.9	98.1	97.7	68.9	69.3	68.7	68.5	68.9	68.9	68.9	68.5	68.7	69.3	69.5	69.1	69.8	69.3	69.1	69.5	68.7	98.8																												
HQ025979/RVA/Human-wt/KOR/CAU-195/2006/G12P[6]	68.7	69.1	97.7	97.7	69.1	98.4	97.9	69.1	69.5	68.9	68.7	69.1	69.1	69.1	68.7	68.9	69.8	70.0	69.3	70.0	69.5	69.8	69.8	69.3	99.0	98.6																												
KJ753566/RVA/Human-wt/ZAF/MRC-DPRU4079-11/2011/G1P[8]	69.3	69.3	96.9	96.9	69.3	97.5	97.1	69.3	69.8	69.1	68.9	69.1	69.3	69.3	68.9	69.1	69.8	70.0	69.5	70.2	69.8	69.5	70.0	69.5	97.7	96.9	97.5																												
KJ751708/RVA/Human-wt/GMB/MRC-DPRU3176/2010/G1P[8]	68.9	69.3	97.1	97.1	69.3	97.7	97.3	69.3	69.8	69.1	68.9	69.3	69.3	68.9	69.1	69.8	70.0	69.5	70.2	69.8	69.5	70.0	69.1	98.4	98.8	97.7	96.5																												
KJ752233/RVA/Human-wt/ZMB/MRC-DPRU1648/2009/G1P[8]	68.3	68.7	97.1	97.1	68.7	97.7	97.3	68.7	69.1	68.5	68.3	68.7	68.7	68.7	68.3	68.5	69.1	69.3	68.9	69.5	69.1	68.9	69.3	68.9	98.4	99.2	98.1	96.5	97.9																												
KJ751928/RVA/Human-wt/SWZ/MRC-DPRU5119/2010/G1P[8]	69.1	69.5	97.7	97.7	69.5	98.4	97.9	69.5	70.0	69.3	69.1	69.5	69.5	69.5	69.1	69.3	69.8	70.0	69.5	70.2	69.8	69.5	70.0	69.5	99.0	98.1	98.8	97.5	97.7	97.7																												
KP[8]82753/RVA/Human-wt/MLI/Mali-021/2008/G1P[8]	68.9	69.3	97.3	97.3	69.3	97.9	97.5	69.3	69.3	69.1	68.9	69.3	69.3	69.3	68.9	69.1	69.8	70.0	69.5	70.0	69.5	69.5	70.0	69.5	98.1	98.1	97.5	96.3	98.1	97.7	97.5																												
KF636273/RVA/Human-wt/ZAF/MRC-DPRU2052/2010/G1P[8]	69.1	69.5	98.1	98.1	69.5	98.8	100.0	69.5	69.5	69.3	69.1	69.5	69.5	69.5	69.1	69.3	70.0	70.2	69.8	70.4	70.0	69.8	70.2	69.8	98.6	97.7	97.9	97.1	97.3	97.3	97.9	97.5																												
KC769377/RVA/Human-wt/AUS/CK00066/2007/G1P[8]	68.1	68.5	96.7	96.7	68.5	97.3	96.9	68.5	68.9	68.3	68.1	68.5	68.5	68.5	68.1	68.7	68.9	69.1	68.7	69.3	68.9	68.7	69.1	68.7	97.9	97.1	97.7	96.5	96.7	96.7	97.7	96.5	96.9																												
KJ753463/RVA/Human-wt/ZWE/MRC-DPRU1102/2012/G9P[8]	68.3	68.5	96.5	96.5	68.5	97.1	96.7	68.5	68.9	68.3	68.1	68.5	68.5	68.5	68.1	68.7	68.9	69.1	68.7	69.3	68.9	68.7	69.1	68.3	97.7	97.3	97.5	96.5	96.9	96.5	97.5	96.3	96.7	98.6																												
KX954620/RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P[8]	68.7	68.9	82.7	82.7	68.7	82.5	82.9	68.7	69.1	68.5	68.3	68.7	68.5	68.3	68.3	68.3	69.3	69.3	68.9	69.5	69.1	68.9	69.3	68.9	82.9	82.5	83.1	82.5	82.1	82.3	82.9	82.5	82.9	83.1	82.9																												
HQ392247/RVA/Human-wt/BEL/BE00030/2008/G1P[8]	67.3	67.9	82.9	82.9	67.7	82.7	83.1	67.7	68.1	67.5	67.3	67.7	67.5	67.3	67.3	67.3	68.1	68.1	67.7	68.3	67.9	67.7	68.1	67.9	83.1	82.7	83.3	82.3	82.3	82.5	83.1	82.7	83.1	83.1	82.9	97.3																												
JQ069436/RVA/Human-wt/CAN/RT004-09/2009/G3P[8]	69.1	69.5	82.5	82.5	69.3	81.9	81.9	69.3	69.8	69.1	68.9	69.3	69.1	68.9	68.9	68.9	69.5	69.5	69.1	69.3	68.9	69.1	69.8	69.1	82.3	81.9	82.5	81.3	81.5	81.7	82.5	82.1	81.9	82.1	81.9	94.9	94.7																												
KP752785/RVA/Human-wt/ETH/MRC-DPRU4970/2010/G12P[8]	69.3	69.5	82.5	82.5	69.3	82.1	82.1	69.3	69.8	69.3	69.1	68.9	69.1	68.9	68.9	68.9	69.8	69.8	69.5	69.8	69.3	69.5	70.0	69.3	82.5	82.1	82.7	82.5	82.5	81.9	82.1	82.7	82.1	81.9	82.1	90.7	89.9	88.7		

Appendix 17o-p: Nucleotide and amino acid identities for the NSP2 of the four Zambian reassortants

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NSP2 nucleotide identities among strains calculated using the p-distance algorithm in MEGA 6 (Tamura et al., 2013)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
RVA/Human-wt/ZMB/UFS-NGS-MRC-DRPU4749/2014/G2P[8]																																										
RVA/Human-wt/ZMB/UFS-NGS-MRC-DRPU13541/2016/G1P[8]	99.5																																									
RVA/Human-wt/ZMB/UFS-NGS-MRC-DRPU13327/2016/G2P[4]	82.7	82.5																																								
RVA/Human-wt/ZMB/UFS-NGS-MRC-DRPU13232/2016/G1P[8]	99.5	99.8	82.7																																							
MG181828/RVA/Human-wt/MWI/BID11E/2012/G2P[4]	99.8	99.5	82.8	99.5																																						
MG181630/RVA/Human-wt/MWI/BID225/2013/G1P[8]	99.7	99.4	82.6	99.4	99.7																																					
MG926743/RVA/Human-wt/MOZ/0440/2013/G2P[4]	98.8	98.5	82.0	98.5	98.8	98.7																																				
LC227895/RVA/Human-wt/IND/Kol-063/2013/G9P[4]	99.5	99.2	82.6	99.2	99.5	99.4	98.7																																			
JX965148/RVA/Human-wt/AUS/WAPC703/2010/G2P[4]	99.1	98.7	82.2	98.7	99.1	99.0	99.8	99.0																																		
LC477585/RVA/Human-wt/JPN/Tokyo18-41/2018/G2P[4]	98.5	98.2	82.3	98.2	98.5	98.4	99.3	98.4	99.5																																	
MK302413/RVA/Human-wt/IND/NIV1323769/2013/G1P[6]	82.9	82.7	99.5	82.9	83.0	82.8	82.2	82.8	82.4	82.5																																
KC822938/RVA/Human-wt/RUS/Nov12-N4489/2012/GXP[8]	83.0	82.8	99.4	83.0	83.1	82.9	82.3	82.9	82.5	82.6	99.7																															
KU048685/RVA/Human-wt/ITA/ME659-14/2014/G1P[8]	82.8	82.6	99.3	82.8	82.9	82.7	82.1	82.7	82.3	82.4	99.8	99.5																														
DQ492676/RVA/Human-wt/BGD/Dhaka16/2003/G1P[8]	82.3	82.1	98.4	82.3	82.4	82.2	81.5	82.2	81.7	81.8	98.5	98.4	98.3																													
LC374045/RVA/Human-wt/NPL/09N3012/2009/G12P[6]	82.8	82.6	98.1	82.8	82.9	82.7	82.1	82.7	82.3	82.4	98.4	98.3	98.2	98.4																												
KJ751929/RVA/Human-wt/SWZ/MRC-DPRU5119/2010/G1P[8]	82.7	82.5	98.6	82.7	82.8	82.6	81.7	82.6	82.0	82.1	98.7	98.6	98.5	98.5	98.2																												
KJ751863/RVA/Human-wt/UGA/MRC-DPRU3713/2010/G12P[6]	82.7	82.5	97.2	82.7	82.8	82.6	82.0	82.6	82.2	82.3	97.5	97.4	97.3	97.4	97.1	97.4																												
KJ870918/RVA/Human-wt/COD/KisB521/2008/G12P[6]	82.8	82.6	97.2	82.8	82.9	82.7	82.1	82.5	82.3	82.4	97.5	97.4	97.3	97.7	97.4	97.5	98.2																												
KJ751687/RVA/Human-wt/ZAF/MRC-DPRU1270/2009/G1P[8]	82.7	82.5	98.7	82.7	82.8	82.6	81.7	82.6	82.0	82.1	98.8	98.7	98.6	98.6	98.3	99.9	97.3	97.4																										
KJ752032/RVA/Human-wt/ETH/MRC-DPRU1843/2009/G1P[8]	82.7	82.5	98.1	82.7	82.8	82.6	82.4	82.6	82.6	82.7	98.4	98.3	98.2	98.4	98.5	98.4	97.2	97.4	98.5																								
KF812769/RVA/Human-wt/KOR/SeoulO291/2008/G1P[8]	82.5	82.3	98.2	82.5	82.6	82.4	82.0	82.4	82.2	82.3	98.3	98.2	98.1	98.1	98.0	98.5	96.7	97.1	98.6	98.2																						
LC086765/RVA/Human-wt/THA/SKT-98/2013/G1P[8]	82.6	82.4	97.0	82.4	82.7	82.5	81.8	82.5	82.1	82.2	97.3	97.4	97.3	97.3	97.6	97.5	96.3	96.4	97.6	97.6	97.1																					
MF184832/RVA/Human-wt/USA/CNMC123/2011/G2P[4]	82.8	82.6	97.4	82.8	82.9	82.7	82.3	82.7	82.5	82.6	97.7	97.6	97.5	97.9	98.4	97.9	96.7	97.3	97.8	98.4	97.7	96.9																				
JQ069293/RVA/Human-wt/CAN/RT006-07/2007/G1P[8]	82.2	82.0	97.8	82.2	82.3	82.1	81.6	82.1	81.8	82.0	98.1	98.0	97.9	98.3	98.2	98.1	97.2	97.3	98.2	98.2	97.7	97.1	97.7																		
KX954621/RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P[8]	82.4	82.3	89.9	82.5	82.4	82.3	82.6	82.2	82.6	82.7	90.1	90.2	90.1	90.3	90.5	90.5	90.1	90.9	90.3	90.2	90.1	89.9	89.9	90.3																	
JX027869/RVA/Human-wt/AUS/CK00088/2009/G1P[8]	82.7	82.5	99.2	82.7	82.8	82.6	82.0	82.6	82.2	82.3	99.5	99.4	99.3	98.6	98.3	98.8	97.5	97.6	99.0	98.5	98.4	97.4	97.8	98.2	90.2																
KJ454642/RVA/Human-wt/BRA/MA20306/2011/G9P[8]	82.5	82.3	97.9	82.5	82.6	82.4	81.7	82.4	82.0	82.1	98.2	98.1	98.0	98.6	98.3	98.4	97.3	97.6	98.3	98.3	97.8	97.4	98.0	98.2	90.5	98.3															
KJ752234/RVA/Human-wt/ZMB/MRC-DPRU1648/2009/G1P[8]	82.7	82.5	97.3	82.7	82.8	82.6	82.2	82.4	82.4	82.5	97.6	97.5	97.4	97.8	97.5	97.6	96.3	98.8	97.5	97.5	97.2	96.5	97.4	97.6	90.7	97.7	97.7													
KM660135/RVA/Human-wt/CMR/BA368/2010/G2P[4]	96.1	95.8	82.4	95.8	96.1	96.0	96.1	95.9	96.3	95.8	82.6	82.9	82.5	82.0	82.7	82.2	82.2	82.5	82.2	82.8	82.4	82.1	82.5	81.7	82.1	82.4	82.1	82.4													
KP752689/RVA/Human-wt/GMB/MRC-DPRU3199/2010/G2P[4]	95.9	95.6	82.9	95.6	95.9	95.8	95.9	95.7	96.1	95.6	83.1	83.4	83.0	82.5	83.2	82.7	82.7	83.2	82.7	83.3	82.9	82.6	83.0	82.3	82.6	82.9	82.6	83.1	98.5												
KP[8]82380/RVA/Human-wt/GHA/Ghan-010/2009/G2P[4]	96.2	95.9	82.7	95.9	96.2	96.1	96.2	96.0	96.4	95.9	82.9	83.2	82.8	82.3	83.0	82.5	82.5	83.0	82.5	83.1	82.7	82.4	82.8	82.1	82.4	82.7	82.4	82.9	98.8	99.7											
KJ752157/RVA/Human-wt/TGO/MRC-DPRU5124/2010/G2P[4]	96.1	95.8	82.7	95.8	96.1	96.0	96.1	95.9	96.3	95.8	82.9	83.2	82.8	82.3	83.0	82.5	82.5	83.0	82.5	83.1	82.7	82.4	82.8	82.1	82.4	82.7	82.4	82.9	98.7	99.8	99.9										
KP752895/RVA/Human-wt/ETH/MRC-DPRU1862/2009/G1P[8]	86.3	86.1	84.4	86.4	86.3	86.1	86.9	86.3	86.7	86.8	84.2	84.1	84.1	84.1	84.8	84.1	84.1	83.8	84.2	84.4	84.7	83.4	84.2	84.1	83.7	83.9	84.1	83.8	87.1	86.7	86.6	86.5									
KP753174/RVA/Human-wt/UGA/MRC-DPRU3710/2009/G2P[4]	97.9	97.6	82.8	97.6	97.9	97.8	98.0	97.8	98.2	97.9	83.0	83.1	82.9	82.4	82.9	82.6	82.8	82.9	82.6	83.2	82.8	82.5	83.1	82.1	82.7	82.8	82.6	82.8	96.4	96.2	96.5	96.4	87.1								
KF636318/RVA/Human-wt/ZAF/MRC-DPRU1061/2009/G2P[4]	97.3	97.0	83.1	97.0	97.3	97.2	97.4	97.2	97.6	97.1	83.3	83.6	83.4	82.7	83.2	82.9	82.7	83.2	82.9	83.5	83.1	82.8	83.4	82.4	82.9	83.1	82.8	83.1	96.9	97.1	97.4	97.3	86.4	97.7							
LC066646/RVA/Human-wt/THA/PCB-180/2013/G1P[8]	97.1	96.7	83.2	96.7	97.1	97.0	97.2	97.0	97.4	97.1	83.4	83.5	83.3	82.8	83.3	83.0	83.2	83.3	83.0	83.6	83.2	82.9	83.5	82.5	82.7	83.2	83.0	83.2	95.7	96.0	95.9	95.8	87.6	98.7	97.1						
LC086743/RVA/Human-wt/THA/LS-04/2013/G2P[8]	86.3	85.9	82.5	85.9	86.3	86.1	86.8	86.1	87.0	86.7	82.3	82.4	82.2	82.3	82.4	82.3	82.1	82.2	82.2	82.4	82.3	82.3	82.2	82.1	82.0	82.2	82.2	82.1	86.9	87.3	87.2	87.1	88.1	87.0	87.0	87.1					
KC822941/RVA/Human-wt/RUS/O1321/2012/G2P[4]	97.4	97.1	82.6	97.1	97.4	97.3	97.5	97.3	97.7	97.4	82.8	82.9	82.7	82.2	82.7	82.4	82.6	82.7	82.4	83.0	82.6	82.3	82.9	81.8	82.5	82.6	82.4	82.6	96.2	95.9	96.2	96.1	87.0	99.1	97.2	98.4	86.9				
JQ069354/RVA/Human-wt/CAN/RT008-09/2009/G2P[4]	98.7	98.4	82.6	98.4	98.7	98.6	98.8	98.6	99.1	98.5	82.8	82.9	82.7	82.2	82.7	82.4	82.6	82.7	82.4	83.0	82.6	82.3	82.9	82.1	82.6	82.6	82.4	82.6	96.0	95.8	96.1	96.0	86.6	97.9	97.3	97.3	86.9	97.4			
MG573360/RVA/Human-wt/BRA/IAL-R3123/2013/G1P[8]	97.4	97.1	83.0	97.1	97.4	97.3	97.5	97.3	97.7	97.4	83.2	83.3	83.1	82.6	83.1	82.8	83.0	83.1	82.8	83.4	83.0	82.7	83.3	82.3	82.7	83.0	82.8	83.0	96.2	96.1	96.2	96.1	87.3	99.1	97.2	99.5	86.8	98.7	97.6		
KP752775/RVA/Human-wt/ZMB/MRC-DPRU1673/2009/G2P[4]	97.5	97.2	83.2	97.2	97.5	97.4	97.6	97.4	97.8	97.3	83.4	83.7	83.5	82.8	83.3	83.0	82.8	83.3	83.0	83.6	83.2	82.9	83.5	82.5	83.0	83.2	82.9	83.2	97.1	97.3	97.6	97.5	86.6	97.9	99.8	97.3	87.0	97.4	97.5	97.4	
JX946175/RVA/Human-wt/CHN/E2451/2011/G3P[9] - outgroup	71.2	71.0	72.6	71.2	71.4	71.1	71.0	71.1	71.0	70.8	72.6	72.7	72.4	73.0	73.1	72.9	72.7	73.0	72.8	72.9	73.6	72.1	73.5	72.6	73.5	72.9	72.8	73.1	71.7	72.0	71.9	72.0	72.9	71.6	71.6	71.6	71.9	71.7	71.5	71.6	71.8	

p.

NSP2 amino acid identities among strains calculated using the p-distance algorithm in MEGA 6 (Tamura et al., 2013)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
RVA/Human-wt/ZMB/UFS-NGS-MRC-DRPU4749/2014/G2P[8]																																										
RVA/Human-wt/ZMB/UFS-NGS-MRC-DRPU13541/2016/G1P[8]	99.0																																									
RVA/Human-wt/ZMB/UFS-NGS-MRC-DRPU13327/2016/G2P[4]	90.1	89.2																																								
RVA/Human-wt/ZMB/UFS-NGS-MRC-DRPU13232/2016/G1P[8]	99.4	99.7	89.5																																							
MG181828/RVA/Human-wt/MWI/BID11E/2012/G2P[4]	99.7	98.7	90.1	99.0																																						
MG181630/RVA/Human-wt/MWI/BID225/2013/G1P[8]	99.7	98.7	89.8	99.0	99.4																																					
MG926743/RVA/Human-wt/MOZ/0440/2013/G2P[4]	99.4	98.4	89.8	98.7	99.0	99.0																																				
LC227895/RVA/Human-wt/IND/Kol-063/2013/G9P[4]	99.7	98.7	89.8	99.0	99.4	99.4	99.0																																			
JX965148/RVA/Human-wt/AUS/WAPC703/2010/G2P[4]	99.4	98.4	89.8	98.7	99.0	99.0	100.0	99.0																																		
LC477585/RVA/Human-wt/JPN/Tokyo18-41/2018/G2P[4]	99.0	98.1	89.5	98.4	98.7	98.7	99.7	98.7	99.7																																	
MK302413/RVA/Human-wt/IND/NIV1323769/2013/G1P[6]	89.8	88.9	99.7	89.2	89.8	89.5	89.5	89.5	89.5	89.2																																
KC822938/RVA/Human-wt/RUS/Nov12-N4489/2012/GXP[8]	90.1	89.2	100.0	89.5	90.1	89.8	89.8	89.8	89.8	89.5	99.7																															
KU048685/RVA/Human-wt/ITA/ME659-14/2014/G1P[8]	89.5	88.5	99.4	88.9	89.5	89.2	89.2	89.2	89.2	88.9	99.7	99.4																														
DQ492676/RVA/Human-wt/BGD/Dhaka16/2003/G1P[8]	90.1	89.2	99.4	89.5	90.1	89.8	89.8	89.8	89.8	89.5	99.0	99.4	98.7																														
LC374045/RVA/Human-wt/NPL/09N3012/2009/G12P[6]	90.1	89.2	99.0	89.5	90.1	89.8	89.8	89.8	89.8	89.5	98.7	99.0	98.4	99.0																														
KJ751929/RVA/Human-wt/SWZ/MRC-DPRU5119/2010/G1P[8]	89.8	88.9	99.4	89.2	89.8	89.5	89.5	89.5	89.5	89.2	99.0	99.4	98.7	98.7	99.0																														
KJ751863/RVA/Human-wt/UGA/MRC-DPRU3713/2010/G12P[6]	89.2	88.2	98.1	88.5	89.2	88.9	88.9	88.9	88.9	88.5	97.8	98.1	97.5	98.1	97.8	97.5																														
KJ870918/RVA/Human-wt/COD/KisB521/2008/G12P[6]	89.8	88.9	99.0	89.2	89.8	89.5	89.5	89.5	89.5	89.2	98.7	99.0	98.4	99.0	98.7	98.4	99.0																														
KJ751687/RVA/Human-wt/ZAF/MRC-DPRU1270/2009/G1P[8]	89.8	88.9	99.4	89.2	89.8	89.5	89.5	89.5	89.5	89.2	99.0	99.4	98.7	98.7	99.0	100.0	97.5	98.4																														
KJ752032/RVA/Human-wt/ETH/MRC-DPRU1843/2009/G1P[8]	90.1	89.2	98.7	89.5	90.1	89.8	89.8	89.8	89.8	89.5	98.4	98.7	98.1	98.7	99.0	98.7	97.5	98.4	98.7																														
KF812769/RVA/Human-wt/KOR/SeoulO291/2008/G1P[8]	89.5	88.5	99.0	88.9	89.5	89.2	89.2	89.2	89.2	88.9	98.7	99.0	98.4	98.4	98.7	99.0	97.1	98.1	99.0	98.4																														
LC086765/RVA/Human-wt/THA/SKT-98/2013/G1P[8]	89.8	88.9	98.4	89.2	89.8	89.5	89.5	89.5	89.5	89.2	98.1	98.4	97.8	98.4	98.7	98.4	97.8	98.1	98.4	99.0	98.1																														
MF184832/RVA/Human-wt/USA/CNMC123/2011/G2P[4]	90.1	89.2	98.7	89.5	90.1	89.8	89.8	89.8	89.8	89.5	98.4	98.7	98.1	98.7	99.0	98.7	97.5	98.4	98.7	99.4	98.4	98.4																														
JQ069293/RVA/Human-wt/CAN/RT006-07/2007/G1P[8]	90.1	89.2	98.4	89.5	90.1	89.8	89.8	89.8	89.8	89.5	98.1	98.4	97.8	98.4	99.0	98.4	97.1	98.1	98.4	98.4	98.1	98.1	98.4																														
KX954621/RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P[8]	90.4	89.5	96.2	89.8	90.1	90.1	90.8	90.1	90.8	90.4	95.9	96.2	95.5	96.2	96.2	95.9	95.9	96.5	95.9	95.9	95.5	96.2	95.9	95.5																														
JX027869/RVA/Human-wt/AUS/CK00088/2009/G1P[8]	89.8	88.9	99.7	89.2	89.8	89.5	89.5	89.5	89.5	89.2	99.4	99.7	99.0	99.0	98.7	99.0	97.8	98.7	99.0	98.4	98.7	98.1	98.4	98.1	95.9																														
KJ454642/RVA/Human-wt/BRA/MA20306/2011/G9P[8]	89.8	88.9	98.7	89.2	89.8	89.5	89.5	89.5	89.5	89.2	98.4	98.7	98.1	98.7	99.0	98.7	97.5	98.4	98.7	98.7	98.4	98.4	98.7	98.4	95.9	98.4																														
KJ752234/RVA/Human-wt/ZMB/MRC-DPRU1648/2009/G1P[8]	89.8	88.9	99.0	89.2	89.8	89.5	89.5	89.5	89.5	89.2	98.7	99.0	98.4	99.0	98.7	98.4	99.0	100.0	98.4	98.4	98.1	98.1	98.4	98.1	96.5	98.7	98.4																														
KM660135/RVA/Human-wt/CMR/BA368/2010/G2P[4]	97.8	96.8	90.1	97.1	97.5	97.5	98.1	97.5	98.1	97.8	89.8	90.1	89.5	90.1	90.1	89.8	89.2	89.8	89.8	90.1	89.5	89.5	90.1	90.4	90.8	89.8	89.8	89.8																														
KP752689/RVA/Human-wt/GMB/MRC-DPRU3199/2010/G2P[4]	98.4	97.5	90.1	97.8	98.1	98.1	98.4	98.1	98.4	98.1	89.8	90.1	89.5	90.1	90.1	89.8	89.2	89.8	89.8	90.1	89.5	89.8	90.1	90.4	91.1	89.8	89.8	89.8	99.4																														
KP[8]82380/RVA/Human-wt/GHA/Ghan-010/2009/G2P[4]	98.4	97.5	90.1	97.8	98.1	98.1	98.4	98.1	98.4	98.1	89.8	90.1	89.5	90.1	90.1	89.8	89.2	89.8	89.8	90.1	89.5	89.8	90.1	90.4	91.1	89.8	89.8	89.8	99.4	100.0																														
KJ752157/RVA/Human-wt/TGO/MRC-DPRU5124/2010/G2P[4]	98.4	97.5	90.1	97.8	98.1	98.1	98.4	98.1	98.4	98.1	89.8	90.1	89.5	90.1	90.1	89.8	89.2	89.8	89.8	90.1	89.5	89.8	90.1	90.4	91.1	89.8	89.8	89.8	99.4	100.0	100.0																														
KP753174/RVA/Human-wt/UGA/MRC-DPRU3710/2009/G2P[4]	98.7	97.8	89.8	98.1	98.4	98.4	98.7	98.4	98.7	98.4	89.5	89.8	89.2	89.8	89.8	89.5	88.9	89.5	89.5	89.8	89.2	89.5	89.8	89.2	90.1	88.9	88.9	88.9	96.2	96.5	96.5	96.5																														
KF636318/RVA/Human-wt/ZAF/MRC-DPRU1061/2009/G2P[4]	98.7	97.8	89.8	98.1	98.4	98.4	98.7	98.4	98.7	98.4	89.5	89.8	89.2	89.8	89.8	89.5	88.9	89.5	89.5	89.8	89.2	89.5	89.8	89.2	90.8	89.5	89.5	89.5	98.4	99.0	99.0	99.0	96.8																														
KF636318/RVA/Human-wt/ZAF/MRC-DPRU1061/2009/G2P[4]	98.1	97.1	89.8	97.5	97.8	97.8	98.1	97.8	98.1	97.8	89.5	89.8	89.8	89.8	89.5	88.9	89.5	89.5	89.8	89.2	89.5	89.8	89.8	91.1	89.5	89.5	89.5	97.8	98.4	98.4	98.4	96.5	98.7																														
LC066646/RVA/Human-wt/THA/PCB-180/2013/G1P[8]	98.4	97.5	89.8	97.8	98.1	98.1	98.7	98.1	98.7	98.4	89.5	89.8	89.2	89.8	89.8	89.5	88.9	89.5	89.5	89.8	89.2	89.5	89.8	89.8	90.8	89.5	89.5	89.5	98.4	98.7	98.7	98.7	96.8	99.7	98.4																														
LC086743/RVA/Human-wt/THA/LS-04/2013/G2P[8]	95.2	94.3	88.9	94.6	94.9	94.9	95.9	94.9	95.9	95.5	88.5	88.9	88.2	88.9	88.9	88.5	87.9	88.5	88.5	89.2	88.9	88.5	89.2	88.9	89.8	88.5	88.5	88.5	95.2	95.5	95.5	95.5	96.5	96.5	95.5	96.5						
KC822941/RVA/Human-wt/RUS/O1321/2012/G2P[4]	97.8	96.8	89.5	97.1	97.5	97.5	98.1	97.5	98.1	97.8	89.2	89.5	88.9	89.5	89.5	89.2	88.5	89.2	89.2	89.5	88.9	89.2	89.5	89.5	90.4	89.2	89.2	89.2	97.8	98.1	98.1	98.1	97.1	99.0	97.8	99.4	96.2					
JQ069354/RVA/Human-wt/CAN/RT008-09/2009/G2P[4]	99.4	98.4	89.5	98.7	99.0	99.0	99.4	99.0	99.4	99.0	89.2	89.5	88.9	89.5	89.5	89.2	88.5	89.2	89.2	89.5	88.9	89.2	89.5	89.5	90.4	89.2	89.2	89.2	97.8	98.4	98.4	98.4	96.5	98.7	98.1	98.4	95.2	97.8				
MG573360/RVA/Human-wt/BRA/IAL-R3123/2013/G1P[8]	98.4	97.5	89.8	97.8	98.1	98.1	98.7	98.1	98.7	98.4	89.5	89.8	89.2	89.8	89.8	89.5	88.9	89.5	89.5	89.8	89.2	89.5	89.8	89.8	90.8	89.5	89.5	89.5	98.4	98.7	98.7	98.7	96.8	99.7	98.4	100.0	96.5	99.4	98.4			
KP752775/RVA/Human-wt/ZMB/MRC-DPRU1673/2009/G2P[4]	98.4	97.5	89.8	97.8	98.1	98.1	98.4	98.1	98.4	98.1	89.5	89.8	89.8	89.8	89.8	89.5	88.9	89.

Appendix 17q-r: Nucleotide and amino acid identities for the NSP3 of the four Zambian reassortants

q.

NSP3 nucleotide identities among strains calculated using the p-distance algorithm in MEGA 6 (Tamura et al., 2013)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4749/2014/G2P[8]																																											
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13232/2016/G1P[8]	79.0																																										
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13327/2016/G2P[4]	98.7	79.3																																									
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13541/2016/G1P[8]	78.9	99.9	79.2																																								
MG181917/RVA/Human-wt/MWI/BID15V/2012/G2P[4]	99.7	79.3	98.6	79.2																																							
MG181763/RVA/Human-wt/MWI/BID2Q//2014/G1P[8]	99.4	78.8	98.3	78.6	99.5																																						
LC374134/RVA/Human-wt/NPL/09N3140/2009/G12P[6]	78.6	98.8	79.0	98.7	79.0	78.4																																					
MG181532/RVA/Human-wt/MWI/BID14A/2012/G1P[8]	78.4	98.7	79.0	98.6	78.8	78.2	99.4																																				
KX536643/RVA/Human-wt/IND/RV09/2009/G9P[4]	78.9	98.7	79.4	98.6	79.2	78.6	99.2	99.2																																			
MG181499/RVA/Human-wt/MWI/BID110/2012/G1P[8]	78.4	98.5	79.0	98.4	78.8	78.2	99.2	99.5	99.4																																		
DQ492677/RVA/Human-wt/BGD/Dhaka16/2003/G1P[8]	78.6	98.4	79.0	98.3	79.0	78.4	98.9	98.7	98.6	98.6																																	
MK302416/RVA/Human-wt/NIV1323769/2013/G1P[6]	78.5	98.5	78.9	98.4	78.9	78.3	99.0	98.8	98.7	98.7	98.6																																
MG926744/RVA/Human-wt/MOZ/0440/2013/G2P[4]	98.9	79.3	99.6	79.2	98.8	98.5	79.0	78.8	79.2	78.8	79.0	78.9																															
KP007156/RVA/Human-wt/PHI/TGO12-003/2012/G2P[4]	99.2	79.3	99.5	79.2	99.1	98.8	79.0	78.8	79.2	78.8	79.0	78.9	99.7																														
MG891990/RVA/Human-wt/MOZ/0126/2013/G2P[4]	98.9	79.3	99.6	79.2	98.8	98.5	79.0	78.8	79.2	78.8	79.0	78.9	100.0	99.7																													
LC227906/RVA/Human-wt/IND/Kol-063/2013/G9P[4]	98.5	78.6	98.9	78.5	98.4	98.2	78.3	78.1	78.5	78.1	78.3	78.2	99.4	99.2	99.4																													
JX965151/RVA/Human-wt/AUS/WAPC703/2010/G2P[4]	99.0	79.1	99.0	79.0	98.9	98.6	78.8	78.5	79.0	78.5	78.8	78.6	99.5	99.6	99.5	99.0																												
MG181323/RVA/Human-wt/MWI/BID1JK/2013/G2P[4]	99.7	79.2	98.6	79.1	99.8	99.5	78.9	78.6	79.1	78.6	78.9	78.8	98.8	99.1	98.8	98.4	98.9																											
HQ641370/RVA/Human-wt/BGD/MMC88/2005/G2P[4]	99.2	79.1	98.8	79.0	99.1	98.8	78.8	78.5	79.0	78.5	78.8	78.6	99.2	99.4	99.2	98.8	99.4	99.1																											
KX954622/RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P[8]	78.0	95.4	78.4	95.3	78.3	78.2	95.9	95.7	95.6	95.6	95.7	95.4	78.3	78.3	78.3	77.7	78.1	78.2	78.1																										
KP[8]82667/RVA/Human-wt/GHA/Ghan-147/2008/G1P[8]	78.2	97.9	78.5	97.7	78.5	78.0	98.4	98.2	98.1	98.1	98.8	98.3	78.5	78.5	78.5	77.9	78.3	78.4	78.3	95.5																								
KP753209/RVA/Human-wt/TGO/MRC-DPRU5153/2010/G1P[8]	78.1	97.5	78.4	97.4	78.4	77.9	98.2	98.0	98.1	97.9	98.6	97.9	78.4	78.4	78.4	77.8	78.2	78.3	78.2	95.2	98.9																						
JQ069271/RVA/Human-wt/CAN/RT010-09/2009/G3P[8]	78.3	97.3	78.8	97.2	78.6	78.1	98.1	97.9	97.7	97.7	97.6	97.5	78.6	78.6	78.6	78.0	78.4	78.5	78.4	97.0	97.4	97.1																					
KJ752703/RVA/Human-wt/ETH/MRC-DPRU1840/2007/G1P[8]	78.1	98.3	78.4	98.2	78.4	77.9	99.2	98.8	98.7	98.7	98.4	98.5	78.4	78.4	78.4	77.8	78.2	78.3	78.2	95.4	97.9	97.6	97.5																			
KJ870919/RVA/Human-wt/COD/KisB521/2008/G12P[6]	77.9	93.9	78.2	93.8	78.2	77.9	94.2	94.0	94.1	93.9	94.2	94.1	78.1	78.1	78.1	77.7	77.9	78.1	77.9	96.7	93.8	93.5	94.7	93.7																		
KJ752235/RVA/Human-wt/ZMB/MRC-DPRU1648/2009/G1P[8]	78.5	97.1	79.0	97.0	78.6	78.1	97.9	97.4	97.3	97.3	97.6	97.3	78.9	78.9	78.9	78.2	78.6	78.5	78.6	96.4	97.2	96.9	98.1	97.1	94.5																	
KJ751688/RVA/Human-wt/ZAF/MRC-DPRU1270/2009/G1P[8]	79.0	97.6	79.3	97.5	79.3	78.8	98.4	98.2	98.3	98.1	98.4	98.1	79.3	79.3	79.3	78.6	79.1	79.2	79.1	95.8	98.1	98.1	97.7	97.9	94.3	98.0																
KP752863/RVA/Human-wt/ZMB/MRC-DPRU1660/2008/G12P[6]	78.3	98.3	78.6	98.2	78.6	78.1	99.0	98.6	98.7	98.5	98.2	98.5	78.6	78.6	78.6	78.0	78.4	78.5	78.4	95.2	97.6	97.4	97.3	98.9	93.8	96.9	97.6															
KJ751930/RVA/Human-wt/SWZ/MRC-DPRU5119/2010/G1P[8]	79.0	97.6	79.3	97.5	79.3	78.8	98.4	98.2	98.3	98.1	98.4	98.1	79.3	79.3	79.3	78.6	79.1	79.2	79.1	95.8	98.1	98.1	97.7	97.9	94.3	98.0	100.0	97.6														
KP[6]45330/RVA/Human-wt/AUS/CK00108/2011/G1P[8]	78.5	97.7	78.9	97.6	78.9	78.3	98.5	98.3	98.4	98.2	98.3	98.2	78.9	78.9	78.9	78.2	78.6	78.8	78.4	95.9	98.0	98.0	97.9	98.0	94.2	97.9	99.0	97.7	99.0													
KU048714/RVA/Human-wt/ITA/PA525/14/2014/G12P[8]	78.3	98.3	78.6	98.2	78.6	78.1	98.8	98.6	98.5	98.7	98.2	99.1	78.6	78.6	78.6	78.0	78.4	78.5	78.4	95.2	97.9	97.4	97.3	98.3	93.9	96.9	97.6	98.3	97.6	97.7												
KM660170/RVA/Human-wt/CMR/MA104/2011/G2P[4]	97.5	78.5	97.1	78.4	97.4	97.3	78.2	78.0	78.4	78.0	78.2	78.1	97.3	97.4	97.3	96.9	97.4	97.4	97.9	78.3	77.8	77.7	78.0	77.7	78.0	78.6	78.8	77.9	78.8	78.1	77.9											
JQ069270/RVA/Human-wt/CAN/RT008-09/2009/G2P[4]	97.4	79.2	97.4	79.1	97.3	97.0	78.9	78.6	79.1	78.6	78.9	78.8	97.6	97.7	97.6	97.6	97.7	97.3	98.0	78.8	78.2	78.1	78.9	78.3	78.8	79.3	79.2	78.5	79.2	78.8	78.5	97.1										
LC086777/RVA/Human-wt/THA/BD-20/2013/G2P[4]	97.2	79.2	97.2	79.1	97.1	96.8	78.9	78.6	79.1	78.6	78.9	78.8	97.4	97.5	97.4	97.4	97.5	97.1	97.7	78.5	78.2	78.1	78.6	78.3	78.5	79.1	79.4	78.5	79.4	79.0	78.5	97.1	98.9									
KF716409/RVA/Human-wt/USA/VU10-11-11/2011/G2P[4]	99.5	79.1	98.6	79.0	99.4	99.0	78.8	78.5	79.0	78.5	78.8	78.6	99.0	99.1	99.0	98.6	99.1	99.4	99.4	78.1	78.3	78.2	78.4	78.2	77.9	78.6	79.1	78.4	79.1	78.6	78.4	97.6	97.5	97.3								
MG573363/RVA/Human-wt/BRA/IAL-R3122/2013/G1P[8]	97.0	79.5	96.8	79.4	96.9	96.6	79.2	79.0	79.4	79.0	79.2	79.1	97.0	97.1	97.0	96.8	97.1	96.9	97.5	79.1	78.5	78.4	79.0	78.6	79.1	79.4	79.5	78.9	79.5	79.1	78.9	97.3	97.2	97.2	97.3							
LC086744/RVA/Human-wt/THA/LS-04/2013/G2P[8]	97.0	79.2	97.0	79.1	96.9	96.6	78.9	78.6	79.1	78.6	78.9	78.8	97.2	97.1	97.2	96.8	97.3	96.9	97.5	78.8	78.6	78.3	78.6	78.3	78.8	79.1	79.4	78.5	79.4	78.8	78.5	97.3	97.0	97.0	97.3	98.7						
KJ918989/RVA/Human-wt/HUN/ERNS044/2012/G2P[4]	98.2	79.1	98.0	79.0	98.1	97.7	78.8	78.5	79.0	78.5	78.8	78.6	98.2	98.3	98.2	97.7	98.3	98.1	98.7	78.6	78.3	78.2	78.5	78.2	78.4	79.2	79.3	78.4	79.3	78.9	78.4	98.1	98.4	98.2	98.3	98.0	98.0					
KP[8]82920/RVA/Human-wt/MLI/Mali-038/2008/G1P[8]	97.5	78.5	97.3	78.4	97.4	97.3	78.4	78.2	78.6	78.2	78.4	78.3	97.5	97.6	97.5	97.1	97.6	97.4	98.1	78.5	78.0	77.9	78.2	77.9	78.1	78.9	79.0	78.1	79.0	78.3	78.1	99.4	97.3	97.1	97.9	97.5	97.5	98.3				
KP752776/RVA/Human-wt/ZMB/MRC-DPRU1673/2009/G2P[4]	97.3	79.1	97.4	79.0	97.2	97.1	78.8	78.5	79.0	78.5	78.8	78.5	97.6	97.7	97.6	97.2	97.7	97.2	97.9	78.3	78.3	78.2	78.5	78.2	77.8	79.0	79.3	78.4	79.3	78.6	78.4	98.5	97.3	97.3	97.6	97.3	97.3	98.1	98.7			
MT674498/RVA/Human-wt/BRA/TO-243/2015/G3P[8]	78.6	99.1	79.0	99.0	79.0	78.4	99.7	99.5	99.4	99.4	99.0	99.4	79.0	79.0	79.0	78.3	78.8	78.9	78.8	96.0	98.7	98.3	98.2	99.1	94.3	97.7	98.5	98.9	98.5	98.6	99.1	78.2	78.9	78.9	78.8	79.2	78.9	78.8	78.4	78.8			
MT674485/RVA/Human-wt/BRA/TO-186/2014/G12P[8]	78.6	99.1	79.0	99.0	79.0	78.4	99.7	99.5	99.4	99.4	99.0	99.4	79.0	79.0	79.0	78.3	78.8	78.9	78.8	96.0	98.7	98.3	98.2	99.1	94.3	97.7	98.5	98.9	98.5	98.6	99.1	78.2	78.9	78.9	78.8	79.2	78.9	78.8	78.4	78.8	100.0		
JX946176/RVA/Human-wt/CHN/E2451/2011/G3P[9] - outgroup	81.4	75.4	81.2	75.3	81.5	81.3	75.3	74.9	75.3	74.8	75.5	75.4	81.3	81.4	81.3	81.1	81.4	81.4	81.8	75.3	75.8	74.9	74.8	74.7	76.1	75.4	75.5	75.2	75.5	75.0	75.0	81.5	80.9	81.0	81.5	82.2	82.4	81.1	82.0	81.4	75.4	75.4

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NSP3 amino acid identities among strains calculated using the p-distance algorithm in MEGA 6 (Tamura et al., 2013)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4749/2014/G2P[8]																																											
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13232/2016/G1P[8]	82.3																																										
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13327/2016/G2P[4]	99.7	82.6																																									
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13541/2016/G1P[8]	81.9	99.7	82.3																																								
MG181917/RVA/Human-wt/MWI/BID15V/2012/G2P[4]	100.0	82.3	99.7	81.9																																							
MG181763/RVA/Human-wt/MWI/BID2Q//2014/G1P[8]	99.7	81.9	99.4	81.6	99.7																																						
LC374134/RVA/Human-wt/NPL/09N3140/2009/G12P[6]	81.9	99.7	82.3	99.4	81.9	81.6																																					
MG181532/RVA/Human-wt/MWI/BID14A/2012/G1P[8]	81.3	99.0	81.6	98.7	81.3	81.0	99.0																																				
KX536643/RVA/Human-wt/IND/RV09/2009/G9P[4]	81.9	99.7	82.3	99.4	81.9	81.6	100.0	99.0																																			
MG181499/RVA/Human-wt/MWI/BID110/2012/G1P[8]	81.3	99.0	81.6	98.7	81.3	81.0	99.4	99.0	99.4																																		
DQ492677/RVA/Human-wt/BGD/Dhaka16/2003/G1P[8]	82.3	99.4	82.6	99.0	82.3	81.9	99.7	98.7	99.7	99.0																																	
MK302416/RVA/Human-wt/IND/NIV1323769/2013/G1P[6]	81.6	99.0	81.9	98.7	81.6	81.3	99.4	98.4	99.4	98.7	99.0																																
MG926744/RVA/Human-wt/MOZ/0440/2013/G2P[4]	100.0	82.3	99.7	81.9	100.0	99.7	81.9	81.3	81.9	81.3	82.3	81.6																															
KP007156/RVA/Human-wt/PHI/TGO12-003/2012/G2P[4]	100.0	82.3	99.7	81.9	100.0	99.7	81.9	81.3	81.9	81.3	82.3	81.6	100.0																														
MG891990/RVA/Human-wt/MOZ/0126/2013/G2P[4]	100.0	82.3	99.7	81.9	100.0	99.7	81.9	81.3	81.9	81.3	82.3	81.6	100.0	100.0																														
LC227906/RVA/Human-wt/IND/Kol-063/2013/G9P[4]	99.4	81.6	99.0	81.3	99.4	99.7	81.3	80.6	81.3	80.6	81.6	81.0	99.4	99.4	99.4																														
JX965151/RVA/Human-wt/AUS/WAPC703/2010/G2P[4]	100.0	82.3	99.7	81.9	100.0	99.7	81.9	81.3	81.9	81.3	82.3	81.6	100.0	100.0	100.0	99.4																													
MG181323/RVA/Human-wt/MWI/BID1JK/2013/G2P[4]	99.7	82.3	99.4	81.9	99.7	99.4	81.9	81.3	81.9	81.3	82.3	81.6	99.7	99.7	99.7	99.0	99.7																													
HQ641370/RVA/Human-wt/BGD/MMC88/2005/G2P[4]	100.0	82.3	99.7	81.9	100.0	99.7	81.9	81.3	81.9	81.3	82.3	81.6	100.0	100.0	100.0	99.4	100.0	99.7																												
KX954622/RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P[8]	82.6	97.4	82.9	97.1	82.6	82.3	97.7	96.8	97.7	97.1	98.1	97.1	82.6	82.6	82.6	81.9	82.6	82.6	82.6																											
KP[8]82667/RVA/Human-wt/GHA/Ghan-147/2008/G1P[8]	81.3	99.0	81.6	98.7	81.3	81.0	99.4	98.4	99.4	98.7	99.0	98.7	81.3	81.3	81.3	80.6	81.3	81.3	81.3	97.7																								
KP753209/RVA/Human-wt/TGO/MRC-DPRU5153/2010/G1P[8]	81.3	99.0	81.6	98.7	81.3	81.0	99.4	98.4	99.4	98.7	99.0	98.7	81.3	81.3	81.3	80.6	81.3	81.3	81.3	97.7	99.4																						
JQ069271/RVA/Human-wt/CAN/RT010-09/2009/G3P[8]	82.6	99.0	82.9	98.7	82.6	82.3	99.4	98.4	99.4	98.7	99.0	98.7	82.6	82.6	82.6	81.9	82.6	82.6	82.6	98.4	98.7	98.7																					
KJ752703/RVA/Human-wt/ETH/MRC-DPRU1840/2007/G1P[8]	81.9	99.4	82.3	99.0	81.9	81.6	99.7	98.7	99.7	99.0	99.4	99.0	81.9	81.9	81.9	81.3	81.9	81.9	81.9	97.7	99.0	99.0	99.4																					
KJ870919/RVA/Human-wt/COD/KisB521/2008/G12P[6]	82.3	96.8	82.6	96.5	82.3	81.9	97.1	96.1	97.1	96.5	97.4	97.1	82.3	82.3	82.3	81.6	82.3	82.3	82.3	98.1	96.5	96.5	97.7	97.1																			
KJ752235/RVA/Human-wt/ZMB/MRC-DPRU1648/2009/G1P[8]	82.6	98.7	82.9	98.4	82.6	82.3	99.0	98.1	99.0	98.4	98.7	98.7	82.6	82.6	82.6	81.9	82.6	82.6	82.6	98.1	98.4	98.4	99.7	99.0	97.7																	
KJ751688/RVA/Human-wt/ZAF/MRC-DPRU1270/2009/G1P[8]	82.3	99.0	82.6	98.7	82.3	81.9	99.4	98.4	99.4	98.7	99.0	98.7	82.3	82.3	82.3	81.6	82.3	82.3	82.3	97.7	98.7	98.7	99.4	99.4	97.1	99.0																	
KP752863/RVA/Human-wt/ZMB/MRC-DPRU1660/2008/G12P[6]	81.9	99.0	82.3	98.7	81.9	81.6	99.4	98.4	99.4	98.7	99.0	98.7	81.9	81.9	81.9	81.3	81.9	81.9	81.9	97.1	98.7	98.7	98.7	99.0	96.5	98.4	98.7																
KJ751930/RVA/Human-wt/SWZ/MRC-DPRU5119/2010/G1P[8]	82.3	99.0	82.6	98.7	82.3	81.9	99.4	98.4	99.4	98.7	99.0	98.7	82.3	82.3	82.3	81.6	82.3	82.3	82.3	97.7	98.7	98.7	99.4	99.4	97.1	99.0	100.0	98.7															
KP[6]45330/RVA/Human-wt/AUS/CK00108/2011/G1P[8]	81.9	99.0	82.3	98.7	81.9	81.6	99.4	98.4	99.4	98.7	99.0	98.7	81.9	81.9	81.9	81.3	81.9	81.9	81.9	97.7	98.7	98.7	99.4	99.4	97.1	99.0	99.4	98.7	99.4														
KU048714/RVA/Human-wt/ITA/PA525/14/2014/G12P[8]	81.3	99.0	81.6	98.7	81.3	81.0	99.4	98.4	99.4	98.7	99.0	99.4	81.3	81.3	81.3	80.6	81.3	81.3	81.3	97.1	98.7	98.7	98.7	99.0	96.5	98.4	98.7	98.7	98.7	98.7													
KM660170/RVA/Human-wt/CMR/MA104/2011/G2P[4]	98.7	82.3	98.7	81.9	98.7	98.4	81.9	81.3	81.9	81.3	82.3	81.6	98.7	98.7	98.7	98.1	98.7	98.4	98.7	82.6	81.3	81.3	82.6	81.9	82.3	82.6	82.3	81.9	82.3	81.9	81.3												
JQ069270/RVA/Human-wt/CAN/RT008-09/2009/G2P[4]	98.1	82.3	97.7	81.9	98.1	97.7	81.9	81.3	81.9	81.3	82.3	81.6	98.1	98.1	98.1	97.4	98.1	97.7	98.1	82.6	81.3	81.3	82.6	81.9	82.3	82.6	82.3	81.9	82.3	81.9	81.3	97.1											
LC086777/RVA/Human-wt/THA/BD-20/2013/G2P[4]	98.4	82.9	98.1	82.6	98.4	98.1	82.6	81.9	82.9	82.3	98.4	98.4	98.4	98.4	98.4	97.7	98.4	98.1	98.4	82.6	81.9	81.9	82.6	82.6	82.3	82.6	82.9	82.6	82.9	82.6	82.9	97.1	98.4										
KF716409/RVA/Human-wt/USA/VU10-11-11/2011/G2P[4]	100.0	82.3	99.7	81.9	100.0	99.7	81.9	81.3	81.9	81.3	82.3	81.6	100.0	100.0	100.0	99.4	100.0	99.7	100.0	82.6	81.3	81.3	82.6	81.9	82.3	82.6	82.3	81.9	82.3	81.9	81.3	98.7	98.1	98.4									
MG573363/RVA/Human-wt/BRA/IAL-R3122/2013/G1P[8]	99.4	82.3	99.0	81.9	99.4	99.0	81.9	81.3	81.9	81.3	82.3	81.6	99.4	99.4	99.4	98.7	99.4	99.0	99.4	82.6	81.3	81.3	82.6	81.9	82.3	82.6	82.3	81.9	82.3	81.9	81.3	98.1	97.4	97.7	99.4								
LC086744/RVA/Human-wt/THA/LS-04/2013/G2P[8]	99.0	81.9	98.7	81.6	99.0	98.7	81.6	81.0	81.6	81.0	81.9	81.3	99.0	99.0	99.0	98.4	99.0	98.7	99.0	82.3	81.6	81.0	82.3	81.6	81.9	82.3	81.9	81.6	81.9	81.6	81.0	97.7	97.1	97.4	99.0	99.0							
KJ918989/RVA/Human-wt/HUN/ERNS044/2012/G2P[4]	99.4	82.6	99.0	82.3	99.4	99.0	82.3	81.6	82.3	81.6	82.6	81.9	99.4	99.4	99.4	98.7	99.4	99.0	99.4	82.9	81.6	81.6	82.9	82.3	82.6	82.9	82.6	82.3	82.6	82.3	81.6	98.1	98.7	99.0	99.4	98.7	98.4						
KP[8]82920/RVA/Human-wt/MLI/Mali-038/2008/G1P[8]	98.7	82.3	98.7	81.9	98.7	98.4	81.9	81.3	81.9	81.3	82.3	81.6	98.7	98.7	98.7	98.1	98.7	98.4	98.7	82.6	81.3	81.3	82.6	81.9	82.3	82.6	82.3	81.9	82.3	81.9	81.3	100.0	97.1	97.1	98.7	98.1	97.7	98.1	97.7	98.1			
KP752776/RVA/Human-wt/ZMB/MRC-DPRU1673/2009/G2P[4]	98.7	82.6	98.4	82.3	98.7	98.4	82.3	81.6	82.3	81.6	82.6	81.6	98.7	98.7	98.7	98.1	98.7	98.4	98.7	82.9	81.6	81.6	82.9	82.3	82.3	82.6	82.6	82.3	82.6	82.3	82.3	81.9	99.4	97.1	97.1	98.7	98.1	97.7	98.1	99.4			
MT674498/RVA/Human-wt/BRA/TO-243/2015/G3P[8]	81.9	99.7	82

Appendix 17s-t: Nucleotide and amino acid identities for the NSP4 of the four Zambian reassortants

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NSP4 nucleotide identities among strains calculated using the p-distance algorithm in MEGA 6 (Tamura et al., 2013)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
RVA/Human-wt/ZMB/UFS-NGS-MRC-DRPU4749/2014/G2P[8]																																										
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13232/2016/G1P[8]	79.1																																									
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13327/2016/G2P[4]	90.9	79.5																																								
RVA/Human-wt/ZMB/UFS-NGS-MRC-DRPU13541/2016/G1P[8]	79.1	100.0	79.5																																							
MG181588/RVA/Human-wt/MWI/BID1KY/2013/G1P[8]	98.9	79.5	90.9	79.5																																						
KU248403/RVA/Human-wt/BGN/J266/2010/G2P[4]	93.4	80.5	96.4	80.5	93.4																																					
LC477642/RVA/Human-wt/JPN/Tokyo18-38/2018/G9P[8]	93.2	81.4	96.2	81.4	93.2	98.7																																				
JX965154/RVA/Human-wt/AUS/WAPC703/2010/G2P[4]	93.0	80.3	97.2	80.3	93.0	98.9	98.7																																			
MG181511/RVA/Human-wt/MWI/BID111/2012/G1P[8]	80.8	96.4	80.8	96.4	80.8	81.8	82.7	81.6																																		
JF766587/RVA/Human-wt/KOR/CAU09-371/2009/G9P[8]	79.9	96.4	79.9	96.4	80.3	80.8	81.8	80.6	97.5																																	
KP007157/RVA/Human-wt/PHI/TGO12-003/2012/G2P[4]	92.6	79.3	97.2	79.3	92.2	98.1	97.9	98.9	80.6	79.7																																
KF636210/RVA/Human-wt/ZAF/MRC-DPRU1544/2010/G1P[8]	79.5	98.5	79.3	98.5	79.9	80.3	81.2	80.1	97.5	97.2	79.1																															
KF636276/RVA/Human-wt/ZAF/MRC-DPRU2052/2010/G1P[8]	79.5	98.5	79.3	98.5	79.9	80.3	81.2	80.1	97.5	97.2	79.1	100.0																														
KJ753290/RVA/Human-wt/ZWE/MRC-DPRU1844-11/2011/G1P[8]	79.7	98.3	79.9	98.3	80.1	80.8	81.8	80.6	97.7	97.3	79.7	99.4	99.4																														
JX027817/RVA/Human-wt/AUS/CK00083/2008/G1P[8]	79.5	97.0	79.5	97.0	79.5	80.5	81.4	80.3	98.3	97.9	79.3	97.7	97.7	97.9																														
MF184775/RVA/Human-wt/USA/CNMC25/2011/G1P[8]	79.7	96.8	79.7	96.8	79.7	80.6	81.6	80.5	98.5	98.1	79.5	97.9	97.9	98.1	99.1																														
LC439280/RVA/Human-wt/GHA/M0094/2010/G9P[8]	80.5	96.8	80.5	96.8	80.5	81.4	82.4	81.2	98.9	98.3	80.3	97.9	97.9	98.1	99.1	99.2																														
KU361020/RVA/Human-wt/BRA/QUI-150-F1/2010/G1P[8]	80.1	96.4	80.5	96.4	80.1	81.4	82.4	81.2	97.7	97.7	80.3	97.2	97.2	97.3	98.3	98.5	98.5																														
KP752635/RVA/Human-wt/SEN/MRC-DPRU2051/2009/G9P[8]	81.0	92.0	81.4	92.0	81.2	82.0	82.9	82.2	92.0	92.4	81.2	91.8	91.8	92.0	92.2	92.0	92.0	92.2																														
KP[8]82701/RVA/Human-wt/KEN/Keny-057/2009/G1P[8]	79.7	95.1	79.9	95.1	79.3	80.6	81.6	80.6	96.8	97.2	79.7	96.2	96.2	96.4	97.3	97.5	97.5	97.5	91.5																														
HG917361/RVA/Human-wt/FRA/E8997/2013/G1P[8]	80.1	91.8	80.1	91.8	79.9	80.3	81.2	80.5	91.8	91.8	79.9	92.0	92.0	92.2	92.0	91.8	91.8	91.7	96.8	91.3																														
LC367298/RVA/Human-wt/NPL/09N3589/2009/G12P[6]	80.3	96.2	80.3	96.2	80.3	80.8	81.8	80.6	97.5	98.7	80.1	97.0	97.0	97.2	98.1	98.3	98.3	98.3	92.2	97.7	92.0																														
KJ752282/RVA/Human-wt/GMB/MRC-DPRU3174/2010/G1P[8]	81.0	91.7	80.6	91.7	81.2	82.0	82.5	81.8	91.7	92.0	80.8	91.8	91.8	92.0	91.8	91.7	91.7	91.5	98.1	91.1	96.8	91.8																														
DQ492678/RVA/Human-wt/BGD/Dhaka16/2003/G1P[8]	79.3	96.0	79.7	96.0	79.3	80.6	81.6	80.5	97.3	97.7	79.5	96.8	96.8	97.0	97.9	98.1	98.1	99.2	92.2	97.5	91.7	98.3	91.5																														
JQ069125/RVA/Human-wt/CAN/RT006-07/2007/G1P[8]	79.9	96.2	79.9	96.2	79.9	80.8	81.8	80.6	97.5	98.3	79.7	97.0	97.0	97.2	98.1	98.3	98.3	99.4	92.4	97.7	91.8	98.9	91.7	99.4																														
KJ752024/RVA/Human-wt/ETH/MRC-DPRU1843/2009/G1P[8]	80.6	92.2	80.6	92.2	80.8	81.6	82.2	81.4	92.2	92.6	80.5	92.4	92.4	92.6	92.4	92.2	92.2	92.0	98.5	91.7	96.8	92.4	98.9	92.0	92.2																														
KP752669/RVA/Human-wt/SWZ/MRC-DPRU4550/2010/G1P[8]	79.3	96.4	79.3	96.4	79.3	80.3	81.2	80.1	97.7	97.3	79.1	97.2	97.2	97.3	99.1	98.5	98.5	97.7	92.0	96.8	91.8	97.5	91.7	97.3	97.5	92.2																														
KP752571/RVA/Human-wt/TGO/MRC-DPRU4562/2011/G1P[8]	79.9	94.7	80.1	94.7	79.5	80.8	81.8	80.8	96.4	96.8	79.9	95.8	95.8	96.0	97.0	97.2	97.2	97.2	91.1	99.2	90.9	97.3	90.7	97.2	97.3	91.3	96.4																														
KJ752236/RVA/Human-wt/ZMB/MRC-DPRU1648/2009/G1P[8]	80.6	97.0	80.3	97.0	81.0	81.2	82.2	81.0	98.7	98.1	80.1	98.1	98.1	98.3	98.5	98.7	99.1	98.3	92.2	97.0	91.7	97.7	91.8	97.5	97.7	92.4	97.9	96.6																														
MG573369/RVA/Human-wt/BRA/IAL-R3165/2013/G1P[8]	92.4	80.3	95.8	80.3	92.8	98.3	98.1	98.3	81.8	80.6	97.5	80.1	80.1	80.6	80.3	80.5	81.2	81.2	82.0	80.1	80.3	80.6	81.6	80.5	80.6	81.2	80.1	80.3	81.0																														
LC066659/RVA/Human-wt/THA/SKT-109/2013/G1P[8]	92.4	80.3	95.8	80.3	92.8	98.3	98.1	98.3	81.8	80.6	97.5	80.1	80.1	80.6	80.3	80.5	81.2	81.2	82.0	80.1	80.3	80.6	81.6	80.5	80.6	81.2	80.1	80.3	81.0	100.0																														
LC086778/RVA/Human-wt/THA/BD-20/2013/G2P[4]	93.4	80.6	96.4	80.6	93.4	98.5	98.7	98.5	82.2	81.0	97.7	80.6	80.6	81.0	80.6	80.8	81.6	81.6	82.5	80.8	80.8	81.0	82.2	80.8	81.0	81.8	80.5	81.0	81.4	98.7	98.7																														
KX758593/RVA/Human-wt/RUS/NN439/2014/G1P[8]	92.6	79.9	95.6	79.9	93.0	98.5	97.9	98.1	81.4	80.3	97.3	79.7	79.7	80.3	79.9	80.1	80.8	80.8	81.6	79.7	79.9	80.3	81.2	80.1	80.3	80.8	79.7	79.9	80.6	99.1	99.1	98.5																														
KP753176/RVA/Human-wt/UGA/MRC-DPRU3710/2009/G2P[4]	93.0	80.5	96.4	80.5	93.4	98.9	98.7	98.9	81.4	80.8	98.1	80.3	80.3	80.8	80.1	80.3	81.0	81.0	82.4	80.3	80.3	80.5	82.0	80.3	80.5	81.6	79.9	80.5	81.2	98.3	98.3	98.5	98.1																														
KJ753521/RVA/Human-wt/SEN/MRC-DPRU1915/2008/G2P[4]	93.0	80.8	96.4	80.8	93.4	98.9	98.7	98.9	81.8	81.2	98.1	80.6	80.6	81.2	80.5	80.6	81.4	81.4	82.4	80.6	80.6	80.8	82.0	80.6	80.8	81.6	80.3	80.8	81.6	98.3	98.3	98.5	98.1	99.6																														
LC086789/RVA/Human-wt/THA/NP-M51/2013/G2P[4]	92.6	81.0	95.6	81.0	92.6	98.1	99.4	98.1	82.4	81.4	97.3	80.8	80.8	81.4	81.0	81.2	82.0	82.0	82.5	81.2	80.8	81.4	82.2	81.6	81.4	81.8	80.8	81.4	81.8	98.3	97.5	97.5	98.1	97.3	98.1	98.1																														
MG181918/RVA/Human-wt/MWI/BID15V/2012/G2P[4]	98.9	79.1	90.9	79.1	99.6	93.4	93.2	93.0	80.5	79.9	92.2	79.5	79.5	79.7	79.1	79.3	80.1	79.7	81.0	79.3	79.7	79.9	81.0	78.9	79.5	80.6	78.9	79.5	80.6	92.4	92.4	93.4	92.6	93.4	93.4	92.6																														
MG181599/RVA/Human-wt/MWI/BID1LN/2013/G1P[8]	98.9	79.5	90.9	79.5	100.0	93.4	93.2	93.0	80.8	80.3	92.2	79.9	79.9	80.1	79.5	79.7	80.5	80.1	81.2	79.3	79.9	80.3	81.2	79.3	79.9	80.8	79.3	79.5	81.0	92.8	92.8	93.4	93.0	93.4	93.4	92.6	99.6																														
MG181489/RVA/Human-wt/MWI/OPS-001/2008/G1P[8]	81.0	96.6	80.3	96.6	81.0	81.2	82.2	81.0	99.1	97.7	80.1	97.9	97.9	97.9	98.5	98.7	99.1	97.9	91.8	97.0	91.7	97.7	91.5	97.5	97.7	92.0	97.9	96.6	99.2	81.0	81.0	81.8	80.6	80.8	81.2	81.8	80.6	81.0																														
KX638741/RVA/Human-wt/IND/RV1206/2012/G2P[4]	92.8	79.5	97.7	79.5	92.8	98.3	98.1	99.1	80.8	79.9	99.1	79.3	79.3	79.9	79.5	79.7	80.5	80.5	81.4	79.9	80.1	80.3	81.0	79.7	79.9	80.6	79.3	80.1	80.3	97.7	97.7	97.9	97.5	98.3	98.3	97.5	92.8	92.8	80.3																														
KX954623/RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P[8]	80.3	92.0	80.3	92.0	80.1	80.5	81.4	80.6	92.0	92.0	80.1	92.2	92.2	92.4	92.2	92.0	92.0	91.8	97.0	91.5	99.8	92.2	97.0	91.8	92.0	97.0	92.0	91.1	91.8	80.5	80.5	81.0	80.1	80.5	80.8	81.0	79.9	80.1	91.8	80.3																														
JX946177/RVA/Human-wt/CHN/E2451/2011/G3P[9] - outgroup	78.2	79.7	78.7	79.7	78.0	78.7	79.3	79.3	80.6	80.1	78.6	79.7	79.7	79.9	79.7	80.3	79.9	79.9	80.6	79.5	80.1	79.9	80.6	79.9	80.1	80.8	80.3	80.3	79.7	79.3	79.3	79.3	78.9	79.1	79.5	78.7	77.8	78.0	80.1	78.7	80.3	

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NSP4 amino acid identities among strains calculated using the p-distance algorithm in MEGA 6 (Tamura et al., 2013)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
RVA/Human-wt/ZMB/UFS-NGS-MRC-DRPU4749/2014/G2P[8]																																										
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13232/2016/G1P[8]	82.3																																									
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13327/2016/G2P[4]	96.0	81.7																																								
RVA/Human-wt/ZMB/UFS-NGS-MRC-DRPU13541/2016/G1P[8]	82.3	100.0	81.7																																							
MG181588/RVA/Human-wt/MWI/BID1KY/2013/G1P[8]	99.4	81.7	95.4	81.7																																						
KU248403/RVA/Human-wt/BGN/J266/2010/G2P[4]	97.7	82.9	97.1	82.9	97.1																																					
LC477642/RVA/Human-wt/JPN/Tokyo18-38/2018/G9P[8]	98.3	83.4	97.7	83.4	97.7	99.4																																				
JX965154/RVA/Human-wt/AUS/WAPC703/2010/G2P[4]	97.7	83.4	98.3	83.4	97.1	98.9	99.4																																			
MG181511/RVA/Human-wt/MWI/BID111/2012/G1P[8]	84.0	96.0	82.3	96.0	83.4	83.4	84.0	84.0																																		
JF766587/RVA/Human-wt/KOR/CAU09-371/2009/G9P[8]	84.0	95.4	81.7	95.4	83.4	82.9	83.4	83.4	97.1																																	
KP007157/RVA/Human-wt/PHI/TGO12-003/2012/G2P[4]	96.6	82.9	97.1	82.9	96.0	97.7	98.3	98.9	83.4	82.9																																
KF636210/RVA/Human-wt/ZAF/MRC-DPRU1544/2010/G1P[8]	83.4	97.7	81.7	97.7	82.9	82.9	83.4	83.4	98.3	97.7	82.9																															
KF636276/RVA/Human-wt/ZAF/MRC-DPRU2052/2010/G1P[8]	83.4	97.7	81.7	97.7	82.9	82.9	83.4	83.4	98.3	97.7	82.9	100.0																														
KJ753290/RVA/Human-wt/ZWE/MRC-DPRU1844-11/2011/G1P[8]	84.0	97.1	82.3	97.1	83.4	83.4	84.0	84.0	98.9	98.3	83.4	99.4	99.4																														
JX027817/RVA/Human-wt/AUS/CK00083/2008/G1P[8]	84.0	97.1	82.3	97.1	83.4	83.4	84.0	84.0	98.9	98.3	83.4	99.4	99.4	100.0																														
MF184775/RVA/Human-wt/USA/CNMC25/2011/G1P[8]	84.0	97.1	82.3	97.1	83.4	83.4	84.0	84.0	98.9	98.3	83.4	99.4	99.4	100.0	100.0																														
LC439280/RVA/Human-wt/GHA/M0094/2010/G9P[8]	84.6	96.6	82.9	96.6	84.0	84.0	84.6	84.6	98.3	97.7	84.0	98.9	98.9	99.4	99.4																														
KU361020/RVA/Human-wt/BRA/QUI-150-F1/2010/G1P[8]	84.0	97.1	83.4	97.1	83.4	84.6	85.1	85.1	97.7	97.1	84.6	98.3	98.3	98.9	98.9	98.9	98.3																														
KP752635/RVA/Human-wt/SEN/MRC-DPRU2051/2009/G9P[8]	83.4	93.7	82.9	93.7	82.9	84.0	84.6	84.6	93.7	93.1	83.4	94.3	94.3	94.9	94.9	94.9	94.9	96.0																														
KP[8]82701/RVA/Human-wt/KEN/Keny-057/2009/G1P[8]	83.4	94.9	81.7	94.9	82.9	82.9	83.4	83.4	96.6	96.0	82.9	97.1	97.1	97.7	97.7	97.7	97.1	97.7	93.7																														
HG917361/RVA/Human-wt/FRA/E8997/2013/G1P[8]	82.3	92.6	80.6	92.6	81.7	81.7	82.3	82.3	93.7	93.1	81.1	94.3	94.3	94.9	94.9	94.9	94.3	94.9	96.6	93.7																														
LC367298/RVA/Human-wt/NPL/09N3589/2009/G12P[6]	85.1	96.0	82.9	96.0	84.6	84.0	84.6	84.6	97.7	98.3	84.0	98.3	98.3	98.9	98.9	98.9	98.3	98.9	94.9	97.7	94.9																														
KJ752282/RVA/Human-wt/GMB/MRC-DPRU3174/2010/G1P[8]	84.0	92.6	82.3	92.6	83.4	83.4	84.0	84.0	93.7	93.1	82.9	94.3	94.3	94.9	94.9	94.9	94.3	94.9	98.3	93.7	96.6	94.9																														
DQ492678/RVA/Human-wt/BGD/Dhaka16/2003/G1P[8]	83.4	96.6	82.9	96.6	82.9	84.0	84.6	84.6	97.1	96.6	84.0	97.7	97.7	98.3	98.3	98.3	97.7	99.4	95.4	97.1	94.3	98.3	94.3																														
JQ069125/RVA/Human-wt/CAN/RT006-07/2007/G1P[8]	84.6	96.6	83.4	96.6	84.0	84.6	85.1	85.1	97.1	97.7	84.6	97.7	97.7	98.3	98.3	98.3	97.7	99.4	95.4	97.1	94.3	99.4	94.3	98.9																														
KJ752024/RVA/Human-wt/ETH/MRC-DPRU1843/2009/G1P[8]	84.0	92.6	82.3	92.6	83.4	83.4	84.0	84.0	93.7	93.1	82.9	94.3	94.3	94.9	94.9	94.9	94.3	94.9	98.3	93.7	96.6	94.9	98.9	94.3	94.3																														
KP752669/RVA/Human-wt/SWZ/MRC-DPRU4550/2010/G1P[8]	83.4	96.6	81.7	96.6	82.9	82.9	83.4	83.4	98.3	97.7	82.9	98.9	98.9	99.4	99.4	98.9	98.3	99.4	94.9	97.1	94.3	98.3	94.3	97.7	97.7	94.3																														
KP752751/RVA/Human-wt/TGO/MRC-DPRU4562/2011/G1P[8]	84.0	95.4	82.3	95.4	83.4	83.4	84.0	84.0	97.1	96.6	83.4	97.7	97.7	98.3	98.3	98.3	97.7	98.3	94.3	99.4	94.3	98.3	94.3	97.7	97.7	94.3	97.7																														
KJ752236/RVA/Human-wt/ZMB/MRC-DPRU1648/2009/G1P[8]	84.0	97.1	82.3	97.1	83.4	83.4	84.0	84.0	98.9	98.3	83.4	99.4	99.4	100.0	100.0	100.0	99.4	98.9	94.9	97.7	94.9	98.9	94.9	98.3	98.3	94.9	99.4	98.3																														
MG573369/RVA/Human-wt/BRA/IAL-R3165/2013/G1P[8]	97.7	82.9	97.1	82.9	97.1	98.9	99.4	98.9	83.4	82.9	97.7	82.9	82.9	83.4	83.4	83.4	84.0	84.6	84.0	82.9	81.7	84.0	83.4	84.0	84.6	83.4	82.9	83.4	83.4																														
LC066659/RVA/Human-wt/THA/SKT-109/2013/G1P[8]	97.7	82.9	97.1	82.9	97.1	98.9	99.4	98.9	83.4	82.9	97.7	82.9	82.9	83.4	83.4	83.4	84.0	84.6	84.0	82.9	81.7	84.0	83.4	84.0	84.6	83.4	82.9	83.4	83.4	100.0																														
LC086778/RVA/Human-wt/THA/BD-20/2013/G2P[4]	98.3	83.4	97.7	83.4	97.7	99.4	100.0	99.4	84.0	83.4	98.3	83.4	83.4	84.0	84.0	84.0	84.6	85.1	84.6	83.4	82.3	84.6	84.0	84.6	85.1	84.0	83.4	84.0	84.0	99.4	99.4																														
KX758593/RVA/Human-wt/RUS/NN439/2014/G1P[8]	97.1	82.9	96.6	82.9	96.6	98.3	98.9	98.3	82.9	82.3	97.1	82.3	82.3	82.9	82.9	82.9	83.4	84.0	83.4	82.3	81.1	83.4	82.9	83.4	84.0	82.9	82.3	82.9	82.9	98.3	98.3	98.9																														
KP753176/RVA/Human-wt/UGA/MRC-DPRU3710/2009/G2P[4]	97.7	82.9	97.1	82.9	97.1	98.9	99.4	98.9	83.4	82.9	97.7	82.9	82.9	83.4	83.4	83.4	84.0	84.6	84.0	82.9	81.7	84.0	83.4	84.0	84.6	83.4	82.9	83.4	83.4	98.9	98.9	99.4	98.3																														
KJ753521/RVA/Human-wt/SEN/MRC-DPRU1915/2008/G2P[4]	97.7	83.4	97.1	83.4	97.1	98.9	99.4	98.9	84.0	83.4	97.7	83.4	83.4	84.0	84.0	84.0	84.6	85.1	84.6	83.4	82.3	84.6	83.4	84.6	85.1	83.4	83.4	84.0	84.0	98.9	98.9	99.4	98.3	98.9																														
LC086789/RVA/Human-wt/THA/NP-M51/2013/G2P[4]	98.3	83.4	97.7	83.4	97.7	99.4	100.0	99.4	84.0	83.4	98.3	83.4	83.4	84.0	84.0	84.0	84.6	85.1	84.6	83.4	82.3	84.6	84.0	84.6	85.1	84.0	83.4	84.0	84.0	99.4	99.4		98.9	99.4	99.4																														
MG181918/RVA/Human-wt/MWI/BID15V/2012/G2P[4]	99.4	81.7	95.4	81.7	100.0	97.1	97.7	97.1	83.4	83.4	96.0	82.9	82.9	83.4	83.4	83.4	84.0	83.4	82.9	82.9	81.7	84.6	83.4	82.9	84.0	83.4	82.9	83.4	83.4	97.1	97.1	97.7	96.6	97.1	97.1	97.7																														
MG181599/RVA/Human-wt/MWI/BID1LN/2013/G1P[8]	99.4	81.7	95.4	81.7	100.0	97.1	97.7	97.1	83.4	83.4	96.0	82.9	82.9	83.4	83.4	83.4	84.0	83.4	82.9	82.9	81.7	84.6	83.4	82.9	84.0	83.4	82.9	83.4	83.4	97.1	97.1	97.7	96.6	97.1	97.1	97.7	100.0																														
MG181489/RVA/Human-wt/MWI/OPS-001/2008/G1P[8]	84.0	97.1	82.3	97.1	83.4	83.4	84.0	84.0	98.9	98.3	83.4	99.4	99.4	100.0	100.0	100.0	99.4	98.9	94.9	97.7	94.9	98.9	94.9	98.3	98.3	94.9	99.4	98.3	100.0	83.4	83.4	84.0

Appendix 17u-v: Nucleotide and amino acid identities for the NSP5 of the four Zambian reassortants

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NSP5 nucleotide identities among strains calculated using the p-distance algorithm in MEGA 6 (Tamura et al., 2013)

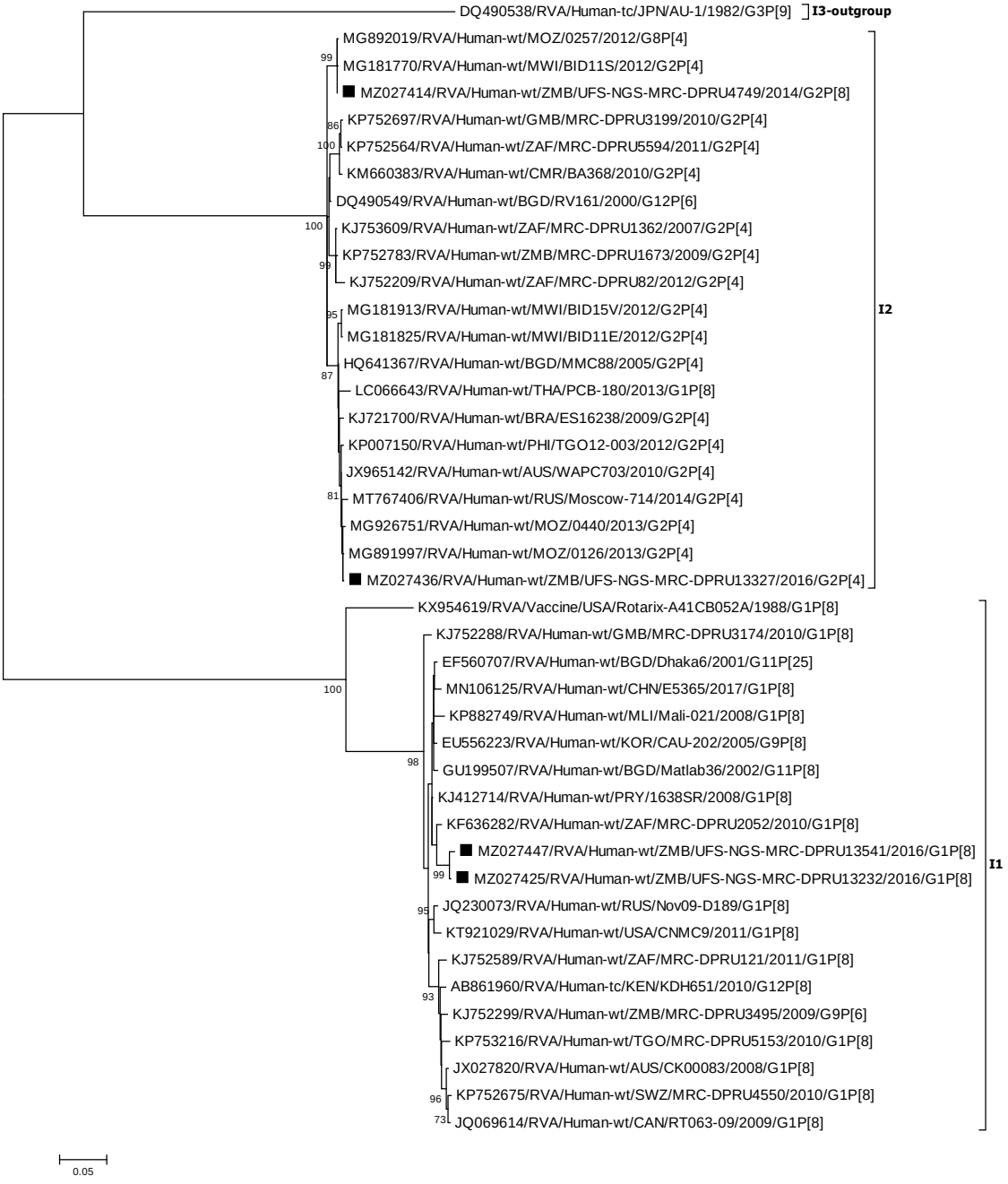
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4749/2014/G2P[8]																																								
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13232/2016/G1P[8]	82.8																																							
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13327/2016/G2P[4]	97.9	82.9																																						
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13541/2016/G1P[8]	82.8	100.0	82.9																																					
MG181776/RVA/Human-wt/MWI/BID115/2012/G2P[4]	99.2	82.9	98.5	82.9																																				
KF636321/RVA/Human-wt/ZAF/MRC-DPRU1061/2009/G2P[4]	99.3	83.2	98.6	83.2	99.8																																			
MG181897/RVA/Human-wt/MWI/BID1BI/2012/G2P[4]	98.6	83.2	98.3	83.2	99.2	99.3																																		
MG181852/RVA/Human-wt/MWI/BID1AW/2012/G2P[6]	98.8	82.9	98.1	82.9	99.7	99.5	98.8																																	
LC477660/RVA/Human-wt/JPN/Tokyo17-16/2017/G2P[4]	98.8	83.8	98.1	83.8	99.3	99.5	98.8	99.0																																
KU361040/RVA/Human-wt/BRA/QUI-59-F3/2010/G1P[8]	83.0	99.0	83.2	99.0	83.2	83.4	83.4	83.2	84.0																															
MG926713/RVA/Human-wt/MOZ/0289/2012/G12P[6]	83.0	98.8	83.2	98.8	83.2	83.4	83.4	83.2	84.1	99.8																														
DQ146659/RVA/Human-wt/BGD/Dhaka25/2002/G12P[8]	82.8	98.8	82.9	98.8	82.9	83.2	83.2	82.9	83.8	99.8	99.7																													
KT919380/RVA/Human-wt/USA/VU11-12-66/2012/G12P[8]	83.4	98.6	83.6	98.6	83.6	83.8	83.8	83.6	84.0	99.7	99.5	99.5																												
LC477673/RVA/Human-wt/JPN/Tokyo18-39/2018/G9P[8]	83.0	98.3	83.2	98.3	83.1	83.4	83.4	83.1	84.0	99.3	99.1	99.1	99.0																											
JF766599/RVA/Human-wt/KOR/CAU09-376/2009/G9P[8]	82.5	98.6	82.7	98.6	82.7	82.9	82.9	82.7	83.6	99.7	99.5	99.5	99.3	99.3																										
MG926746/RVA/Human-wt/MOZ/0440/2013/G2P[4]	98.3	82.9	99.7	82.9	98.8	99.0	98.6	98.5	98.5	83.2	83.2	82.9	83.6	83.2	82.7																										
MG891992/RVA/Human-wt/MOZ/0126/2013/G2P[4]	98.3	82.9	99.7	82.9	98.8	99.0	98.6	98.5	98.5	83.2	83.2	82.9	83.6	83.2	82.7	100.0																								
MK302420/RVA/Human-wt/IND/NIV1416591/2014/G9P[4]	98.3	83.2	99.3	83.2	98.8	99.0	98.6	98.5	98.5	83.4	83.4	83.1	83.8	83.4	82.9	99.7	99.7																							
MG181325/RVA/Human-wt/MWI/BID1JK/2013/G2P[4]	98.1	83.4	99.1	83.4	98.6	98.8	98.5	98.3	98.6	83.6	83.6	83.4	84.0	83.6	83.2	99.5	99.5	99.5																						
KP007180/RVA/Human-wt/PHI/TGO12-007/2012/G2P[4]	98.1	83.2	99.1	83.2	98.6	98.8	98.5	98.3	98.3	83.4	83.4	83.2	83.8	83.4	82.9	99.5	99.5	99.5	99.3																					
JX965157/RVA/Human-wt/AUS/WAPC703/2010/G2P[4]	98.1	82.9	99.1	82.9	98.6	98.8	98.5	98.3	98.3	83.2	83.2	82.9	83.6	83.1	82.7	99.5	99.5	99.5	99.7	99.3																				
KJ751556/RVA/Human-wt/SEN/MRC-DPRU2130-09/2009/G1P[8]	83.2	96.9	82.9	96.9	83.4	83.6	83.6	83.4	84.3	97.2	97.1	97.4	96.9	96.5	96.9	83.4	83.4	83.6	83.8	83.6	83.4																			
KJ752025/RVA/Human-wt/ETH/MRC-DPRU1843/2009/G1P[8]	82.8	97.8	83.0	97.8	83.0	83.2	83.2	83.0	83.8	98.5	98.3	98.6	98.1	97.8	98.1	83.0	83.0	83.2	83.4	83.2	83.0	98.1																		
KJ751932/RVA/Human-wt/SWZ/MRC-DPRU5119/2010/G1P[8]	81.8	97.6	82.0	97.6	82.0	82.2	82.3	82.0	82.5	97.9	98.1	98.1	97.9	97.2	97.6	82.0	82.0	82.2	82.5	82.7	82.0	96.5	97.8																	
KJ751712/RVA/Human-wt/GMB/MRC-DPRU3176/2010/G1P[8]	83.0	97.9	83.2	97.9	83.2	83.4	83.4	83.2	84.0	98.6	98.5	98.8	98.3	98.3	98.3	83.2	83.2	83.4	83.6	83.4	83.2	97.9	99.2	97.9																
KJ753467/RVA/Human-wt/ZWE/MRC-DPRU1102/2012/G9P[8]	82.5	97.9	82.7	97.9	82.7	82.9	83.0	82.7	83.6	98.6	98.5	98.8	98.3	97.9	98.3	82.7	82.7	82.9	83.2	82.9	82.7	98.3	99.5	98.3	99.3														
KM660211/RVA/Human-wt/CMR/MA01/2010/G12P[8]	82.5	97.9	82.7	97.9	82.7	82.9	82.9	82.7	83.6	98.6	98.5	98.8	98.3	97.9	98.3	82.7	82.7	82.9	83.2	82.9	82.7	97.9	99.2	97.9	99.7	99.3														
JQ069040/RVA/Human-wt/CAN/RT005-07/2007/G1P[8]	82.8	98.8	82.9	98.8	82.9	83.2	83.2	82.9	83.8	99.8	99.7	99.7	99.5	99.5	99.8	82.9	82.9	83.1	83.4	83.2	82.9	97.1	98.3	97.8	98.5	98.5	98.5												
KJ752639/RVA/Human-wt/ZMB/MRC-DPRU3488/2009/G12P[6]	82.8	98.1	82.9	98.1	82.9	83.2	83.2	82.9	83.8	98.5	98.3	98.6	98.5	97.8	98.1	82.9	82.9	83.1	83.4	83.2	82.9	97.8	99.0	98.1	99.1	99.1	99.1	98.3												
JQ069102/RVA/Human-wt/CAN/RT008-09/2009/G2P[4]	98.1	82.7	98.8	82.7	98.6	98.8	98.5	98.3	98.3	82.9	82.9	82.7	83.4	82.9	82.5	99.1	99.1	99.2	99.3	99.0	99.3	83.2	82.7	81.8	82.9	82.5	82.5	82.7	82.7											
KU361049/RVA/Human-wt/BRA/QUI-73-F2/2010/G12P[6]	98.6	82.7	98.3	82.7	99.1	99.3	98.6	98.8	99.1	82.9	82.9	82.7	83.4	82.9	82.5	98.6	98.6	98.6	98.8	98.5	98.5	83.2	82.7	81.8	82.9	82.5	82.5	82.7	82.7	98.5										
KP752559/RVA/Human-wt/ZAF/MRC-DPRU5594/2011/G2P[4]	97.6	81.6	97.2	81.6	98.1	98.3	97.6	97.8	97.8	81.3	81.3	81.1	81.8	81.3	80.9	97.6	97.6	97.6	97.4	97.8	97.4	81.6	81.1	81.1	81.3	80.9	80.9	81.1	81.6	97.8	97.9									
KP752692/RVA/Human-wt/GMB/MRC-DPRU3199/2010/G2P[4]	97.8	81.8	97.4	81.8	98.3	98.5	97.8	97.9	97.9	81.6	81.6	81.3	82.0	81.6	81.1	97.8	97.8	97.8	97.6	97.9	97.6	81.8	81.4	81.3	81.6	81.1	81.1	81.3	81.8	97.9	98.1	99.8								
KP752778/RVA/Human-wt/ZMB/MRC-DPRU1673/2009/G2P[4]	99.2	83.0	98.5	83.0	99.7	99.8	99.2	99.3	99.3	83.2	83.2	82.9	83.6	83.2	82.7	98.8	98.8	98.8	98.6	98.6	98.6	83.4	83.0	82.0	83.2	82.7	82.7	82.9	82.9	98.6	99.2	98.1	98.3							
KJ752160/RVA/Human-wt/TGO/MRC-DPRU5124/2010/G2P[4]	97.2	81.1	96.9	81.1	97.8	97.9	97.2	97.4	97.4	80.9	80.9	80.6	81.3	80.9	80.4	97.2	97.2	97.2	97.0	97.4	97.0	81.1	80.7	80.6	80.9	80.4	80.4	80.6	81.6	97.4	97.6	99.0	99.2	97.8						
KP[8]82306/RVA/Human-wt/GHA/Ghan-002/2008/G2P[4]	98.1	82.3	97.8	82.3	98.6	98.8	98.1	98.3	98.3	82.0	82.0	81.8	82.5	82.0	81.6	98.1	98.1	98.1	97.9	97.9	97.9	82.3	81.8	81.3	82.0	81.6	81.6	81.8	82.3	97.9	98.5	99.1	99.3	98.6	98.8					
KP753177/RVA/Human-wt/UGA/MRC-DPRU3710/2009/G2P[4]	97.9	82.5	98.6	82.5	98.5	98.6	98.3	98.1	98.1	82.7	82.7	82.5	83.2	82.7	82.2	99.0	99.0	99.0	99.1	98.8	99.1	82.9	82.5	81.5	82.7	82.2	82.2	82.5	82.5	99.1	98.3	97.2	97.4	98.5	96.9	97.8				
MH171474/RVA/Human-wt/ESP/SS453194/2010/G12P[8]	83.0	99.0	83.2	99.0	83.2	83.4	83.4	83.2	84.0	100.0	99.8	99.8	99.7	99.3	99.7	83.2	83.2	83.4	83.6	83.4	83.2	97.2	98.5	97.9	98.6	98.6	98.6	99.8	98.5	82.9	82.9	81.3	81.6	83.2	80.9	82.0	82.7			
KU361041/RVA/Human-wt/BRA/QUI-89-F4/2010/G1P[8]	83.0	99.0	83.2	99.0	83.2	83.4	83.4	83.2	84.0	100.0	99.8	99.8	99.7	99.3	99.7	83.2	83.2	83.4	83.6	83.4	83.2	97.2	98.5	97.9	98.6	98.6	98.6	99.8	98.5	82.9	82.9	81.3	81.6	83.2	80.9	82.0	82.7	100.0		
KX954624/RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P[8]	82.1	92.4	82.3	92.4	82.2	82.5	82.5	82.2	82.5	93.1	92.9	92.9	92.9	93.1	92.8	82.7	82.7	82.9	82.7	82.9	82.7	91.6	92.2	91.8	93.1	92.4	93.1	92.9	92.2	82.5	82.0	81.3	81.6	82.3	80.9	82.5	82.7	93.1	93.1	
AB971770/RVA/SugarGlider-tc/JPN/SG385/2012/G27P[36] - outgroup	79.5	80.4	80.0	80.4	79.7	80.0	80.0	79.2	80.2	80.7	80.7	80.9	81.1	80.0	80.2	80.4	80.4	80.2	80.2	80.7	79.7	81.1	80.7	80.7	80.9	80.9	80.9	80.4	81.1	79.5	80.2	78.8	79.0	79.7	79.0	79.0	79.5	80.7	80.7	79.9

v.

NSP5 amino acid identities among strains calculated using the p-distance algorithm in MEGA 6 (Tamura et al., 2013)

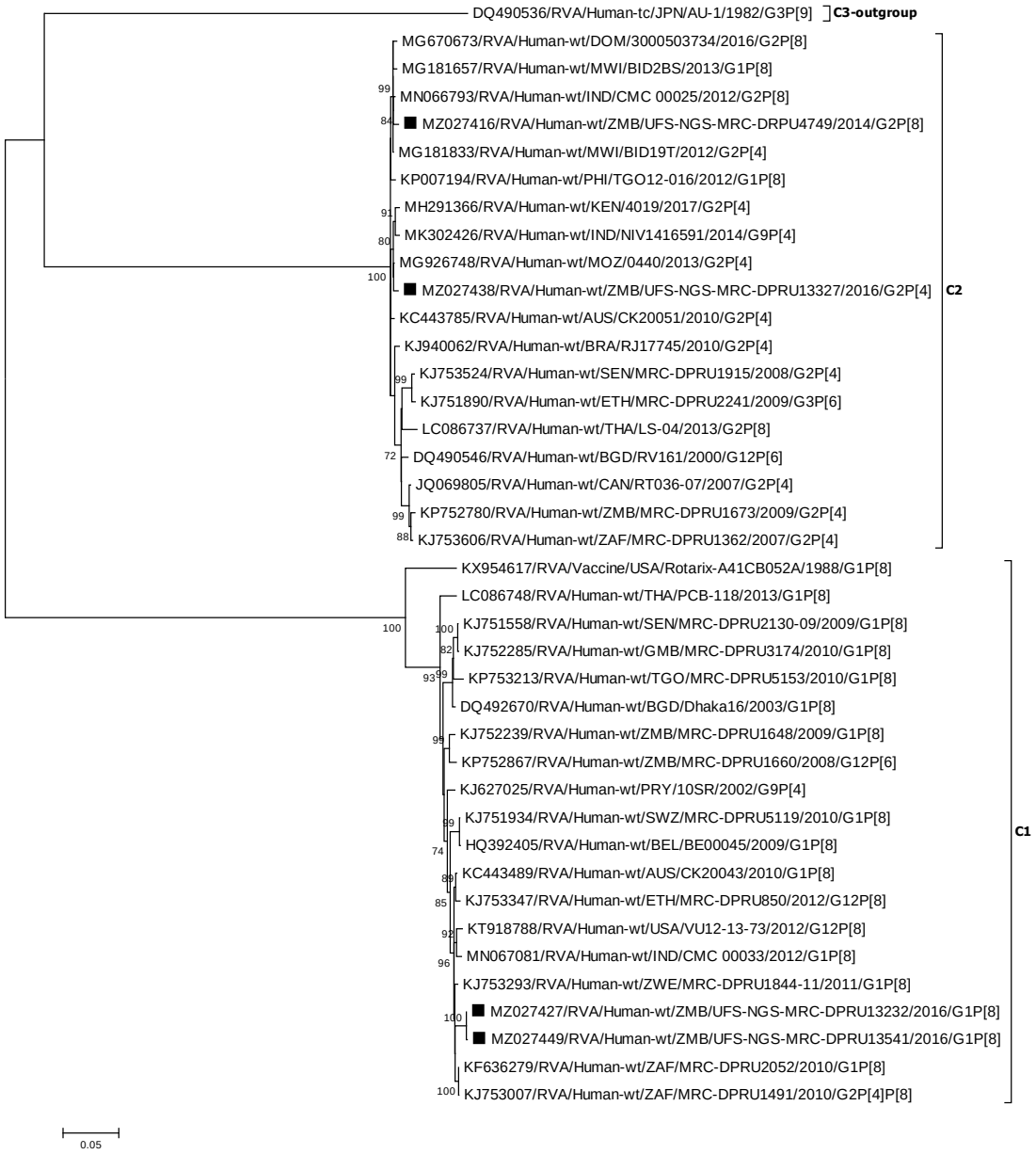
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU4749/2014/G2P[8]																																								
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13232/2016/G1P[8]	83.2																																							
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13327/2016/G2P[4]	99.0	83.8																																						
RVA/Human-wt/ZMB/UFS-NGS-MRC-DPRU13541/2016/G1P[8]	83.2	100.0	83.8																																					
MG181776/RVA/Human-wt/MWI/BID115/2012/G2P[4]	99.5	83.8	99.5	83.8																																				
KF636321/RVA/Human-wt/ZAF/MRC-DPRU1061/2009/G2P[4]	99.5	83.8	99.5	83.8	100.0																																			
MG181897/RVA/Human-wt/MWI/BID1BI/2012/G2P[4]	99.5	83.8	99.5	83.8	100.0	100.0																																		
MG181852/RVA/Human-wt/MWI/BID1AW/2012/G2P[6]	99.0	83.8	99.0	83.8	99.5	99.5	99.5																																	
LC477660/RVA/Human-wt/JPN/Tokyo17-16/2017/G2P[4]	99.0	84.3	99.0	84.3	99.5	99.5	99.5	99.0																																
KU361040/RVA/Human-wt/BRA/QUI-59-F3/2010/G1P[8]	84.8	98.5	85.3	98.5	85.3	85.3	85.3	85.3	85.8																															
MG926713/RVA/Human-wt/MOZ/0289/2012/G12P[6]	84.8	98.5	85.3	98.5	85.3	85.3	85.3	85.3	85.8	100.0																														
DQ146659/RVA/Human-wt/BGD/Dhaka25/2002/G12P[8]	84.8	98.5	85.3	98.5	85.3	85.3	85.3	85.3	85.8	100.0	100.0																													
KT919380/RVA/Human-wt/USA/VU11-12-66/2012/G12P[8]	84.8	98.5	85.3	98.5	85.3	85.3	85.3	85.3	85.8	100.0	100.0	100.0																												
LC477673/RVA/Human-wt/JPN/Tokyo18-39/2018/G9P[8]	84.3	98.0	84.8	98.0	84.8	84.8	84.8	84.8	84.8	85.3	99.5	99.5	99.5	99.5																										
JF766599/RVA/Human-wt/KOR/CAU09-376/2009/G9P[8]	84.8	98.5	85.3	98.5	85.3	85.3	85.3	85.3	85.8	100.0	100.0	100.0	100.0	99.5																										
MG926746/RVA/Human-wt/MOZ/0440/2013/G2P[4]	99.5	83.8	99.5	83.8	100.0	100.0	100.0	99.5	99.5	85.3	85.3	85.3	85.3	84.8	85.3	100.0																								
MG891992/RVA/Human-wt/MOZ/0126/2013/G2P[4]	99.5	83.8	99.5	83.8	100.0	100.0	100.0	99.5	99.5	85.3	85.3	85.3	85.3	84.8	85.3	100.0	100.0																							
MK302420/RVA/Human-wt/IND/NIV1416591/2014/G9P[4]	99.5	83.8	99.5	83.8	100.0	100.0	100.0	99.5	99.5	85.3	85.3	85.3	85.3	84.8	85.3	100.0	100.0																							
MG181325/RVA/Human-wt/MWI/BID1JK/2013/G2P[4]	99.5	83.8	99.5	83.8	100.0	100.0	100.0	99.5	99.5	85.3	85.3	85.3	85.3	84.8	85.3	100.0	100.0	100.0																						
KP007180/RVA/Human-wt/PHI/TGO12-007/2012/G2P[4]	99.5	83.8	99.5	83.8	100.0	100.0	100.0	99.5	99.5	85.3	85.3	85.3	85.3	84.8	85.3	100.0	100.0	100.0	100.0																					
JX965157/RVA/Human-wt/AUS/WAPC703/2010/G2P[4]	99.5	83.8	99.5	83.8	100.0	100.0	100.0	99.5	99.5	85.3	85.3	85.3	85.3	84.8	85.3	100.0	100.0	100.0	100.0	100.0																				
KJ751556/RVA/Human-wt/SEN/MRC-DPRU2130-09/2009/G1P[8]	84.3	96.4	84.8	96.4	84.8	84.8	84.8	84.8	85.3	98.0	98.0	98.0	98.0	97.5	98.0	84.8	84.8	84.8	84.8	84.8	84.8																		
KJ752025/RVA/Human-wt/ETH/MRC-DPRU1843/2009/G1P[8]	84.3	98.0	84.8	98.0	84.8	84.8	84.8	84.8	85.3	99.5	99.5	99.5	99.5	99.0	99.5	84.8	84.8	84.8	84.8	84.8	84.8	97.5																		
KJ751932/RVA/Human-wt/SWZ/MRC-DPRU5119/2010/G1P[8]	83.8	98.5	84.3	98.5	84.3	84.3	84.3	84.3	84.8	99.0	99.0	99.0	99.0	98.5	99.0	84.3	84.3	84.3	84.3	84.3	84.3	97.0	98.5																	
KJ751712/RVA/Human-wt/GMB/MRC-DPRU3176/2010/G1P[8]	84.8	98.5	85.3	98.5	85.3	85.3	85.3	85.3	85.8	100.0	100.0	100.0	100.0	99.5	100.0	85.3	85.3	85.3	85.3	85.3	85.3	98.0	99.5	99.0																
KJ753467/RVA/Human-wt/ZWE/MRC-DPRU1102/2012/G9P[8]	84.8	98.5	85.3	98.5	85.3	85.3	85.3	85.3	85.8	100.0	100.0	100.0	100.0	99.5	100.0	85.3	85.3	85.3	85.3	85.3	85.3	98.0	99.5	99.0	100.0															
KM660211/RVA/Human-wt/CMR/MA01/2010/G12P[8]	84.3	98.0	84.8	98.0	84.8	84.8	84.8	84.8	85.3	99.5	99.5	99.5	99.5	99.0	99.5	84.8	84.8	84.8	84.8	84.8	84.8	97.5	99.0	98.5	99.5	99.5														
JQ069040/RVA/Human-wt/CAN/RT005-07/2007/G1P[8]	84.8	98.5	85.3	98.5	85.3	85.3	85.3	85.3	85.8	100.0	100.0	100.0	100.0	99.5	100.0	85.3	85.3	85.3	85.3	85.3	85.3	98.0	99.5	99.0	100.0	100.0	99.5													
KJ752639/RVA/Human-wt/ZMB/MRC-DPRU3488/2009/G12P[6]	84.3	99.0	84.8	99.0	84.8	84.8	84.8	84.8	85.3	99.5	99.5	99.5	99.5	99.0	99.5	84.8	84.8	84.8	84.8	84.8	84.8	97.5	99.0	99.5	99.5	99.5	99.0	99.5												
JQ069102/RVA/Human-wt/CAN/RT008-09/2009/G2P[4]	99.0	83.2	99.0	83.2	99.5	99.5	99.5	99.0	99.0	84.8	84.8	84.8	84.8	84.3	84.8	99.5	99.5	99.5	99.5	99.5	99.5	84.3	84.3	83.8	84.8	84.8	84.3	84.8	84.3											
KU361049/RVA/Human-wt/BRA/QUI-73-F2/2010/G12P[6]	99.5	83.8	99.5	83.8	100.0	100.0	100.0	99.5	99.5	85.3	85.3	85.3	85.3	84.8	85.3	100.0	100.0	100.0	100.0	100.0	100.0	84.8	84.8	84.3	85.3	85.3	84.8	85.3	84.8	99.5										
KP752559/RVA/Human-wt/ZAF/MRC-DPRU5594/2011/G2P[4]	97.5	83.2	97.5	83.2	98.0	98.0	98.0	97.5	97.5	83.2	83.2	83.2	83.2	82.7	83.2	98.0	98.0	98.0	98.0	98.0	98.0	82.7	82.7	83.2	83.2	83.2	82.7	83.2	83.8	97.5	98.0									
KP752692/RVA/Human-wt/GMB/MRC-DPRU3199/2010/G2P[4]	97.5	83.2	97.5	83.2	98.0	98.0	98.0	97.5	97.5	83.2	83.2	83.2	83.2	82.7	83.2	98.0	98.0	98.0	98.0	98.0	98.0	82.7	82.7	83.2	83.2	83.2	82.7	83.2	83.8	97.5	98.0	100.0								
KP752778/RVA/Human-wt/ZMB/MRC-DPRU1673/2009/G2P[4]	99.0	83.2	99.0	83.2	99.5	99.5	99.5	99.0	99.0	84.8	84.8	84.8	84.8	84.3	84.8	99.5	99.5	99.5	99.5	99.5	99.5	84.3	84.3	83.8	84.8	84.8	84.3	84.8	84.3	99.5	99.5	97.5	97.5							
KJ752160/RVA/Human-wt/TGO/MRC-DPRU5124/2010/G2P[4]	97.0	82.7	97.0	82.7	97.5	97.5	97.5	97.0	97.0	82.7	82.7	82.7	82.7	82.2	82.7	97.5	97.5	97.5	97.5	97.5	97.5	82.2	82.2	82.7	82.7	82.7	82.2	82.7	83.2	97.0	97.5	98.5	98.5	97.0						
KP[8]82306/RVA/Human-wt/GHA/Ghan-002/2008/G2P[4]	97.5	83.2	97.5	83.2	98.0	98.0	98.0	97.5	97.5	83.2	83.2	83.2	83.2	82.7	83.2	98.0	98.0	98.0	98.0	98.0	98.0	83.2	82.7	83.2	83.2	83.2	82.7	83.2	83.8	97.5	98.0	99.0	99.0	97.5	98.5					
KP753177/RVA/Human-wt/UGA/MRC-DPRU3710/2009/G2P[4]	98.5	82.7	98.5	82.7	99.0	99.0	99.0	98.5	98.5	84.3	84.3	84.3	84.3	83.8	84.3	99.0	99.0	99.0	99.0	99.0	99.0	83.8	83.8	83.2	84.3	84.3	83.8	84.3	83.8	98.5	99.0	97.0	97.0	98.5	96.4	97.0				
MH171474/RVA/Human-wt/ESP/SS453194/2010/G12P[8]	84.8	98.5	85.3	98.5	85.3	85.3	85.3	85.3	85.8	100.0	100.0	100.0	100.0	99.5	100.0	85.3	85.3	85.3	85.3	85.3	85.3	98.0	99.5	99.0	100.0	100.0	99.5	100.0	99.5	84.8	85.3	83.2	83.2	84.8	82.7	83.2	84.3	100.0		
KU361041/RVA/Human-wt/BRA/QUI-89-F4/2010/G1P[8]	84.8	98.5	85.3	98.5	85.3	85.3	85.3	85.3	85.8	100.0	100.0	100.0	100.0	99.5	100.0	85.3	85.3	85.3	85.3	85.3	85.3	98.0	99.5	99.0	100.0	100.0	99.5	100.0	99.5	84.8	85.3	83.2	83.2	84.8	82.7	83.2	84.3	100.0	100.0	
KX954624/RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P[8]	83.2	92.9	83.8	92.9	83.8	83.8	83.8	83.8	84.3	94.4	94.4	94.4	94.4	93.9	94.4	83.8	83.8	83.8	83.8	83.8	83.8	92.9	93.9	93.4	94.4	94.4	94.9	94.4	93.9	83.2	83.8	81.7	81.7	83.2	81.2	82.7	83.2	94.4	94.4	
AB971770/RVA/SugarGlider-tc/JPN/SG385/2012/G27P[36] - outgroup	81.7	82.7	82.2	82.7	82.2	82.2	82.2	82.2	82.7	83.8	83.8	83.8	83.8	83.2	83.8	82.2	82.2	82.2	82.2	82.2	82.2	83.2	83.2	82.7	83.8	83.8	83.2	83.8	83.2	81.7	82.2	80.2	80.2	81.7	80.2	80.2	81.7	83.8	83.8	82.2

Appendix 18. VP6 phylogenetic tree of four Zambian study strains along with representative strains.



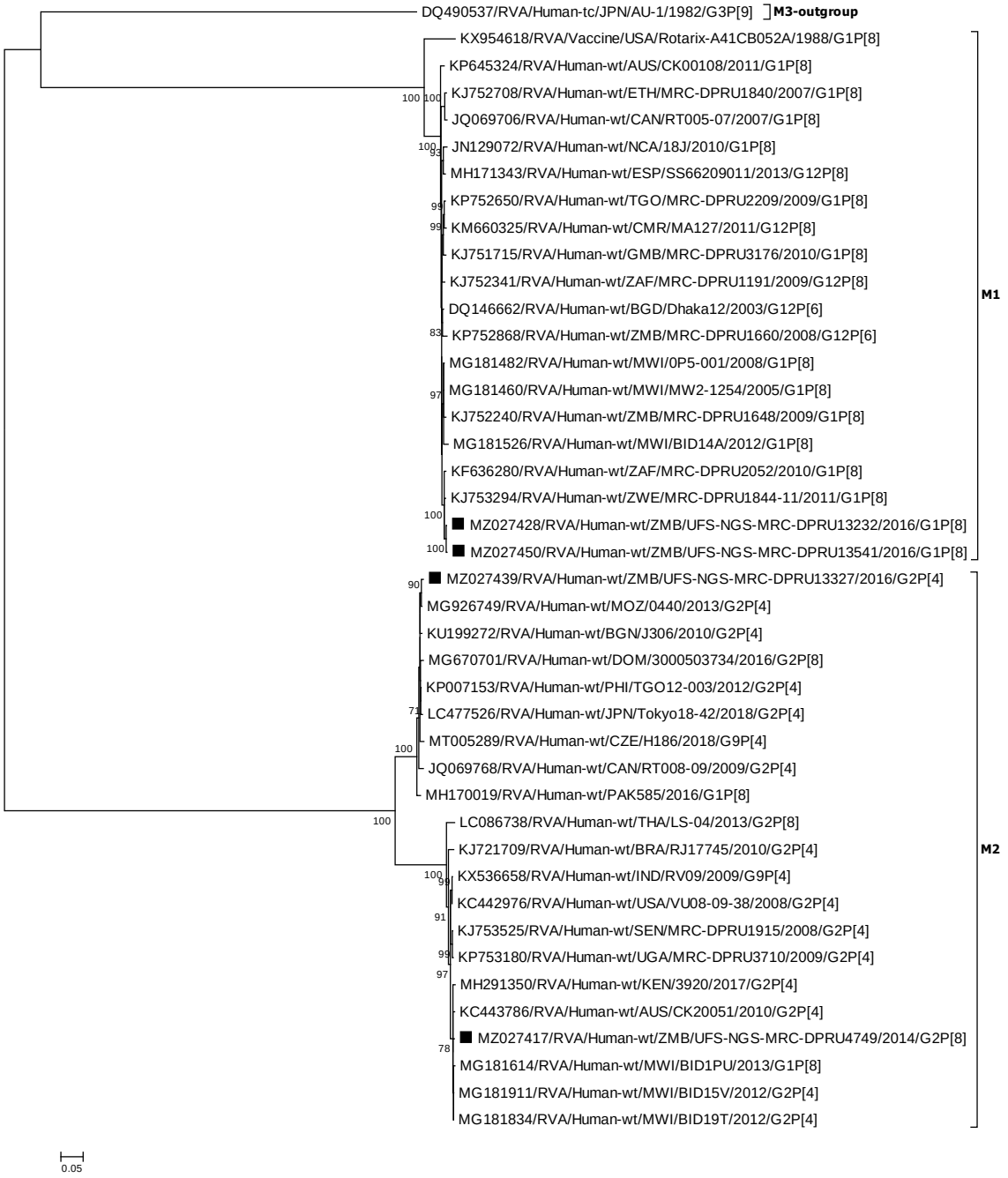
VP6 phylogenetic tree of the four Zambian strains indicated by black squares along with representative strains. Phylogenetic analysis was conducted using the maximum likelihood method with bootstrap values of 1000 replicates. The scale is indicated at the bottom. Percent values of bootstrap values greater than or equal to 70 is indicated on the branch nodes.

Appendix 19: VP2 phylogenetic tree of four Zambian study strains along with representative strains.



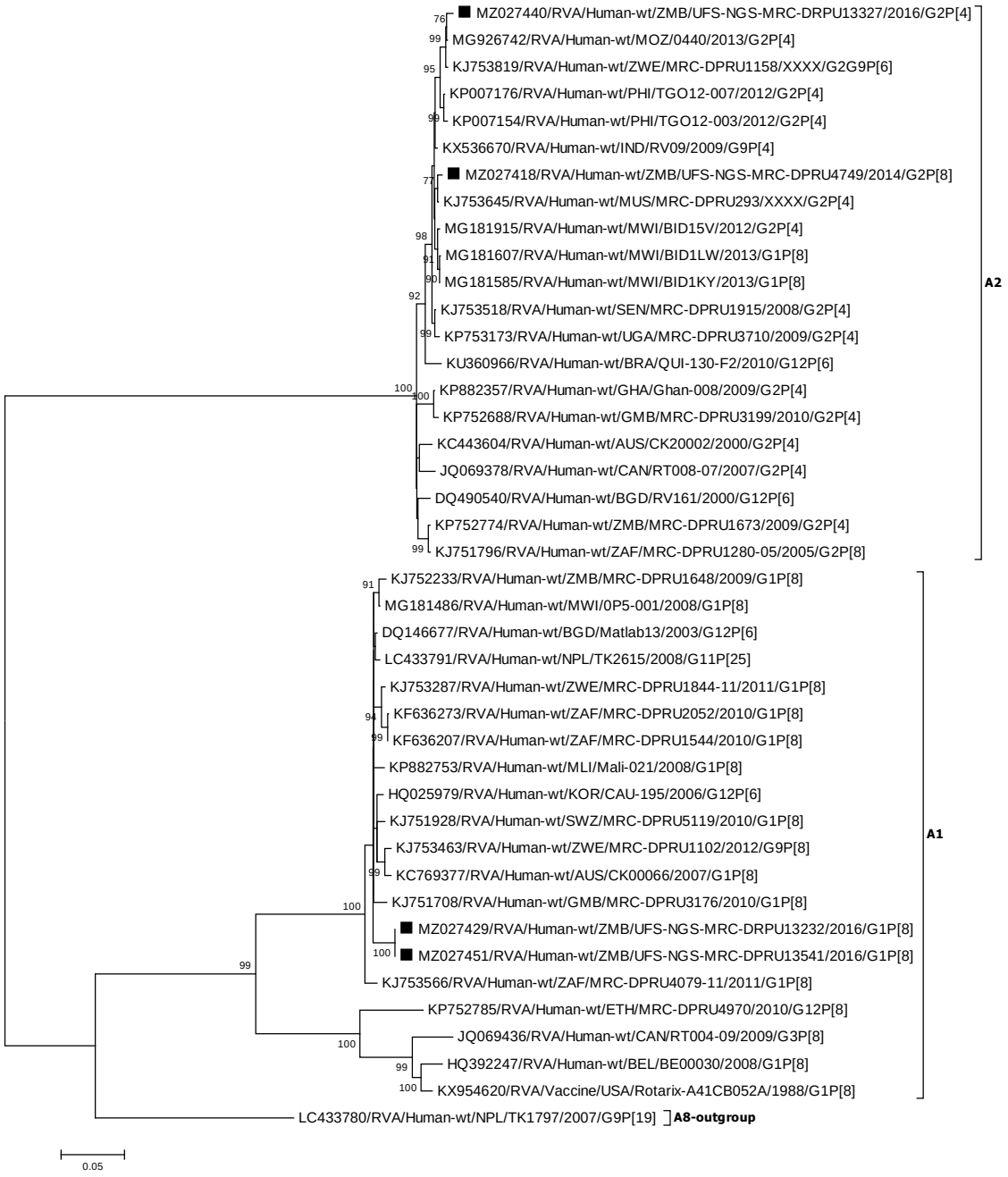
VP2 phylogenetic tree of the four Zambian strains indicated by black squares along with representative strains. Phylogenetic analysis was conducted using the maximum likelihood method with bootstrap values of 1000 replicates. The scale is indicated at the bottom. Percent values of bootstrap values greater than or equal to 70 is indicated on the branch nodes.

Appendix 20: VP3 phylogenetic tree of four Zambian study strains along with representative strains.



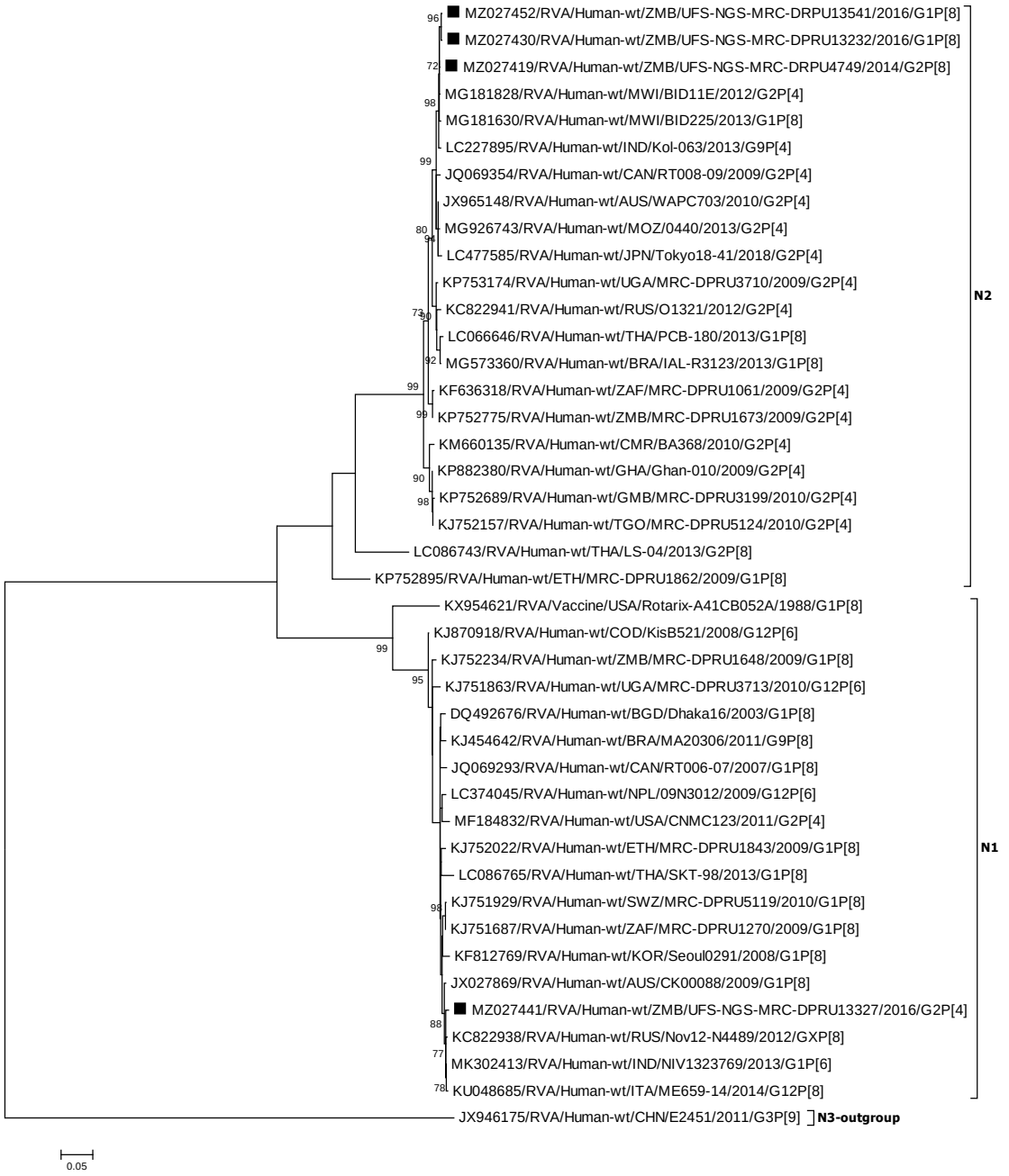
VP3 phylogenetic tree of the four Zambian strains indicated by black squares along with representative strains. Phylogenetic analysis was conducted using the maximum likelihood method with bootstrap values of 1000 replicates. The scale is indicated at the bottom. Percent values of bootstrap values greater than or equal to 70 is indicated on the branch nodes.

Appendix 21: NSP1 phylogenetic tree of four Zambian study strains along with representative strains.



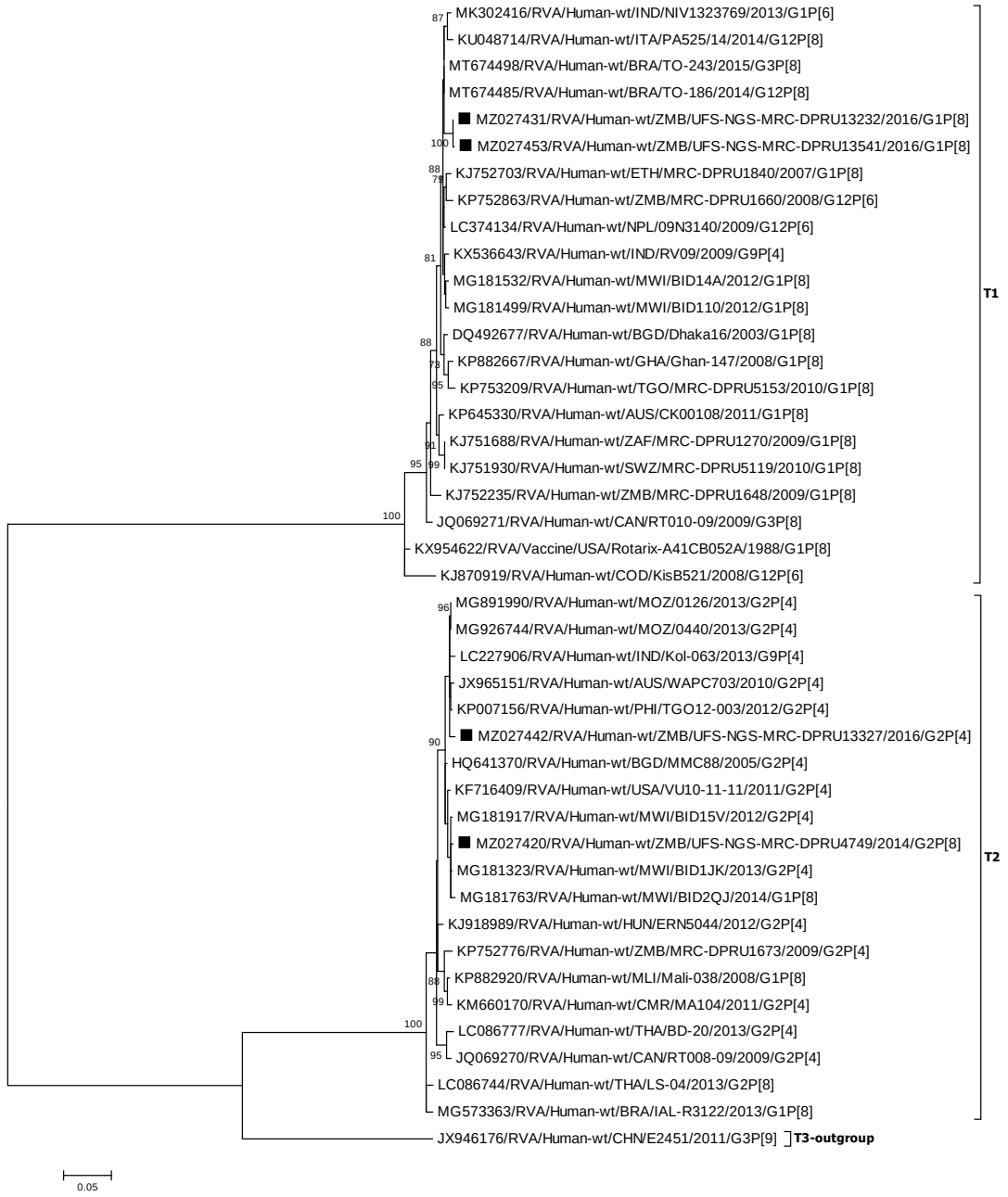
NSP1 phylogenetic tree of the four Zambian strains indicated by black squares along with representative strains. Phylogenetic analysis was conducted using the maximum likelihood method with bootstrap values of 1000 replicates. The scale is indicated at the bottom. Percent values of bootstrap values greater than or equal to 70 is indicated on the branch nodes.

Appendix 22: NSP2 phylogenetic tree of four Zambian study strains along with representative strains.



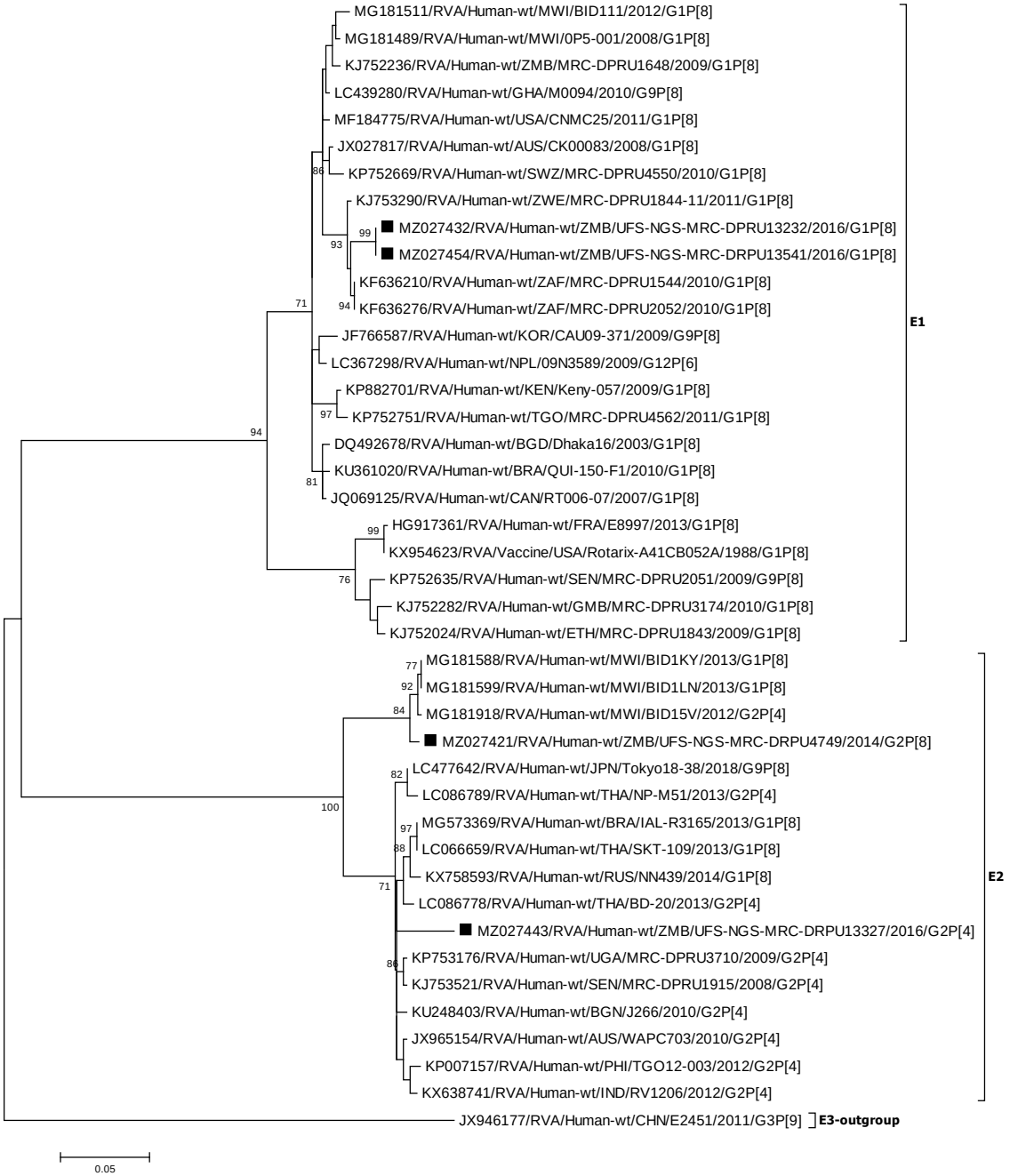
NSP2 phylogenetic tree of the four Zambian strains indicated by black squares along with representative strains. Phylogenetic analysis was conducted using the maximum likelihood method with bootstrap values of 1000 replicates. The scale is indicated at the bottom. Percent values of bootstrap values greater than or equal to 70 is indicated on the branch nodes.

Appendix 23: NSP3 phylogenetic tree of four Zambian study strains along with representative strains.



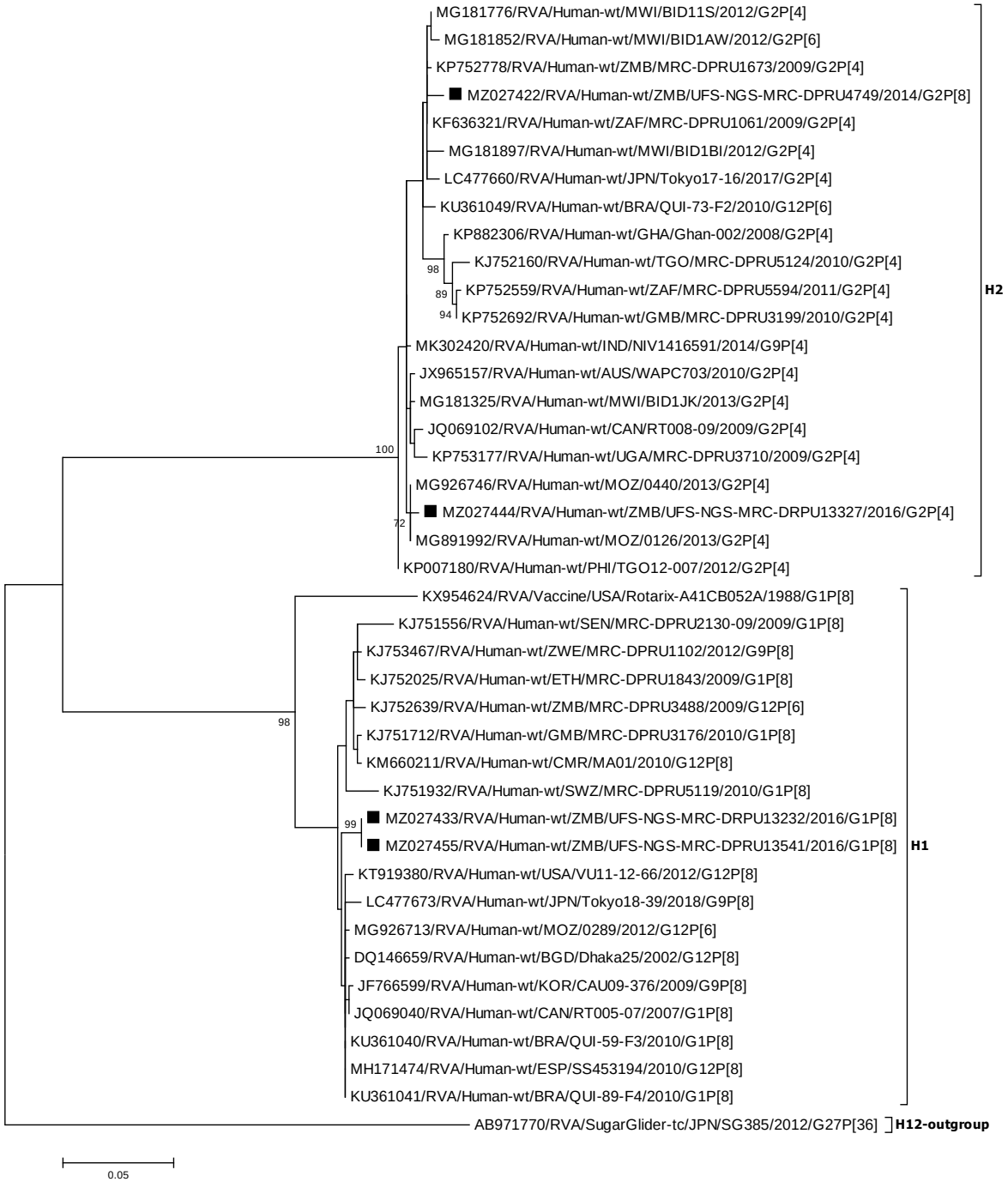
NSP3 phylogenetic tree of the four Zambian strains indicated by black squares along with representative strains. Phylogenetic analysis was conducted using the maximum likelihood method with bootstrap values of 1000 replicates. The scale is indicated at the bottom. Percent values of bootstrap values greater than or equal to 70 is indicated on the branch nodes.

Appendix 24: NSP4 phylogenetic tree of four Zambian study strains along with representative strains.



NSP4 phylogenetic tree of the four Zambian strains indicated by black squares along with representative strains. Phylogenetic analysis was conducted using the maximum likelihood method with bootstrap values of 1000 replicates. The scale is indicated at the bottom. Percent values of bootstrap values greater than or equal to 70 is indicated on the branch nodes.

Appendix 25: NSP5 phylogenetic tree of four Zambian study strains along with representative strains.



NSP5 phylogenetic tree of the four Zambian strains indicated by black squares along with representative strains. Phylogenetic analysis was conducted using the maximum likelihood method with bootstrap values of 1000 replicates. The scale is indicated at the bottom. Percent values of bootstrap values greater than or equal to 70 is indicated on the branch nodes.